This International Student Edition is for use outside of the U.S.

Marjorie Kelly Cowan
Heidi Smith
with Jennifer Lusk
BSN RN CCRN

Third Edition

Microbiology FUNDAMENTALS

A Clinical Approach

Meet The Microbiome
What you need to know about the microbiome as it relates to each chapter.

Epidemiology
Disease tables have epidemiology statistics for every organism.

NCLEX®-Style Questions
Inside and Online!

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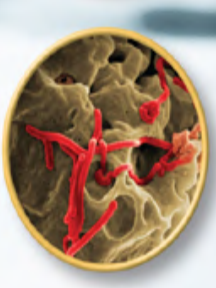

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MICROBIOLOGY FUNDAMENTALS

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Brief Contents

CHAPTER 1 Introduction to Microbes and Their Building Blocks
CHAPTER 2 Tools of the Laboratory: Methods for the Culturing and Microscopic Analysis of Microorganisms
CHAPTER 3 Bacteria and Archaea
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CHAPTER 5 Viral Structure and Multiplication
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CHAPTER 21 Infectious Diseases Affecting the Genitourinary System

CHAPTER 22 One Health: The Interconnected Health of the Environment, Humans, and Other Animals

With contributions from Ronald M. Atlas and Marjorie Kelly Cowan

About the Authors

Courtesy Kelly Cowan

Kelly Cowan, PhD, started teaching microbiology at Miami University in 1993. Her specialty is teaching microbiology for pre-nursing/allied health students at the university's Middletown campus, a regional open-admissions campus. She started life as a dental hygienist. She then went on to attain her PhD at the University of Louisville, and later worked at the University of Maryland's Center of Marine Biotechnology and the University of Groningen in The Netherlands. Kelly has published (with her students) 24 research articles stemming from her work on bacterial adhesion mechanisms and plant-derived antimicrobial compounds. But her first love is teaching—both doing it and studying how to do it better. She is past chair of the Undergraduate Education Committee of the American Society for Microbiology (ASM). Her current research focuses on the student achievement gap associated with economic disparities, as well as literacy in the science classroom. In her spare time, Kelly hikes, reads, and still tries to (s)mother her three grown kids.

©Heidi Smith

Heidi Smith leads the microbiology discipline at Front Range Community College, Fort Collins, Colorado. Collaboration with other faculty across the nation, the development and implementation of new digital learning tools, and her focus on student learning outcomes have revolutionized her face-to-face and online teaching approaches and student performance in her classes. Outside of the classroom, Heidi oversees a federal grant program designed to train and support underrepresented students for undergraduate research and transfer into four-year STEM degree programs. She is also an active member of the American Society for Microbiology and participated as a task force member for the development of their Curriculum Guidelines for Undergraduate Microbiology Education. Off campus, Heidi enjoys the beautiful Colorado outdoors with her husband and three young children.

©Tia Brayman

Jennifer Lusk, BSN, RN, CCRN, is a registered nurse at a large academic children's hospital in Denver, Colorado. She has practiced in pediatric intensive care for 10 years in large inner-city pediatric hospitals. Jennifer has spent her nursing career caring for critically ill children as a bedside nurse, charge nurse, and Continuous Renal Replacement Therapy (CRRT) specialist. She is the CRRT Clinical Program Coordinator, providing oversight and program development for the critical care dialysis therapy. She enjoys her diverse clinical role, which involves educating nurses and physicians, mentoring, researching, writing policies, and quality improvement work. In her time away from work,

Jennifer enjoys spending time outdoors with her husband and dog, especially hiking and exploring national parks.

Preface

Students:

Welcome! I am so glad you are here. I am very excited for you to try this book. I wrote it after years of frustration, teaching from books that didn't focus on the right things that my students needed. My students (and, I think, you) need a solid but not overwhelming introduction to microbiology and infectious diseases. I asked myself: What are the major concepts I want my students to remember five years from now? And then I worked backward from there, making sure everything pointed to the big picture.

While this book has enough detail to give you context, there is not so much detail that you will lose sight of the major principles. Biological processes are described right next to the illustrations that illustrate them. The format is easier to read than most books, because there is only one column of text on a page and wider margins. The margins gave me space to add interesting illustrations and clinical content. A working nurse, Jennifer Lusk, brings her experience to life on the pages and shows you how this information will matter to you when you are working as a health care provider. We have interesting and up-to-the-moment Case Files, Medical Moments, Microbiome selections, and NCLEX® questions in every chapter. My coauthor, Heidi Smith, has brought so much to the book and online material. I don't think you'll find a better online set of learning tools anywhere.

I really wanted this to be a different kind of book. I've started using it in my own classes and my students love it! Well, maybe they have to say that, but I hope you truly do enjoy it and find it to be a refreshing kind of science book.

—Kelly Cowan

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I dedicate this book to every hard-working student studying in a health care field.—Kelly

I dedicate this book to my favorite people, Ryan, Noah, Ryleigh, and Jake.—Heidi

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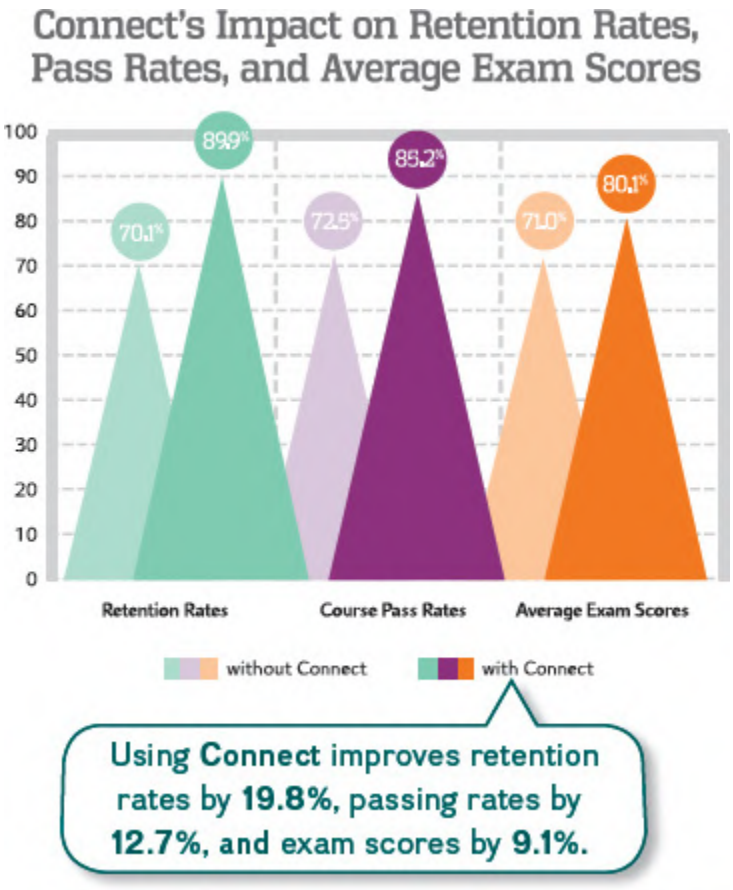

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- Connect automatically grades assignments and quizzes, providing easy-to-read reports on individual and class performance.

More students earn **As** and **Bs** when they use McGraw-Hill Education **Connect**.

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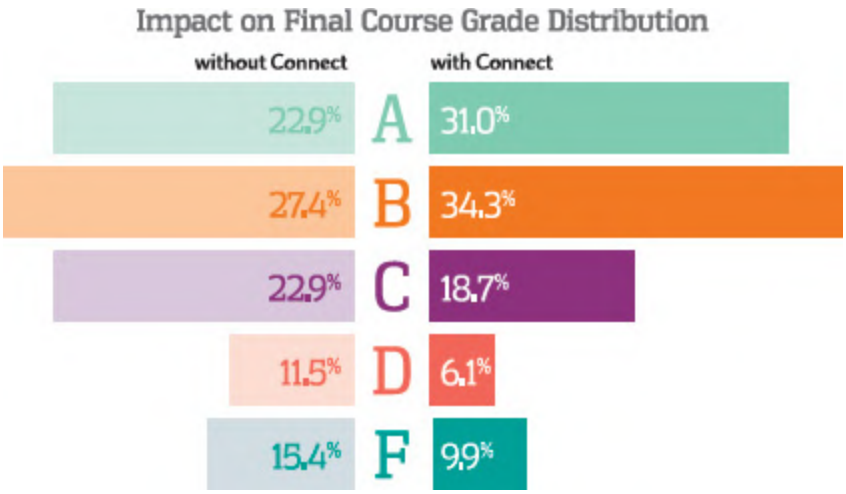

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www.mheducation.com/connect

UNIQUE INTERACTIVE QUESTION TYPES

Source: CDC/Janice Haney Carr

Unique Interactive Question Types in Connect, Tagged to ASM's Curriculum Guidelines for Undergraduate Microbiology

1. **Case Study:** Case studies come to life in a learning activity that is interactive, self-grading, and assessable. The integration of the cases with videos and animations adds depth to the content, and the use of integrated questions forces students to stop, think, and evaluate their understanding. Pre- and post-testing allow instructors and students to assess their overall comprehension of the activity.

2. **Concept Maps:** Concept maps allow students to manipulate terms in a hands-on manner in order to assess their understanding of chapter-wide topics. Students become actively engaged and are given immediate feedback, enhancing their understanding of important concepts within each chapter.

3. **What's the Diagnosis:** Specifically designed for the disease chapters of the text, this is an integrated learning experience designed to assess the student's ability to utilize information learned in the preceding chapters to successfully culture, identify, and treat a disease-causing microbe in a simulated patient scenario. This question type is true experiential learning and allows the students to think critically through a real-life clinical situation.

4. **SmartGrid Questions:** New to this edition, SmartGrid questions replace the traditional end-of-chapter questions, and all of these questions are available for assignment in Connect. These questions were carefully constructed to assess chapter material as it relates to all six concepts outlined in the American Society of Microbiology curriculum guidelines plus the competency of "Scientific Thinking." The questions are cross-referenced with Bloom's taxonomy of learning level. Seven concepts/competencies x three increasing Bloom's levels = a robust assessment tool.

5. **Animations:** Animation quizzes pair our high-quality animations with questions designed to probe student understanding of the illustrated concepts.

6. **Animation Learning Modules:** Animations, videos, audio, and text all combine to help students understand complex processes. These tutorials take a stand-alone, static animation and turn it into an interactive learning experience for your students with real-time remediation. Key topics have an Animated Learning Module assignable through Connect.

7. **Labeling:** Using the high-quality art from the textbook, check your students' visual understanding as they practice interpreting figures and learning structures and relationships.

8. **Classification:** Ask students to organize concepts or structures into categories by placing them in the correct "bucket."

9. **Sequencing:** Challenge students to place the steps of a complex process in the correct order.

10. **Composition:** Fill in the blanks to practice vocabulary, and then reorder the sentences to form a logical paragraph (these exercises may qualify as "writing across the curriculum" activities!).

All McGraw-Hill Connect content is tagged to Learning Outcomes for each chapter as well as topic, section, Bloom's Level, ASM topic, and ASM Curriculum Guidelines to assist you in customizing assignments and in reporting on your students' performance against these points. This will enhance your ability to assess student learning in your courses by allowing you to align your learning activities to peer-reviewed standards from an international organization.

NCLEX®

NCLEX® Prep Questions: Sample questions are available in Connect to assign to students, and there are questions throughout the book as well.

Source: CDC/Janice Haney Carr (*S. aureus* and *Legionella*); Source: CDC/Dr. Erskine Palmer & Byron Skinner (Rotavirus); Source: CDC/Dr. Stan Erlandsen (*Giardia* cyst); ©Science Photo Library/Getty Images (white blood cells); ©Steve Gschmeissner/Science Source (fallopian tube surface); NIAID, NIH/Rocky Mountain Laboratories (*Salmonella typhimurium*)

ADDITIONAL RESOURCES

Create what you've only imagined.

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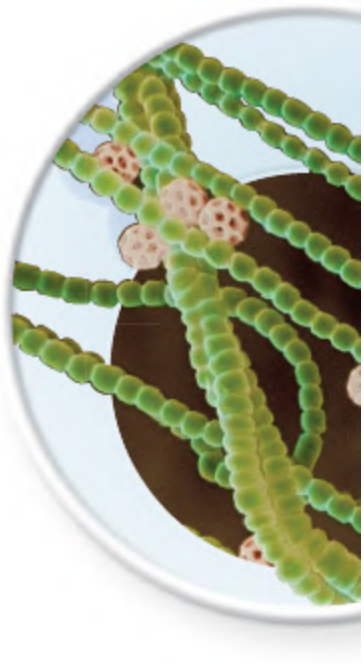

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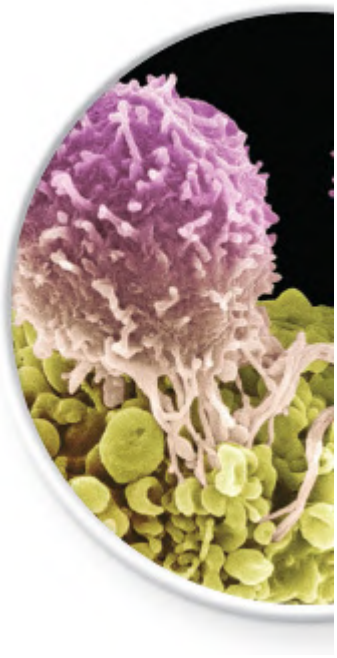
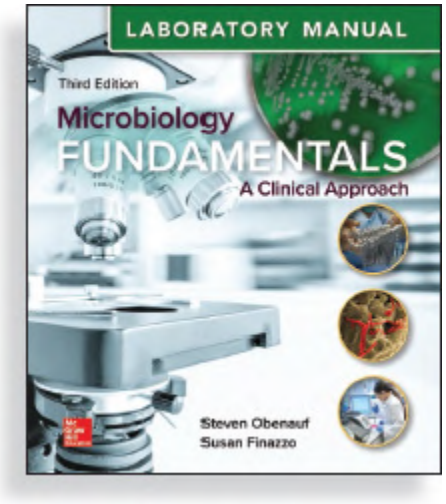
McGraw-Hill Campus® is a groundbreaking service that puts world-class digital learning resources just a click away for all your faculty and students. All your faculty—whether or not they use a McGraw-Hill Education title—can instantly browse, search, and access the entire library of McGraw-Hill Education instructional resources and services including eBooks, test banks, PowerPoint slides, animations, and learning objects—from any Learning Management System (LMS), at no additional cost to your institution. Users also have single sign-on access to McGraw-Hill Education digital platforms, including Connect, ALEKS®, Create, and Tegrity.

LearnSmart® Prep is designed to get students ready for a forthcoming course by quickly and effectively addressing prerequisite knowledge gaps that may cause problems down the road. LearnSmart Prep maintains a continuously adapting learning path individualized for each student, and tailors content to focus on what the student needs to master in order to have a successful start in the new class.





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Microbiology Fundamentals Laboratory Manual, Third Edition

Steven Obenauf, Broward College
Susan Finazzo, Perimeter College, Georgia State University

Written specifically for pre-nursing and allied health microbiology students, this manual features brief, visual exercises with a clinical emphasis.

CLINICAL

Clinical applications help students see the relevance of microbiology.

Case File Each chapter begins with a case written from the perspective of a former microbiology student.

These high-interest introductions provide a specific example of how the chapter content is relevant to real life and future health care careers.

CASE FILE

Wound Care

I was an RN working in a large city hospital on a medical floor. A lot of our patients had diabetes and were suffering various complications of the disease, particularly diabetic wounds caused by poor circulation. Wound care was a large part of my job. After 2 years on the unit, I decided to pursue wound care certification. Once I became a wound care specialist, I continued to work in the same hospital and saw patients with complicated and/or chronic wounds.

Mr. Jones was one of the first patients I consulted about after I became certified. He was an elderly gentleman who had lost his sight due to diabetes.

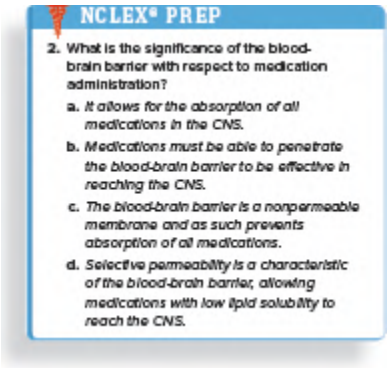
SmartGrid: From Knowledge to Critical Thinking

SmartGrid

In place of traditional end-of-chapter questions, Kelly Cowan has created a grid made up of three columns and seven rows, for a total of 21 questions. The rows contain the six major curricular guidelines (and the competency of *scientific thinking*) from the American Society for Microbiology. The columns represent increasing levels of Bloom's taxonomy of learning. Each question is carefully constructed of material from the chapter that meets both the ASM guideline and the Bloom's level indicated. Instructors can assign a row (to emphasize a curriculum guideline) or a column (asking a variety of questions at a particular Bloom's level). The questions in column 3 (Bloom's level 5 and 6) can easily be used for group problem solving and other higher-order learning activities.

NCLEX® Prep Questions Found throughout the chapter, these multiple-choice questions are application-oriented and designed to help students learn the microbiology information they will eventually need to pass the NCLEX® examination. Students will begin learning to think critically, apply information, and over time, prep themselves for the examination.

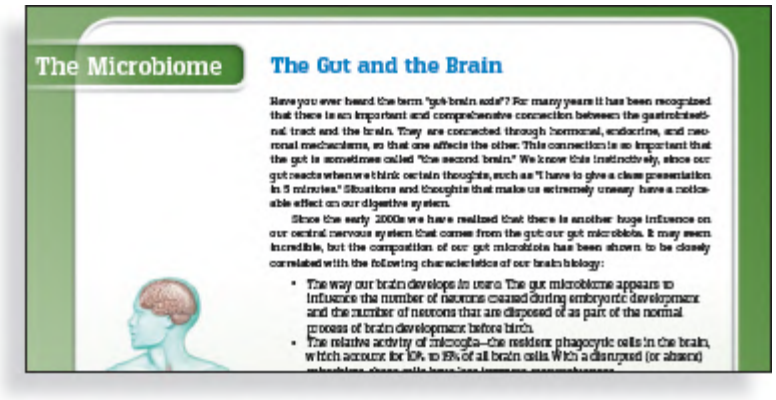
Additional questions are available in Connect for homework and assessment.



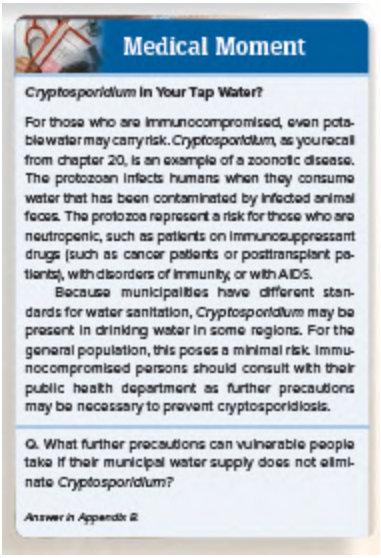
NCLEX® PREP

2. What is the significance of the blood-brain barrier with respect to medication administration?
   a. *It allows for the absorption of all medications in the CNS.*
   b. *Medications must be able to penetrate the blood-brain barrier to be effective in reaching the CNS.*
   c. *The blood-brain barrier is a nonpermeable membrane and as such prevents absorption of all medications.*
   d. *Selective permeability is a characteristic of the blood-brain barrier, allowing medications with low lipid solubility to reach the CNS.*

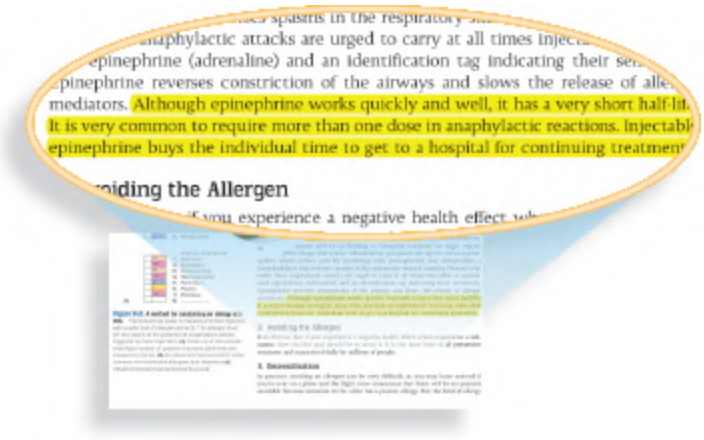
The Microbiome Each chapter ends with a reading about a microbiome discovery or story that is relevant to that chapter.



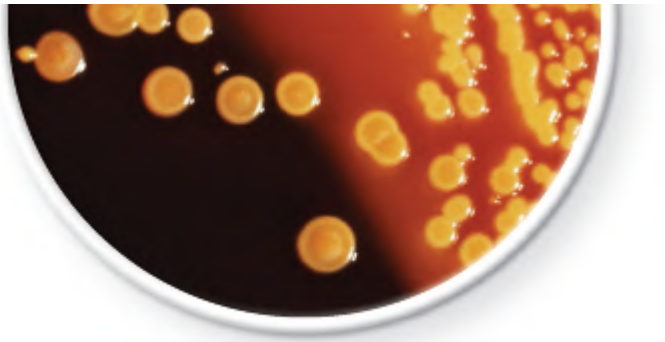
Medical Moment These boxes give students a more detailed clinical application of a nearby concept in the chapter. New in this edition: Each Medical Moment ends with a question. Answers appear in Appendix B.



Clinical Examples Throughout Clinical insights and examples are woven throughout the chapter—not just in boxed elements.


spasms in the respiratory
anaphylactic attacks are urged to carry at all times injec
epinephrine (adrenaline) and an identification tag indicating their se
epinephrine reverses constriction of the airways and slows the release of alle
mediators. Although epinephrine works quickly and well, it has a very short half-li
It is very common to require more than one dose in anaphylactic reactions. Injectabl
epinephrine buys the individual time to get to a hospital for continuing treatmen
oiding the Allergen
you experience a negative health effect wh

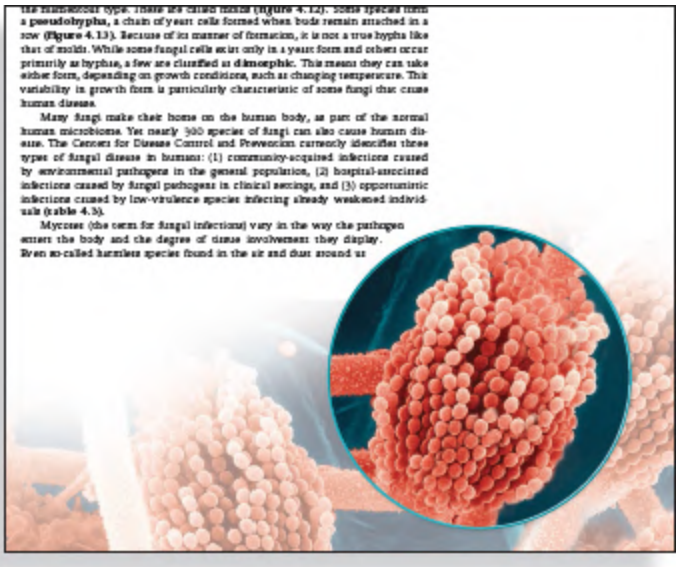
VISUAL



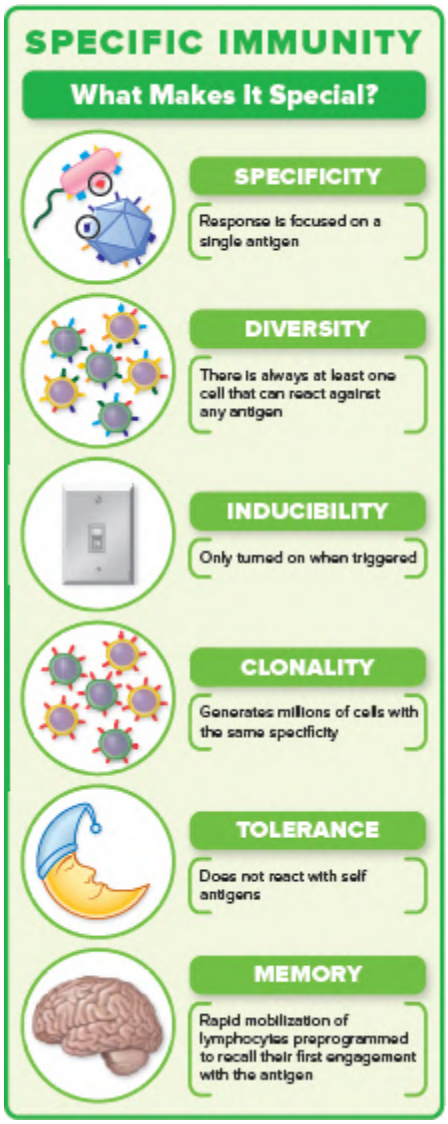
Source: CDC

Visually appealing layouts and vivid art closely linked to narrative for easier comprehension.

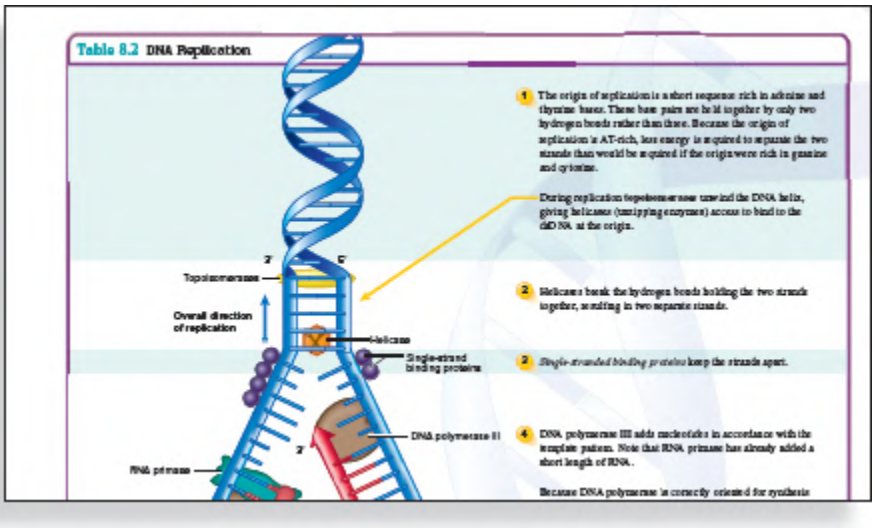
Engaging, Accurate, and Educational Art Single column of text is easier to read and leaves space for eye-catching art to keep students engaged.



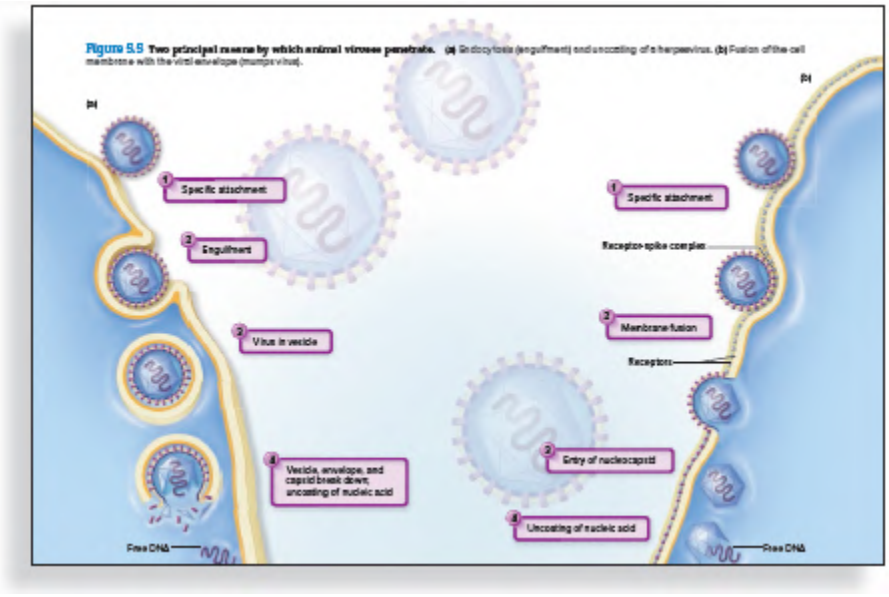
Infographics New infographic-style visual summaries that students can relate to.



Visual Tables The most important points explaining a concept are distilled into table format and paired with explanatory art.



Process Figures Complex processes are broken into easy-to-follow steps. Numbered steps in the art coordinate with numbered text boxes to walk students through the figure.



BRIEF

Streamlined coverage of core concepts help students retain the information they will need for advanced courses.

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Chemistry topics required for understanding microbiology are combined with the foundation content found in chapter 1.

Genetics content is synthesized into one chapter covering the concepts that are key to microbiology students.

A chapter in microbiology textbooks that is often not used in health-related classes becomes relevant because it presents the 21st-century idea of "One Health"—that the environment and animals influence human health and infections.

Brief Contents

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CHAPTER 22 One Health: The Interconnected Health of the Environment, Humans, and Other Animals 678

With contributions from Ronald M. Atlas and Marjorie Kelly Cowan

Duplication Eliminated Detail is incorporated into figures so students can learn in context with the art. This allows a more concise narrative flow while still retaining core information.

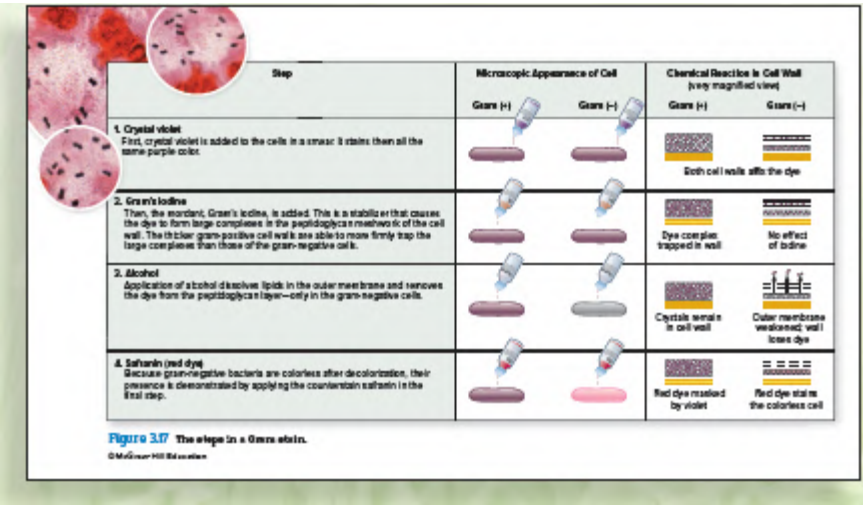

Figure 3.17 The steps in a Gram stain.

Changes to the Third Edition

Significant Changes

New end-of-chapter feature organizes 21 questions in a grid. The rows contain the six major curricular guidelines (and the competency of *scientific thinking*) from the American Society for Microbiology. The columns represent increasing levels of Bloom's Taxonomy of learning. Each question is carefully constructed of material from the chapter that meets both the ASM guideline and the Bloom's level indicated. Instructors can assign a row (to emphasize a curriculum guideline) or a column (asking a variety of questions at a particular Bloom's level). The questions in column three (Bloom's level 5 and 6) can easily be used for group problem solving and other higher-order learning activities.

Microbiome information in every chapter and also as a separate boxed feature at the end of the chapter. These features also walk students through how to critically analyze the onslaught of studies and findings.

Infographic-style tables present material in a way that students can connect with.

The Medical Moments now each end with an open-ended question. Answers to the questions are in Appendix B.

Chapter 12 (Host Defenses I: Overview and Nonspecific Defenses) is almost completely reorganized and rewritten based on feedback from reviewers and students.

Category A bioterror threat organisms are indicated in disease tables.

Disease chapter feature A new visual feature in each disease chapter (chapters 16 through 21) places the microbes from that chapter in context with respect to ***communicability*** and ***deadliness*****.**

Organisms denoted In all disease tables, each organism is denoted as "B, V, F, P, or H"—indicating bacterium, virus, etc. When bacterial, the table also shows G+ or G−.

Chapter Highlights

Chapter 1 More explicit explanation of the scientific method.

Chapter 2 Illustrations made simpler and clearer.

Chapter 3 Addition of nanotube/nanowires as bacterial appendages; additional discussion of bacterial cytoskeletal proteins.

Chapter 4 Added endosymbiotic theory (incorporating last common ancestor and role of DNA viruses); neglected parasitic infections in the United States; and a table with examples of community-acquired, hospital-acquired, and opportunistic infections.

Chapter 5 New prion disease (MSA).

Chapter 6 Expansion of the binary "saprobe" and "parasite"—so that nonsaprobes can be seen as normal biota as well as parasites. Changes to discussion of "commensal" versus "mutualistic" in light of importance of microbiome to human health.

Chapter 7 Simplified the language and added discussion of "rock-eating" bacteria.

Chapter 8 Added single-nucleotide polymorphisms, deep DNA sequencing, synthetic biology, gene therapy, and CRISPR.

Chapter 9 Added new FDA ban on triclosan and other chemicals.

Chapter 10 Comprehensive updates of currently used antibiotics; new antibiotic strategies from noncultivable microorganisms, peptides, RNA interference, CRISPR; new discussion of allylamine class of antifungals; and of colistin-resistant bacteria.

Chapter 11 Extensively rewritten to include newest understanding of human microbiome, epigenetic host changes that microbes cause, big data in epidemiology, more emphasis on polymicrobial infections, new epidemiological feature.

Chapter 12 Changed overall organization for greater understanding; simplified cytokine section, added role of microbiome in nonspecific immunity, rewrote antimicrobial products section and added host restriction factors; simplified the figure showing origins of blood cells; added diagram of lymph node.

Chapter 13 Enhanced emphasis on clonal deletion; changed T-cell terminology to include T follicular helper cells; added infographic on how vaccines are prepared, tested, and come to market.

Chapter 14 Cancer's ability to dampen immune response; new ways to desensitize against allergens, role of the gut microbiome in hypersensitivities and in autoimmune

disease.

Chapter 15 Decreased immunology section to accommodate more genetic testing; included MGIT testing, more point-of-care diagnostics, PCR, qPCR, pan bacterial qPCR, new strategies in development such as identifying all the viruses a person has ever encountered in a single drop of blood; new infographics summarize the testing procedures for phenotypic, genotypic, and immunological methods in a visually consistent manner.

Chapter 16 Updated normal biota sections.

Chapter 17 Zika added as highlight disease; another opportunity (in Microbiome feature) to discuss difference between correlation and causation and how media report it.

Chapter 18 Information on new children's malaria vaccine; updates on Ebola epidemic and lessons learned; added *Ehrlichia, Anaplasma*, and *Babesia* to nonhemorrhagic diseases.

Chapter 19 Added human metapneumovirus as agent of pneumonia.

Chapter 20 More information about other Shiga-toxin-producing *E. coli* (in addition to O157:H7); non-cholera *Vibrio* species.

Chapter 21 Carbapenem-resistance in UTIs; the significant increase in STIs in the United States (2015 CDC report). All updated statistics; table clearly outlining who should get HPV vaccine.

Chapter 22 New list of emerging and reemerging diseases; new infographic of WHO predictions about health effects of climate change.

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I am always most grateful to my students in my classes. They teach me every darned day how to do a better job helping them understand these concepts that are familiar to me but new to them. All the instructors who reviewed the manuscript were also great allies. I thank them for lending me some of their microbiological excellence. I had several contributors to the book and digital offerings—Jennifer Smith, Ronald Atlas, Kathleen Sandman, Dorothy Wood, Nicole Frank, and Connie Fisk chief among them. Heidi Smith improves everything she touches, especially this book. Jennifer Lusk added very meaningful medical insights and clinical content. My handlers at McGraw-Hill Education make the wheels go around. They include Marija Magner, Valerie Kramer, Jessica Portz, Matt Backhaus, Christina Nelson, and Lori Hancock. Darlene Schueller, my day-to-day editor, is a wonderful human being and taskmaster, in that order. In short, I'm just a lucky girl surrounded by talented people.

—Kelly Cowan

I, too, am thankful for the many students that enter my classroom every year. My work was greatly inspired by their questions, their ideas, and their unique way of looking at complex things. I am filled with gratitude to Kelly Cowan for her willingness to partner with me; I constantly learn from her about better ways to reach all students. Writing a solid digital package requires more than just one mind; for this work, Dorothy Wood brought her expertise and creativity in writing critical thinking questions for every chapter. Finally, I appreciate the entire microbiology team at McGraw-Hill Education who truly believes in producing materials that lead to student success.

—Heidi Smith

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We are very pleased to have been able to incorporate real student data points and input, derived from thousands of our SmartBook users, to help guide our revision. SmartBook heat maps provided a quick visual snapshot of usage of portions of the text and the relative difficulty students experienced in mastering the content. With these data, we were able to hone not only our text content but also the SmartBook questions.

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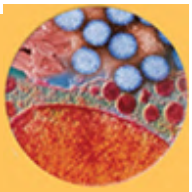

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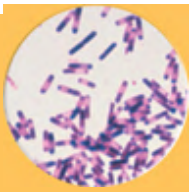

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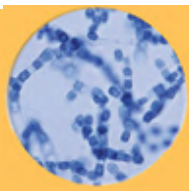

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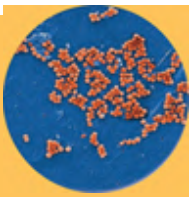

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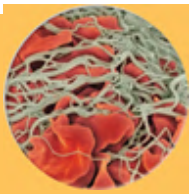

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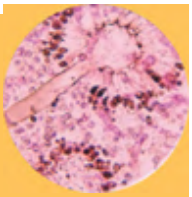

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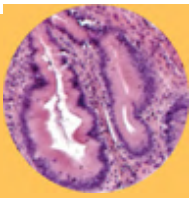

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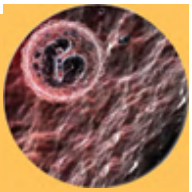

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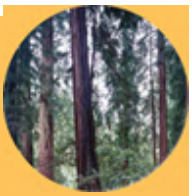

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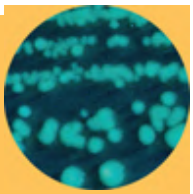

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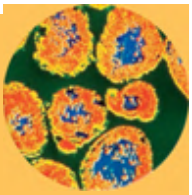

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APPENDIX B Answers to the Medical Moments and NCLEX® Questions

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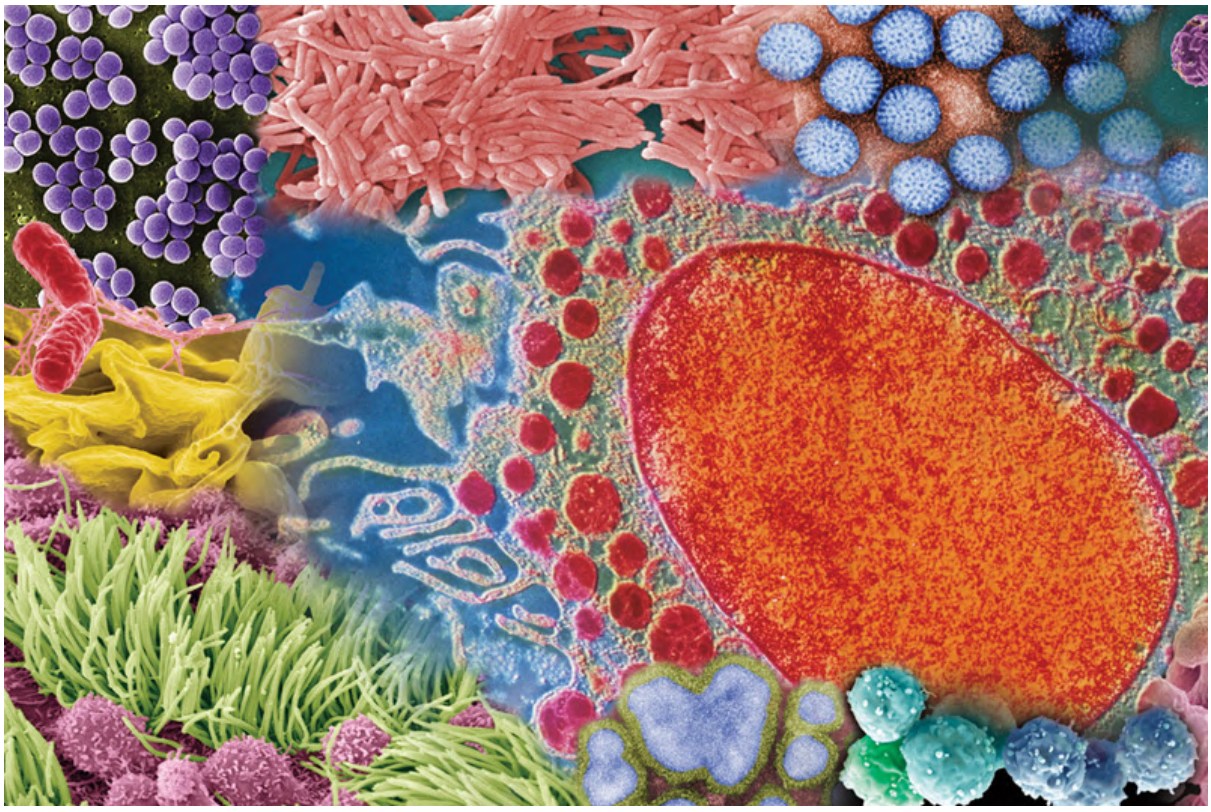


Source: CDC/Janice Haney Carr (*Staphylococcus aureus* and *Legionella*); Source: CDC/Dr. Erskine Palmer & Byron Skinner (rotavirus); Source: CDC/Dr. Stan Erlandsen (*Giardia* sp. cyst); ©Science Photo Library/Getty Images (white blood cell); ©Steve Gschmeissner/Science Source (fallopian tube, SEM); NIAID, NIH/Rocky Mountain Laboratories (*Salmonella typhimurium*); ©Michael Williams (photo of Kelly Cowan with student); Jerome Wexler/Science Source (algae)

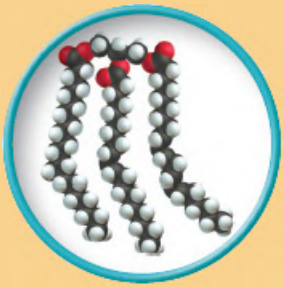
IN THIS CHAPTER…

1.1 Microbes: Tiny but Mighty

1. List the various types of microorganisms that can colonize humans.
2. Describe the role and impact of microbes on the earth.
3. Explain the theory of evolution and why it is called a theory.
4. Explain the ways that humans manipulate organisms for their own uses.
5. Summarize the relative burden of human disease caused by microbes.
6. Differentiate among bacteria, archaea, and eukaryotic microorganisms.
7. Identify an acellular infectious agent that is studied in microbiology.
8. Compare and contrast the relative sizes of the different microbes.

1.2 Microbes in History

9. Make a time line of the development of microbiology from the 1600s to today.
10. List some recent microbiology discoveries of great impact.
11. Identify the important features of the scientific method.



1.3 Macromolecules: Superstructures of Life

12. Name the four main families of biochemicals.
13. Provide examples of cell components made from each of the families of biochemicals.
14. Differentiate among primary, secondary, tertiary, and quaternary levels of protein structure.
15. List the three components of a nucleotide.
16. Name the nitrogen bases of DNA and RNA.
17. List the three components of ATP.
18. Recall three characteristics common to all cells.

1.4 Naming, Classifying, and Identifying Microorganisms

19. Differentiate among the terms *nomenclature, taxonomy*, and *classification*.
20. Create a mnemonic device for remembering the taxonomic categories.
21. Correctly write the binomial name for a microorganism.
22. Draw a diagram of the three major domains.
23. Explain the difference between traditional and molecular approaches to taxonomy.

CASE FILE

The Subject Is You!

At the beginning of every chapter in this book a different health care worker will tell you a story about something "microbiological" that happened to him or her in the line of duty. For this first chapter, though, I am claiming "dibs" as author and am going to introduce myself to you by telling you about the first day of class in my course.

Long ago I noticed that students have a lot of anxiety about their microbiology course. I know that starts you out with one strike against you, as attitudes are such powerful determinants of our success. So on the first day of class I often spend some time talking with students about how much they already know about microbiology.

Sometimes I start with "How many of you have taken your kids for vaccinations?" since in the classes I teach very many students are parents. Right away students will tell me why they or friends they know have not vaccinated their children and I can tell them there's a sophisticated microbiological concept they are referencing, even if they aren't naming it: *herd immunity*, discussed in chapter 11 of this book.

My favorite question (now that we're all warmed up) is "Who knows someone—whom you don't have to identify—who has had a really unusual or scary infection?" A surprising number of people have known someone who has had malaria, or leptospirosis, or endocarditis, or encephalitis. Then the conversation gets interesting as students begin to

understand how much they already know about microbiology, and the class is not going to be as intimidating as they thought.

- Think about how many times you have taken antibiotics in the past few years. What is special about antibiotics that they are only given to treat infections?
- What is the most unusual infection you have ever encountered among family or friends or patients you have cared for?

Case File Wrap-Up appears at the end of the chapter.

1.1 Microbes: Tiny but Mighty

Microbiology is a specialized area of biology that deals with living things ordinarily too small to be seen without magnification. Such **microscopic** organisms are collectively referred to as **microorganisms** (my″-kroh-or′-gun-izms), **microbes**, or several other terms depending on the kind of microbe or the purpose. There are several major groups of microorganisms that we'll be studying. They are **bacteria, archaea, protozoa, fungi, helminths**, and **viruses**. There is another very important group of organisms called **algae**. They are critical to the health of the biosphere but do not directly infect humans, so we will not consider them in this book. Each of the other six groups contains members that colonize humans, so we will focus on them.

The nature of microorganisms makes them both very easy and very difficult to study—easy because they reproduce so rapidly and we can quickly grow large populations in the laboratory, and difficult because we usually can't see them directly. We rely on a variety of indirect means of analyzing them in addition to using microscopes.

Mycobacterium tuberculosis bacteria
Source: CDC/Janice Carr

Microbes and the Planet

For billions of years, microbes have extensively shaped the development of the earth's habitats and the evolution of other life forms. It is understandable that scientists searching for life on

other planets first look for signs of microorganisms.

Single-celled organisms appeared on this planet about 3.8 billion years ago according to the fossil record. One of these organisms—referred to as LCA, or the Last Common Ancestor—eventually led to the appearance of two newer single cell types, called bacteria and archaea. A little bit later this single-celled ancestor gave rise to **eukaryotic** (yoo-kar′-ee-ot-ic) cells. The type of cell known as LCA no longer exists. Only its "offspring"—bacteria, archaea, and eukaryotes—remain. *Eu-kary* means "true nucleus," and these were the only cells containing a nucleus. Bacteria and archaea have no true nucleus. For that reason, they have traditionally been called **prokaryotes** (pro-kar′-ee-otes), meaning "prenucleus." But researchers are suggesting we no longer use the term *prokaryote* to lump them together because archaea and bacteria are so distinct genetically. If you consider the six types of microorganisms we will be dealing with in this book, you will recognize bacteria and archaea as each having their own domain. The protozoa, fungi, and helminths are all in the domain Eukarya. Viruses do not appear on the tree of life since they are not cells, and not considered living. That sounds strange, but we will delve into that in the virus chapter, which comes later.

Figure 1.1 depicts the resulting tree of life—a diagram of all organisms on the planet. There are two important things to note about this figure. First, all of biologic life falls into these three categories, known as domains. Most of the organisms you are familiar with (animals, plants, etc.) are in one category, Eukarya. Second, these three domains all emerged from a single common cell type (the "stem" at the bottom).

Important Note to Students!

This is your author here. I wanted to alert you right up front that you should look at the figures and read the tables in the chapters. I know that it is human nature to skip these, when you see the reference in the main text (like "**figure 1.4**") and just move on with the next sentence. But in this book I made a real point to put a lot of information in the figures and tables, because it is easier to digest things such as processes and categories when they are presented in a more visual format. And there are a lot of "processes" and "categories" in biology! So I opted for a bit less text, and a bit more pictures and tables. So be sure to make it a point to stop by and examine these visual features. Thanks! Kelly

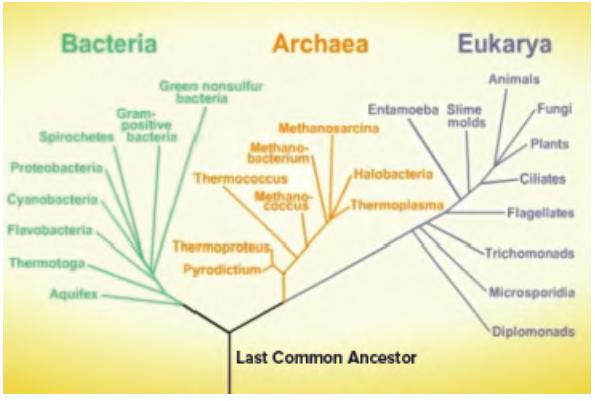


Figure 1.1 **Current view of evolutionary relatedness of all organisms.**
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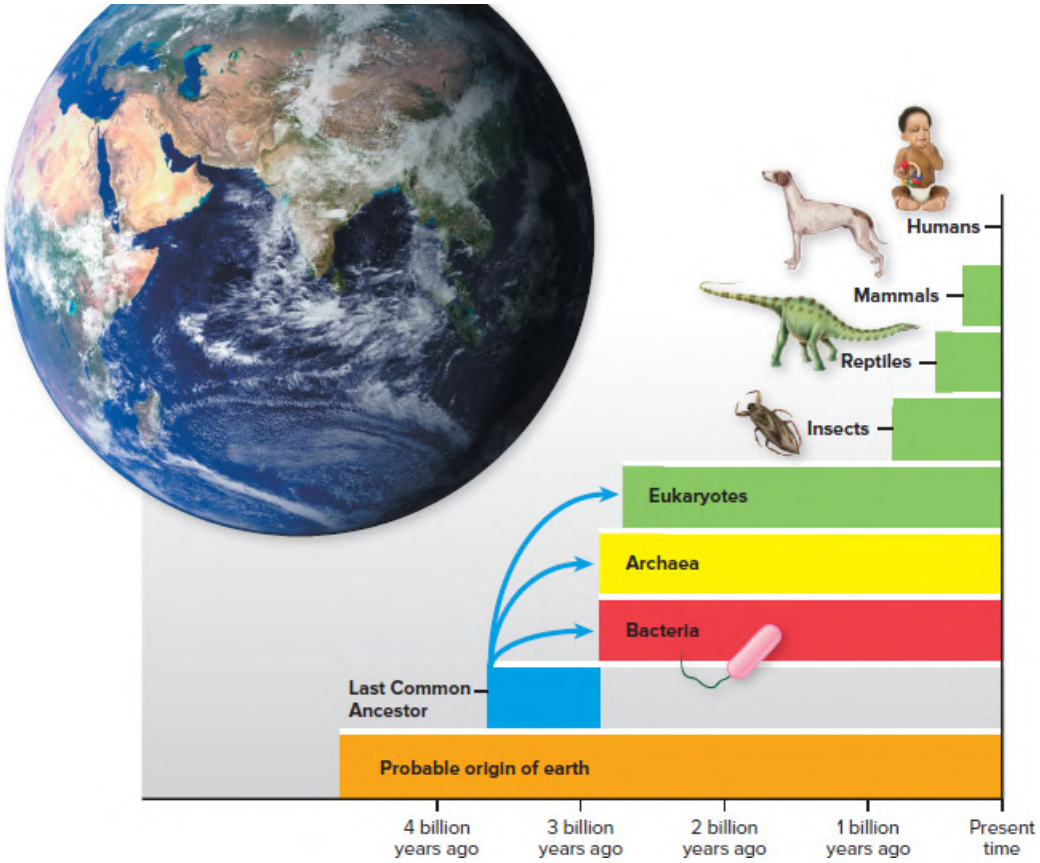


Figure 1.2 **Evolutionary time line**.
(photo): Source: NASA GSFC image by Robert Simmon and Reto Stöckli

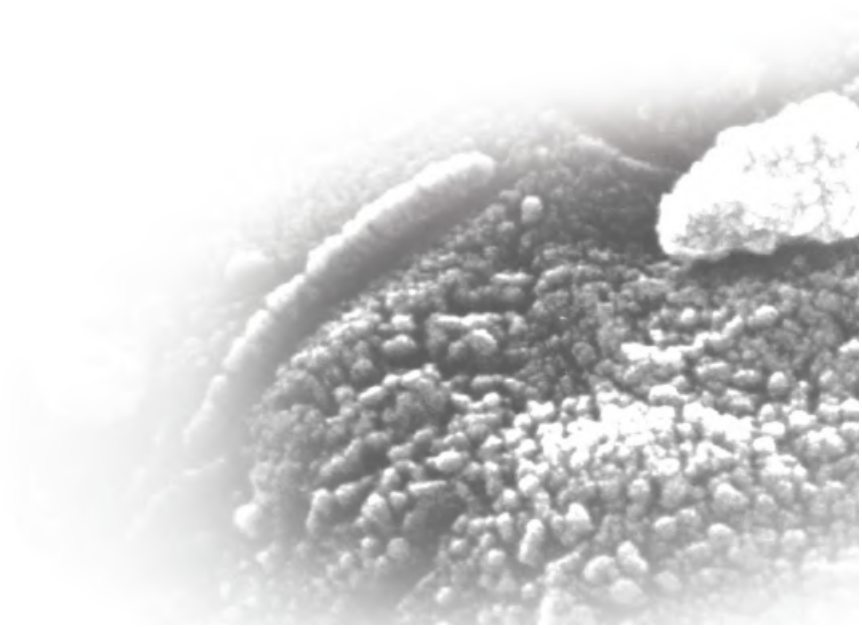
Bacteria and archaea are predominantly single-celled organisms. Many, many eukaryotic organisms are also single-celled; but the eukaryotic cell type also developed into highly complex multicellular organisms, such as worms and humans. In terms of numbers, eukaryotic cells are a small minority compared to the bacteria and archaea, but their larger size (and our own status as eukaryotes!) makes us perceive them as dominant to—and more important than—bacteria and archaea.

Figure 1.2 depicts the time line of appearances of different types of organisms on earth. Starting on the left, you see that the ancestor cell type was here alone for quite a while before giving rise to the three domains of life. Eukaryotes came along last, and it took a very long time for single-celled eukaryotes to develop into more complex eukaryotic organisms (insects, reptiles, and mammals). On the scale pictured in the figure, humans just barely appeared in very recent earth history. Bacteria and archaea preceded even the earliest animals by more than 2 billion years. This is a good indication that humans are not likely to—nor should we try to—eliminate bacteria from our environment. They have survived and adapted to many catastrophic changes over the course of our geologic history.

Another indication of the huge influence bacteria exert is how **ubiquitous** they are. Microbes can be found nearly everywhere, from deep in the earth's crust, to the polar ice caps and oceans, to inside the bodies of plants and animals. Being mostly invisible, the actions of microorganisms are usually not as obvious or familiar as those of larger plants and animals.

They make up for their small size by their immense numbers and by living in places that many other organisms cannot survive. Above all, they play central roles in the earth's landscape that are essential to life.

When we point out that single-celled organisms have adapted to a wide range of conditions over the 3.5 billion years of their presence on this planet, we are talking about evolution. The presence of life in its present form would not be possible if the earliest life forms had not changed constantly, adapting to their environment and circumstances. Getting from the far left in figure 1.2 to the far right, where humans appeared, involved billions and billions of tiny changes, starting with the first cell that appeared about a billion years after the planet itself was formed.



Source: NASA

You have no doubt heard this concept described as the **theory of evolution**. Let's clarify some terms. **Evolution** is the accumulation of changes that occur in organisms as they adapt to their environments. It is documented every day in all corners of the planet, an observable phenomenon testable by science. Scientists use the term *theory* in a different way than the general public does, which often leads to great confusion. In science, a theory begins as a hypothesis, or an educated guess to explain an observation. By the time a hypothesis has been labeled a *theory* in science, it has undergone years and years of testing and not been disproved. It is taken as fact. This is much different from the common usage, as in "My theory is that he overslept and that's why he was late." The theory of evolution, like the germ theory and many other scientific theories, refers to a well-studied and well-established natural phenomenon, not just a random guess.

Medical Moment

Medications from Microbes

Penicillin is a worthy example of how microorganisms can be used to improve human life. Alexander Fleming, a Scottish bacteriologist, discovered penicillin quite by accident in 1928. While growing several bacterial cultures in Petri dishes, he accidentally forgot to cover them. They remained uncovered for several days. When Fleming checked the Petri dishes, he found them covered with mold. Just before Fleming went to discard the Petri dishes, he happened to notice that there were no bacteria to be seen around the mold—in other words, the mold was killing all of the bacteria in its vicinity.

Recognizing the importance of this discovery, Fleming experimented with the mold (of the genus *Penicillium*) and discovered that it effectively stopped or slowed the growth of several bacteria. The chemical that was eventually isolated from the mold—penicillin—became widely used during the Second World War and saved many soldiers' lives, in addition to cementing Fleming's reputation.

Q. Can you think of a logical reason that a microbe (the fungus) would produce a chemical that harms another microbe (the bacteria)?

Answer in Appendix B.

How Microbes Shape Our Planet

Microbes are deeply involved in the flow of energy and food through the earth's ecosystems. Most people are aware that plants carry out **photosynthesis**, which is the light-fueled conversion of carbon dioxide to organic material, accompanied by the formation of oxygen (called oxygenic photosynthesis). However, bacteria invented photosynthesis long before the first plants appeared, first as a process that did not produce oxygen (*anoxygenic photosynthesis*). This anoxygenic photosynthesis later evolved into oxygenic photosynthesis, which not only produced oxygen but also was much more efficient in extracting energy from sunlight. Hence, these ancient, single-celled microbes were responsible for changing the atmosphere of the earth from one without oxygen to one with oxygen. The production of oxygen also led to the use of oxygen for aerobic respiration and the formation of ozone, both of which set off an explosion in species diversification. Today, photosynthetic microorganisms (mainly bacteria and algae) account for more than 70% of the earth's photosynthesis, contributing the majority of the oxygen to the atmosphere **(figure 1.3)**.

In the long-term scheme of things, microorganisms are the main forces that drive the structure and content of the soil, water, and atmosphere. For example:

- The temperature of the earth is regulated by gases emitted by living organisms. These gases include carbon dioxide, nitrous oxide, and methane, which create an insulation layer in the atmosphere and help retain heat. Many of these gases are produced by microbes living in the environment and the digestive tracts of animals.
- The most abundant cellular organisms in the oceans are not fish but bacteria. Think of a 2-liter soda bottle. Two liters of surface ocean water contains approximately 1,000,000,000 (1 billion) bacteria. Each of these bacteria likely harbors thousands of viruses inside of it, making viruses the most abundant inhabitants of the oceans. The bacteria and their viruses are major contributors to photosynthesis and other important processes that create our environment. (Be careful here. The first sentence in this paragraph said that bacteria are the most abundant *cellular* organisms in oceans. But viruses, which are not cellular, far outnumber them.)

- Bacteria and fungi live in complex associations with plants that assist the plants in obtaining nutrients and water and may protect them against disease. Microbes form similar interrelationships with animals, notably, in the stomach of cattle, where a rich assortment of bacteria digests the complex carbohydrates of the animals' diets and causes the animals to release large amounts of methane into the atmosphere.

Figure 1.3 **A rich photosynthetic community**. Summer pond with a thick mat of algae.
©Jerome Wexler/Science Source

Microbes and Humans

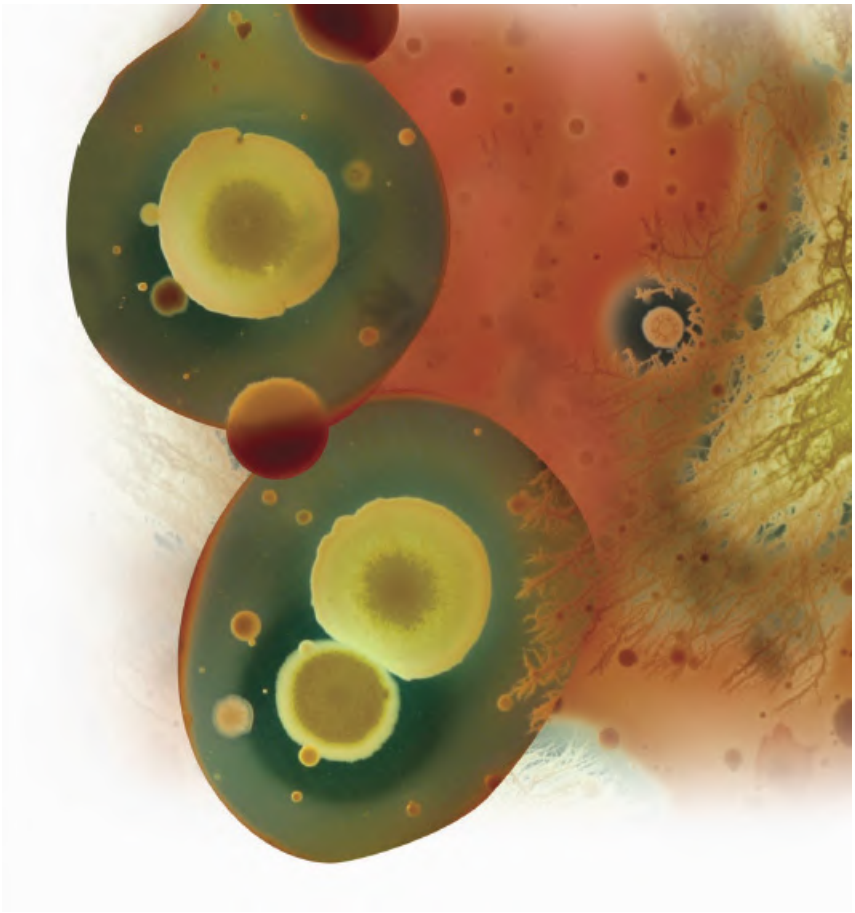
Microorganisms clearly have monumental importance to the earth's operation. Their diversity and versatility make them excellent candidates for being used by humans for our own needs, and for them to "use" humans for their needs, sometimes causing disease along the way. We'll look at both of these kinds of microbial interactions with humans in this section.

By accident or choice, humans have been using microorganisms for thousands of years to improve life and even to shape civilizations. Baker's and brewer's yeasts, types of single-celled fungi, cause bread to rise and ferment sugar into alcohol to make wine and beers. Other fungi are used to make special cheeses such as Roquefort or Camembert. Historical records show that households in ancient Egypt kept moldy loaves of bread to apply directly to wounds and lesions. When humans manipulate microorganisms to make products in an industrial setting, it is called **biotechnology**. For example, some specialized bacteria have unique capacities to mine precious metals or to clean up human-created contamination.

Genetic engineering is an area of biotechnology that manipulates the genetics of microbes, plants, and animals for the purpose of creating new products and genetically modified organisms (GMOs). One powerful technique for designing GMOs is termed **recombinant**

DNA technology. This technology makes it possible to transfer genetic material from one organism to another and to deliberately alter DNA. Bacteria and fungi were some of the first organisms to be genetically engineered. This was possible because they are single-celled organisms and they are so adaptable to changes in their genetic makeup. Recombinant DNA technology has unlimited potential in terms of medical, industrial, and agricultural uses. Microbes can be engineered to synthesize desirable products such as drugs, hormones, and enzymes. It has become popular to dislike GMOs. As with any technological advance, the capacity to create GMOs can have both positive and negative aspects. Your job is to learn about them, so that you can have an informed opinion.

Another way of tapping into the unlimited potential of microorganisms is the science of **bioremediation** (by′-oh-ree-mee-dee-ay″-shun). This term refers to the ability of microorganisms—ones already present or those introduced intentionally—to restore stability or to clean up toxic pollutants. Microbes have a surprising capacity to break down chemicals that would be harmful to other organisms **(figure 1.4)**. This includes even human-made chemicals that scientists have developed and for which there are no natural counterparts.



Colonies of bacteria and fungi on solid agar-based medium.
©McGraw-Hill Education/Don Rubbelke, photographer

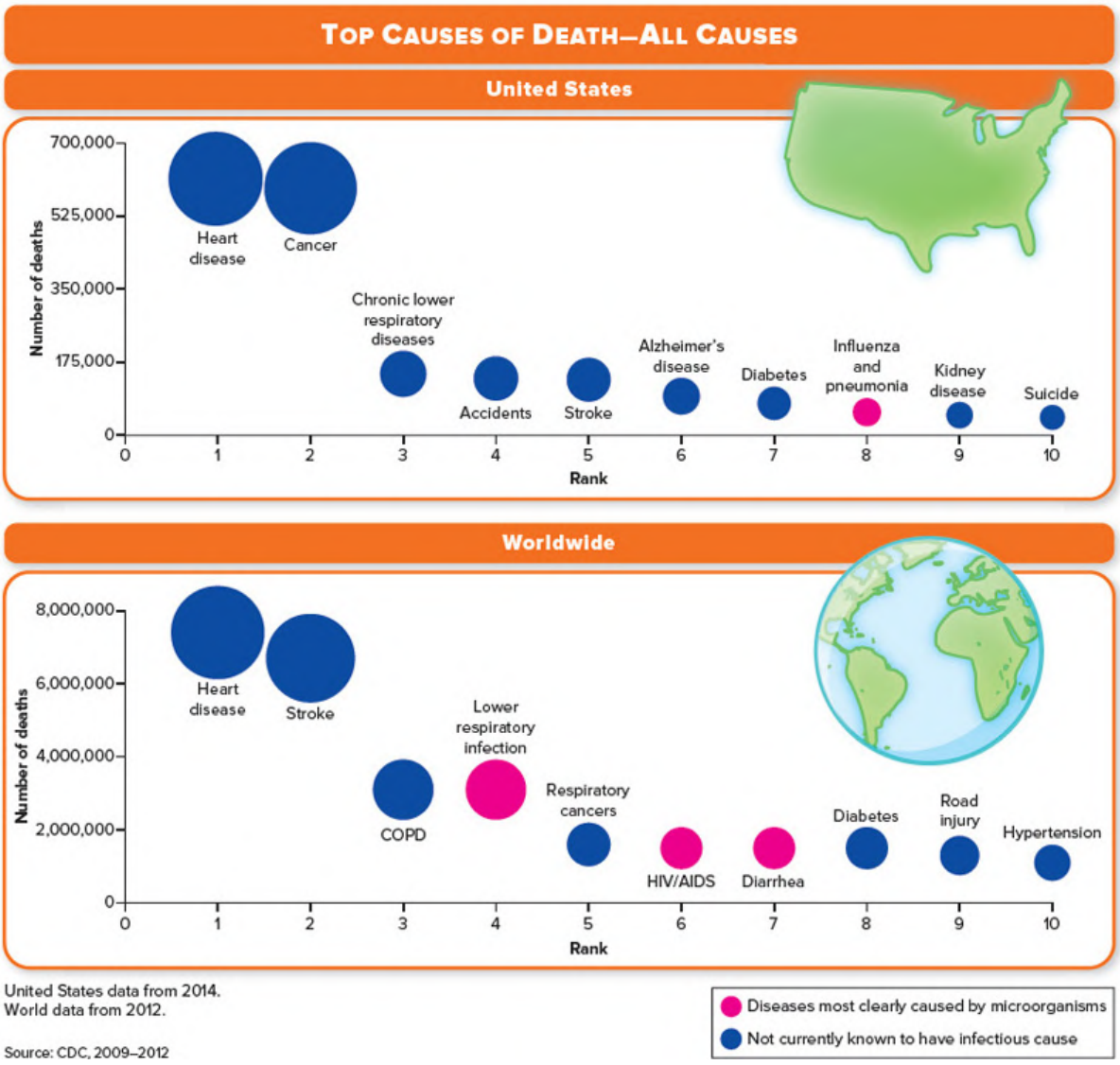
Microbes Harming Humans

One of the most fascinating aspects of the microorganisms with which we share the earth is that, despite all of the benefits they provide, they also contribute significantly to human misery as **pathogens** (path′-oh-jenz). The vast majority of microorganisms that associate with humans cause no harm. In fact, they provide many benefits to their human hosts. Note that a diverse microbial biota living in and on humans is an important part of human well-being. However, humankind is also plagued by nearly 2,000 different microbes that can cause various types of disease. Any disease caused by a microorganism is termed an **infectious disease**. Many diseases are not caused by microorganisms, but by genetic defects, imbalances in body systems, exposure to chemicals in the environment, among others. Infectious diseases still devastate human populations worldwide, despite significant strides in understanding and treating them. The World Health Organization (WHO) estimates there are a total of 10 billion new infections across the world every year. Infectious diseases are also among the most common causes of death in much of humankind, and they still kill a significant percentage of the U.S. population. **Table 1.1** depicts the 10 top causes of death per year (by all causes, infectious and noninfectious) in the United States and worldwide.

We are also witnessing an increase in the number of new (emerging) and older (reemerging) diseases. AIDS, hepatitis C, Zika virus, West Nile virus, and tuberculosis are examples. It is becoming clear that human actions in the form of deforestation, industrial farming techniques, and chemical and antibiotic usage can foster the emergence or reemergence of particular infectious diseases. These patterns will be discussed in chapter 22.

Figure 1.4 **The 2011 Gulf oil spill**. There is evidence that ocean bacteria metabolized (“chewed up”) some of the spilled oil.
Source: U.S. Coast Guard/Chief Petty Officer John Kepsimelis, Atlantic Strike Team

Table 1.1 Top Causes of Death—All Causes



One of the most eye-opening discoveries in recent years is that many diseases that used to be considered noninfectious probably do involve microbial infection. One well-known example is that of gastric ulcers, now known to be caused by a bacterium called *Helicobacter*. But there are more. Diseases as different as multiple sclerosis, obsessive compulsive disorder, coronary artery disease, and even obesity have been linked to chronic infections with microbes. It seems that the golden age of microbiological discovery, during which all of the "obvious" diseases were

characterized and cures or preventions were devised for them, should more accurately be referred to as the *first* golden age. We're now discovering the subtler side of microorganisms.

Later in this chapter we will introduce the human microbiome—the microbes that call the human body home from birth onward. We will see that variations in the microbiome also determine a person's tendency to develop both infectious and noninfectious conditions.

Another important development in infectious disease trends is the increasing number of patients with weakened defenses, who, because of welcome medical advances, are living active lives instead of enduring long-term disability or death from their conditions. They are subject to infections by common microbes that are not pathogenic to healthy people. There is also an increase in microbes that are resistant to drugs. It appears that even with the most modern technology available to us, microbes still have the "last word," as the great French scientist Louis Pasteur observed.

NCLEX® PREP

1. For which of the following disease processes has microbial infection been implicated? Select all that apply.
   - **a.** *gastric ulcers*
   - **b.** *diabetes type 1*
   - **c.** *renal artery stenosis*
   - **d.** *schizophrenia*
   - **e.** *obesity*
   - **f.** *deep vein thrombosis*

What Are They Exactly?

Cellular Organization

As discussed earlier, two basic cell types appeared during evolutionary history. The bacteria and archaea, along with eukaryotic cells, differ not only in the complexity of their cell structure but also in contents and function.

In general, bacterial and archaeal cells are about 10 times smaller than eukaryotic cells, and they lack many of the eukaryotic cell structures such as **organelles**. Organelles are small, double-membrane-bound structures in the eukaryotic cell that perform specific functions and include the nucleus, mitochondria, and chloroplasts. Examples of bacteria, archaea, and eukaryotic microorganisms are covered in more detail in chapters 3 and 4.

All bacteria and archaea are microorganisms, but only some eukaryotes are microorganisms **(figure 1.5)**. Also, of course, humans are eukaryotes. Certain small eukaryotes—such as helminths (worms), many of which can be seen with the naked eye—are also included in the study of infectious diseases because of the way they are transmitted and the way the body responds to them, though they are not microorganisms.

Let's look at viruses. As stated previously, they are not independently living cellular organisms. Instead, they are small particles that are at a level of complexity somewhere between large molecules and cells. Viruses are much simpler than cells. Outside their host, they are composed of a small amount of hereditary material (either DNA or RNA but never both) wrapped up in a protein covering. Some viruses have an additional layer, a lipid membrane that is exterior to the protein part. When inside their host organism, in the intracellular state, viruses usually exist only in the form of genetic material.

1.1 LEARNING OUTCOMES—Assess Your Progress

1. List the various types of microorganisms that can colonize humans.
2. Describe the role and impact of microbes on the earth.
3. Explain the theory of evolution and why it is called a theory.
4. Explain the ways that humans manipulate organisms for their own uses.
5. Summarize the relative burden of human disease caused by microbes.
6. Differentiate among bacteria, archaea, and eukaryotic microorganisms.
7. Identify an acellular infectious agent that is studied in microbiology.
8. Compare and contrast the relative sizes of the different microbes.

1.2 Microbes in History

If not for the extensive interest, curiosity, and devotion of thousands of microbiologists over the last 300 years, we would know little about the microscopic realm that surrounds us. Many of the discoveries in this science have resulted from the prior work of men and women who toiled long hours in dimly lit laboratories with the crudest of tools. Each additional insight, whether large or small, has added to our current knowledge of living things and processes. And the discoveries continue. Every day brings new surprises and insights into the microbial world. This section summarizes the prominent discoveries made in the past 300 years.

Spontaneous Generation

From very earliest history, humans noticed that when certain foods spoiled, they became inedible or caused illness, and yet other "spoiled" foods did no harm and even had enhanced flavor. Indeed, several centuries ago, there was already a sense that diseases such as the Black Plague and smallpox were caused by some sort of transmissible matter. But the causes of such phenomena were vague and obscure because, frankly, we couldn't *see* anything amiss. Consequently, they remained cloaked in mystery and regarded with superstition—a trend that led even well-educated scientists to believe in a concept called **spontaneous generation**. This was the belief that invisible vital forces present in matter led to the creation of life. The belief was continually reinforced as people observed that meat left out in the open soon "produced" maggots, that mushrooms appeared on rotting wood, seemingly out of nowhere, that rats and mice emerged from piles of litter, and other similar phenomena. Though some of these early ideas seem quaint and ridiculous in light of modern

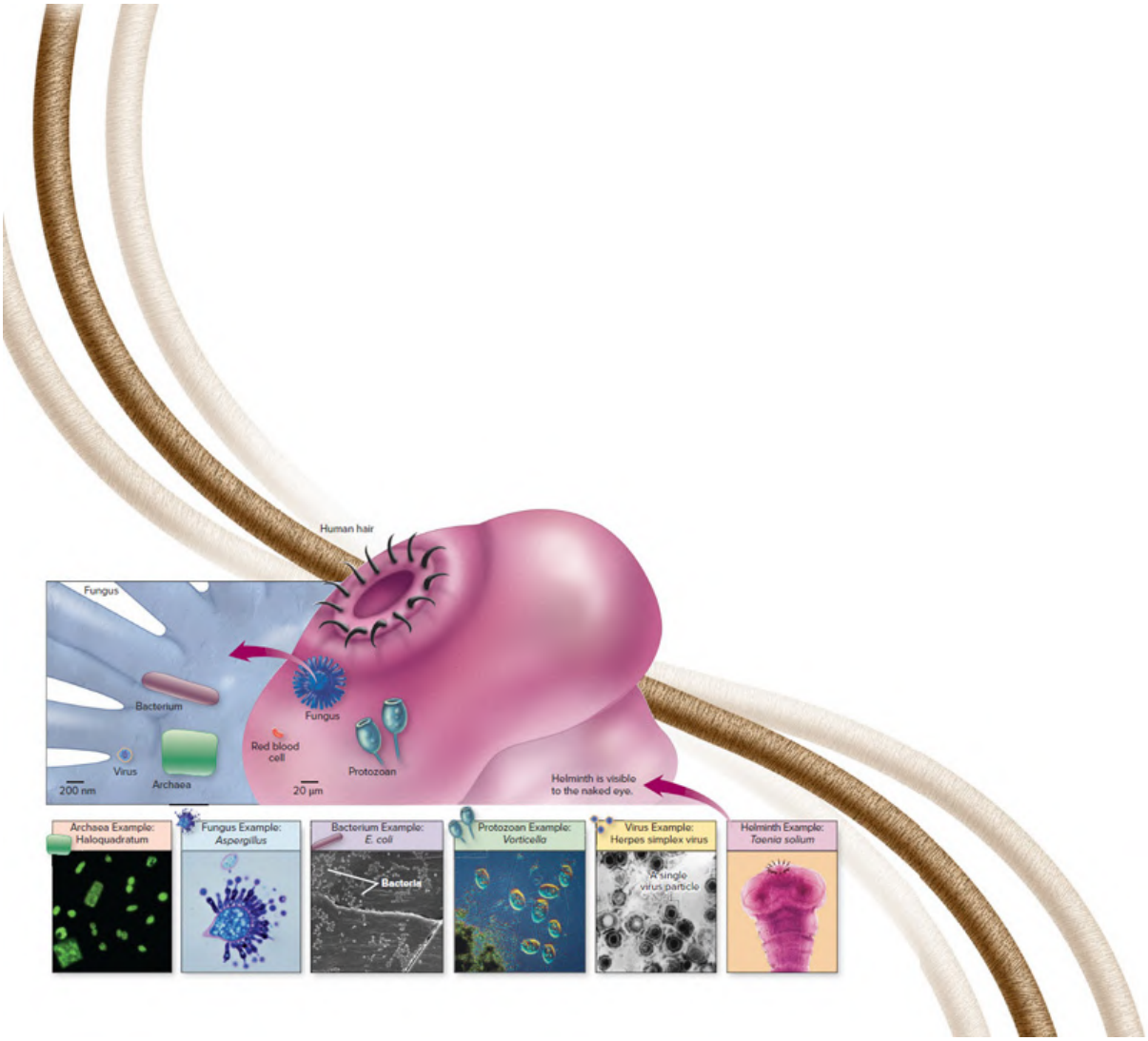


Figure 1.5 **Six types of microorganisms**.
©Dr. Mike Dyall-Smith (Haloquadratum); ©Michael Abbey/Science Source (*Aspergillus*); Source: CDC/Janice Haney Carr (*E. coli*); ©Nuridsany et Perennou/Science Source (*Vorticella*); Source: CDC/Dr. Erskine Palmer (Herpes simplex); Source: CDC/Dr. Mae Melvin (*Taenia solium*)

knowledge, we must remember that, at the time, mysteries in life were accepted and the scientific method was not widely practiced. Even after single-celled organisms were discovered during the mid-1600s, the idea of spontaneous generation continued to exist. Some scientists assumed that microscopic beings were an early stage in the development of more complex ones.

Over the subsequent 200 years, scientists waged an experimental battle over the two hypotheses that could explain the origin of simple life forms. Some tenaciously clung to the idea of **abiogenesis** (*a* = "without"; *bio* = "life"; *genesis* = "beginning"—"beginning in absence of life"), which embraced spontaneous generation. On the other side were advocates of **biogenesis** ("beginning with life") saying that living things arise only from others of their same

kind. There were serious advocates on both sides, and each side put forth what appeared on the surface to be plausible explanations of why its evidence was more correct. Finally in the mid-1800s, the acclaimed chemist and microbiologist Louis Pasteur entered the arena. He had recently been studying the roles of microorganisms in the fermentation of beer and wine, and it was clear to him that these processes were brought about by the activities of microbes introduced into the beverage from air, fruits, and grains. The methods he used to discount spontaneous generation were simple yet brilliant.

To demonstrate that air and dust were the source of microbes, Pasteur filled flasks with broth and fashioned their openings into long, swan-neck-shaped tubes **(figure 1.6)**. The flasks' openings were freely open to the air but were curved so that gravity would cause any airborne dust particles to deposit in the lower part of the necks. He heated the flasks to sterilize the broth and then incubated them. As long as the flask remained intact, the broth remained sterile; but if the neck was broken off so that dust fell directly down into the container, microbial growth immediately commenced.

Pasteur summed up his findings, "For I have kept from them, and am still keeping from them, that one thing which is above the power of man to make; I have kept from them the germs that float in the air, I have kept from them life."

Wine, cheese, and bread are each made using bacteria and fungi.
©lynx/iconotec.com/Glow Images

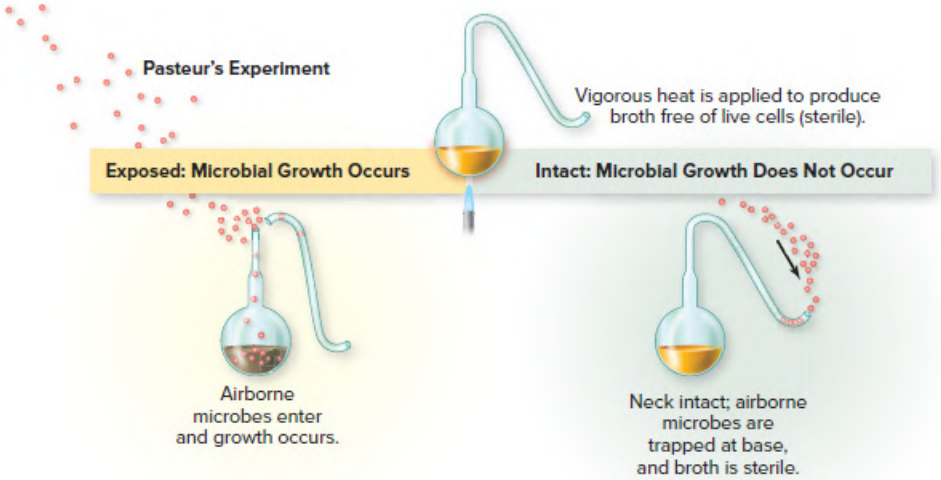


Figure 1.6 **Pasteur's swan-neck flask experiment disproving spontaneous generation**. He left the flask open to air but bent the neck so that gravity would trap any airborne microbes.

The Role of the Microscope

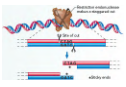
True awareness of the widespread distribution of microorganisms and some of their characteristics was finally made possible by the development of the first microscopes. These devices revealed microbes as discrete entities sharing many of the characteristics of larger, visible plants and animals. Probably the earliest record of microbes is in the works of Englishman Robert Hooke. In the 1660s, Hooke studied many different materials, including household objects, plants, and trees. He described cellular structures for the first time (in tree bark); and drew

sketches of "little structures" that seemed to be alive. Hooke paved the way for even more exacting observations of microbes by Antonie van Leeuwenhoek (lay′-oo-wun-hook), a Dutch linen merchant and self-made microbiologist.

Leeuwenhoek taught himself to grind glass lenses to ever-finer specifications so he could see the threads in his fabrics with better clarity. Eventually, he became interested in things other than thread counts. He took rainwater from a clay pot, smeared it on his specimen holder, and peered at it through his finest lens. He found "animals appearing to me ten thousand times less than those which may be perceived in the water with the naked eye."

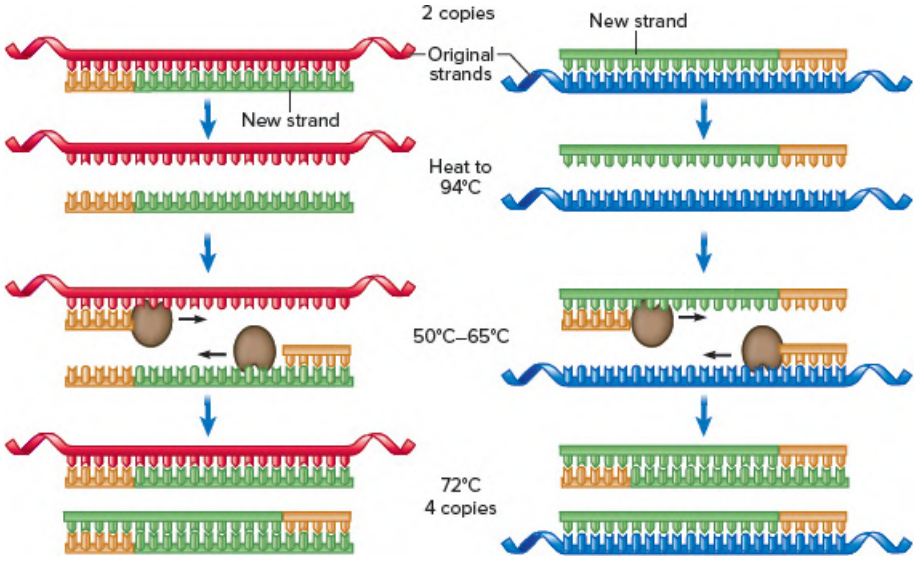
He didn't stop there. He scraped the plaque from his teeth and from the teeth of some volunteers who had never cleaned their teeth in their lives and took a good close look at that. He recorded: "In the said matter there were many very little living animalcules, very prettily a-moving. . . . Moreover, the other animalcules were in such enormous numbers, that all the water . . . seemed to be alive." Leeuwenhoek started sending his observations to the Royal Society of London, and eventually he was recognized as a scientist of great merit.

Table 1.2 Recent Advances in Microbiology



Discovery of restriction enzymes—1970s. Three scientists, Daniel Nathans, Werner Arber, and Hamilton Smith, discovered these little molecular "scissors" inside bacteria. They chop up DNA in specific ways. This was a huge moment that led to the ability of scientists to use these enzymes to cut DNA in tailor-made ways. This opened the floodgates to genetic engineering—and all that has meant for the treatment of diseases, the investigation into biological processes, and the biological "revolution" of the 21st century.

The invention of the PCR technique—1980s. The polymerase chain reaction (PCR) was a breakthrough in our ability to detect tiny amounts of DNA and then amplify them into quantities sufficient for studying. It has provided a new and powerful method for discovering new organisms, diagnosing infectious diseases, and for forensic work such as crime scene investigation. Its inventor is Kary Mullis, a scientist working at a company in California at the time. He won the Nobel Prize for this invention in 1993.



©Steve Gschmeissner/SPL/Getty Images

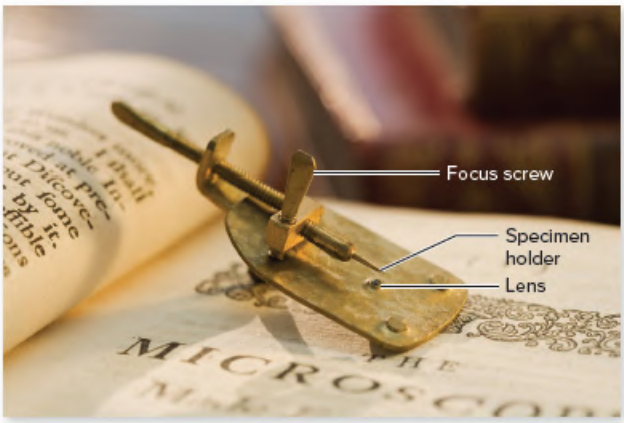


Figure 1.7 **Leeuwenhoek's microscope**. A brass replica of a Leeuwenhoek microscope. The lens is held in front of one eye with the specimen holder facing outward.
©Tetra Images/Alamy

Leeuwenhoek constructed more than 250 small, powerful microscopes that could magnify objects up to 300 times **(figure 1.7)**. Considering that he had no formal training in science, his descriptions of bacteria and protozoa (which he called "animalcules") were astute and precise.

These events marked the beginning of our understanding of microbes and the diseases they can cause. Discoveries continue at a breakneck pace, however. In fact, the 2000s are being widely called the Century of Biology, fueled by our new abilities to study genomes and harness biological processes.

To give you a feel for what has happened most recently, **table 1.2** provides a glimpse of some recent discoveries that have had huge impacts on our understanding of microbiology.

The importance of small RNAs—2000s. Once we were able to sequence entire genomes (another big move forward), scientists discovered something that turned a concept we literally used to call "dogma" on its head. The previously held "Central Dogma of Biology" was that DNA makes RNA which leads to the creation of proteins. Genome sequencing has revealed that perhaps only 2% of DNA actually codes for a protein. Much RNA doesn't end up with a protein counterpart. These pieces of RNA are usually small. It now appears that they have critical roles in regulating what happens in the cell. It has led to new approaches to how diseases are treated. For example, if the small RNAs are important in bacteria that infect humans, they can be new targets for antimicrobial therapy.

Genetic identification of the human microbiome—2010s and beyond. The first detailed information produced by the Human Microbiome Project (HMP) was astounding: Even though the exact types of microbes found in and on different people are highly diverse, the overall set of metabolic capabilities the bacterial communities possess is remarkably similar among people. This and other groundbreaking discoveries have set the stage for new knowledge of our microbial guests and their role in our overall health and disease.

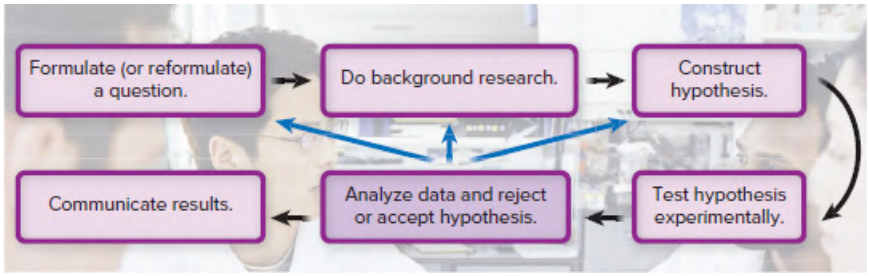


Figure 1.8 An overview of the scientific method.
©Ryan McVay/Getty Images

The changes to our view of the role of RNAs that you see in table 1.2 highlight a feature of biology—and all of science—that is perhaps underappreciated. Because we have thick textbooks containing all kinds of assertions and "facts," many people think science is an ironclad collection of facts. Wrong! Science is an ever-evolving collection of new information, gleaned from observable phenomena and combined with old information to come up with the current understandings of nature. Some of the hypotheses explaining these observations have been confirmed so many times over such a long period of time that they are, if not "fact," very close to fact. Many other hypotheses will be altered over and over again as new findings emerge. And that is the beauty of science.

It is important to understand that modern science is conducted according to a set of widely accepted "rules," termed the *scientific method* **(figure 1.8)**. Researchers form a hypothesis, and then perform experiments or other types of studies that allow them to either accept or reject the hypothesis. In real life it can also get much more complicated than that. Note the blue arrows in the figure indicating that the process can stop and revisit earlier steps. After the results are communicated (last step in the figure), other scientists review and repeat the studies in order to verify them or bring them into question. This is a process that distinguishes true research from even the most educated guesses or opinions.

The Beginnings of Medical Microbiology

Early experiments on the sources of microorganisms led to the profound realization that microbes are everywhere: Not only are air and dust full of them, but the entire surface of the earth, its waters, and all objects are inhabited by them. This discovery led to immediate applications in medicine. Thus the seeds of medical microbiology were sown in the mid to latter half of the 19th century with the introduction of the germ theory of disease and the resulting use of sterile, aseptic, and pure culture techniques.

The Discovery of Spores and Sterilization

The discovery and detailed description of heat-resistant bacterial endospores by Ferdinand Cohn, a German botanist, clarified the reason that heat would sometimes fail to completely eliminate all microorganisms. The modern sense of the word **sterile**, meaning completely free of all life forms (including spores) and virus particles, was established from that point on. The capacity to sterilize objects and materials is an absolutely essential part of microbiology, medicine, dentistry, and many industries.

The Development of Aseptic Techniques

At the same time that spontaneous generation was being hotly debated, a few physicians began to suspect that microorganisms could cause not only spoilage and decay but also infectious diseases. It occurred to these rugged individualists that even the human body itself was a source of infection. In 1843, Dr. Oliver Wendell Holmes, an American physician, published an article in which he observed that mothers who gave birth at home experienced fewer infections than did mothers who gave birth in the hospital. A few years later, the Hungarian Dr. Ignaz Semmelweis showed quite clearly that women became infected in the maternity ward after being examined by physicians coming directly from the autopsy room—without washing their hands.

In the 1860s, the English surgeon Joseph Lister took notice of these observations and was the first to introduce **aseptic** (ay-sep′-tik) **techniques** aimed at reducing

microbes in a medical setting. Lister's concept of asepsis was much more limited than our modern precautions. It mainly involved disinfecting the hands and the air with strong antiseptic chemicals, such as phenol, prior to surgery. It is hard for us to believe, but as recently as the late 1800s surgeons wore street clothes in the operating room and had little idea that hand washing was important **(figure 1.9)**. Lister's techniques and the application of heat for sterilization became the foundations for microbial control by physical and chemical methods, which are still in use today.

The Germ Theory of Disease

Louis Pasteur made enormous contributions to our understanding of the microbial role in wine and beer formation. He invented pasteurization and conducted some of the first studies showing that human diseases could arise from infection. These studies, supported by the work of other scientists, became known as the **germ theory of disease**. Pasteur's contemporary, Robert Koch, established *Koch's postulates*, a series of logical steps that verified the germ theory and could establish whether an organism was pathogenic and which disease it caused (see chapter

11). About 1875, Koch used this experimental system to show that anthrax was caused by a bacterium called *Bacillus anthracis*. So useful were his postulates that the causative agents of 20 other diseases were discovered between 1875 and 1900, and even today, they are the standard for identifying pathogens of plants and animals.

Figure 1.9 **An artist's depiction of Joseph Lister's operating theater in the mid-1800s.**
©Bettmann/Corbis/Getty Images

1.2 LEARNING OUTCOMES—Assess Your Progress

9. Make a time line of the development of microbiology from the 1600s to today.
10. List some recent microbiology discoveries of great impact.
11. Identify the important features of the scientific method.

1.3 Macromolecules: Superstructures of Life

In this book, we won't be presenting the basics of chemistry, though of course it is important to understand chemical concepts to understand all of biology. But that is what chemistry textbooks are for! However, there will be so much emphasis on some important *bio*chemicals in this book and in your course that we want to present a concise description of cellular macromolecules.

All microorganisms—indeed, all organisms—are constructed from just a few major types of biological molecules, called **macromolecules**—"macro" because they are often very large. They include four main families: carbohydrates, lipids, proteins, and nucleic acids **(table 1.3)**. All macromolecules except lipids are formed by polymerization, a process in which repeating subunits termed **monomers** are bound into chains of various lengths termed **polymers**. For example, proteins (polymers) are composed of a chain of amino acids (monomers). In the following section and in later chapters, we consider numerous concepts relating to the roles of macromolecules in cells. **Table 1.4** presents the important structural features of the four main macromolecules.

The green specks are microorganisms in the stomach of a tube worm.
©Stephen Durr

Carbohydrates: Sugars and Polysaccharides

The term **carbohydrate** originates from the composition of members of this class: They are combinations of carbon (*carbo-*) and water. Carbohydrates can be generally represented by the formula $(CH_2O)_n$, in which *n* indicates the number of units of this

Table 1.3 Macromolecules and Their Functions

Macromolecule	Basic Structure	Examples	Notes About the Examples
Carbohydrates			
Monosaccharides	3- to 7-carbon sugars	Glucose, fructose	Sugars involved in metabolic reactions; building block of disaccharides and polysaccharides
Disaccharide	Two monosaccharides	Maltose (malt sugar)	Composed of two glucoses; an important breakdown product of starch
		Lactose (milk sugar)	Composed of glucose and galactose
		Sucrose (table sugar)	Composed of glucose and fructose
Polysaccharides	Chains of monosaccharides	Starch, cellulose, glycogen	Cell wall, food storage
Lipids			
Triglycerides	Fatty acids + glycerol	Fats, oils	Major component of cell membranes; storage
Phospholipids	Fatty acids + glycerol + phosphate	Membrane components	
Waxes	Fatty acids, alcohols	Mycolic acid	Cell wall of mycobacteria
Steroids	Ringed structure	Cholesterol, ergosterol	In membranes of eukaryotes and some bacteria
Proteins			

Macromolecule	Basic Structure	Examples	Notes About the Examples
	Chains of amino acids	Enzymes; part of cell membrane, cell wall, ribosomes, antibodies	Serve as structural components and perform metabolic reactions
Nucleic acids	Nucleotides (pentose sugar + phosphate + nitrogen base) Nitrogen bases Purines: adenine (A), guanine (G) Pyrimidines: cytosine (C), thymine (T), uracil (U)		
Deoxyribonucleic acid (DNA)	Contains deoxyribose sugar and thymine, not uracil	Chromosomes; genetic material of viruses	Mediate inheritance
Ribonucleic acid (RNA)	Contains ribose sugar and uracil, not thymine	Ribosomes; mRNA, tRNA, small RNAs, genetic material of viruses	Facilitate expression of genetic traits

combination of atoms. Some carbohydrates also contain additional atoms of sulfur or nitrogen **(figure 1.10)**.

Monosaccharides and disaccharides are specified by combining a prefix that describes some characteristic of the sugar with the suffix *-ose*. For example, **hexoses** are composed of 6 carbons, and **pentoses** contain 5 carbons. **Glucose** (Gr. *glyko*, "sweet") is the most common and universally important hexose; **fructose** is named for fruit (one place where it is found); and xylose, a pentose, derives its name from the Greek word for wood. Disaccharides are named similarly: **lactose** (L. *lacteus*, "milk") is an important component of milk; **maltose** means malt sugar; and **sucrose** (Fr. *sucre*, "sugar") is common table sugar or cane sugar.

Medical Moment

Delivering Essential Nutrients

It is important to maintain homeostasis in ill patients. Supplemental nutrition is often necessary. For some patients, intake of calories by mouth or feeding tube is not possible. In order to supply essential minerals, vitamins, electrolytes, amino acids, glucose, and fluid, nutrition via intravenous (IV) line may be initiated. Total parenteral nutrition (TPN) is prescribed to meet the entirety of the patient's daily nutritional needs, accounting for their specific disease process and organ function. In addition, fatty acids might be prescribed alongside TPN, because they provide essential energy stores and help with the absorption of some vitamins. This intravenous nutrition provides the body with energy patients need for maintenance of cellular processes and promotion of healing.

Q. Use context in the paragraph above to deduce what the word "parenteral" means.

Answer in Appendix B.

The Functions of Polysaccharides

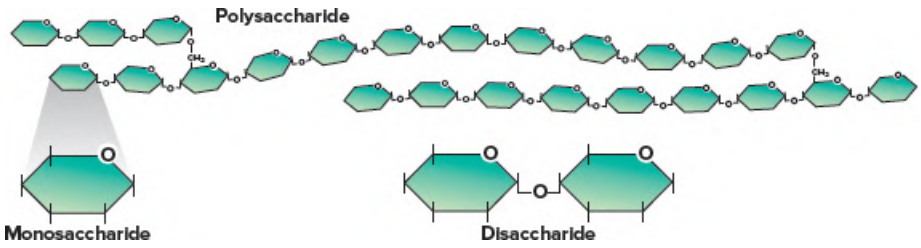
Polysaccharides contribute to structural support and protection and also serve as nutrient and energy stores. The cell walls in plants and many microscopic algae derive their strength and rigidity from **cellulose**, a long, fibrous polysaccharide. Because of this role, cellulose is probably one of the most common organic substances on the earth. Interestingly, it is digestible

only by certain bacteria, fungi, and protozoa. These microbes, called decomposers, play an essential role in breaking down and recycling plant materials.

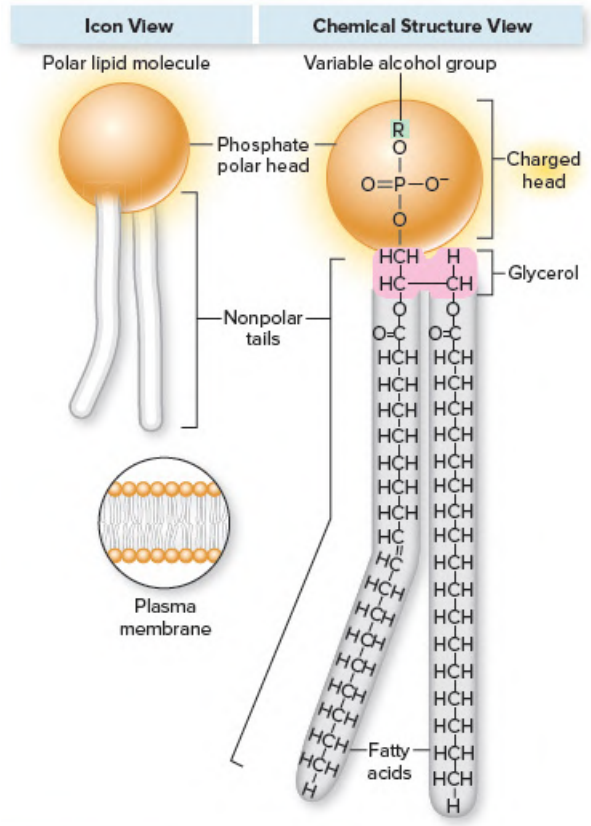
Structural polysaccharides can be conjugated (chemically bonded) to amino acids, nitrogen bases, lipids, or proteins. **Agar**, an indispensable polysaccharide in preparing solid culture media, is a natural component of certain seaweeds. It is a

Table 1.4 Macromolecules in the Cell

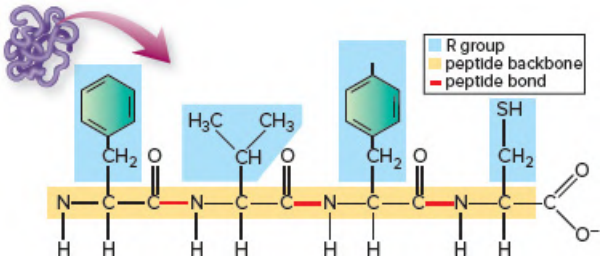
Carbohydrates. Another word for *sugar* is **saccharide**. A **monosaccharide** is a simple sugar containing from 3 to 7 carbons; a **disaccharide** is a combination of two monosaccharides; and a **polysaccharide** is a polymer of five or more monosaccharides bound in linear or branched chain patterns.



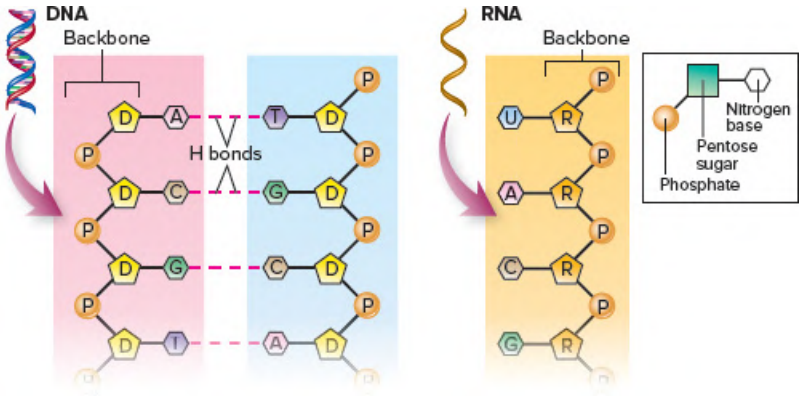
Lipids. The term **lipid**, derived from the Greek word *lipos*, meaning fat, is not a chemical designation but an operational term for a variety of substances that are not soluble in polar solvents such as water but will dissolve in nonpolar solvents such as benzene and chloroform. Here we see a model of a single molecule of a phospholipid. The phosphate-alcohol head leads a charge to one end of the molecule; its long trailing hydrocarbon chain is uncharged.



Proteins. Proteins are chains of amino acids. Amino acids have a basic skeleton consisting of a carbon (called the α carbon) linked to an amino group (NH_2), a carboxyl group (COOH), a hydrogen atom (H), and a variable R group. The variations among the amino acids occur at the R group, which is different in each amino acid and confers the unique characteristics to the molecule and to the proteins that contain it. A covalent bond called a **peptide bond** forms between the amino group on one amino acid and the carboxyl group on another amino acid.



Nucleic acids. Both DNA and RNA are polymers of repeating units called **nucleotides**, each of which is composed of three smaller units: a **nitrogen base**, a **pentose** (5-carbon) sugar, and a **phosphate**. The nitrogen base is a cyclic compound that comes in two forms: *purines* (two rings) and *pyrimidines* (one ring). There are two types of purines—**adenine (A)** and **guanine (G)**—and three types of pyrimidines—**thymine (T)**, **cytosine (C)**, and **uracil (U)**. The nitrogen base is covalently bonded to the sugar *ribose* in RNA and *deoxyribose* (because it has one less oxygen than ribose) in DNA. The backbone of a nucleic acid strand is a chain of alternating phosphate-sugar-phosphate-sugar molecules, and the nitrogen bases branch off the side of this backbone.



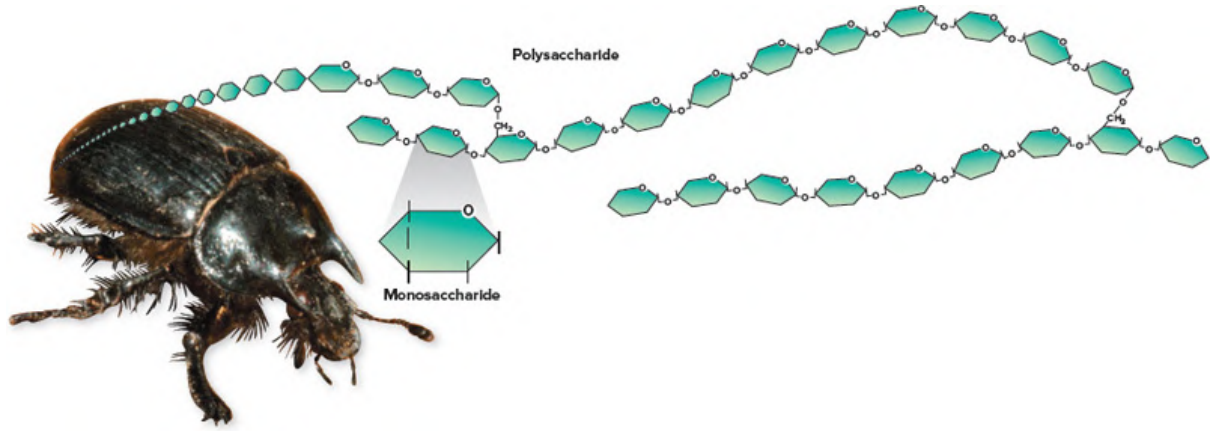


Figure 1.10 **Carbohydrates**. Polysaccharides are built of monomer sugars. They are present in many substances in nature, including chitin, which makes up the exoskeleton of some insects.
©H Lansdown/Alamy Stock Photo

complex polymer of galactose and sulfur-containing carbohydrates. The exoskeletons of certain fungi contain **chitin** (ky′-tun), a polymer of glucosamine (a sugar with an amino functional group). **Peptidoglycan** (pep-tih-doh-gly′-kan) is one special class of compounds in which polysaccharides (glycans) are linked to peptide fragments (a short chain of amino acids). This molecule provides the main source of structural support to the bacterial cell wall. The outer covering of gram-negative bacteria also contains **lipopolysaccharide**, a complex of lipid and polysaccharide responsible for symptoms such as fever and shock (see chapters 3 and 11).

The outer surface of many cells has a "sugar coating" composed of polysaccharides bound in various ways to proteins (this combination is termed a glycoprotein). This structure, called the **glycocalyx**, serves as a protective outer layer, and also can play a role in attachment of the cells to other cells or surfaces. Small sugar molecules on cell surfaces also account for the differences in human blood types. Viruses also have glycoproteins on their surface with which they bind to and invade their host cells.

Lipids: Fats, Phospholipids, Steroids, and Waxes

There are four main types of compounds classified as lipids: triglycerides, phospholipids, steroids, and waxes.

The **triglycerides** are an important storage lipid. This category includes fats and oils. Triglycerides are composed of a single molecule of glycerol bound to three fatty acids **(figure 1.11). Glycerol** is a 3-carbon alcohol with three OH groups, and fatty acids are long-chain hydrocarbon molecules with a carboxyl group (COOH) at one end that is free to bind to the glycerol. The hydrocarbon portion of a fatty acid can vary in length from 4 to 24 carbons—and, depending on the fat, it may be saturated or unsaturated. If all carbons in the chain are single-bonded to 2 other carbons and 2 hydrogens, the fat is saturated; if there is at least one C=C double bond in the chain, it is unsaturated. The structure of fatty acids is what gives fats and oils (liquid fats) their greasy, insoluble nature. In general, solid fats (such as butter) are more saturated, and liquid fats (such as oils) are more unsaturated.

In most cells, triglycerides are stored in long-term concentrated form as droplets or globules. When they are acted on by digestive enzymes called lipases, the fatty acids and glycerol are freed to be used in metabolism. Fatty acids are a superior source of energy, yielding twice as much per gram as other storage molecules (carbohydrates). Soaps are K^+ or Na^+ salts of fatty acids whose qualities make them excellent grease removers and cleaners (see chapter 9).

Oils on duck feathers keep these two canvasback ducks insulated and dry, no matter how much time they spend in the water.

©Guy Crittenden/Getty Images

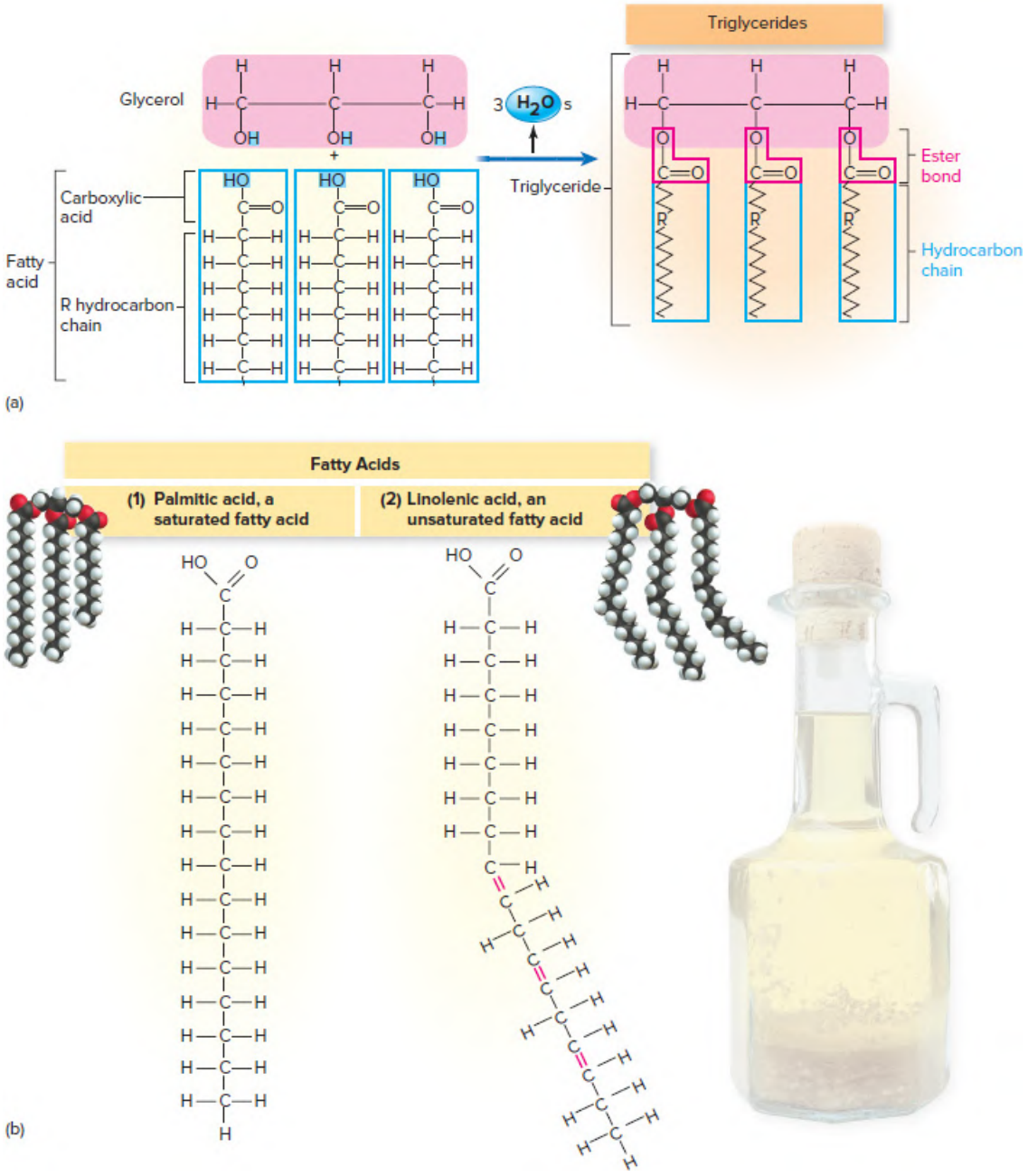


Figure 1.11 **Synthesis and structure of a triglyceride**. **(a)** Because a water molecule is released at each ester bond, this is an example of dehydration synthesis. The jagged lines and R symbol represent the hydrocarbon chains of the fatty acids, which are commonly very long. **(b)** Structural and three-dimensional models of fatty acids and triglycerides. (1) A saturated fatty acid has long, straight chains that readily pack together and form solid fats. (2) An unsaturated fatty acid—here a polyunsaturated one with 3 double bonds—has a bend in the chain that prevents packing and produces oils (right).

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Membrane Lipids

Phospholipids in membranes have a hydrophilic ("water-loving") region and a hydrophobic ("water-fearing") region. The hydrophilic region carries a negative charge due to a phosphate group attached to an alcohol group. The long fatty acid chains are uncharged and make that portion of the molecule hydrophobic **(figure 1.12*a*)**. When exposed to an aqueous solution, the charged heads are attracted to the water phase, and the nonpolar tails are repelled from the water phase **(figure 1.12*b*)**. This property causes lipids to naturally form into single and double layers (bilayers). When

two single layers of polar lipids come together to form a double layer, the outer hydrophilic face of each single layer will orient itself toward the solution, and the hydrophobic portions will become immersed in the core of the bilayer. This behavior allows phospholipids to be the main constituent of all cell membranes.

Table 1.5 Twenty Amino Acids and Their Abbreviations

Acid	Abbreviation	Characteristic of R Groups
Alanine	Ala	nonpolar
Arginine	Arg	+
Asparagine	Asn	polar
Aspartic acid	Asp	−
Cysteine	Cys	polar
Glutamic acid	Glu	−
Glutamine	Gln	polar
Glycine	Gly	polar
Histidine	His	+
Isoleucine	Ile	nonpolar
Leucine	Leu	nonpolar
Lysine	Lys	+
Methionine	Met	nonpolar
Phenylalanine	Phe	nonpolar
Proline	Pro	nonpolar
Serine	Ser	polar
Threonine	Thr	polar
Tryptophan	Trp	nonpolar
Tyrosine	Tyr	polar
Valine	Val	nonpolar

+ = positively charged; − = negatively charged.

Steroids and Waxes

Steroids are complex ringed compounds commonly found in cell membranes and as animal hormones. The best known of these is the sterol called **cholesterol (figure 1.13)**. (A sterol is a steroid that has an OH group.) Cholesterol reinforces the structure of the cell membrane in animal cells and in an unusual group of cell-wall-deficient bacteria called the mycoplasmas. The cell membranes of fungi also contain a sterol, called ergosterol.

Chemically, a *wax* is an ester formed between a long-chain alcohol and a saturated fatty acid. The resulting material is typically pliable and soft when warmed but hard and water resistant when cold (paraffin, for example). Among living things, fur, feathers, fruits, leaves, human skin, and insect exoskeletons are naturally waterproofed with a coating of wax. Bacteria that cause tuberculosis and leprosy produce a wax that repels ordinary laboratory stains and contributes to their pathogenicity.

Proteins: Shapers of Life

The predominant organic molecules in cells are **proteins**. To a large extent, the structure, behavior, and unique qualities of each living thing are a consequence of the proteins they contain. The building blocks of proteins are **amino acids**, which exist in 20 different naturally occurring forms **(table 1.5)**. Various combinations of these amino acids account for the nearly infinite variety of proteins.

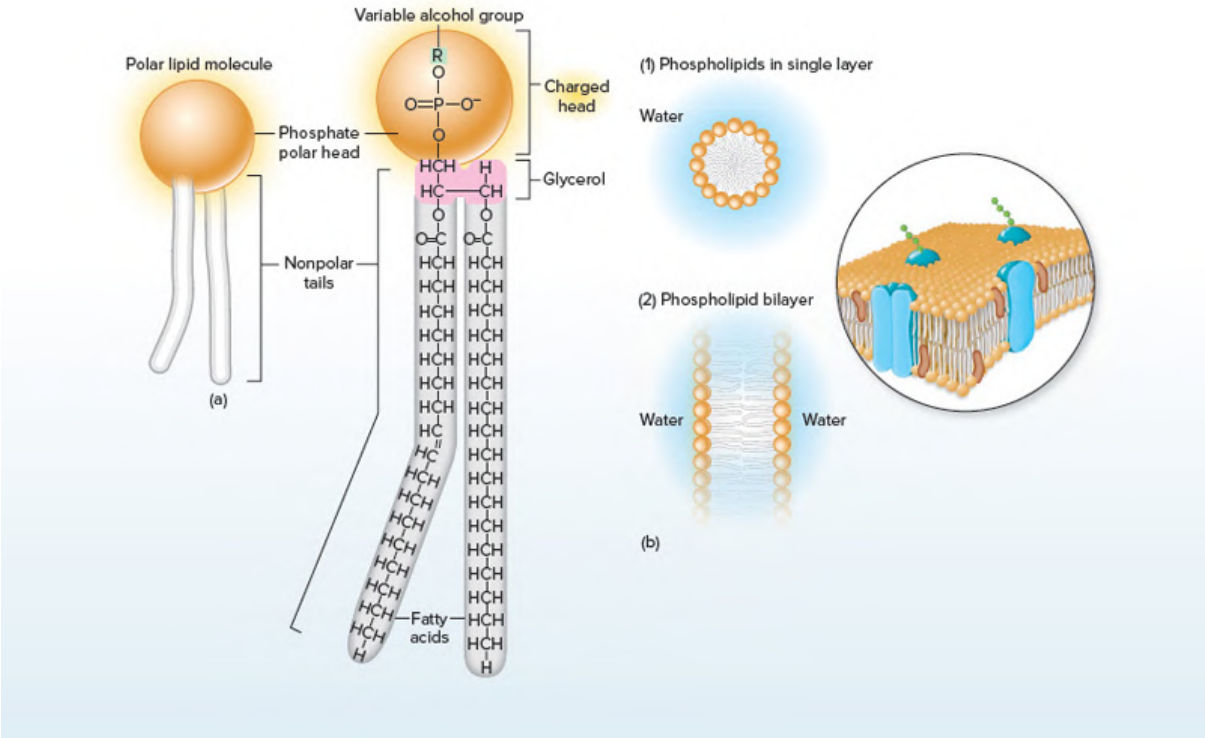

Figure 1.12 **Phospholipids—membrane molecules**. **(a)** A model of a single molecule of a phospholipid. The phosphate-alcohol head lends a charge to one end of the molecule; its long, trailing hydrocarbon chain is uncharged. **(b)** The behavior of phospholipids in water-based solutions causes them to become arranged (1) in single layers called micelles, with the charged head oriented toward the water phase and the hydrophobic nonpolar tail buried away from the water phase, or (2) in double-layered phospholipid systems with the hydrophobic tails sandwiched between two hydrophilic layers.

Various terms are used to denote the nature of proteins. **Peptide** usually refers to a molecule composed of short chains of amino acids, such as a dipeptide (two amino acids), a tripeptide (three), and a tetrapeptide (four). A **polypeptide** contains an unspecified number of amino acids but usually has more than 20 and is often a smaller subunit of a protein. A protein is the

largest of this class of compounds and usually contains a minimum of 50 amino acids. It is common for the term *protein* to be used to describe all of these molecules. In chapter 8, we see that protein synthesis is not just a random connection of amino acids; it is directed by information provided in DNA.

Protein is a major component of meats, eggs, and nuts.
©Comstock/Jupiter Images

Protein Structure and Diversity

The reason that proteins are so varied and specific is that the way they are folded makes all the difference with respect to their function. A protein has a natural tendency to assume more complex levels of organization, called the secondary, tertiary, and quaternary structures **(figure 1.14)**. The **primary (1°) structure** is the type, number, and order of amino acids in the chain, which varies extensively from protein to protein. The **secondary (2°) structure** arises when various functional groups exposed on the outer surface of the molecule interact by forming hydrogen bonds. This interaction causes the amino acid chain to twist into a coiled configuration called the *alpha helix* (α helix) or to fold into an accordion pattern called a *beta-pleated sheet* (β-pleated sheet). Many proteins contain both types of secondary configurations. Proteins at the secondary level undergo a third degree of torsion called the **tertiary (3°) structure** created by additional bonds between functional groups **(figure 1.14*c*)**. In proteins with the sulfur-containing amino acid **cysteine**, further tertiary stability is achieved through covalent disulfide bonds between sulfur atoms on two different parts of the molecule. Some complex proteins also participate in a **quaternary (4°) structure**, in which more than one polypeptide forms a large, multiunit protein. This is typical of antibodies and some enzymes that act in cell synthesis.

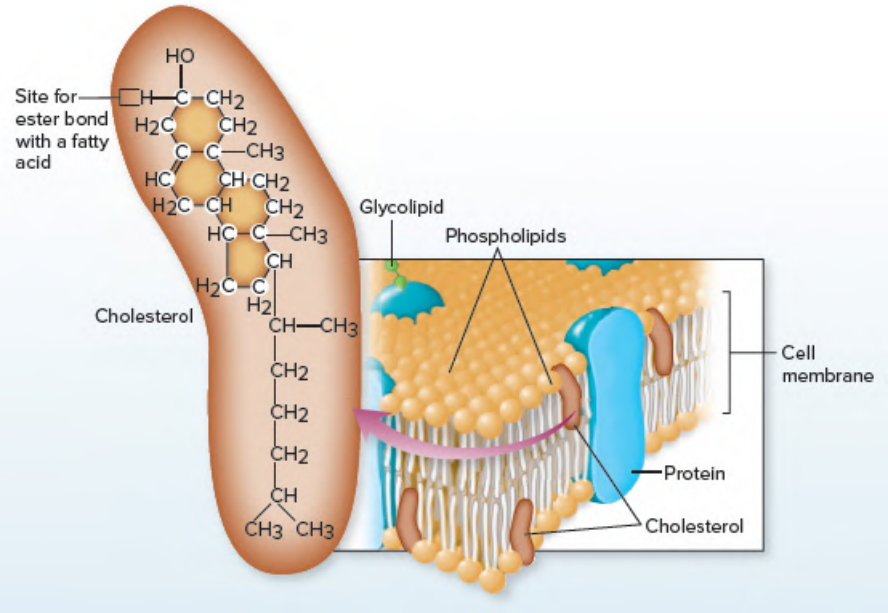

Figure 1.13 **Cutaway view of a membrane with its bilayer of lipids**. The primary lipid is phospholipid—however, cholesterol is inserted in some membranes. Other structures are protein and glycolipid molecules.

The most important outcome of the various forms of bonding and folding is that each different type of protein develops a unique shape, and its surface displays a distinctive pattern of pockets and bulges. As a result, a protein can react only with molecules that complement or fit its particular surface features like a lock and key. Such a degree of specificity can provide the functional diversity required for many thousands of different cellular activities. For example, **enzymes** serve as the catalysts for all chemical reactions in cells, and nearly every reaction requires a different enzyme (see chapter 7). This specificity comes from the architecture of the binding site, which determines which molecules fit it. The same is true of antibodies: **Antibodies** are complex glycoproteins with specific regions of attachment for bacteria, viruses, and other microorganisms; certain bacterial toxins (poisonous products) react with only one specific organ or tissue. The functional three-dimensional form of a protein is termed the *native state*. If it is disrupted by some means, the protein is said to be *denatured*. Agents such as heat, acid, alcohol, and some disinfectants disrupt (and thus denature) the stabilizing bonds within the chains and cause the molecule to become nonfunctional, as described in chapter 9.

Curly hair is the result of particular protein folding patterns as described in figure 1.14.
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The Nucleic Acids: A Cell Computer and Its Programs

The fourth type of macromolecule is **nucleic acid**. DNA and RNA are two major representatives of this group. DNA contains a special coded genetic program with detailed and specific instructions for each organism's heredity. It transfers the details of its program to RNA, "helper" molecules responsible for carrying out DNA's instructions and translating the DNA program into proteins that can perform life

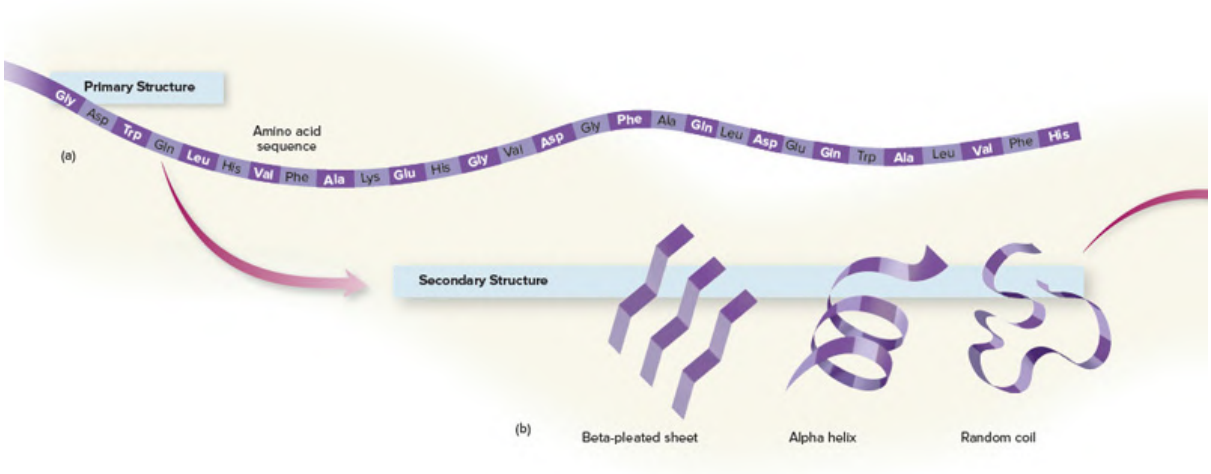


Figure 1.14 Stages in the formation of a functioning protein. **(a)** Its primary structure is a series of amino acids bound in a chain. **(b)** Its secondary structure develops when the chain forms hydrogen bonds that fold it into one of several configurations such as an *α helix* or *β-pleated sheet*. Some proteins have several configurations in the same molecule. **(c)** A protein's tertiary structure is due to further folding of the molecule into a three-dimensional mass that is stabilized by hydrogen, ionic, and disulfide bonds between functional groups. **(d)** The quaternary structure exists only in proteins that consist of more than one polypeptide chain. The chains in this protein each have a different color.

functions. For now, let us briefly consider the structure and some functions of DNA, RNA, and a close relative, adenosine triphosphate (ATP).

The Double Helix of DNA

DNA is a huge molecule formed by two long nucleotide strands linked along their length by hydrogen bonds between nitrogen bases. The pairing of the nitrogen bases occurs according to a predictable pattern: Adenine always pairs with thymine, and cytosine with guanine. The bases are attracted in this way because each pair shares oxygen, nitrogen, and hydrogen atoms exactly positioned to align perfectly for hydrogen bonds **(figure 1.15)**.

Owing to the manner of nucleotide pairing and stacking of the bases, the actual configuration of DNA is a *double helix* that looks somewhat like a spiral staircase. Just as with proteins, the structure of DNA is intimately related to its function. DNA molecules are usually extremely long.

NCLEX® PREP

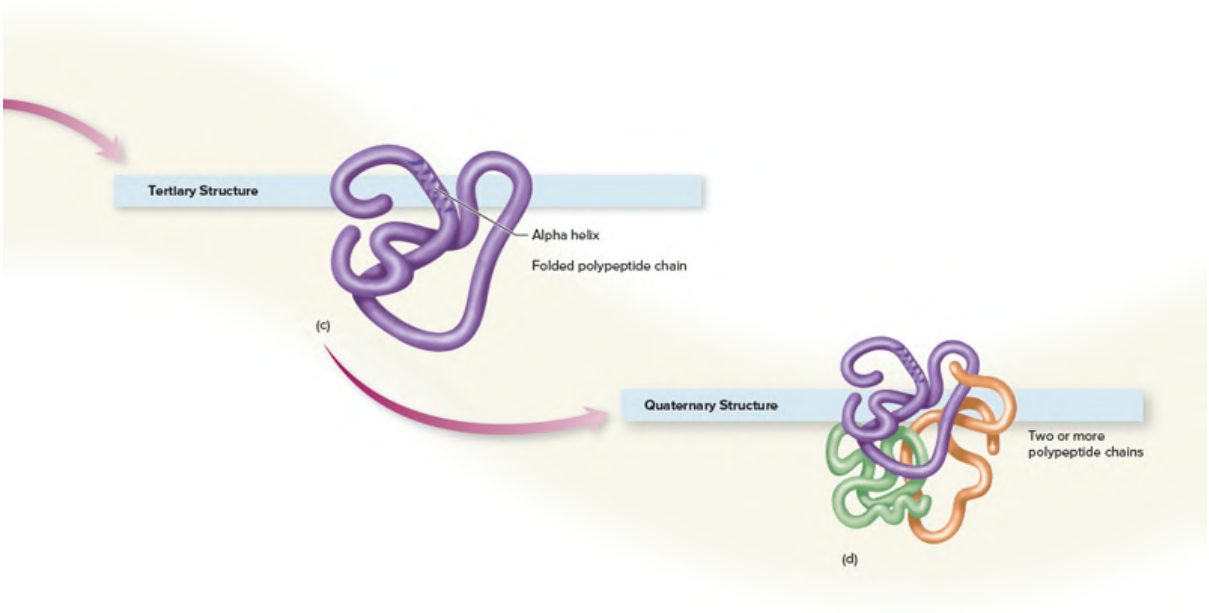
2. A nurse is caring for a patient with refractory epilepsy. To enhance medical management, a ketogenic diet has been prescribed. In educating the patient about dietary energy sources, all of the following statements are true, except:
- **a.** *Proteins consist of polymers of nucleic acids*.
- **b.** *Fat provides more kilocalories per gram than protein*.
- **c.** *A high-fat diet may cause increased serum cholesterol levels*.
- **d.** *Starch is an example of a carbohydrate*.

RNA: Organizers of Protein Synthesis

Like DNA, RNA consists of a long chain of nucleotides. However, RNA is usually a single strand, except in some viruses. It contains ribose sugar instead of deoxyribose and uracil instead of thymine (see table 1.4). Several functional types of RNA are formed using the DNA template through a process called transcription. Three major types of RNA are directly used for protein synthesis. Messenger RNA (mRNA) is a copy of a gene (a single functional part of the DNA) that provides the order and type of amino acids in a protein; transfer RNA (tRNA) is a carrier that delivers the correct amino acids for protein assembly; and ribosomal RNA (rRNA) is a major component of ribosomes (described in chapter 3). A fourth type of RNA is the RNA that acts to regulate the genes and gene expression. More information on these important processes is presented in chapter 8.

ATP: The Energy Molecule of Cells

A relative of RNA involved in an entirely different cell activity is **adenosine triphosphate (ATP)**. ATP is a nucleotide containing adenine, ribose, and three



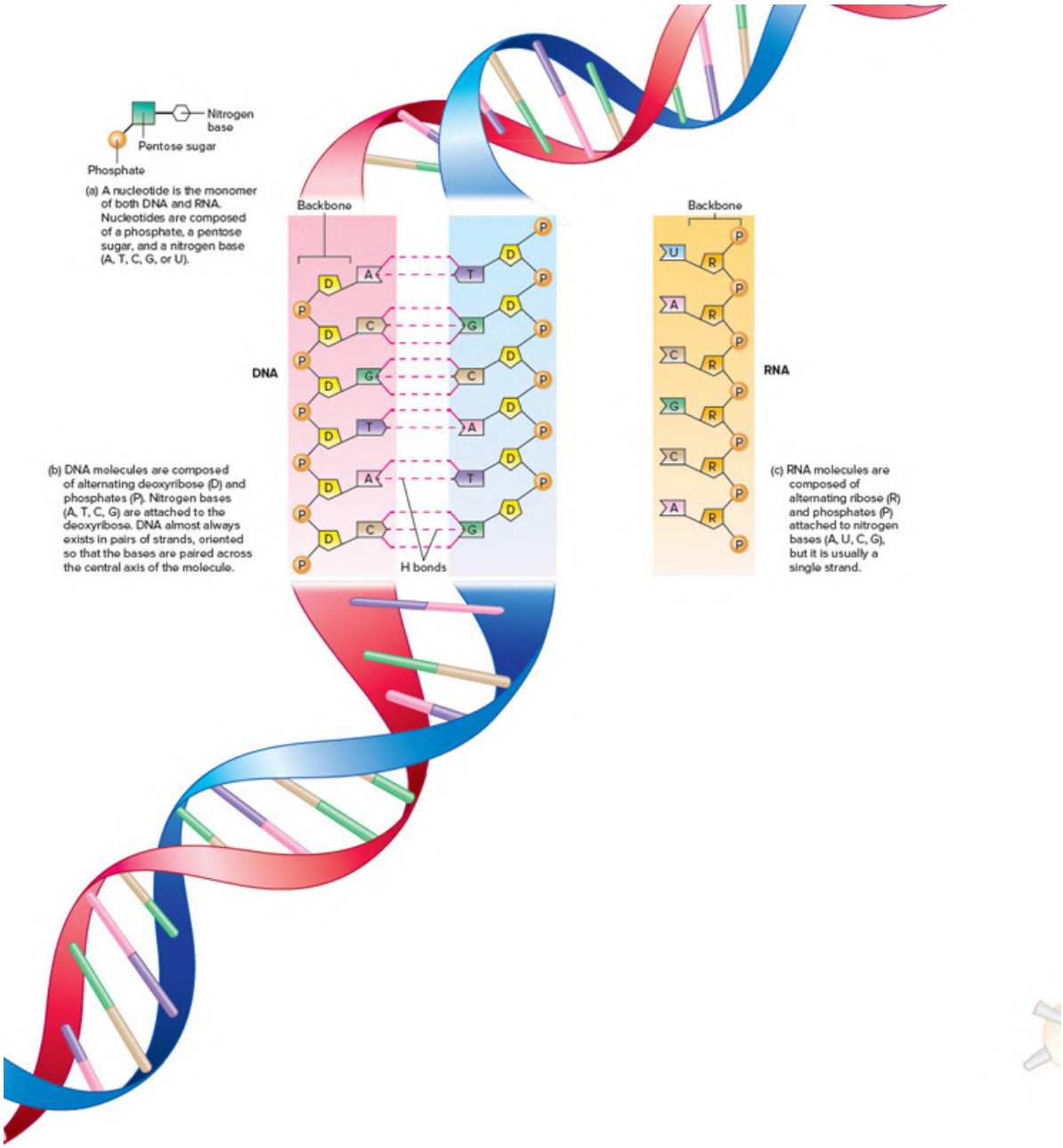


Figure 1.15 **The general structure of nucleic acids**.

phosphates rather than just one **(figure 1.16)**. It belongs to a category of high-energy compounds (also including guanosine triphosphate [GTP]) that gives off energy when the bond is broken between the second and third (outermost) phosphate. The presence of these high-energy bonds makes it possible for ATP to release and store energy for cellular chemical reactions. Breakage of the bond of the terminal phosphate releases energy to do cellular work and also generates adenosine diphosphate (ADP). ADP can be converted back to ATP when the third phosphate is restored, thereby serving as an energy depot. Carriers for oxidation-reduction

activities (nicotinamide adenine dinucleotide [NAD], for instance) are also derivatives of nucleotides (see chapter 8).

Cells: Where Chemicals Come to Life

As we proceed in this chemical survey from the level of simple molecules to increasingly complex levels of macromolecules, at some point we cross a line from the realm of lifeless molecules and arrive at the fundamental unit of life called a **cell**. A cell is indeed a huge aggregate of carbon, hydrogen, oxygen, nitrogen, and many other atoms, and it follows the basic laws of chemistry and physics, but it is much more. The combination of these atoms produces characteristics, reactions, and products that can only be described as *living*.

Fundamental Characteristics of Cells

The bodies of some living things, such as bacteria and protozoa, consist of only a single cell, whereas those of animals and plants contain trillions of cells. Regardless of the organism, all cells have a few common characteristics. They tend to be spherical, polygonal, cubical, or cylindrical; and their protoplasm (internal cell

contents) is encased in a cell or cytoplasmic membrane. They have chromosomes containing DNA, and ribosomes for protein synthesis, and they are exceedingly complex in function. Aside from these few similarities, the contents and structure of the three different cell types—bacterial, archaeal, and eukaryotic—differ significantly.

Animals, plants, fungi, and protozoa are all made up of eukaryotic cells. Such cells contain a number of complex internal parts called organelles that perform useful functions for the cell involving growth, nutrition, or metabolism. Organelles are distinct cell components that perform specific functions and are enclosed by membranes. Organelles also partition the eukaryotic cell into smaller compartments. The most visible organelle is the nucleus, a roughly ball-shaped mass surrounded by a double membrane that contains the DNA of the cell. Other organelles include the Golgi apparatus, endoplasmic reticulum, vacuoles, and mitochondria.

Bacterial and archaeal cells may seem to be the cellular “have nots” because, for the sake of comparison, they are described by what they lack. They have no nucleus and generally no other organelles. This apparent simplicity is misleading, however, because the fine structure of these cells is complex. Overall, bacterial and archaeal cells can engage in nearly every activity that eukaryotic cells can, and many can function in ways that eukaryotes cannot. Chapters 3 and 4 delve deeply into the properties of bacterial, archaeal, and eukaryotic cells.

A poultry egg is a single large cell.
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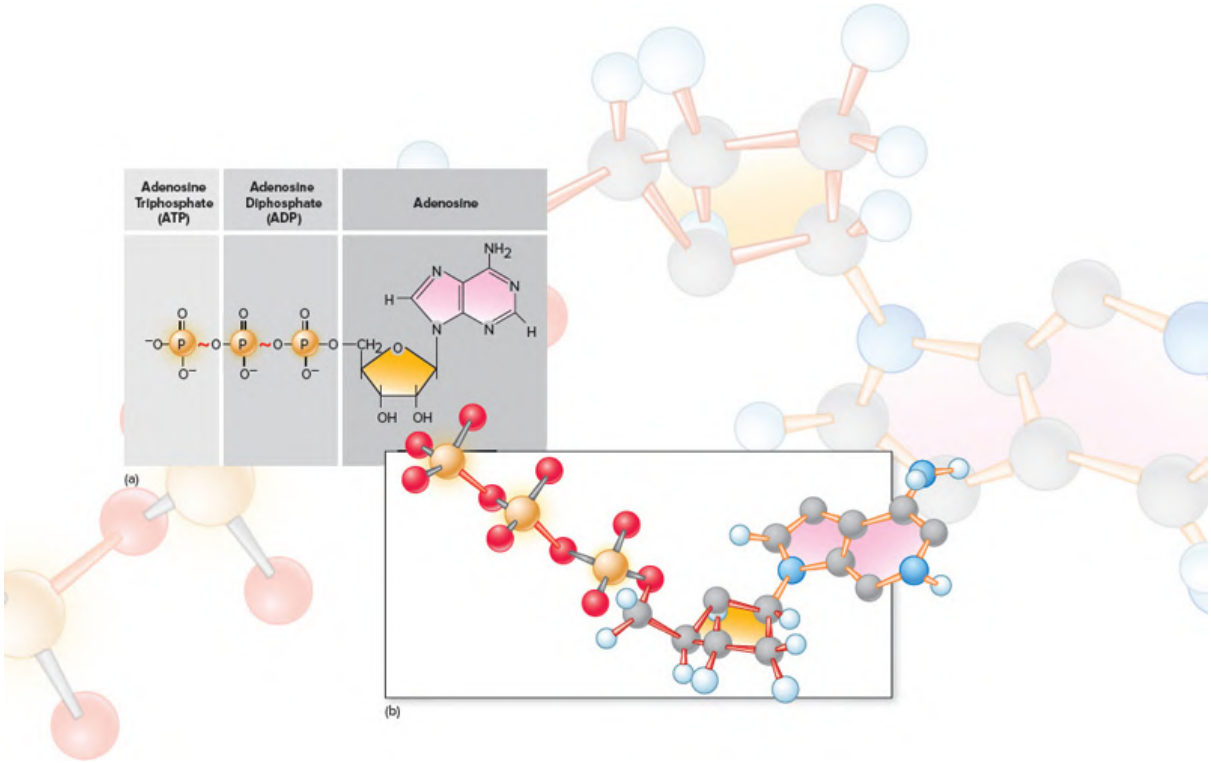


Figure 1.16 **An ATP molecule**. **(a)** The structural formula. Wavy lines connecting the phosphates represent bonds that release large amounts of energy. **(b)** A ball-and-stick model.

1.3 LEARNING OUTCOMES—Assess Your Progress

12. Name the four main families of biochemicals.
13. Provide examples of cell components made from each of the families of biochemicals.
14. Differentiate among primary, secondary, tertiary, and quaternary levels of protein structure.
15. List the three components of a nucleotide.
16. Name the three nitrogen bases of DNA and RNA.
17. List the three components of ATP.
18. Recall three characteristics common to all cells.

1.4 Naming, Classifying, and Identifying Microorganisms

The science of classifying living beings is **taxonomy**. It originated more than 250 years ago when Carl von Linné (also known as Linnaeus; 1701–1778), a Swedish botanist, laid down the basic rules for *classification* and established taxonomic categories, or **taxa** (singular, *taxon*).

Von Linné realized early on that a system for recognizing and defining the properties of living beings would prevent chaos in scientific studies by providing each organism with a unique name and an exact "slot" in which to catalog it. This classification would then serve as a means for future identification of that same organism and permit workers in many biological fields to know if they were indeed discussing the same organism.

The primary concerns of modern taxonomy are still naming, classifying, and identifying. These three areas are interrelated and play a vital role in keeping a dynamic inventory of the extensive array of living and extinct beings. In general,

Nomenclature (naming) is the assignment of scientific names to the various taxonomic categories and to individual organisms.

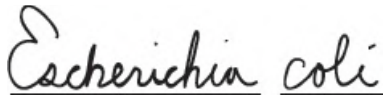
Classification is the orderly arrangement of organisms into a hierarchy.

Identification is the process of discovering and recording the traits of organisms so that they may be recognized or named and placed in an overall taxonomic scheme.

Nomenclature

Many macroorganisms are known by a common name suggested by their dominant features. For example, a bird species may be called a "red-headed blackbird" or a flowering plant species a "black-eyed Susan." Some species of microorganisms are also called by informal names, including human pathogens such as "gonococcus" (*Neisseria gonorrhoeae*) or fermenters such as "brewer's yeast" (*Saccharomyces cerevisiae*), or the recent "Iraqiabacter" (*Acinetobacter baumannii*), but this is not the usual practice. If we were to adopt common names such as the "little yellow coccus," the terminology would become even more cumbersome and challenging than scientific names.

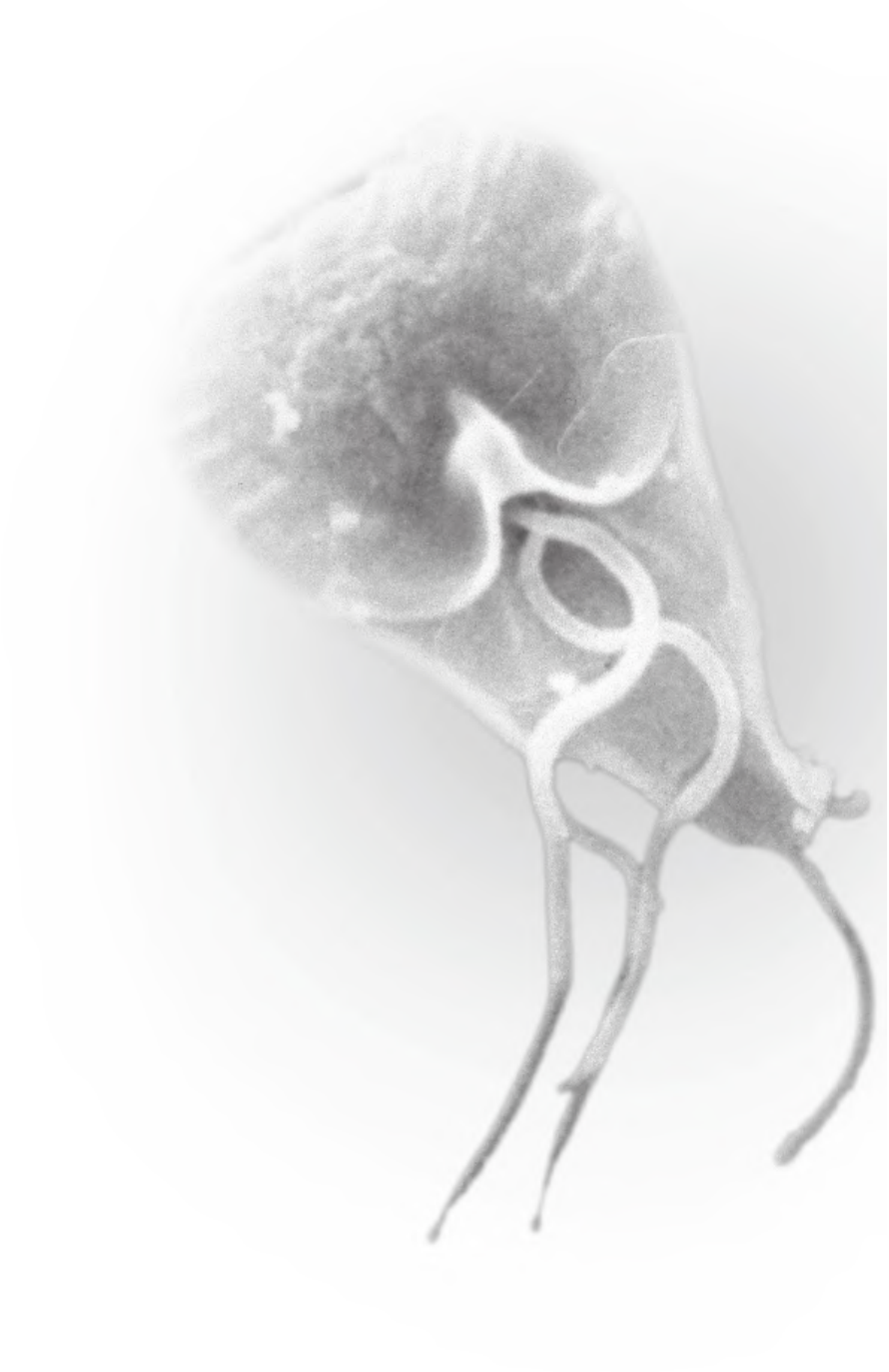
The method of assigning a scientific or specific name is called the **binomial** (two-name) **system** of nomenclature. The scientific name is always a combination of the genus name followed by the species name. The genus part of the scientific name is capitalized, and the species part begins with a lowercase letter. Both should be italicized (or underlined if using handwriting), as follows:



The two-part name of an organism is sometimes abbreviated to save space, as in *E. coli*, but only if the genus name has already been stated. The inspiration for names is extremely varied and often rather imaginative. Some species have been named in honor of a microbiologist who originally discovered the microbe or who has made outstanding contributions to the field. Other names may designate a characteristic of the microbe (shape, color), a location where it was found, or a disease it causes. Some examples of specific names, their pronunciations, and their origins are

- *Staphylococcus aureus* (staf′-i-lo-kok′-us ah′-ree-us) Gr. *staphule*, "bunch of grapes," *kokkus*, "berry," and Gr. *aureus*, "golden." The species looks like a bunch of grapes under a microscope, and its colonies are golden yellow on agar. It is a common bacterial pathogen of humans.
- *Lactobacillus sanfrancisco* (lak′-toh-bass-ill′-us san-fran-siss′-koh) L. *lacto*, "milk," and *bacillus*, "little rod." A bacterial species used to make sourdough bread, for which San Francisco is known.
- *Giardia lamblia* (jee-ar′-dee-uh lam′-blee-uh) for Alfred Giard, a French microbiologist, and Vilem Lambl, a Bohemian physician, both of whom worked on the organism, a protozoan that causes a severe intestinal infection.

Here's a helpful hint: These names may seem difficult to pronounce and the temptation is to simply "slur over them." But when you encounter the names of microorganisms in the chapters ahead, it will be extremely useful to take the time to sound them out and repeat them until they seem familiar. Even experienced scientists stumble the first few times through new names. Stumbling out loud is a great way to figure them out and you are much more likely to remember them that way—they are less likely to end up in a tangled heap with all of the new language you will be learning.



Giardia lamblia
Source: CDC/Janice Haney Carr

Classification

Classification schemes are organized into several descending ranks, beginning with the most general all-inclusive taxonomic category and ending with the smallest and most specific category. This means that all members of the highest category share only one or a few general characteristics, whereas members of the lowest category are essentially the same kind of

organism—that is, they share the majority of their characteristics. The taxonomic categories from top to bottom are **domain**, **kingdom**, **phylum** or **division**, class, order, **family**, **genus**, and **species**. Thus, each kingdom can be subdivided into a series of phyla or divisions, each phylum is made up of several classes, each class contains several orders, and so on. Because taxonomic schemes are to some extent artificial, certain groups of organisms may not exactly fit into the main categories. In such a case, additional taxonomic levels can be imposed above (super) or below (sub) a taxon, giving us such categories as "superphylum" and "subclass."

Let's compare the taxonomic breakdowns of a human and a protozoan (pro-tuh-zoh′-un) to illustrate the fine points of this system **(figure 1.17)**. Humans and protozoa are both organisms with nucleated cells (eukaryotes); therefore, they are in the same domain (Eukarya) but they are in different kingdoms. Humans are multicellular animals (kingdom Animalia), whereas protozoa are single-cellular organisms that, together with algae, belong to the kingdom Protista. To emphasize just how broad the category "kingdom" is, ponder the fact that we humans belong to the same kingdom as jellyfish. Of the several phyla within this kingdom, humans belong to the phylum Chordata, but even a phylum is rather all-inclusive, considering that humans share it with other vertebrates as well as with creatures called sea squirts. The next level, class Mammalia, narrows the field considerably by grouping only those vertebrates that have hair and suckle their young. Humans belong to the order Primates, a group that also includes apes, monkeys, and lemurs. Next comes the family Hominoidea, containing only humans and apes. The final levels are our genus, *Homo* (all modern and ancient humans), and our species, *sapiens* (meaning "wise"). Notice

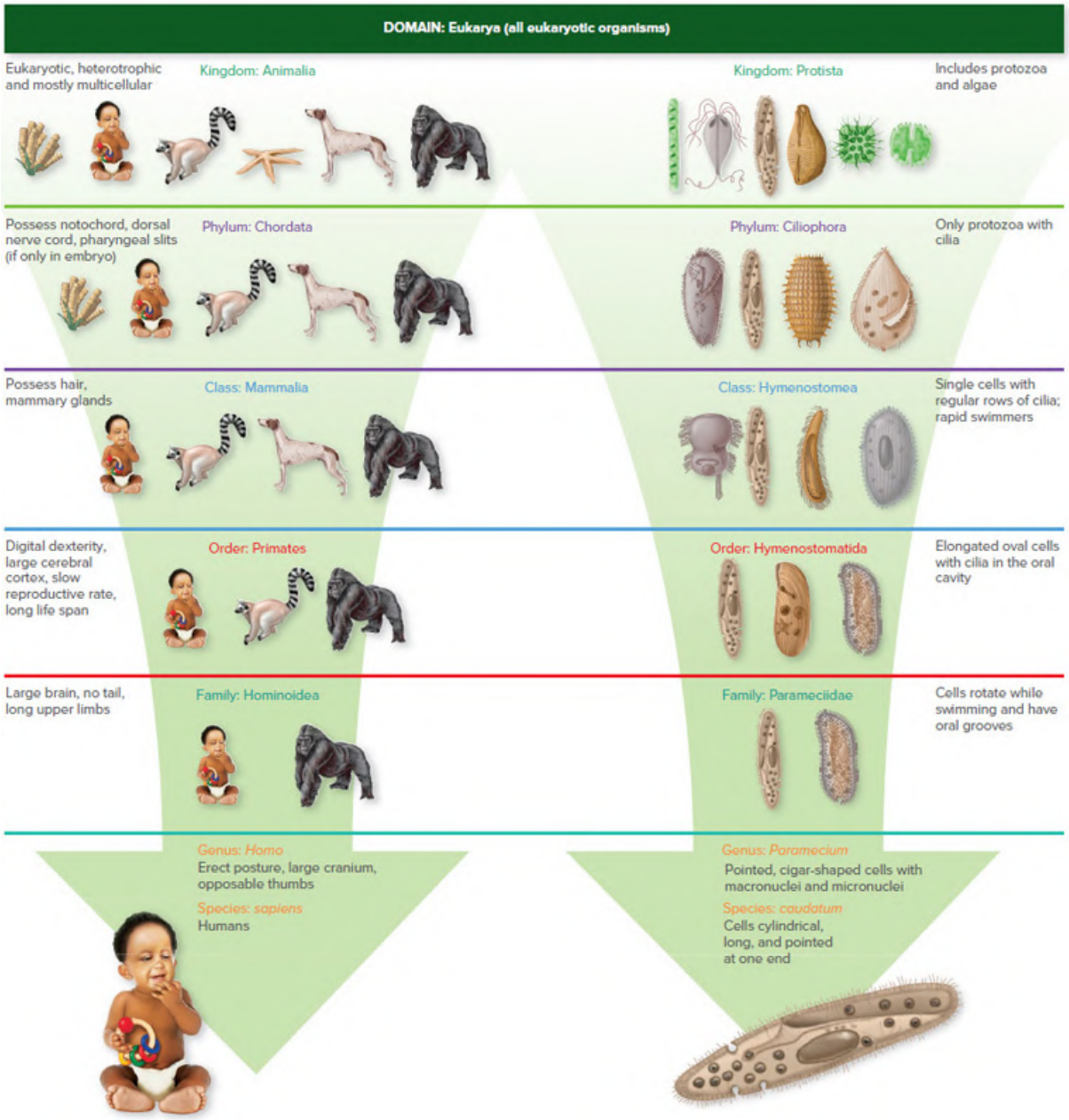


Figure 1.17 **Sample taxonomy**. Two organisms belonging to the Eukarya domain, traced through their taxonomic series. On the left, modern humans, *Homo sapiens*. On the right, a common protozoan, *Paramecium caudatum*.

that for the human as well as the protozoan, the taxonomic categories in descending order become less inclusive and the individual members more closely related. In this text, we are usually concerned with only the most general (domain, kingdom, phylum) and specific (genus, species) taxonomic levels.

Identification

Discovering the identity of microbes we find in the environment or in diseases is an art and a science. The methods used in this process are extensively described in chapter 2 and in chapter

15.

The Origin and Evolution of Microorganisms

As we indicated earlier, *taxonomy*, the science of classification of biological species, is used to organize all of the forms of modern and extinct life. In biology today, there are different methods for deciding on taxonomic categories, but they all rely on the degree of relatedness among organisms. The scheme that represents the natural relatedness (relation by descent) between groups of living beings is called their *phylogeny* (Gr. *phylon*, "race or class"; L. *genesis*, "origin or beginning"). Biologists use phylogenetic relationships to refine the system of taxonomy.

To understand the natural history of and the relatedness among organisms, we must understand some fundamentals of the process of evolution. Evolution is an important theme that underlies all of biology, including the biology of microorganisms. As we said earlier, evolution states that the hereditary information in living beings changes gradually through time and that these changes result in various structural and functional changes through many generations. The process of evolution is selective in that those changes that most favor the survival and reproduction of a particular organism or group of organisms tend to be retained, whereas those that are less beneficial to survival tend to be lost. This is not always the case, but it often is. Charles Darwin called this process *natural selection*.

Usually, evolution progresses toward greater complexity but there are many examples of evolution toward lesser complexity (reductive evolution). This is because individual organisms never evolve in isolation but as populations of organisms in their specific environments, which exert the functional pressures of selection. Because of the nature of the evolutionary process, the phylogeny, or relatedness by descent, of organisms is often represented by a diagram of a tree. The trunk of the tree represents the origin of ancestral lines, and the branches show offshoots into specialized groups of organisms. This sort of arrangement places taxonomic groups with less divergence (less change in the heritable information) from the common ancestor closer to the root of the tree and taxa with lots of divergence closer to the top.

A Universal Web of Life

The first trees of life were constructed a long time ago on the basis of just two kingdoms—plants and animals—by Charles Darwin and Ernst Haeckel. These trees were chiefly based on visible morphological (shape) characteristics. It became clear that certain (micro)organisms such as algae and protozoa, which only existed as single cells, did not truly fit either of those categories, so a third kingdom was added. It was named Protista (or Protozoa). Eventually, when significant differences became evident among even the unicellular organisms, a fourth kingdom was established in the 1870s by Haeckel and named Monera. Almost a century passed before Robert Whittaker extended this work and added a fifth kingdom for fungi during the period of 1959 to 1969. The relationships that were used in Whittaker's tree were those based on structural similarities and differences, and the way these organisms obtained their nutrition. These criteria indicated that there were five major taxonomic units, or kingdoms: the monera, protists, plants, fungi, and animals. Each organism in these

A handful of soil is home to thousands of different kinds of organisms, including a wide diversity of fungi, bacteria, viruses, and protozoa.
©Pixtal/age fotostock

five categories consisted of one of the two cell types, those cells lacking a nucleus and the eukaryotic cells. Whittaker's five-kingdom system quickly became the standard.

With the rise of genetics as a molecular science, newer methods for determining phylogeny have led to the development of a differently shaped tree—with important implications for our understanding of evolutionary relatedness. Molecular genetics allowed an in-depth study of the structure and function of the genetic material at the molecular level. In 1975, Carl Woese discovered that one particular macromolecule, the *ribonucleic acid in the small subunit of the ribosome* (abbreviated "ssu rRNA") was highly conserved—meaning that it was nearly identical in organisms within the smallest taxonomic category, the species. Based on a vast amount of experimental data, Woese hypothesized that ssu rRNA provides a "biological chronometer" or a "living record" of the evolutionary history of a given organism. Extended analysis of this molecule in prokaryotic and eukaryotic cells indicated that all members in a certain group of bacteria, then known as archaebacteria, had ssu rRNA with a sequence that was significantly different from the ssu rRNA found in other bacteria and in eukaryotes. This discovery led Carl Woese and collaborator George Fox to propose a separate taxonomic unit for the archaebacteria, which they named **Archaea**. Under the microscope, they resembled the structure of bacteria, but molecular biology has revealed that the archaea, though seemingly bacterial in nature, were actually more closely related to eukaryotic cells. To reflect these relationships, Woese and Fox proposed an entirely new system that assigned all known organisms to one of the three major taxonomic units, the domains, each being a different type of cell. Turn to the beginning of this chapter and reexamine figure 1.1, which is a depiction of the three-domain system.

The domains are the highest level in hierarchy and can contain many kingdoms and superkingdoms. Cell types lacking a nucleus are represented by the domains Archaea and **Bacteria**, whereas eukaryotes are all placed in the domain **Eukarya**. Analysis of the ssu rRNAs from all organisms in these three domains suggests that all modern and extinct

organisms on earth arose from a common ancestor. Therefore, eukaryotes did not emerge from bacteria and archaea. Instead, it appears that bacteria, archaea, and eukaryotes all emerged separately from a different, now extinct, cell type.

To add another level of complexity, the most current data suggest that "trees" of life do not truly represent the relatedness of organisms in their totality. It has become obvious that genes travel horizontally—meaning from one species to another in nonreproductive ways—and that the tidy generation-to-generation changes are complicated by neighbor-to-neighbor exchanges of DNA. For example, it is estimated that 40% to 50% of human DNA has been carried to humans from other species (by viruses). For these reasons, most scientists like to think of a *web* as the proper representation of life these days. Nevertheless, this new scheme does not greatly affect our presentation of most microbes, because we will discuss them at the genus or species level. Keep in mind that our methods of classification or evolutionary schemes reflect our current understanding and will change as new information is uncovered.

Please note that viruses are not included in any of the classification or evolutionary schemes, because they are not cells or organisms. The special taxonomy of viruses is discussed in chapter 5.

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1.4 LEARNING OUTCOMES—Assess Your Progress

19. Differentiate among the terms *nomenclature, taxonomy*, and *classification*.
20. Create a mnemonic device for remembering the taxonomic categories.
21. Correctly write the binomial name for a microorganism.
22. Draw a diagram of the three major domains.
23. Explain the difference between traditional and molecular approaches to taxonomy.

CASE FILE WRAP-UP

©Michael Williams (photo of Kelly Cowan with student); ©Science Photo Library/Getty Images (white blood cell)

If you have a bacterial infection, your doctor is likely (but not in all cases) to prescribe an antibiotic. Antibiotics are drugs that are designed to harm microbes but not harm the human host. That is their specific job—to target the microorganism. So if you have an illness that is not caused by a microorganism, you should not take antibiotics.

The second question asked in the chapter opening, "What is the most unusual infection you have ever seen?" will, of course, have a different response for every student. If you decide to go into health care as a profession, you will see a few common infections very frequently, but there will also be a wide variety of infections that you will likely only encounter once or a few times in your career. No one expects you to remember everything about every possible infection you study here. What's important is that you become familiar with important patterns of disease and the ways that our body—and the treatments we apply—affect them.

©Stephen Durr

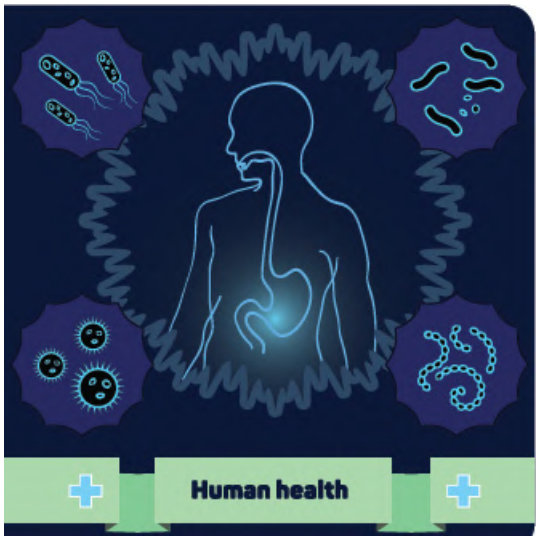
Meet Your Microbiome

In the past few years a new word has popped up on newsfeeds and websites: *microbiome*. It refers to the sum total of all the microbes in a certain environment. The human microbiome consists of all the viruses, bacteria, fungi, and protozoa that call the human body home. Trillions of these microorganisms live on our body, as part of our natural biology. Unless something goes wrong they do not cause disease, and, in fact, are necessary parts of human development and ongoing life.

The Human Microbiome Project (HMP) began in 2008, using techniques to identify body microbes that did not require growing the microbes separately in the lab (a technique scientists have relied on since the mid-1800s), but instead identified them on the basis of their genetic material. The HMP produced a staggering array of results, and they keep coming at breakneck pace.

We have learned that the microbiome differs whether you were delivered via cesarean or vaginal birth. We have learned that the gut microbiome—the microbes living in your intestinal tract—may influence not just your intestinal health but also your propensity to experience autoimmune disease, your weight, and even your mood! We know how the composition of the microbiome of different body systems (your skin, your eyes, your lungs) differs in health and in disease. We have learned that the microbiome *in utero* influences your embryonic development.

In short, we have learned that the characteristics of your microbiome determine your own, human, biology—and what types of experiences you will have as an organism. Right here at the end of every chapter in this book, we will tell a short story about the microbiome as it pertains to the subject matter in the chapter.



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Chapter Summary

1.1 Microbes: Tiny but Mighty

- Microorganisms are defined as "living organisms too small to be seen with the naked eye." Members of this huge group of organisms are bacteria, archaea, protozoa, fungi, parasitic worms (helminths), and viruses.
- Microorganisms live nearly everywhere and influence many biological and physical activities on earth.
- There are many kinds of relationships between microorganisms and humans; most are beneficial, but some are harmful.
- Groups of organisms constantly evolve to produce new forms of life.
- Microbes are crucial to the cycling of nutrients and energy necessary for all life on earth.
- Humans have learned how to manipulate microbes to do important work for them in industry and medicine and in caring for the environment.
- In the last 160 years, microbiologists have identified the causative agents for many infectious diseases. They have discovered distinct connections between microorganisms and diseases whose causes were previously unknown.
- Excluding the viruses, there are three types of microorganisms: bacteria and archaea, which are small and lack a nucleus and (usually) organelles, and eukaryotes, which are larger and have both a nucleus and organelles.
- Viruses are not cellular and are therefore sometimes called particles rather than organisms. They are included in microbiology because of their small size and close relationship with cells.

1.2 Microbes in History

- The theory of spontaneous generation of living organisms from "vital forces" in the air was disproved finally by Louis Pasteur.
- Our current understanding of microbiology is the cumulative work of thousands of microbiologists, many of whom literally gave their lives to advance knowledge in this field.
- The microscope made it possible to see microorganisms and thus to identify their widespread presence, particularly as agents of disease.
- Medical microbiologists developed the germ theory of disease and introduced the critically important concept of aseptic technique to control the spread of disease agents.

1.3 Macromolecules: Superstructures of Life

- Macromolecules are very large organic molecules (polymers) usually built up by polymerization of smaller molecular subunits (monomers).
- Carbohydrates are biological molecules whose polymers are monomers linked together by glycosidic bonds. Their main functions are protection and support (in organisms with cell walls) and also nutrient and energy stores.
- Lipids are biological molecules such as fats that are insoluble in water. Their main functions are as cell components and nutrient and energy stores.
- Proteins are biological molecules whose polymers are chains of amino acid monomers linked together by peptide bonds.
- Proteins are called the "shapers of life" because of the many biological roles they play in cell structure and cell metabolism.
- Protein structure determines protein function. Structure and shape are dictated by amino acid composition and by the pH and temperature of the protein's immediate environment.
- Nucleic acids are biological molecules whose polymers are chains of nucleotide monomers linked together by phosphate–pentose sugar covalent bonds. Double-stranded nucleic acids are linked together by hydrogen bonds. Nucleic acids are information molecules that direct cell metabolism and reproduction. Nucleotides such as ATP also serve as energy-transfer molecules in cells.
- As the atom is the fundamental unit of matter, so is the cell the fundamental unit of life.

1.4 Naming, Classifying, and Identifying Microorganisms

- The taxonomic system has three primary functions: naming, classifying, and identifying species.
- The major groups in the most advanced taxonomic system are (in descending order): domain, kingdom, phylum or division, class, order, family, genus, and species.
- Evolutionary patterns show a treelike or weblike branching, thereby describing the diverging evolution of all life forms from the gene pool of a common ancestor.
- The Woese-Fox classification system places all organisms into three domains: Eukarya, Bacteria, and Archaea.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. Which of the following is an acellular microorganism lacking a nucleus? **a**. bacterium **b**. helminth **c**. protozoan **d**. virus	**2**. Name six types of microorganisms that we are studying in this book and use each one in a sentence.	**3**. Defend the argument that a web of life is a more accurate representation of evolutionary relatedness than a tree of life.
Cell Structure and Function	**4**. Which of the following is a macromolecule that assembles into bilayers? **a**. protein **b**. phospholipid **c**. nucleic acid **d**. carbohydrate	**5**. Often when there is a local water main break, a town will post an advisory to boil water before ingesting it. Identify the biological basis behind the effectiveness of this procedure in minimizing illness.	**6**. Imagine a way you might design a drug to destroy microbes that will not harm human cells.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Metabolic Pathways	**7**. Identify the process or environment in this list that is not affected by microorganisms. **a**. oxygen cycles **b**. global temperatures **c**. human health **d**. all of the above have microbial involvement	**8**. Provide an argument about why metabolic capabilities are so much more diverse in single-celled organisms like bacteria and archaea than they are in multicellular eukaryotes.	**9**. Provide a possible interpretation of the finding that the identity of microbes found in different people may differ, but the metabolic pathways that they bring are similar in different people.
Information Flow and Genetics	**10**. DNA leads to RNA which can lead to the creation of **a**. proteins. **b**. lipids. **c**. cells. **d**. oxygen.	**11**. Compare and contrast the RNA molecule with the DNA molecule.	**12**. Suggest an argument for why eukaryotic cells have developed an enclosed nucleus unlike bacteria and archaea.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Microbial Systems	**13**. Microbes are found in which habitat? **a**. human body **b**. earth's crust **c**. oceans **d**. all of the above	**14**. Defend or refute this statement: *Microbes intend to cause human disease*.	**15**. Coevolution is a term describing the influence that two organisms occupying the same niche have on each other. Sketch a scenario for coevolution between a bacterium and a human living in the same environment.

Impact of Microorganisms	**16**. Which of the following processes can be the result of human manipulation of microbial genes? **a**. the central dogma **b**. natural selection **c**. bioremediation **d**. abiogenesis	**17**. Speculate about why scientists believe there are more microbial species that we do not yet know about than those that we do know about.	**18**. Imagine you are a guest speaker in a middle school science class. Explain to the students why human life would not be possible without microorganisms.
Scientific Thinking	**19**. When a hypothesis has been thoroughly supported by long-term study	**20**. Defend the use of complicated-sounding names for identifying microorganisms.	**21**. Identify the most important component of the scientific method and

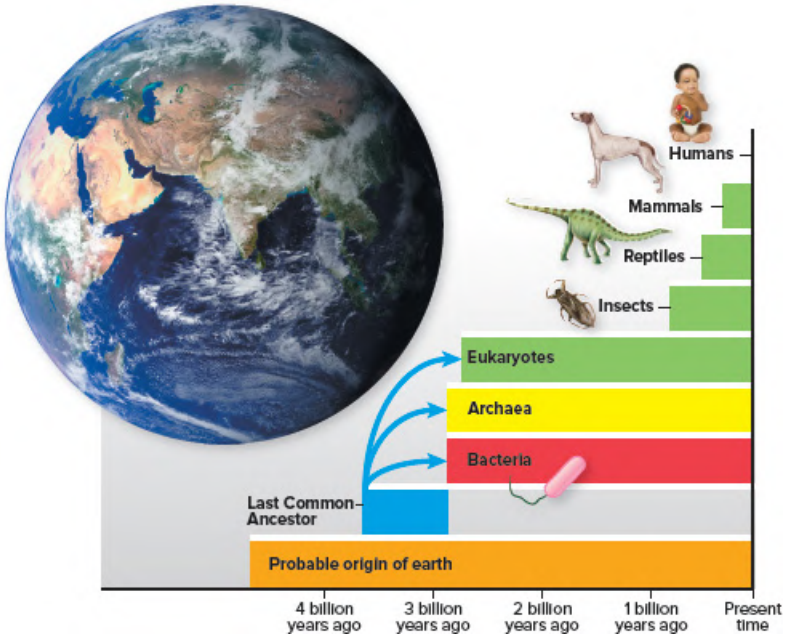
	and data, it is considered **a**. a law. **b**. a speculation. **c**. a theory. **d**. proven.		defend your answer.

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **Figure 1.2**. Look at the red bar (the time that bacteria have been on earth) and at the time that humans appeared. Speculate on the probability that we will be able to completely eliminate all bacteria from our planet, and discuss whether or not this would even be a beneficial action.



Source: NASA GSFC image by Robert Simmon and Reto Stöckli (Earth)

connect Resources are available through Connect, including interactive learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

2

Tools of the Laboratory

Methods for the Culturing and Microscopic Analysis of Microorganisms

©BSIP/Universal Images Group/Getty Images (Urine cultured in petri dish); ©Peter Skinner/Science Source (compound light microscope); ©Terry Vine/Blend Images, LLC (health care worker)

IN THIS CHAPTER…

2.1 How to Culture Microorganisms

1. Explain what the Five I's are and what each step entails.
2. Discuss three physical states of media and when each is used.
3. Compare and contrast selective and differential media, and give an example of each.
4. Provide brief definitions for *defined media* and *complex media*.

2.2 The Microscope

5. Convert among the different units of the metric system.
6. List and describe the three elements of good microscopy.
7. Differentiate between the principles of light microscopy and the principles of electron microscopy.
8. Give examples of simple, differential, and special stains.

CASE FILE

Treating the Unknown

As a nurse in a hospital inpatient surgical unit, an important part of my role is to assess patients for any complications related to their procedure. Many of my patients have incisions from their surgery. Some have implanted equipment, drains, and intravenous (IV) lines. Others are predisposed to developing infection because of the injury that caused them to need surgery. Because of these risks, one of the most important things I look for in my patients is any sign of infection.

I was caring for a 24-year-old male after his orthopedic surgery. He had been involved in a car accident and had a complex fracture in his lower leg. The surgery team had repaired the injury in the operating room. The patient now had implanted rods and screws to stabilize the bones. He had a large incision from the procedure.

Two days after his surgery, the patient developed a high fever. He was reporting increased pain in his leg. I called the provider and we discussed my concern that the patient may have an infection. Blood work was sent to the lab to look at the patient's complete blood count (CBC) with white blood cell differential, electrolyte panel, and a sample to be cultured for microorganism growth. I collected the blood culture in a sterile manner to ensure there was no contamination from the patient or environment that would alter the results.

While I waited for the lab results, I carefully assessed the patient for other signs of infection. His heart rate was high but his blood pressure was normal. The surgical incision appeared red and was warm to the touch. His leg was swollen. There was a small amount of green drainage from the surgical incision.

The patient's white blood cell count was elevated and the differential white blood cell count indicated infection, so the physician ordered the start of IV antibiotics and, to take a closer look at the surgical site itself, an X ray of the leg.

- What are this patient's signs of infection?

- How would the physician decide what type of antibiotics to prescribe?

Case File Wrap-Up appears at the end of the chapter.

Medical Moment

The Making of the Flu Vaccine: An Example of a Live Growth Medium

Have you ever wondered why health care workers ask about allergic reactions to eggs prior to immunizing patients? Live attenuated vaccines are sometimes created by culturing a virus, such as the influenza virus, in live animals, often chick embryos. The virus is inoculated into fertilized eggs, which are then incubated to encourage the replication of large numbers of virus particles. The contents of the eggs are then collected and purified to create the vaccine.

Today, influenza vaccine preparations contain such low levels of egg protein that they can be safely administered even in most individuals with allergies though it is recommended that they be medically monitored after receiving the dose. Vaccines in which the virus has been grown in cell culture, rather than in chicken eggs, are also available.

Q. Based on the information here, do you think that influenza viruses can infect (adult) birds? Why or why not?

Answer in Appendix B.

2.1 How to Culture Microorganisms

The Five I's

When you're trying to study microorganisms, you are confronted by some unique problems. First, most habitats (such as the soil, or the human mouth) contain microbes in complex associations, so it is often necessary to separate the species from one another. Second, to maintain and keep track of such small research subjects, microbiologists usually have to grow them under artificial (and thus distorting) conditions. A third difficulty in working with microbes is that they are not visible to the eye. Fourth, microbes are everywhere, and undesirable ones can be introduced into your experiment, causing misleading results.

Microbiologists use five basic techniques to manipulate, grow, examine, and characterize microorganisms in the laboratory **(figure 2.1):**

1. inoculation,
2. incubation,
3. isolation,
4. inspection, and
5. identification.

These procedures make it possible to handle and maintain microorganisms as discrete entities whose detailed biology can be studied and recorded. Having said that, keep in mind as we

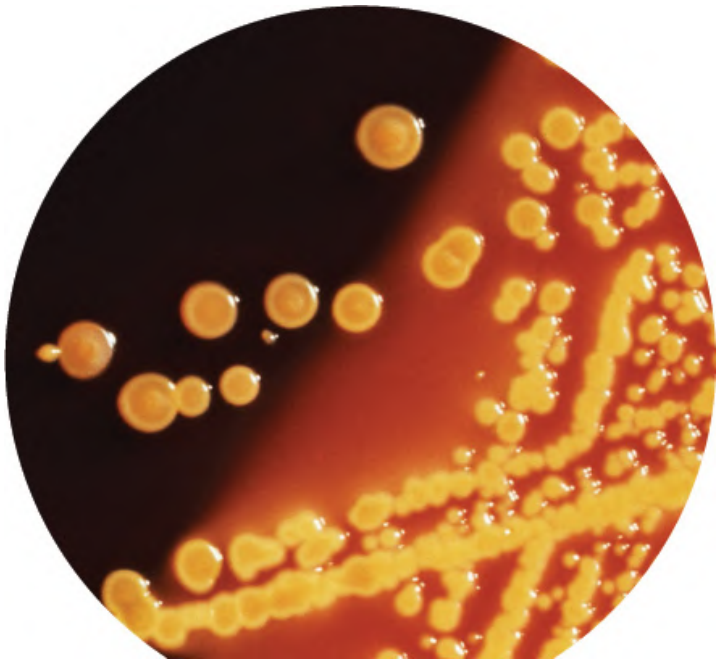
move through this chapter: It is not necessary to cultivate a microorganism to identify it anymore, though it still remains a very common method. You will read about noncultivation methods of identifying microbes in chapter 15. Sometimes growing microbes in isolated cultures can tell you very little about how they act in a mixed species environment, but being able to isolate them and study them is also valuable, as long as you keep in mind that it is an unnatural state for them.

NCLEX® PREP

1. The physician has ordered that a urine culture be taken on a client. What is the most important information the nurse should know in order to complete the collection of this specimen?
   - **a.** *date and time of collection*
   - **b.** *method of collection*
   - **c.** *whether the client is NPO (to have nothing by mouth)*
   - **d.** *age of client*

Inoculation

To grow, or **culture**, microorganisms, one introduces a tiny sample (the inoculum) into a container of nutrient **medium (plural, *media*)**. The medium provides an environment in which they multiply. This process is called **inoculation**. Any instrument used for sampling and inoculation must initially be *sterile*. The observable growth that appears in or on the medium after incubation is known as a culture. (Note that we use "culture" both as a verb and as a noun.) Clinical specimens are obtained from body fluids (blood, cerebrospinal fluid, peritoneal fluid), discharges (sputum, urine, feces), anatomical sites (throat, nose, ear, eye, genital tract), or diseased tissue (such as an abscess or wound). Then the specimens are inoculated into medium in order to identify the microorganisms in them. Other samples subject to microbiological analysis are soil, water, sewage, foods, air, and inanimate objects. Procedures for proper specimen collection are discussed in chapter 15.



Colonies of *Escherichia coli* growing on a plate of selective media.
Source: CDC

Incubation

Once a container of medium has been inoculated, it is **incubated**, which means it is placed in a temperature-controlled chamber (incubator) to encourage multiplication. Although there are microbes that can grow at temperatures ranging from freezing to boiling, the usual temperatures used in laboratories—especially in medical facilities—fall between 20°C and 45°C. Incubators can also control the content of atmospheric gases such as oxygen and carbon dioxide that may be required for the growth of certain microbes. During the incubation period (ranging from a day to several weeks), the microbe multiplies and produces growth that is observable macroscopically. Microbial

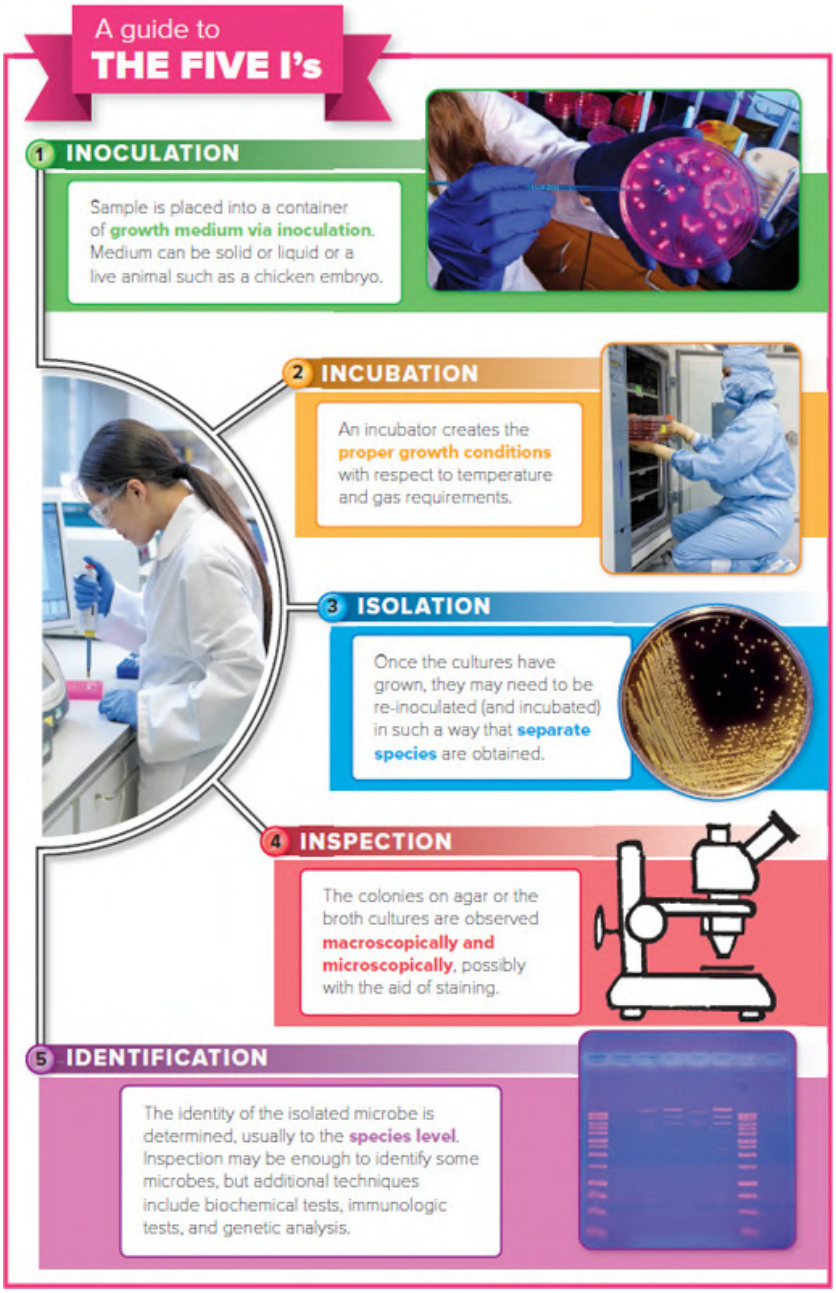


Figure 2.1 **A summary of the general laboratory techniques carried out by microbiologists**. It is not necessary to perform all the steps shown or to perform them exactly in this order, but all microbiologists participate in at least some of these activities. In some cases, one may proceed right from the sample to inspection, and in others, only inoculation and incubation on special media are required.

©Monty Rakusen/Getty Images (scientist in lab); Source: CDC/James Gathany (lab worker holding petri dish); ©Pixtal/age fotostock (kneeling scientist); Source: CDC (*Vibrio cholerae* petri dish); ©McGraw-Hill Education (drawing of microscope); ©McGraw-Hill Education/Lisa Burgess (DNA gel electrophoresis)

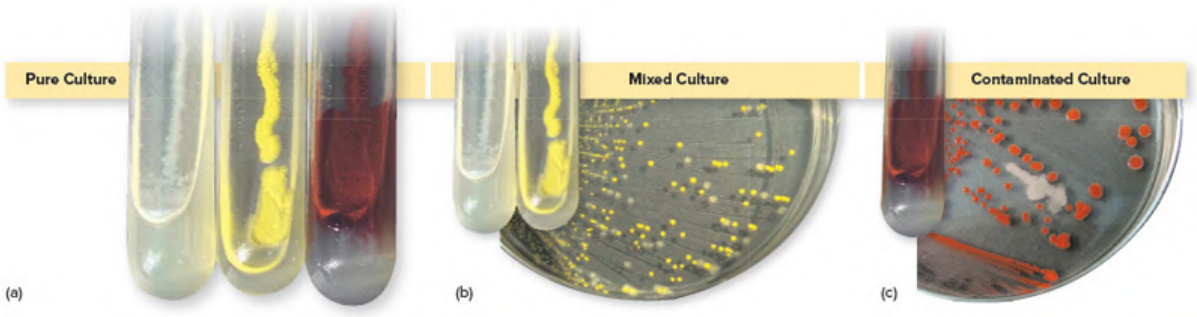


Figure 2.2 Various conditions of cultures. **(a)** Three tubes containing pure cultures of *Escherichia coli* (white), *Micrococcus luteus* (yellow), and *Serratia marcescens* (red). A **pure culture** is a growth medium that contains only a single known species or type of microorganism. This type of culture is most frequently used for laboratory study, because it allows the systematic examination and control of one microorganism by itself.
(b) A mixed culture is a container that holds two or more *identified*, easily differentiated species of microorganisms, not unlike a garden plot containing both carrots and onions. Pictured here is a mixed culture of *M. luteus* (bright yellow colonies) and *E. coli* (faint white colonies).
(c) A contaminated culture was once pure or mixed (and thus a known entity) but has since had **contaminants** (unwanted microbes of uncertain identity) introduced into it, like weeds into a garden. Contaminants get into cultures when the lids of tubes or Petri dishes are left off for too long, allowing airborne microbes to settle into the medium. They can also enter on an incompletely sterilized inoculating loop or on an instrument that you have inadvertently reused or touched to the table or your skin.
This plate of *S. marcescens* was overexposed to room air, and it has developed a large, white colony. Because this intruder is not desirable and not identified, the culture is now contaminated.
All photos: ©Kathleen Talaro

growth in a liquid medium materializes as cloudiness, sediment, scum, or color. The most common manifestation of growth on solid media is the appearance of colonies, especially with bacteria and fungi.

In some ways, culturing microbes is analogous to gardening. Cultures are formed by "seeding" tiny plots (media) with microbial cells. Extreme care is taken to exclude weeds (contaminants). **Figure 2.2** provides a summary of three different types of cultures.

Before we continue to cover information on the Five I's, we will take a side trip to look at media in more detail.

Media: Providing Nutrients in the Laboratory

Some microbes require only a very few simple inorganic compounds for growth; others need a complex list of specific inorganic and organic compounds. This tremendous diversity is evident in the types of media that can be prepared. Culture media are contained in test tubes, flasks, or Petri dishes, and they are inoculated by such tools as loops, needles, pipettes, and swabs. Culturing microbes that cannot grow on artificial media (all viruses and certain bacteria) requires cell cultures or host animals. In this chapter, we will focus on artificial media, because these are the most frequently used type in clinical situations.

Types of Media

Media can be classified according to three properties **(table 2.1)**:

1. physical state,

2. chemical composition, and
3. functional type (purpose).

Most media discussed here are designed for bacteria and fungi, though algae and some protozoa can also be propagated in media.

Agar, the main component of media, is commonly harvested from seaweed.
©Dan Ippolito

Physical States of Media

Figure 2.3 provides a good summary of three physical types of media: liquid, semisolid, and solid.

Agar, a complex polysaccharide isolated from the alga *Gelidium*, is a critical tool in the microbiology lab. The benefits of agar are numerous. It is solid at room temperature, and it melts (liquefies) at the boiling temperature of water (100°C). Once it is liquefied, agar does not resolidify until it cools to 42°C, so it can be inoculated and poured in liquid form at temperatures (45°C to 50°C) that will not harm the microbes or the handler. Agar is flexible and moldable, and it provides a basic framework to hold moisture and nutrients. Importantly, it is not itself a digestible nutrient for most microorganisms.

Table 2.1 Three Categories of Media Classification

Physical State	Chemical Composition	Functional Type
1. Liquid	1. Chemically defined	1. General purpose
2. Semisolid	2. Complex; not chemically defined	2. Enriched
3. Solid (that can be converted to liquid)		3. Selective
4. Solid (that cannot be liquefied)		4. Differential
		5. Anaerobic growth
		6. Specimen transport
		7. Assay
		8. Enumeration

Chemical Content of Media

Media whose compositions are precisely chemically defined are termed *defined* (also known as *synthetic*). Such media contain pure organic and inorganic compounds that vary little from one source to another and have a molecular content specified by means of an exact formula. Defined media may contain nothing more than a few essential compounds such as salts and amino acids dissolved in water or may be composed of a variety of defined organic and inorganic chemicals. Such standardized and reproducible media are most useful for research applications when the exact concentration of components in the media is controlled so that metabolic processes of the microbe can be precisely monitored.

If even one component of a given medium is not precisely defined, the medium belongs in the *complex* category. Complex media contain extracts of animals, plants, or yeasts, including such materials as ground-up cells, tissues, and secretions. Examples are blood, serum, and meat extracts or infusions. These materials are all sure to contain a rich supply of nutrients, although the type and amounts will vary from batch to batch—which is not a problem for many situations. Other possible

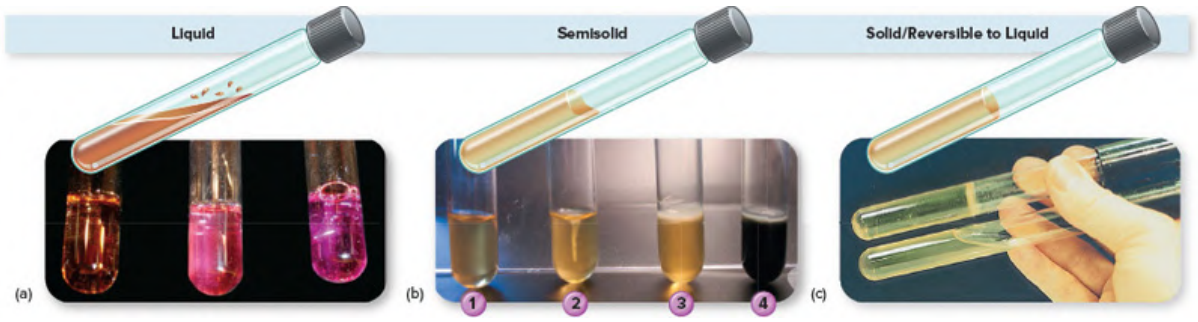


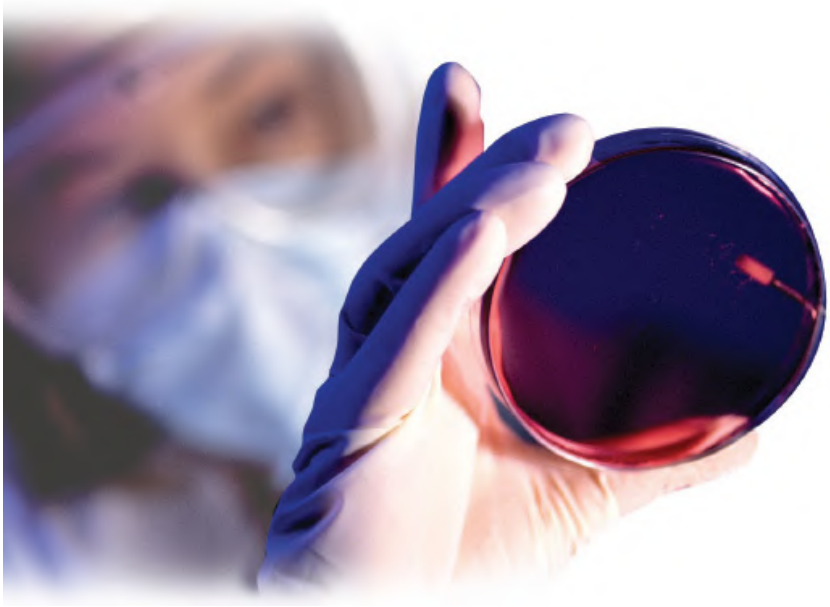
Figure 2.3 Media in different physical forms. (a) Liquid media are water-based solutions that do not solidify at temperatures above freezing and that tend to flow freely when the container is tilted. Growth occurs throughout the container and will appear cloudy, or can appear as flakes or settle to the bottom of the vessel. The tubes here hold urea broth. Urea is added to the broth in order to see if the microbe being cultured contains an enzyme that digests urea and releases ammonium. If this happens, the pH of the broth is raised and the dye becomes increasingly pink. Left: uninoculated broth, pH 7; middle: weak positive, pH 7.5; right: strong positive, pH 8.0.
(b) Liquid media are firmer than liquid media but not as firm as solid media. They do not flow freely and have a soft, clotlike consistency at room temperature. Semisolid media are used to examine the motility of bacteria and to provide a backdrop for visible reactions to occur. Here, sulfur indole motility (SIM) medium is pictured. The (1) medium is stabbed with an inoculum and incubated. Location of growth indicates nonmotility (2) or motility (3). Additionally, if H_2S gas is released, a black precipitate forms (4).

(c) Media containing 1%–5% agar are solid enough to remain in place when containers are tilted or inverted. They are reversibly solid and can be liquefied with heat, poured into a different container, and resolidified. Solid media provide a firm surface on which cells can form discrete colonies. Nutrient gelatin contains enough gelatin (12%) to take on a solid consistency. The top tube shows it as a solid. The bottom tube indicates what happens when it is warmed or when microbial enzymes digest the gelatin and liquefy it.

All photos: ©Kathleen Talaro

ingredients are milk, yeast extract, soybean digests, and peptone. Nutrient broth, blood agar, and MacConkey agar are all complex media.

Tables 2.2A and **2.2B** provide an illustration of the difference between defined and complex media, in this case, for growing *Staphylococcus aureus*.



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Media for Different Purposes

Microbiologists have many types of media at their disposal. Depending on what is added, a microbiologist can fine-tune a medium for nearly any purpose. Until recently, microbiologists knew of only a few species of bacteria or fungi that could not be cultivated artificially. However, newer DNA detection technologies have shown us that there are many more microbes that we don't know how to cultivate in the lab than those that we do. Although we can now study some vital traits of bacteria without actually growing the bacteria, developing new media is still important for growing the bacteria that we are discovering using genomic methods.

General-purpose media are those that will allow the growth of as broad a spectrum of microbes as possible. As a rule, they are of the complex variety. Examples include nutrient agar and broth, brain-heart infusion, and trypticase soy agar (TSA). An **enriched medium** contains complex organic substances such as blood, serum, hemoglobin, or special **growth factors** (specific vitamins, amino acids) that certain species must have in order to grow.

Bacteria that require growth factors and complex nutrients are termed **fastidious**. Blood agar, which is made by adding sterile sheep, horse, or rabbit blood to a sterile agar base **(figure 2.4*a*)** is widely used to grow fastidious streptococci and other pathogens. Pathogenic *Neisseria* (one species causes gonorrhea) are grown on either Thayer-Martin medium or "chocolate" agar, which is a blood agar with added hemin and nicotinamide adenine dinucleotide **(figure 2.4*b*)**. Enriched media are also useful in the clinical laboratory to encourage the growth of pathogens that may be present in very low numbers, such as in urine or blood specimens.

Table 2.2A Defined Medium for Growth and Maintenance of Pathogenic *Staphylococcus aureus*

0.25 Grams Each of These Amino Acids	0.5 Grams Each of These Amino Acids	0.12 Grams Each of These Amino Acids
Cystine	Arginine	Aspartic acid
Histidine	Glycine	Glutamic acid
Leucine	Isoleucine	
Phenylalanine	Lysine	
Proline	Methionine	
Tryptophan	Serine	
Tyrosine	Threonine	
	Valine	

Additional Ingredients

Vitamins:
- 0.005 mole nicotinamide
- 0.005 mole thiamine
- 0.005 mole pyridoxine
- 0.5 micrograms biotin

Salts:
- 1.25 grams magnesium sulfate
- 1.25 grams dipotassium hydrogen phosphate
- 1.25 grams sodium chloride
- 0.125 grams iron chloride

Ingredients dissolved in 1,000 milliliters of distilled water and buffered to a final pH of 7.0.

Table 2.2B Brain-Heart Infusion Broth: A Complex Medium for Growth and Maintenance of Pathogenic *Staphylococcus aureus*

- 27.5 grams brain, heart extract, peptone extract
- 2 grams glucose
- 5 grams sodium chloride
- 2.5 grams disodium hydrogen phosphate

Ingredients dissolved in 1,000 milliliters of distilled water and buffered to a final pH of 7.0.

Selective and Differential Media These media are designed for special microbial groups, and they are extremely useful in isolation and identification. They can permit, in a single step, the preliminary identification of a genus or even a species.

A **selective medium** contains one or more agents that inhibit the growth of certain types of microbe or microbes (call them A, B, and C) but not others (D) and thereby encourages, or *selects*, microbe D and allows it to grow. Selective media are very important in the initial stages of isolating a specific type of microorganism from samples containing dozens of

different species—for example, feces, saliva, skin, water, and soil. They speed up isolation by suppressing the unwanted background organisms and favoring growth of the desired ones.

Media for isolating intestinal pathogens (MacConkey agar, Hektoen enteric [HE] agar) contain bile

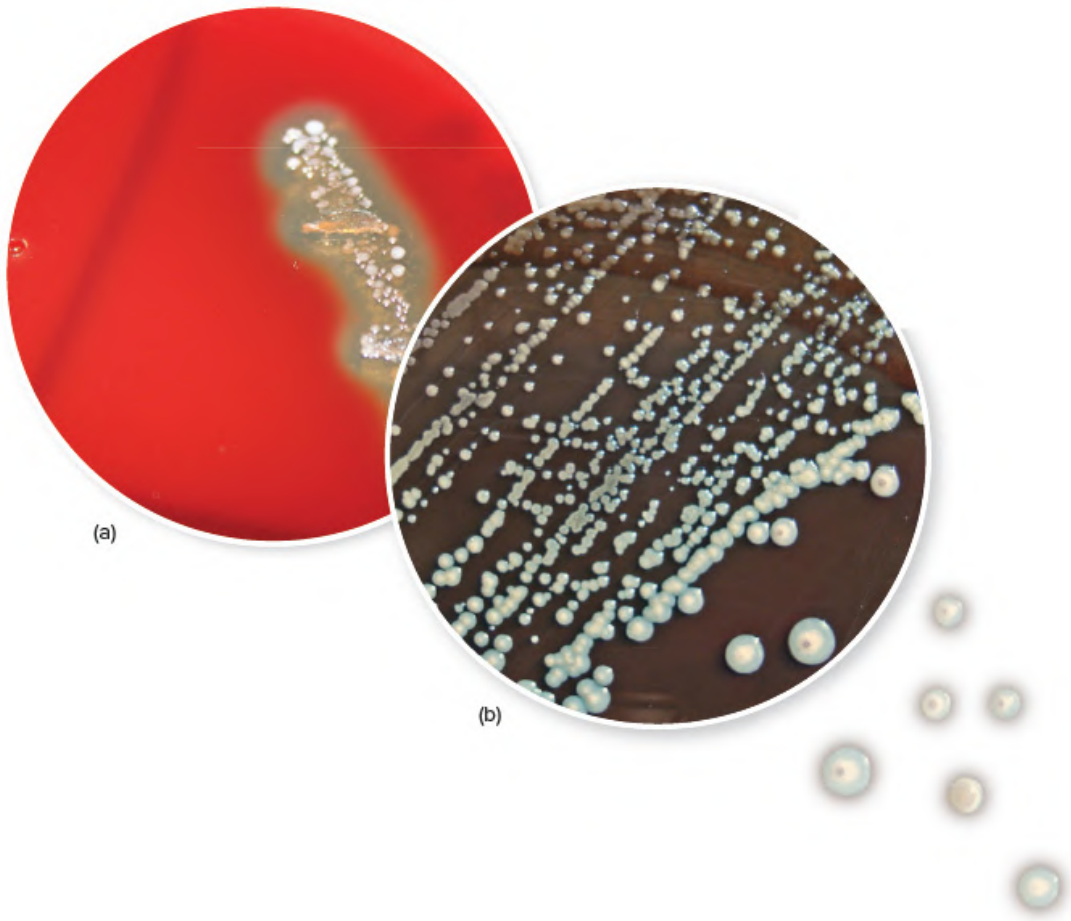

Figure 2.4 **Examples of enriched media**. **(a)** Blood agar plate growing bacteria from the human throat. Enzymes from the bacterial colonies break down the red blood cells in the agar, leaving a clear "halo." **(b)** Culture of *Neisseria* sp. on chocolate agar. Chocolate agar gets its brownish color from cooked blood (not chocolate) and does not produce hemolysis.

©McGraw-Hill Education/Lisa Burgess (a); ©Kathleen Talaro (b)

salts as a selective agent. Other agents that have selective properties are dyes, such as methylene blue and crystal violet, and antimicrobial drugs. **Table 2.3** gives multiple examples of selective media and what they do.

Differential media do not inhibit the growth of any particular microorganisms but are designed to display visible differences in how they grow. Differentiation shows up as variations in colony size or color **(figure 2.5)**, in media color changes, or in the formation of gas bubbles and precipitates. These variations often come from the type of chemicals these media contain and the ways that microbes react to them. For example, when microbe X metabolizes a certain substance not used by organism Y, then X will cause a visible change in

the medium and Y will not **(figure 2.6)**. The simplest differential media show just two reaction types, such as the use or nonuse of a particular nutrient or a color change in some colonies but not in others. Some media are sufficiently complex to allow for three or four different reactions. Importantly, you should know that a single medium can be both selective and differential, owing to its different ingredients. MacConkey agar, for example, appears in table 2.3 (selective media) and **table 2.4** (differential media) due to its ability to suppress the growth of some organisms while producing a visual distinction among the ones that do grow. The agar in figure 2.5

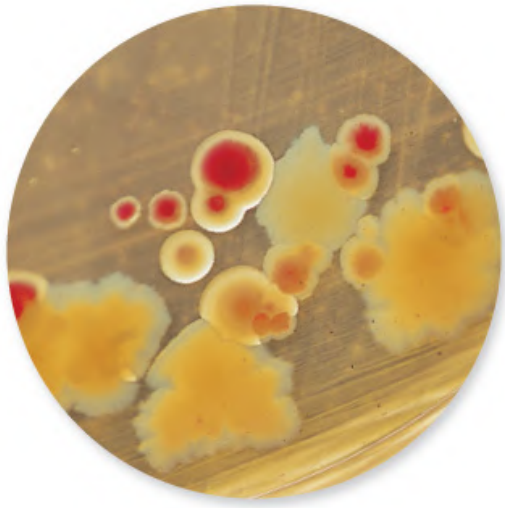

Figure 2.5 A medium that is both selective and differential. MacConkey agar selects against gram-positive bacteria. Therefore, you will not see them here! It also differentiates between lactose-fermenting bacteria (indicated by a pink-red reaction in the center of the colony) and lactose-negative bacteria (indicated by an off-white colony with no dye reaction).
©Kathleen Talaro

Table 2.3 Selective Media, Agents, and Functions

Medium	Selective Agent	Used For
Enterococcus faecalis broth	Sodium azide, tetrazolium	Isolation of fecal enterococci
Tomato juice agar	Tomato juice, acid	Isolation of lactobacilli from saliva
MacConkey agar	Bile, crystal violet	Isolation of gram-negative intestinal bacteria
Salmonella/Shigella (SS) agar	Bile, citrate, brilliant green	Isolation of *Salmonella* and *Shigella*
Lowenstein-Jensen	Malachite green dye	Isolation and maintenance of *Mycobacterium*
Sabouraud's agar	pH of 5.6 (acid)	Isolation of fungi; (inhibits bacteria)

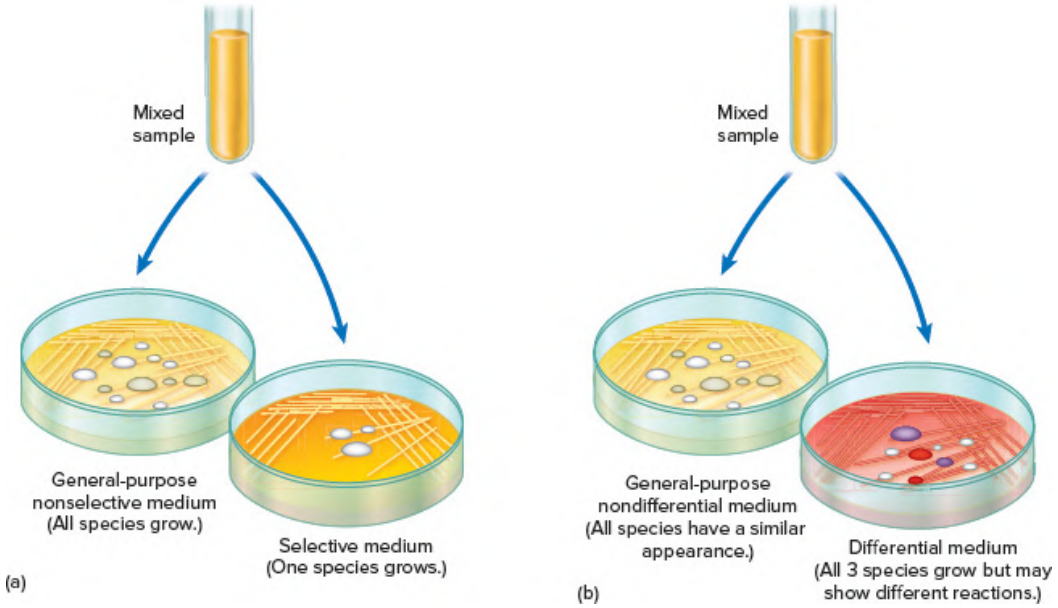

Figure 2.6 **Comparison of selective and differential media with general-purpose media.** **(a)** A mixed sample containing three different species is streaked onto plates of general-purpose nonselective medium and selective medium. **(b)** Another mixed sample containing three different species is streaked onto plates of general-purpose nondifferential medium and differential medium.

illustrates this activity; you just can't see the colonies that were suppressed. Media that are both selective and differential allow for microbial isolation and identification to occur at the same time, which can be very useful in the screening of patient specimens as well as food and water samples.

Dyes are frequently used as differential agents because many of them are pH indicators that change color in response to the production of an acid or a base. For example, MacConkey agar contains neutral red, a dye that is yellow when neutral and pink or red when acidic. A common intestinal bacterium such as *Escherichia coli* that gives off acid when it metabolizes the lactose in the medium develops red to pink colonies, and one like *Salmonella* that does not give off acid remains its natural color (off-white).

NCLEX® PREP

2. A patient with presumed urinary tract infection (UTI) has a urine sample collected and sent to the laboratory for culture. To encourage the growth of scant pathogens in the sample, an enriched medium may be used. Which of the following are substances that may be present in enriched media? Choose all that apply.

a. *serum*
b. *hemoglobin*
c. *vitamins*
d. *growth factors*

Miscellaneous Media A **reducing medium** contains a substance (sodium thioglycollate or cystine) that absorbs oxygen or slows the penetration of oxygen in a medium, thus reducing its

availability. Reducing media are important for growing anaerobic bacteria or for determining oxygen requirements of isolates. **Carbohydrate fermentation media** contain sugars that can be fermented (converted to acids) and a pH indicator to show this reaction **(figure 2.7)**.

Table 2.4 Differential Media

Medium	Substances That Facilitate Differentiation	Differentiates Between or Among
Blood agar	Intact red blood cells	Types of hemolysis displayed by different species of *Streptococcus*
Mannitol salt agar	Mannitol, phenol red	Species of *Staphylococcus*
MacConkey agar	Lactose, neutral red	Bacteria that ferment lactose (lowering the pH) from those that do not
Urea broth	Urea, phenol red	Bacteria that hydrolyze urea to ammonia from those that do not
Sulfur indole motility (SIM)	Thiosulfate, iron	H_2S gas producers from nonproducers
Triple-sugar iron agar (TSIA)	Triple sugars, iron, and phenol red dye	Fermentation of sugars, H_2S production
Birdseed agar	Seeds from thistle plant	*Cryptococcus neoformans* and other fungi

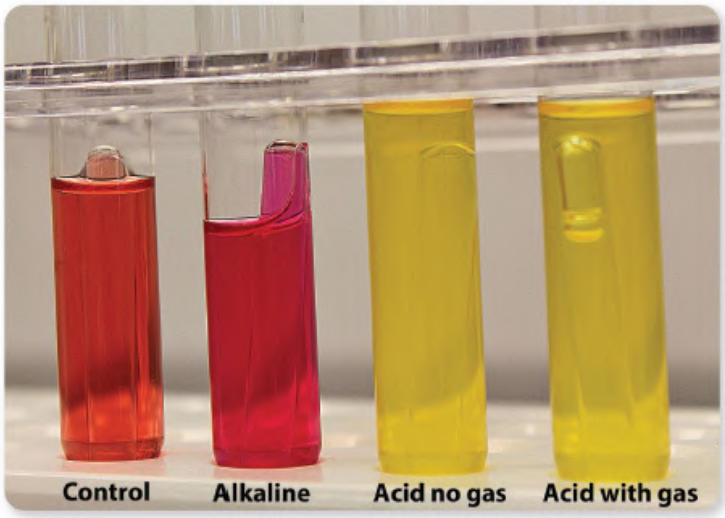

Figure 2.7 Carbohydrate fermentation in broth. This medium is designed to show fermentation (acid production) and gas formation. The medium changes color in the presence of acid (from red to yellow). Gas formation is monitored by placing a small tube upside-down in the medium. If gas bubbles form they are captured inside the small tube.

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Transport media are used to maintain and preserve specimens that have to be held for a period of time before clinical analysis or to sustain delicate species that die rapidly if not held under stable conditions. **Assay media** are used by technologists to test the effectiveness of antimicrobial drugs (see chapter 12) and by drug manufacturers to assess the effect of disinfectants, antiseptics, cosmetics, and preservatives on the growth of microorganisms.

Enumeration media are used by industrial and environmental microbiologists to count the numbers of organisms in milk, water, food, soil, and other samples.

Isolation: Separating One Species from Another

Certain **isolation** techniques are based on the concept that if an individual bacterial cell is separated from other cells and provided adequate space on a solid nutrient surface, it will grow into a discrete mound of cells called a **colony** **(figure 2.8)**. If it was formed from a single cell, a colony consists of just that one species and no other. Proper isolation requires that a small number of cells be inoculated into a relatively large volume or over a large area of medium. It generally requires the following materials: a medium that has a relatively firm surface (see agar in "Physical States of Media," page 41), a Petri dish (a clear, flat dish with a cover), and inoculating tools. With one method, called the streak plate method, a small droplet of culture or sample is spread over the surface of the medium with an *inoculating loop* in a pattern that gradually thins out the sample and separates the cells spatially over several sections of the plate **(figure 2.9*a*)**. The goal here is to allow a single cell to grow into an isolated colony.

In another method, the loop dilution, or pour plate, technique, the sample is inoculated serially into a series of cooled but still liquid agar tubes so as to dilute the number of cells in each successive tube in the series **(figure 2.9*b*)**. Inoculated tubes are then plated out (poured) into sterile Petri dishes and allowed to solidify (harden). The end result (usually in the second or third plate) is that the number of cells per volume is so decreased that cells have ample space to grow into separate colonies. One difference between this and the streak plate method is that in this technique some of the colonies will develop deep in the medium itself. In the streak plate method, the agar is already solidified in the Petri dish before the bacteria are streaked on it.

With the spread plate technique, a small volume of liquid, diluted sample is pipetted onto the surface of the medium and spread around evenly by a sterile

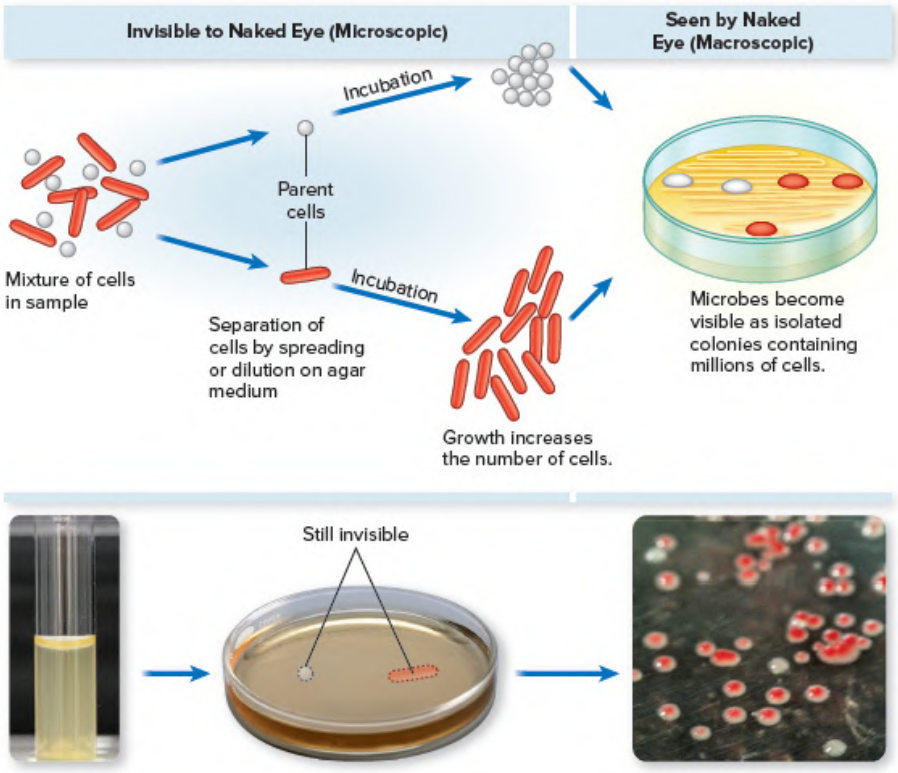

Figure 2.8 Isolation technique. Stages in the formation of an isolated colony, showing the microscopic events and the macroscopic result. Separation techniques such as streaking can be used to isolate single cells. After numerous cell divisions, a macroscopic mound of cells, or a colony, will be formed. This is a relatively simple yet successful way to separate different types of bacteria in a mixed sample.

©McGraw-Hill Education/Lisa Burgess (test tube); ©McGraw-Hill Education/Richard Hutchings (petri dish); ©McGraw-Hill Education/Lisa Burgess (colonies growing on agar medium)

spreading tool (sometimes called a "hockey stick" because of its shape). Like the streak plate, cells are pushed onto separate areas on the surface so that they can form individual colonies **(figure 2.9*c*)**.

Rounding Out the Five I's: Inspection and Identification

How does one determine (i.e., identify) what sorts of microorganisms have been isolated in cultures? Certainly, microscopic appearance can be valuable in differentiating the smaller, simpler bacterial cells from the larger, more complex eukaryotic cells. Appearance can be especially useful in identifying eukaryotic microorganisms to the level of genus or species because of their distinctive morphological features. Unfortunately, bacteria are generally not identifiable by these methods because very different species may appear quite similar. For them, we have to include other techniques, some of which characterize their cellular metabolism. These methods, called biochemical tests, can determine fundamental chemical characteristics such as nutrient requirements, products given off during growth, presence of enzymes, and mechanisms for deriving energy. Their genetic and immunologic characteristics are also used for identification. In chapter 15, we present more detailed examples of the most current genotypic and immunologic identification methods.

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2.1 LEARNING OUTCOMES—Assess Your Progress

1. Explain what the Five I's are and what each step entails.
2. Discuss three physical states of media and when each is used.
3. Compare and contrast selective and differential media, and give an example of each.
4. Provide brief definitions for *defined media* and *complex media*.

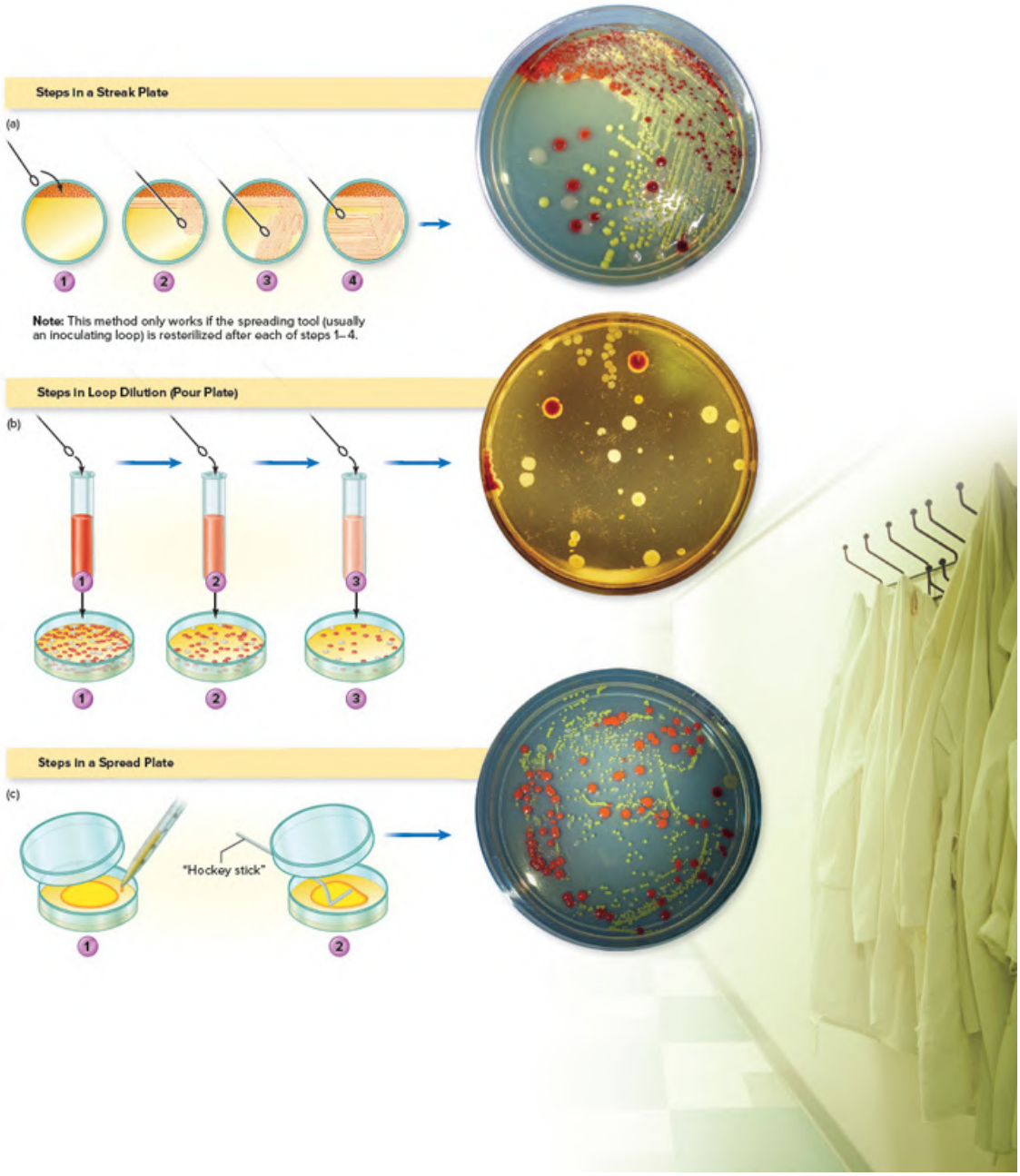


Figure 2.9 **Methods for isolating bacteria**. **(a)** Steps in a quadrant streak plate and resulting isolated colonies of bacteria. **(b)** Steps in the loop dilution method and the appearance of plate 3. **(c)** Spread plate and its result. All photos: ©Kathleen Talaro and Harold Benson

Table 2.5 Conversions Within the Metric System

	Symbol	Factor ("Meter" multiplied by ___)	Numerically	
Giga	Gm	10^9	1 000 000 000	Billion
Mega	Mm	10^6	1 000 000	Million
Kilo	km	10^3	1 000	Thousand
Meter	m		1	
Centi	c	10^{-2}	0.01	Hundredth
Milli	mm	10^{-3}	0.001	Thousandth
Micro	µ	10^{-6}	0.000 001	Millionth
Nano	n	10^{-9}	0.000 000 001	Billionth

2.2 The Microscope

Microbial Size

When we say that microbes are too small to be seen with the unaided eye, what sorts of dimensions are we talking about? Let's compare the size of microbes with the larger organisms of the macroscopic world and on the other hand with the atoms and molecules of the molecular world **(figure 2.10)**. Whereas the dimensions of macroscopic organisms are usually given in centimeters (cm) and meters (m), those of microorganisms fall within the range of millimeters (mm) to micrometers (µm) to nanometers (nm). The very smallest of the microbes are the viruses. They mostly range from 20 nm to about 400 nm, although there are a few types that can be as big as 800 nm or 1,500 nm. (Those viruses are as big as cells.) The smallest bacteria are around 200 nm, and the largest are as large as 750 µm. Yeasts (a single-celled form of fungi) are generally

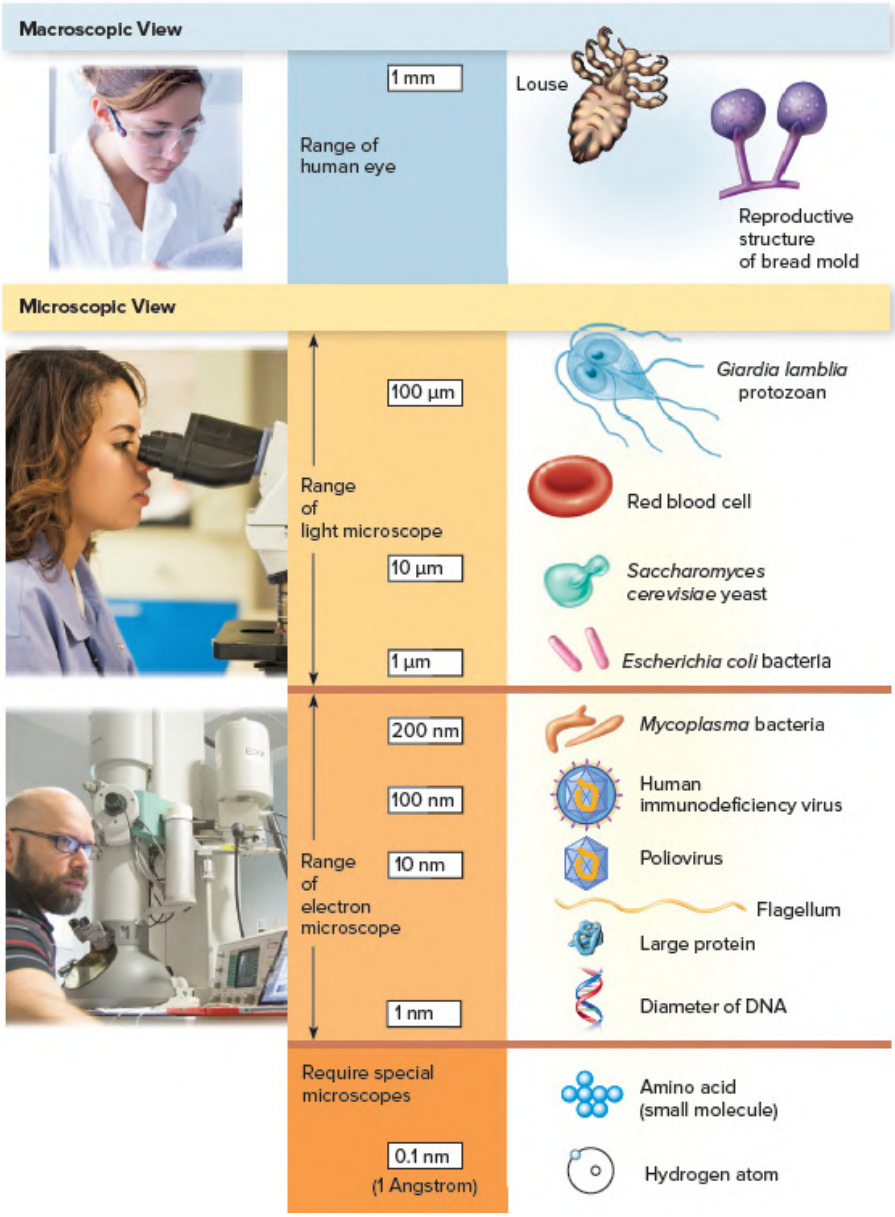


Figure 2.10 **The size of things**. Common measurements encountered in microbiology and a scale of comparison from the macroscopic to the microscopic, molecular, and atomic. Most microbes encountered in our studies will fall between 100 µm and 10 nm in overall dimensions. The microbes shown are more or less to scale within size zone but not between size zones.

©Cultura Creative/Alamy Stock Photo (woman looking at plate); ©Corbis/SuperStock (woman looking in microscope); ©McGraw-Hill Education (A graduate student works with a TEM electron microscope)

3 to 4 µm, though some can be much larger. Protozoa are generally around 3 to 4 mm. It is much easier to get a feel for the relative sizes by looking at a visual (see figure 2.10). Consult **table 2.5** for a reminder of metric measurements and relative size.

Magnification and Microscope Design

The microbial world is of obvious importance, but it would remain largely uncharted without an essential tool: the microscope. The fundamental parts of a modern compound light microscope are illustrated in **figure 2.11**.

Principles of Light Microscopy

Microscopes provide three important qualities:

- magnification,
- resolution,
- and contrast.

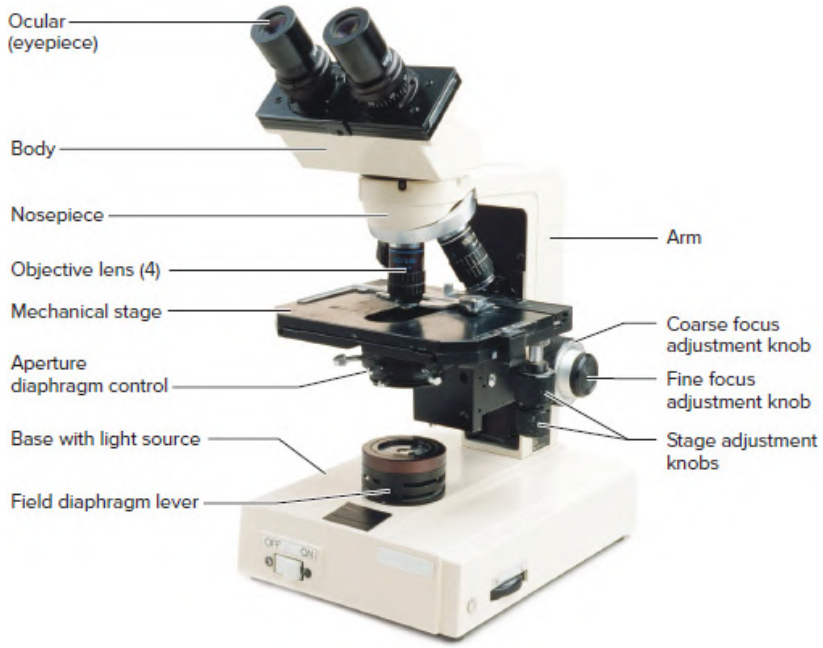


Figure 2.11 **The parts of a student laboratory microscope**. This microscope is a compound light microscope with two oculars (called binocular). It has four objective lenses.
©Peter Skinner/Science Source

Magnification

Magnification occurs in two phases. The first lens in this system (the one closest to the specimen) is the objective lens, and the second (the one closest to the eye) is the ocular lens, or eyepiece **(figure 2.12)**. The objective forms the initial image of the specimen, called the **real image**. When this image is projected up through the microscope body to the plane of the eyepiece, the ocular lens forms a second image, the **virtual image**. The virtual image is the one that will be received by the eye and converted to a retinal and visual image. The magnifying power of the objective lens usually ranges from 4× to 100×, and the power of the ocular lens is usually 10×.

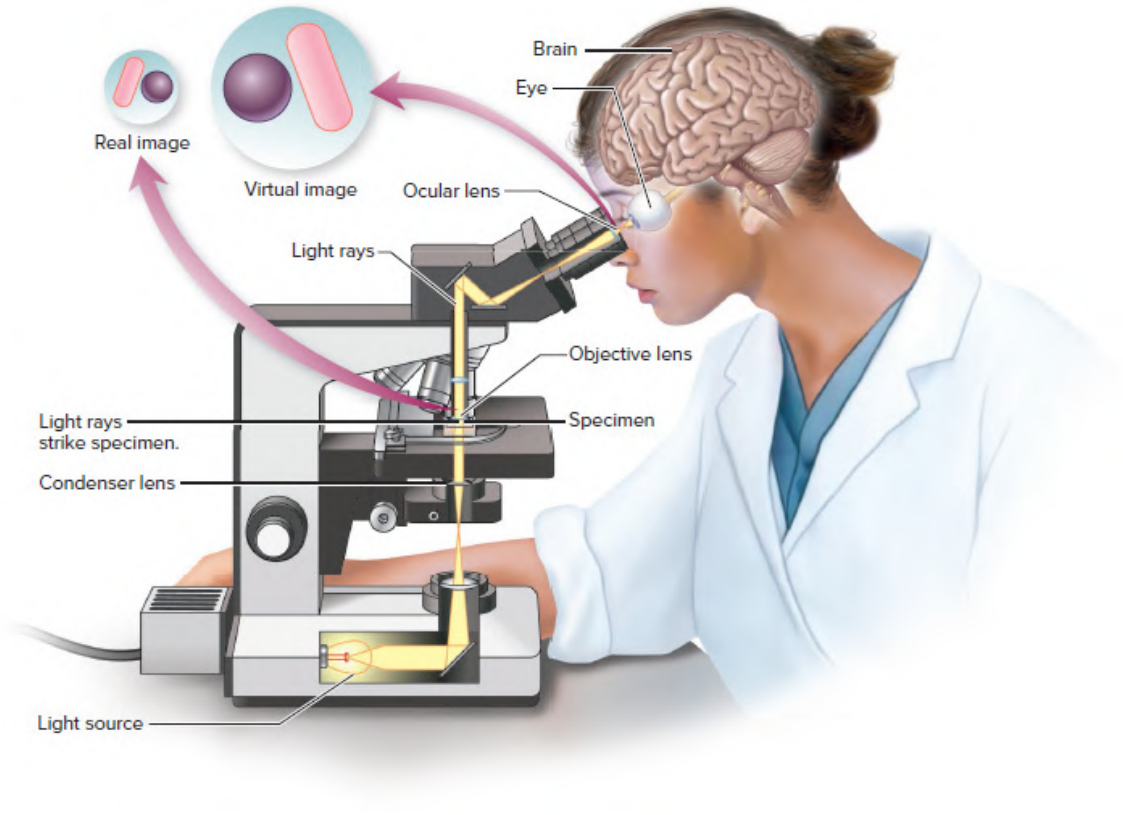


Figure 2.12 The pathway of light and the two stages in magnification of a compound microscope. As light passes through the condenser, it forms a solid beam that is focused on the specimen. Light leaving the specimen that enters the objective lens is refracted so that an enlarged primary image, the real image, is formed. One does not see this image, but its degree of magnification is represented by the smaller circle. The real image is projected through the ocular, and a second image, the virtual image, is formed by a similar process. The virtual image is the final magnified image that is received by the retina and perceived by the brain. Notice that the lens systems cause the image to be reversed.

The total power of magnification of the final image formed by the combined lenses is a product of the separate powers of the two lenses:

Power of objective	*Power of ocular*	*Total magnification*
10× low power objective	× 10×	= 100×
40× high dry objective	× 10×	= 400×
100× oil immersion objective	× 10×	= 1,000×

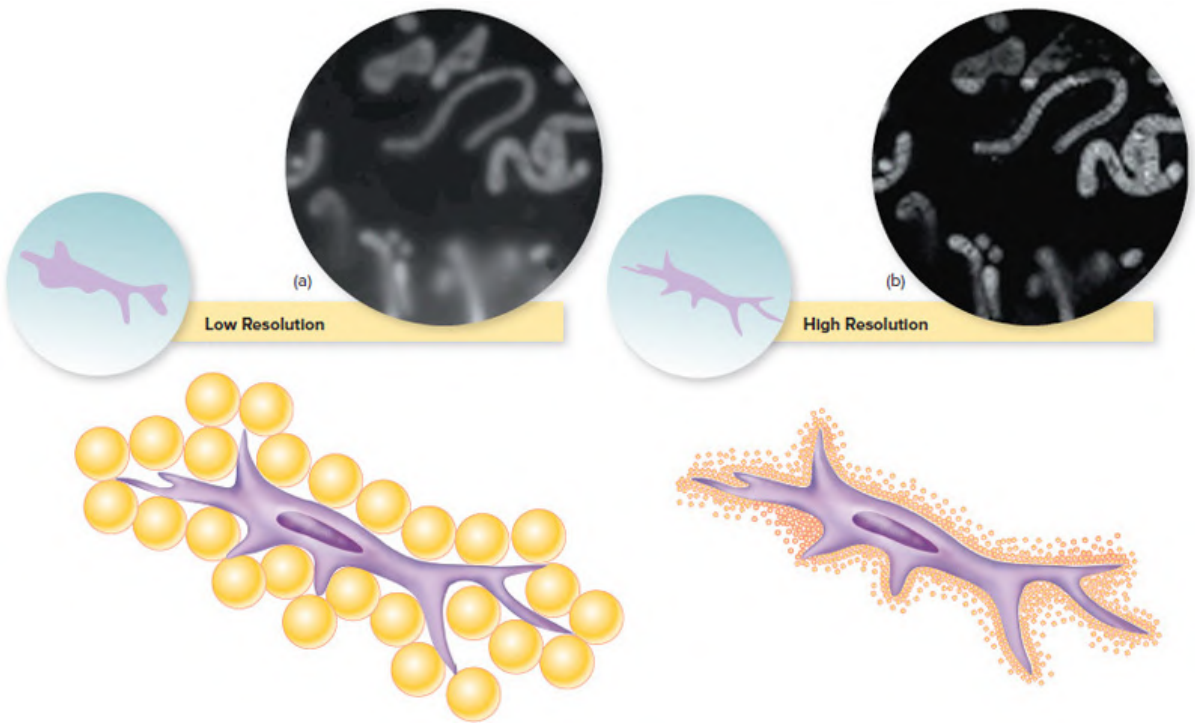
Microscopes are equipped with a nosepiece holding three or more objectives that can be rotated into position as needed. Depending on the power of the ocular, the total magnification of standard light microscopes can vary from 40× with the lowest power objective (called the scanning objective) to 1,000× with the highest power objective (the oil immersion objective).

Resolution: Distinguishing Magnified Objects Clearly

As important as magnification is for visualizing tiny objects or cells, an additional optical property is essential for seeing clearly. That property is resolution, or **resolving power**. Resolution is the capacity of an optical system to distinguish two adjacent objects or points

from one another. For example, at a certain fixed distance, the lens in the human eye can resolve two small objects as separate points as long as the two objects are no closer than 0.2 mm apart. The eye examination given by optometrists is in fact a test of the resolving power of the human eye for various-size letters read at a particular distance. **Figure 2.13** should help you understand the concept of resolution.

The oil immersion lens (100× magnification) uses oil to capture some of the light that would otherwise be lost to scatter **(figure 2.14)**. Reducing this scatter increases resolution. In practical terms, the oil immersion lens can resolve any cell or cell part



Source: CDC

Figure 2.13 **Effect of wavelength on resolution**. A simple model demonstrates how the wavelength of light influences the resolving power of a microscope. The size of the balls illustrates the relative size of the wave. Here, a human cell (fibroblast) is illuminated with long wavelength light **(a)** and short wavelength light **(b)**. In (a), the waves are too large to penetrate the tighter spaces and produce a fuzzy, undetailed image.
Courtesy Nikon Instruments Inc.

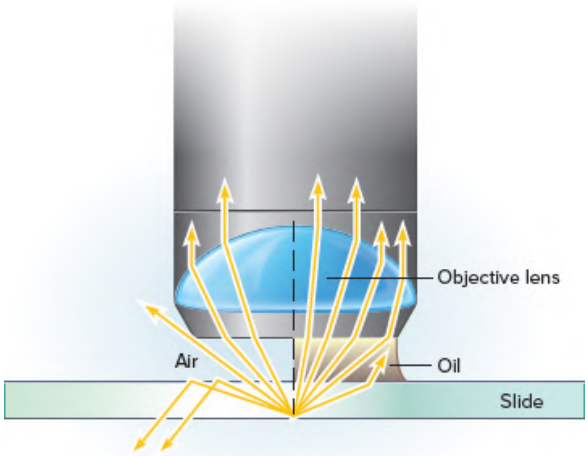


Figure 2.14 **Workings of an oil immersion lens**. Without oil, some of the peripheral light that passes through the specimen is scattered into the air or onto the glass slide; this scattering decreases resolution.

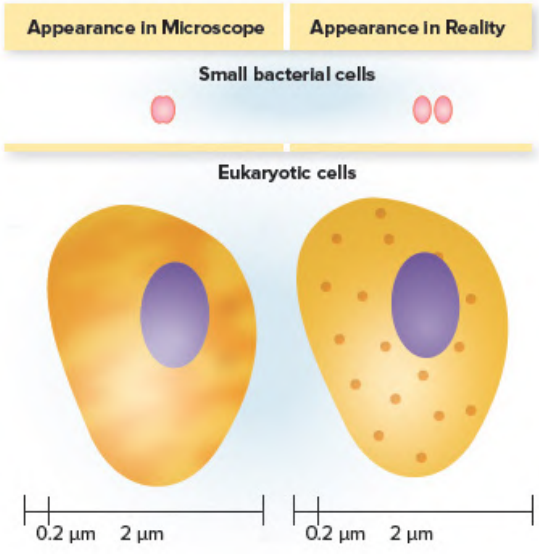


Figure 2.15 **The importance of resolution**. If a microscope has a resolving power of 0.2 µm, then the bacterial cells would not be resolvable as two separate cells. Likewise, the small specks inside the eukaryotic cell will not be visible.

as long as it is at least 0.2 µm in diameter, and it can resolve two adjacent objects as long as they are at least 0.2 µm apart **(figure 2.15)**. In general, organisms that are 0.5 µm or more in diameter are easily seen. This includes fungi and protozoa, some of their internal structures, and most bacteria. However, a few bacteria and most viruses are far too small to be resolved by the optical microscope and require electron microscopy (discussed later in this chapter). In summary, then, the factor that most limits the clarity of a microscope's image is its resolving power. Even if a light microscope were designed to magnify several thousand times, its resolving power could not be increased, and the image it produced would simply be enlarged and fuzzy.

Contrast

The third quality of a well-magnified image is its degree of contrast from its surroundings. The contrast is measured by a quality called the **refractive index**. Refractive index refers to the degree of bending that light undergoes as it passes from one medium, such as water or glass, to another medium, such as bacterial cells. The higher the difference in refractive indexes (the more bending of light), the sharper the contrast that is registered by the microscope and the eye. Because too much light can reduce contrast and burn out the image, an adjustable iris diaphragm on most microscopes controls the amount of light entering the condenser. The lack of contrast in cell components can be compensated for by using special lenses (the phase-contrast microscope) and by adding dyes.

Different Types of Light Microscopes

Optical microscopes that use visible light can be described by the nature of their *field*, meaning the circular area viewed through the ocular lens. We will discuss three types of visible-light microscopes: bright-field, dark-field, and phase-contrast. A fourth type of optical microscope, the fluorescence microscope, uses ultraviolet radiation as the illuminating source; another, the confocal microscope, uses a laser beam. Each of these microscopes is adapted for viewing specimens in a particular way, as described in **table 2.6**.

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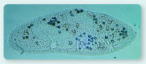
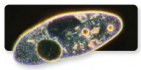
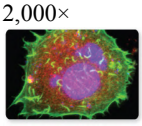
3. The capacity of an optical system to distinguish or separate two adjacent objects or points from one another is known as
- **a.** *the real image*.
- **b.** *the virtual image*.
- **c.** *resolving power*.
- **d.** *numerical aperture*.
- **e.** *power*.

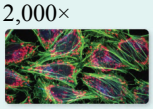
Table 2.6 Comparison of Types of Microscopy

Visible light as source of illumination

Microscope	Maximum Practical Magnification	Resolution
Bright-Field The bright-field microscope is the most widely used type of light microscope. Although we ordinarily view objects (like the words on this page) with light reflected off the surface, a bright-field microscope forms its image when light is transmitted *through* the specimen. The specimen, being denser and more opaque than its surroundings, absorbs some of this light, and the rest of the light is transmitted directly up through the ocular. As a result, the specimen will produce an image that is darker than the surrounding brightly illuminated field. The bright-field microscope is a multipurpose instrument that can be used for both live, unstained material and preserved, stained material.	2,000×  *Paramecium* (400×)	0.2 µm (200 nm)

Visible light as source of illumination		
Microscope	**Maximum Practical Magnification**	**Resolution**
Dark-Field A bright-field microscope can be adapted as a dark-field microscope by adding a special disc called a *stop* to the condenser. The stop blocks all light from entering the objective lens—except peripheral light that is reflected off the sides of the specimen itself. The resulting image is a particularly striking one: brightly illuminated specimens surrounded by a dark (black) field. The most effective use of dark-field microscopy is to visualize living cells that would be distorted by drying or heat or that cannot be stained with the usual methods.	2,000×  *Paramecium* (400×)	0.2 µm
Phase-Contrast If similar objects made of clear glass, ice, cellophane, or plastic are immersed in the same container of water, an observer would have difficulty telling them apart because they have similar optical properties. Internal components of a live, unstained cell also lack contrast and can be difficult to distinguish. But cell structures do differ slightly in density, enough that they can alter the light that passes through them in subtle ways. The phase-contrast microscope has been constructed to take advantage of this characteristic. This microscope contains devices that transform the subtle changes in light waves passing through the specimen into differences in light intensity. For example, denser cell parts such as organelles alter the pathway of light more than less dense regions (the cytoplasm). Light patterns coming from these regions will vary in contrast. The amount of internal detail visible by this method is greater than by either bright-field or dark-field methods. The phase-contrast microscope is most useful for observing intracellular structures such as bacterial endospores, granules, and organelles, as well as the locomotor structures of eukaryotic cells such as cilia.	2,000× *Paramecium* (400×)	0.2 µm
Ultraviolet rays as source of illumination		
Microscope	**Maximum Practical Magnification**	**Resolution**
Fluorescence The fluorescence microscope is a specially modified compound microscope furnished with an ultraviolet (UV) radiation source. The name of this type of microscopy comes from the use of certain dyes (acridine, fluorescein) and minerals that show **fluorescence**. The dyes emit visible light when bombarded by short ultraviolet rays. For an image to be formed, the specimen must first be coated or placed in contact with a source of fluorescence. Subsequent illumination by ultraviolet radiation causes the specimen to give off light that will form its own image, usually an intense red, blue, or green against a black field. Fluorescence microscopy has its most useful applications in diagnosing infections and pinpointing particular cellular structures.	2,000×  Fluorescence image of a eukaryotic cell	0.2 µm

©Science Photo Library/Getty Images (SEM: coccolithophore)

Ultraviolet rays as source of illumination (continued)		
Microscope	**Maximum Practical Magnification**	**Resolution**
Confocal The scanning confocal microscope overcomes the problem of cells or structures being too thick. This is a problem for other types of microscopes, as they are unable to focus on all of their levels. This microscope uses a laser beam of light to scan various depths in the specimen and deliver a sharp image focusing on just a single plane. It is thus able to capture a highly focused view at any level, ranging from the surface to the middle of the cell. It is most often used on fluorescently stained specimens but it can also be used to visualize live unstained cells and tissues.	2,000×  Myofibroblasts, cells involved in tissue repair (400×)	0.2 µm
Electron beam forms image of specimen		
Microscope	**Maximum Practical Magnification**	**Resolution**
Transmission Electron Microscope (TEM) Transmission electron microscopes are the method of choice for viewing the detailed structure of cells and viruses. This microscope produces its image by transmitting electrons through the specimen. Because electrons cannot easily penetrate thick preparations, the specimen must be sectioned into extremely thin slices (20–100 nm thick) and stained or coated with metals that will increase image contrast. The darker areas of TEM micrographs represent the thicker (denser) parts, and the lighter areas indicate the more transparent and less dense parts.	100,000,000× Coronavirus, causative agent of many respiratory infections (100,000×)	0.5 nm

Ultraviolet rays as source of illumination (continued)		
Microscope	**Maximum Practical Magnification**	**Resolution**
Scanning Electron Microscope (SEM) The scanning electron microscope provides some of the most dramatic and realistic images in existence. This instrument is designed to create an extremely detailed three-dimensional view of all kinds of objects—from plaque on teeth to tapeworm heads. To produce its images, the SEM bombards the surface of a metal-coated specimen with electrons while scanning back and forth over it. A shower of electrons deflected from the surface is picked up with great accuracy by a sophisticated detector, and the electron pattern is displayed as an image on a television screen. You will often see these images in vivid colors. The color is always added afterward; the actual microscopic image is black and white.	100,000,000× Algae showing cell walls made of calcium discs (10,000×)	10 nm
©M. I. Walker/Science Source (light microscope image); ©Kent Wood/Science Source (dark-field image); ©Roland Birke/Photolibrary/Getty Images (phase-contrast); ©Dr. Dan Kalman/Science Source (immunofluorescence image); ©Dr. Gopal MurtScience Source (confocal light image micrograph); Source: CDC/Dr. Erskine Palmer (transmission electron micrograph); ©Science Photo Library/Getty Images (scanning electron micrograph)		

Preparing Specimens for the Microscope

A specimen for optical microscopy is generally prepared by placing a sample on a suitable glass slide. This is then placed on the microscope stage between the condenser and the objective lens. The manner in which a slide specimen (also called a mount) is prepared depends upon (1) the condition of the specimen, either in a living or preserved state; (2) the aims of the examiner, whether to observe overall structure, identify the microorganisms, or see movement; and (3) the type of microscopy available, whether it is bright-field, dark-field, phase-contrast, or fluorescence.

Fresh, Living Preparations

Live samples of microorganisms are examined using wet mounts or in hanging drop mounts so that they can be observed as near to their natural state as possible. The cells are suspended in a suitable fluid (water, broth, saline) that temporarily maintains viability and provides space and a medium for locomotion. A wet mount consists of a drop or two of the culture placed on a slide and overlaid with a coverslip. The hanging drop preparation is made with a special concave (depression) slide, a Vaseline adhesive or sealant, and a coverslip from which a tiny drop of sample is suspended **(figure 2.16)**. These short-term mounts provide a true assessment of the size, shape, arrangement, color, and motility of cells. However, if you need to visualize greater cellular detail, you will have to use phase-contrast microscopy, which requires that the specimens are "fixed" and therefore no longer alive.

Fixed, Stained Smears

A more permanent slide for long-term study can be obtained by preparing fixed, stained specimens. "Fixed" in this context means that the organisms are dried and attached to the glass slide. The so-called smear technique, developed by Robert Koch more than 100 years ago,

consists of spreading a thin film made from a liquid suspension of cells on a slide and air-drying it. Next, the air-dried smear is usually heated gently by a process called heat fixation that simultaneously kills the specimen and secures it to the slide.

Stains

Like images on undeveloped photographic film, the unstained cells of a fixed smear are very difficult to see, no matter how great the magnification or how fine the resolving power of the microscope. To solve this problem, stains can be added to the fixed smear, either coloring the objects (microbes) or coloring the background so that the unstained microbes stand out. Staining is any procedure that applies colored chemicals called *dyes* to specimens. Dyes impart a color to cells or cell parts by becoming affixed to them through a chemical reaction. Dyes can be classified as basic (cationic) dyes, which have a positive charge, or acidic (anionic) dyes, which have a negative charge. Because chemicals of opposite charge are attracted to each other, cell parts that are negatively charged will attract basic dyes, and those that are

©Noel Hendrickson/Digital Vision/Getty Images

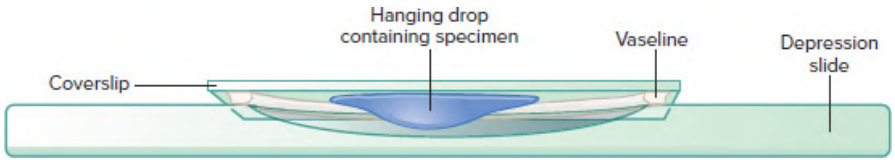
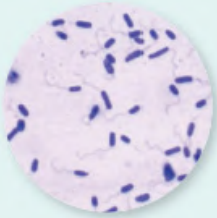


Figure 2.16 **Hanging drop technique**. Cross-section view of slide and coverslip. (Vaseline actually surrounds entire well of slide.)

Table 2.7 Three Categories of Media Classification

	Positive Staining	Negative Staining
Appearance of cell	Colored by dye	Clear and colorless
Background	Not stained (generally white)	Stained (dark gray or black)
Dyes employed	Basic dyes: Crystal violet Methylene blue Safranin Malachite green	Acidic dyes: Nigrosin India ink
Subtypes of stains	Simple stain Differential stains Gram stain Acid-fast stain Spore stain Special stains Capsule Flagella Spore Granules Nucleic acid	Capsule Spore



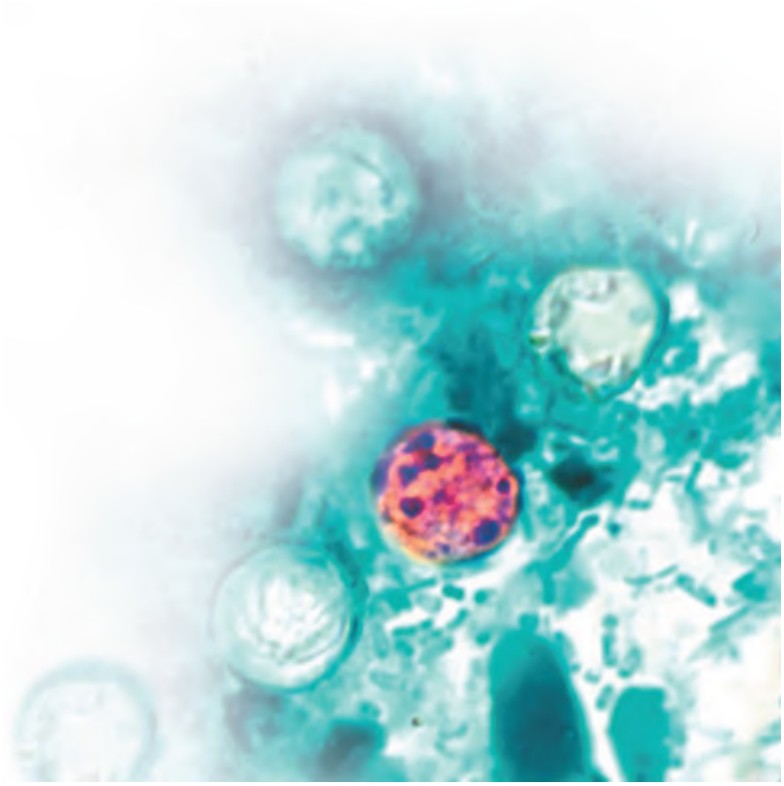
Photos: ©McGraw-Hill Education/Lisa Burgess

positively charged will attract acidic dyes. Many cells, especially those of bacteria, have numerous negatively charged acidic substances on their surfaces and thus stain more readily with basic dyes. Acidic dyes, on the other hand, tend to be repelled by cells, so they are good for negative staining (staining the background).

Negative Versus Positive Staining Two basic types of staining technique are used, depending upon how a dye reacts with the specimen (summarized in **table 2.7**). Most procedures involve a **positive stain**, in which the dye actually sticks to the specimen and gives it color. A **negative stain**, on the other hand, is just the reverse (like a photographic negative). The dye does not stick to the specimen but settles some distance from its outer boundary, forming a silhouette. Nigrosin (blue-black) and India ink (a black suspension of carbon particles) are the dyes most commonly used for negative staining. The cells themselves do not stain because these dyes are negatively charged and are repelled by the negatively charged surface of the cells. The value of negative staining is its simplicity and the reduced shrinkage or distortion of cells, as the smear is not heat fixed. Negative staining is also used to accentuate the capsule that surrounds certain bacteria and yeasts.

Simple Versus Differential Staining Positive staining methods fall into three categories: simple, differential, or special. **Simple stains** require only a single dye. **Differential stains** use two differently colored dyes, called the *primary dye* and the *counterstain*, to distinguish between cell types or parts. Differential staining techniques tend to be more complex and sometimes require additional chemical reagents to produce the desired reaction.

Simple stains cause all cells in a smear to appear more or less the same color, regardless of type, but they can still reveal bacterial characteristics such as shape, size, and arrangement (**figure 2.17**).



Photomicrograph of stool sample stained with acid-fast stain revealing *Cyclospora*.
Source: CDC/DPDx - Melanie Moser

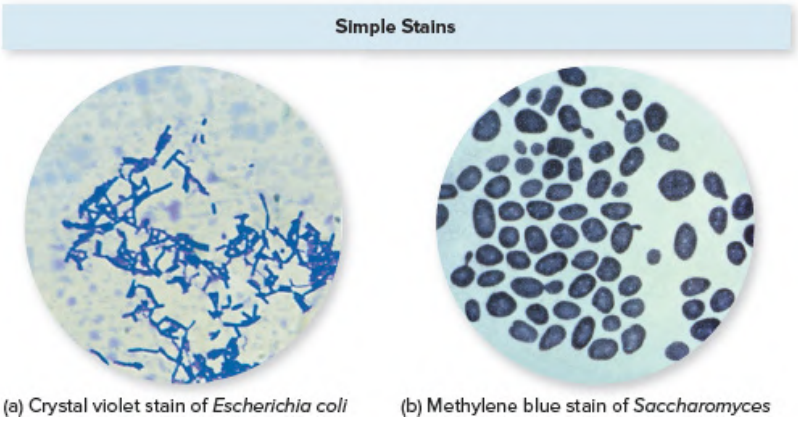


Figure 2.17 **Simple stains**.
©Kathleen Talaro (a); Source: CDC (b)

Medical Moment

Gram-Positive Versus Gram-Negative Bacteria

The Gram stain is one type of differential stain that can help to identify bacterial species and guide treatment decisions. Differentiating between gram-positive and gram-negative organisms is important. One of the main differences between gram-positive and gram-negative bacteria is that gram-negative bacteria have an outer membrane containing LPS (lipopolysaccharide). This molecule is also called endotoxin, which can cause a severe reaction if it enters the circulatory system, causing symptoms of shock (high fever, dangerously low blood pressure, and elevated respiratory rate). This is known as endotoxic shock.

Q. This molecule has two names: lipopolysaccharide and endotoxin. Which of these is most often used by medical doctors and which is more likely to be used by microbiology researchers?

Answer in Appendix B.

Types of Differential Stains A typical differential stain uses differently colored dyes to clearly contrast two cell types or cell parts. Common combinations are red and purple, red and green, or pink and blue **(figure 2.18)**. Typical examples include the Gram stain, the acid-fast, and the endospore stain. Some staining techniques (endospore, capsule), which are differential, are also in the "special" category.

The Gram Stain In 1884, Hans Christian Gram discovered a staining technique that could be used to make bacteria in infectious specimens more visible. His technique consisted of sequential applications of crystal violet (the primary dye), Gram's iodine (the mordant), an alcohol rinse (decolorizer), and a contrasting counterstain. Bacteria that stain purple are called gram-positive, and those that stain red are called gram-negative. Gram-variable organisms produce both pink- and purple-staining cells.

The different results in the Gram stain are due to differences in the structure of the cell envelope and how it reacts to the series of reagents applied to the cells. We will study it in more detail in chapter 3.

This century-old staining method remains a universal basis for bacterial classification and identification. The Gram stain can also be a practical aid in diagnosing infection and in guiding drug treatment. For example, Gram staining a fresh sputum or spinal fluid specimen can help pinpoint the possible cause of infection, and in some cases it is possible to begin drug therapy on the basis of this stain. Even in this day of elaborate and expensive medical technology, the Gram stain remains an important first tool in diagnosis.

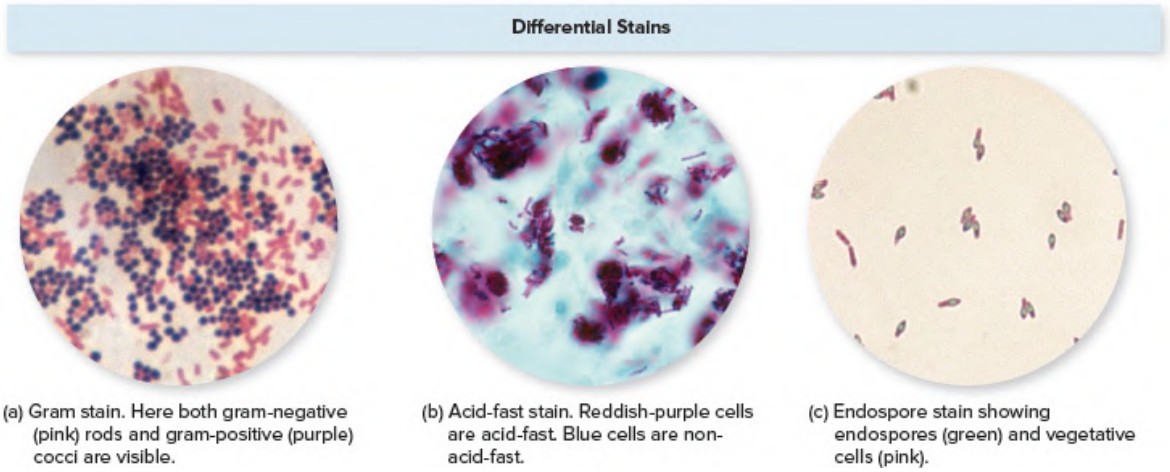


Figure 2.18 **Differential stains**.
©ASM/Science Source (a); ©Richard J. Green/Science Source (b); Source: CDC/Courtesy Larry Stauffer, Oregon State Public Health Laboratory (c)

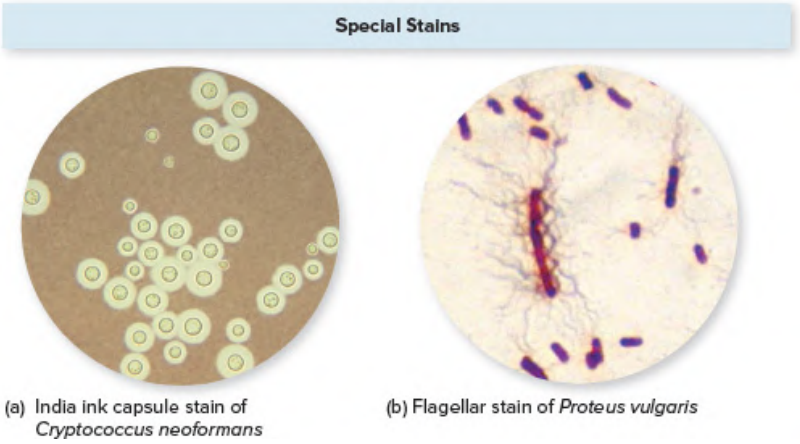


Figure 2.19 **Special stains**.
Source: CDC/Dr. Leanor Haley (a); ©David Fankhauser (b)

Other Differential Stains The **acid-fast stain**, like the Gram stain, is an important diagnostic stain that distinguishes acid-fast bacteria (pink) from non-acid-fast bacteria (blue). (In this context *fast* means "resistant to.") This stain originated as a specific method to detect *Mycobacterium tuberculosis* in specimens. It was determined that these bacterial cells have a particularly impervious outer wall that holds fast (tightly or tenaciously) to the dye (carbol

fuchsin) even when washed with a solution containing acid or acid alcohol. This stain is used for other medically important bacteria, fungi, and protozoa. Often it is performed when a gram-variable result is seen in a specimen.

The **endospore** stain (spore stain) is similar to the acid-fast method in that a dye is forced by heat into resistant bodies called endospores (their characteristics and significance are discussed in chapter 3). This stain is designed to distinguish between endospores and the cells that they come from (so-called **vegetative** cells). Of significance in medical microbiology are the gram-positive, endospore-forming members of the genus *Bacillus* (the cause of anthrax) and *Clostridium* (the cause of botulism and tetanus)—dramatic diseases that we consider in later chapters.

Special Stains Special stains are used to emphasize certain cell parts that are not revealed by conventional staining methods **(figure 2.19). Capsular staining** is a method of observing the microbial capsule, an unstructured protective layer surrounding the cells of some bacteria and fungi. Because the capsule repels most stains, it is often negatively stained with India ink, or it may be demonstrated by special positive stains. One example is *Cryptococcus*, which causes a serious form of fungal meningitis in AIDS patients (see chapter 17).

Flagellar staining is a method of revealing **flagella** (singular, *flagellum*), the tiny, slender filaments used by bacteria for locomotion. Because the width of bacterial flagella lies beyond the resolving power of the light microscope, in order to be seen, they must be enlarged by depositing a coating on the outside of the filament and then staining it. Their presence, number, and arrangement on a cell are useful for identification of the bacteria.

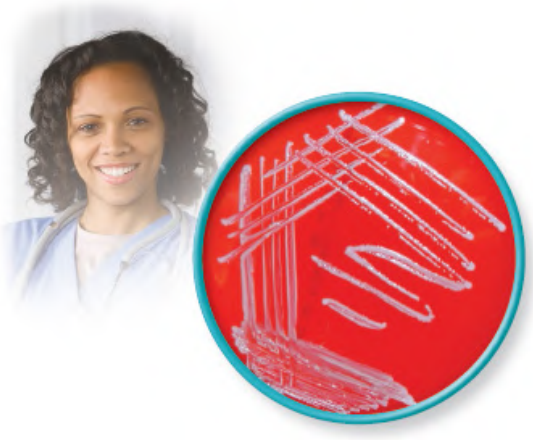
©Flying Colours Ltd/Getty Images

2.2 LEARNING OUTCOMES—Assess Your Progress

5. Convert among the different units of the metric system.

6. List and describe the three elements of good microscopy.
7. Differentiate between the principles of light microscopy and the principles of electron microscopy.
8. Give examples of simple, differential, and special stains.

CASE FILE WRAP-UP



Staphylococcus epidermidis growing on blood agar.
©Terry Vine/Blend Images, LLC (health care worker); ©McGraw-Hill Education/James Redfearn (agar dish)

Identification of microorganisms in the laboratory can take several days to allow for culture growth. Patients with clinical signs of infection, such as fever, rapid heart rate, low blood pressure, high white blood cell count, or localized signs of infection (tenderness, redness, swelling, heat, purulent drainage) are often started on treatment to curb infection. Some antibiotics, referred to as broad-spectrum antibiotics, treat a wide variety of bacterial infections. Based on the suspected source of infection, antifungal or antiviral treatment may also be started. As more information emerges from the lab regarding the culture, the antibiotic treatment is modified to treat more specific types of microorganisms, and eventually, the exact organism may be identified from the culture.

The patient's fever did not initially improve with broad-spectrum antibiotics. The X ray of the patient's leg revealed an abscess, or pocket of pus. The patient returned to the operating room to have the surgical site washed out. A sample of fluid from the abscess was collected and sent to the lab for culture. In just a few hours, the wound culture had already been identified as gram-positive bacteria. The provider changed the antibiotic prescription to one known to specifically treat gram-positive organisms.

After 3 days, the laboratory confirmed that there were no bacteria seen in the patient's blood culture. The specimen in the abscess fluid was identified as *Staphylococcus epidermidis*. Through further testing, the lab was able to recommend the antibiotics that

would best treat the organism. The source of infection had been recognized and effective treatment initiated.

X ray of lower leg with implanted rods and screws
©Thinkstock Images/Getty Images

The Microbiome

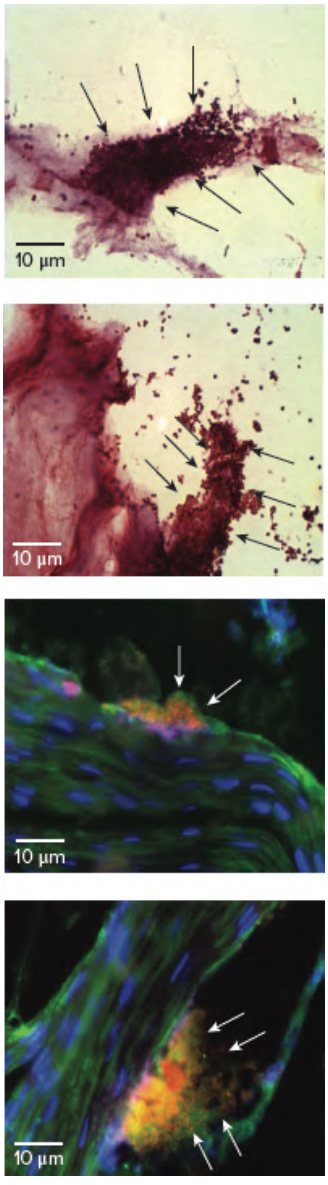
Diabetic Wounds and Their Microbiome as Seen by Microscopy

Diabetic ulcers are an extremely difficult problem in health care. They don't heal well due to reduced circulation, and when they are on extremities, they can even lead to the amputation of limbs. In this chapter, we are learning about ways to (1) sample and isolate microorganisms, and (2) visualize them. A research group in the United Kingdom wanted to look at the microbiome associated with diabetic wounds, and so they used microscopy on tissue samples without isolating and growing the bacteria from them—they just looked directly at the tissue without disturbing it—a process that is easier said than done.

The scientists from Manchester obtained four tissue fragments that had been removed from diabetic foot ulcers by debridement at a diabetic care clinic. The tissues were kept in sterile saline until they were processed for microscopy. The tissues were stained using the differential Gram staining method and then examined with light microscopy, or they were tagged with special molecules that attached only to bacterial (not human) chemicals. Those

molecules had fluorescent dyes linked to them, so that when the tissue was viewed under a fluorescence microscope the bacteria appeared fluorescent red. The resulting images show a robust community of bacteria associated with the wound tissue.

The top images are Gram-stained wound tissues, with clearly visible purple cocci and (fainter) red rods. The bottom images are wound tissue stained with fluorescent probes that caused bacteria to appear red. The green material with blue dots represents human (wound) tissue.

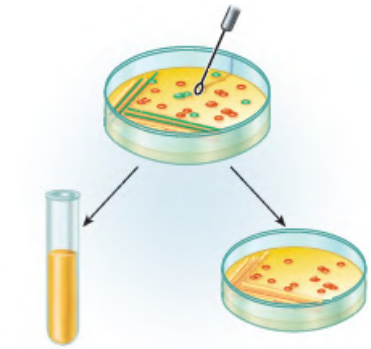


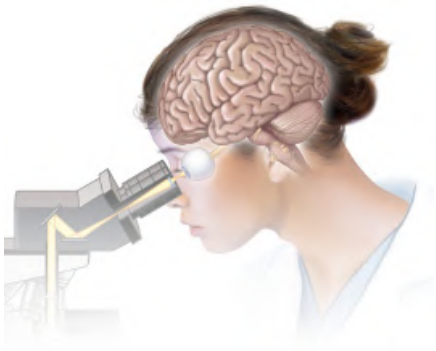
Source: *Journal of Diabetes Research*, 2014.

Chapter Summary

2.1 How to Culture Microorganisms

- The Five I's—inoculation, incubation, isolation, inspection, and identification—summarize the kinds of laboratory procedures used in microbiology.
- Following *inoculation*, cultures are *incubated* at a specified temperature to encourage growth.
- Many microorganisms can be cultured on artificial media, but some can be cultured only in living tissue or in cells.
- Artificial media are classified by their *physical state* as either liquid, semisolid, or solid.
- Artificial media are classified by their *chemical composition* as either *defined* or *complex*, depending on whether the exact chemical composition is known.
- Enriched, selective, differential, transport, assay, and enumerating media are all examples of media designed for specific purposes.
- *Isolated* colonies that originate from single cells are composed of large numbers of cells piled up together.
- A culture may be *pure*, containing only one species or type of microorganism; *mixed*, containing two or more known species; or *contaminated*, containing both known and unknown (unwanted) microorganisms.
- During *inspection*, the cultures are examined and evaluated macroscopically and microscopically.
- Microorganisms are *identified* in terms of their macroscopic or immunologic morphology, their microscopic morphology, their biochemical reactions, and their genetic characteristics.





2.2 The Microscope

- Magnification, resolving power, and contrast all influence the clarity of specimens viewed through the optical microscope.
- The maximum resolving power of the optical microscope is 200 nm, or 0.2 μm. This is sufficient to see the internal structures of eukaryotes and the general shape of most bacteria.
- We described five types of optical microscopes. Three types use visible light for illumination: bright-field, dark-field, and phase-contrast microscopes. The fluorescence microscope uses UV light for illumination. The confocal microscope can use UV light or visible light reflected from specimens.

- Electron microscopes (EM) use electrons, not light waves, as an illumination source to provide high magnification (5,000× to 1,000,000×) and high resolution (0.5 nm).
- Specimens viewed through optical microscopes can be either alive or dead, depending on the type of specimen preparation, but all EM specimens are dead because they must be viewed in a vacuum.
- The Gram stain is an immensely useful differential stain that divides bacteria into two main groups, gram-positive and gram-negative. Some bacteria do not fall in either of these categories.
- Stains increase the contrast of specimens and they can be designed to differentiate cell shape, structure, and biochemical composition of the specimens being viewed.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. The identities of microorganisms on our planet **a**. are mostly known. **b**. have nearly all been identified via microscopy. **c**. have nearly all been identified via culturing techniques. **d**. are still mostly unknown.	**2**. Before the availability of molecular techniques, archaea and bacteria were considered to be very closely related. Explain why that is understandable, based on what you learned about microscopy.	**3**. Often bacteria that are freshly isolated from a patient or the environment have thick sugary outer layers (capsules). After they are transferred many times on laboratory medium, they tend to lose their capsule. Speculate about why that might happen.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Cell Structure and Function	**4**. Which of these types of organisms is least likely to be identified to the genus level with light microscopy? **a**. bacteria **b**. protozoa **c**. fungus **d**. helminth	**5**. Obviously, *structures* of microorganisms can be revealed by various types of microscopy. Can you think of any *functions* that might also be revealed? Which ones, and by which microscopic technique?	**6**. Some bacteria can produce a structure called an endospore, which is used as a survival structure. It is surrounded by a very tough and impervious outer layer, that keeps it protected in adverse environmental conditions. What kinds of things would you need to know about this structure if you wanted to devise a way to stain it and see it under a microscope?

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Metabolic Pathways	**7**. A fastidious organism must be grown on what type of medium? **a**. general-purpose medium **b**. differential medium **c**. defined medium **d**. enriched medium	**8**. Write a short paragraph to differentiate among the following: *selective medium*, *differential medium, enriched medium*.	**9**. Alexander Fleming discovered penicillin in 1928. The first part of the story is that he noticed that agar plates upon which he was growing a bacterium were contaminated with obvious fungal colonies. Instead of throwing the plates out because they were contaminated, he examined them and realized that around the areas where the fungi were growing, there were no bacteria growing. Finish this story.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Information Flow and Genetics	**10**. Viruses are commonly grown in/on **a**. animal cells or tissues. **b**. agar plates. **c**. broth cultures. **d**. all of the above.	**11**. Can you devise a growth medium with ingredients that would detect whether a culture of MRSA (antibiotic-resistant *Staphylococcus aureus)* that you have been storing in your lab is still antibiotic resistant?	**12**. There is a type of differential medium that can reveal whether bacteria growing on it produce and secrete an enzyme that breaks down DNA. Why would a bacterium secrete an enzyme that destroys DNA?

Microbial Systems	**13**. Most of the time, microbes in natural circumstances exist **a**. as single cells relationship. **b**. in relationship with other species. **c**. as single species. **d**. as colonies on agar.	**14**. Several bacteria live naturally in a material on your teeth called *plaque* that contains many different species, which interact with each other in significant ways. Identify some of the problems of studying one	**15**. Archaea often grow naturally in extreme environments. Those called *extreme thermophiles* thrive in hot springs and in high-temperature hydrothermal vents in the ocean. Invent a culturing technique you could use that would be selective for these organisms.

		of these bacterial species after isolating it through a streak plate procedure and examining its behavior.	
Impact of Microorganisms	**16**. Diagnosis of infections in a hospitalized person is often accomplished via **a**. microscopy. **b**. culture of samples from patient. **c**. palpitation of infected area. **d**. two of the above.	**17**. After performing the streak plate procedure on a bacterial specimen, the culture was incubated. When you viewed the plate there was heavy growth with no isolated colonies in the first quadrant. There was no other growth on the plate (in second quadrant and beyond). Discuss possible errors that could have led to this result.	**18**. You are a scientist studying a marsh area contaminated with PCBs, toxic chemical compounds found in industrial waste. You look at a water sample using light microscopy and discover motile single cells that are about 1 micron in circumference. You hypothesize that these microbes might be useful in cleaning up the toxic waste. Since there is no instruction book for how to culture them, how might you go about creating a growth medium for them?
Scientific Thinking	**19**. If a Gram stain result is	**20**. Explain the difference	**21**. You perform the special stain for

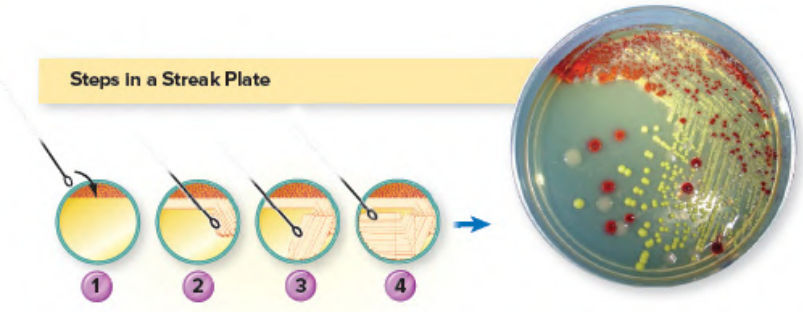
	unclear, which of the following should be considered? **a**. getting a new sample **b**. restaining same sample **c**. identity of microbe may actually be gram-variable **d**. all of the above	between multiplying a meter by 10^9 and multiplying it by 10^{-9} to get a gigameter and a nanometer, respectively.	bacterial flagella on a specimen. You don't know if the bacteria you are staining possess flagella or not. When you examine the specimen you see no flagella on the bacteria. What are all the possibilities for why you didn't see them?

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **Figure 2.9*a***. If you were using the quadrant streak plate method to plate a very dilute broth culture (with many fewer bacteria than the broth used for the plate pictured here), would you expect to see single, isolated colonies in quadrant 4 or quadrant 3? Explain your answer.



Note: This method only works if the spreading tool (usually an inoculating loop) is resterilized after each of steps 1–4. (photo): ©Kathleen Talaro and Harold Benson

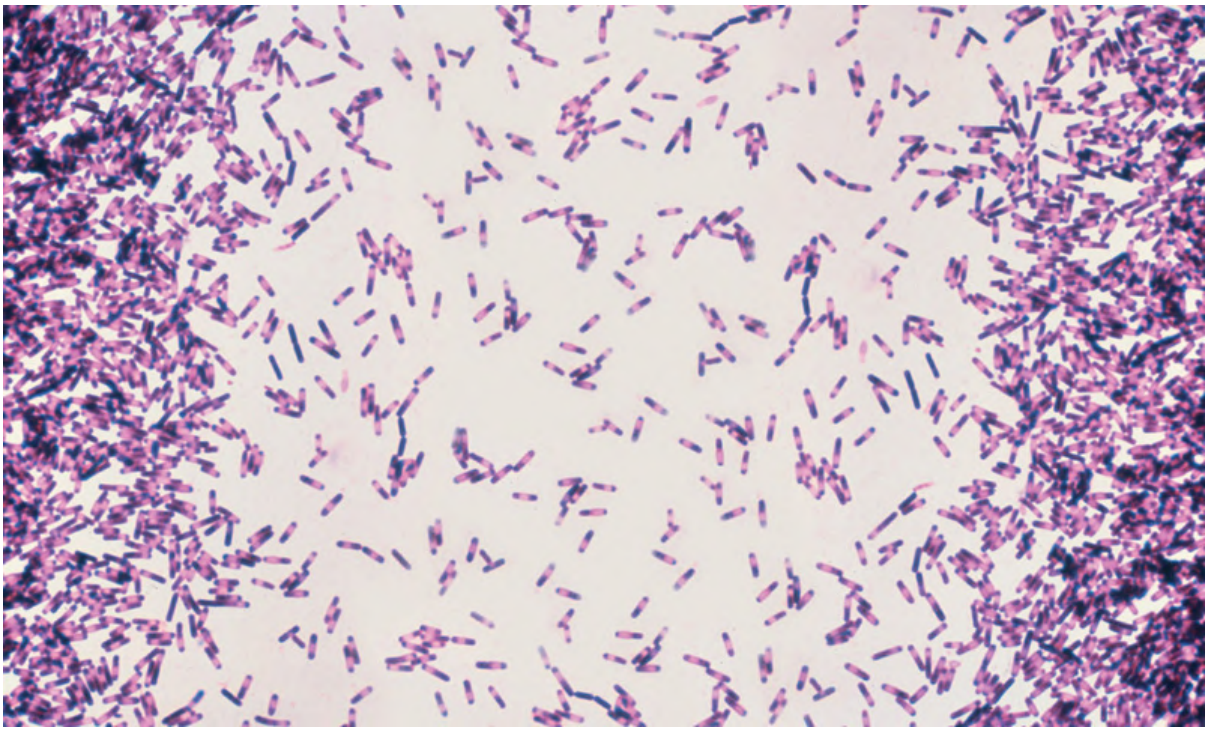
Resources are available through Connect, including interactive

learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

3

Bacteria and Archaea

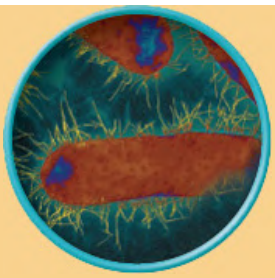


Source: CDC/Dr. Gilda Jones (*C. difficile*); ©Dave and Les Jacobs/Blend Images (nurse); ©Eye of Science/Science Source (*E. coli*); ©Dennis Kunkel Microscopy, Inc./Medical Images (cell wall); ©Corbis (archaea); Courtesy Bergey's Manual Trust (Bergey's Manual)

IN THIS CHAPTER…

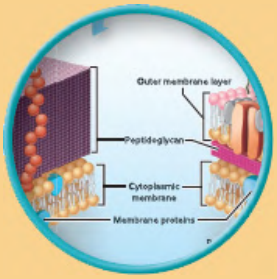
3.1 Form and Function of Bacteria and Archaea

1. List the structures all bacteria possess.
2. Identify three structures some but not all bacteria possess.
3. Describe three major shapes of bacteria.
4. Provide at least four terms to describe bacterial arrangements.



3.2 External Structures

5. Describe the structure and function of six different structures found on the exterior of a bacterial cell.
6. Explain how a flagellum works in the presence of an attractant.



3.3 The Cell Envelope: The Wall and Membrane(s)

7. Differentiate between the two main types of bacterial envelope structure.
8. Discuss why gram-positive cell walls are stronger than gram-negative cell walls.
9. Name a substance in the envelope structure of some bacteria that can cause severe symptoms in humans.

3.4 Bacterial Internal Structure

10. Identify seven structures that may be contained in bacterial cytoplasm.
11. Detail the causes and mechanisms of sporulation and germination.

3.5 The Archaea: The Other "Prokaryotes"

12. Compare and contrast the major features of archaea, bacteria, and eukaryotes.

3.6 Classification Systems for Bacteria and Archaea

13. Differentiate between *Bergey's Manual of Systematic Bacteriology* and *Bergey's Manual of Determinative Bacteriology*.
14. Name four divisions ending in ***–cutes*** and describe their characteristics.
15. Define a *species* in terms of bacteria.

CASE FILE

Extreme Endospores

While working as a newly graduated nurse, I was caring for an elderly female patient from a local nursing home who had been admitted for a hip replacement. The patient seemed to be recovering well until she developed redness, increased swelling, and purulent discharge at the surgical site. The wound was cultured and the patient was started on a cephalosporin antibiotic. The results from microbiological testing revealed that the infection was caused by *Staphylococcus aureus*, and it tested sensitive to the cephalosporin drug she was already taking.

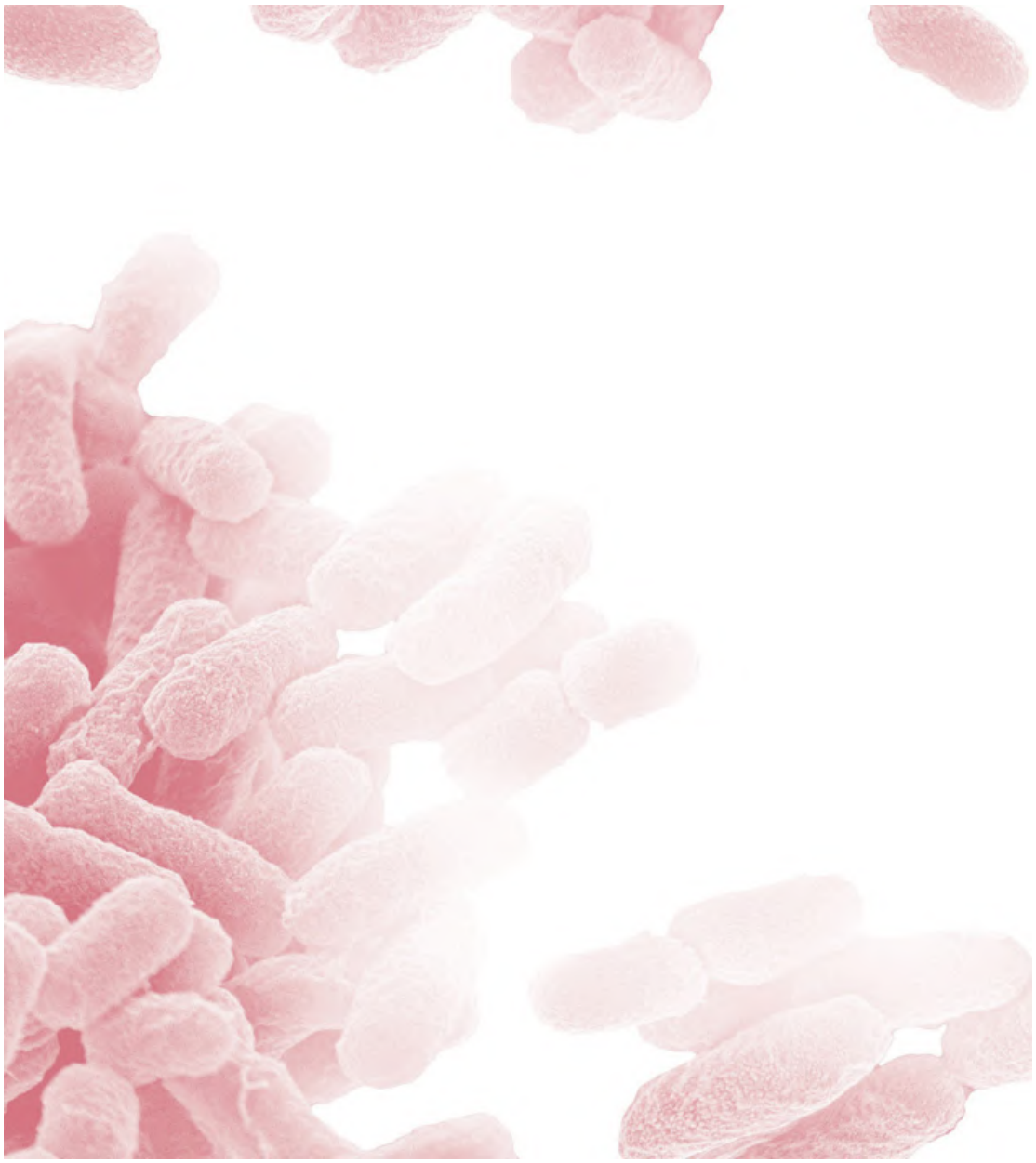
The patient successfully completed the course of antibiotic therapy, and within a few days all signs of infection had subsided. The patient was progressing well with physiotherapy, and we were beginning to plan for discharge back to the nursing home when the patient suddenly

began to experience diarrhea. At first I assumed that the diarrhea was because of an expected side effect from the antibiotic, but it soon became clear that this was something more than a general side effect. On the first day, the patient had two loose bowel movements. By the second day, the episodes of diarrhea were occurring every 2 to 3 hours. The stools were watery and foul-smelling and contained large amounts of mucus. The patient complained of mild abdominal pain and cramping, and she subsequently developed a fever. The physician was notified, and a stool specimen was collected for laboratory testing.

I was surprised when the stool culture came back showing that the patient's diarrhea was actually caused by the bacterium *Clostridium difficile*. The patient was placed on contact isolation and was started on intravenous metronidazole (Flagyl). With this treatment, the diarrhea gradually slowed and finally stopped. Repeat cultures, performed after the metronidazole therapy was completed, showed that the infection had been successfully cleared.

- How is *C. difficile* spread?
- What risk factors made this patient particularly vulnerable to infection with *C. difficile*?

Case File Wrap-Up appears at the end of the chapter.



Escherichia coli
©Science Photo Library/Getty Images

3.1 Form and Function of Bacteria and Archaea

In chapter 1, we described bacteria and archaea as being cells with no true nucleus. Let's look at how bacteria and archaea are different from eukaryotes:

- *The way their DNA is packaged:* Bacteria and archaea have nuclear material that is free inside the cell (i.e., they do not have a nucleus). Eukaryotes have a membrane around their DNA (making up a nucleus). Eukaryotes and some archaea wrap their DNA around **histones**; bacteria do not.

- *The makeup of their cell wall:* Bacteria and archaea have a wall structure that is unique compared to eukaryotes. Bacteria have sturdy walls made of a chemical called peptidoglycan. Archaeal walls are also tough and made of other chemicals, distinct from bacteria and distinct from eukaryotic cells.
- *Their internal structures:* Bacteria and archaea don't have complex, membrane-bounded organelles (eukaryotes do). A few bacteria and archaea have internal membranes, but they don't surround organelles.

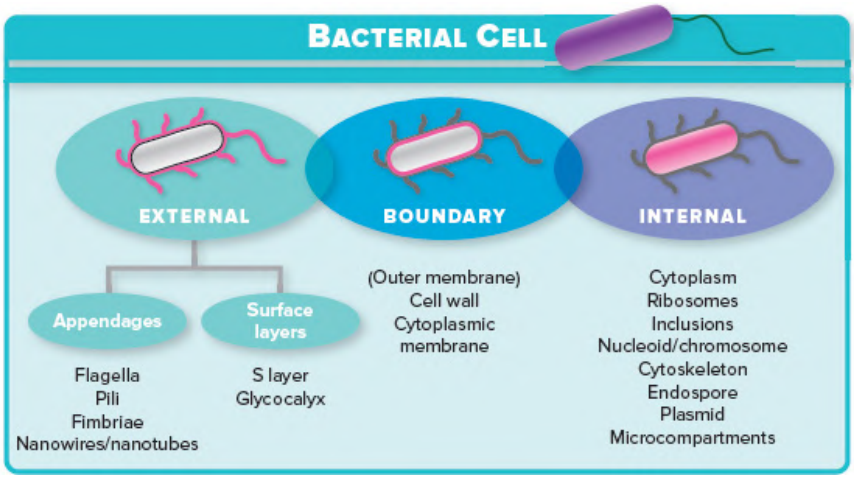
Both non-eukaryotic and eukaryotic microbes are ubiquitous in the world today. Although both can cause infectious diseases, treating them with drugs requires different types of approaches. In this chapter and coming chapters, you'll discover why that is.

The evolutionary history of non-eukaryotic cells extends back almost 4 billion years. The fact that these organisms have endured for so long in such a variety of habitats can be attributed to a cellular structure and function that are amazingly versatile and adaptable.

The Structure of the Bacterial Cell

In this chapter, the descriptions, except where otherwise noted, refer to *bacterial cells*. We will focus on the features of bacteria because you will encounter them more often in a clinical environment. We will analyze the significant ways in which archaea are unique later in the chapter.

The general cellular organization of a bacterial cell can be represented with this infographic:



All bacterial cells invariably have a cytoplasmic membrane, cytoplasm, ribosomes, a cytoskeleton, and one (or a few) chromosome(s). The majority have a cell wall and a surface coating called a glycocalyx. Specific structures that are found in some but not all bacteria are flagella, an outer membrane, pili, fimbriae, nanowires/nanotubes, plasmids, inclusions, endospores, and microcompartments. Most of these structures are observed in archaea as well.

Figure 3.1 presents a three-dimensional anatomical view of a generalized, rod-shaped bacterial cell. As we survey the principal anatomical features of this cell, we

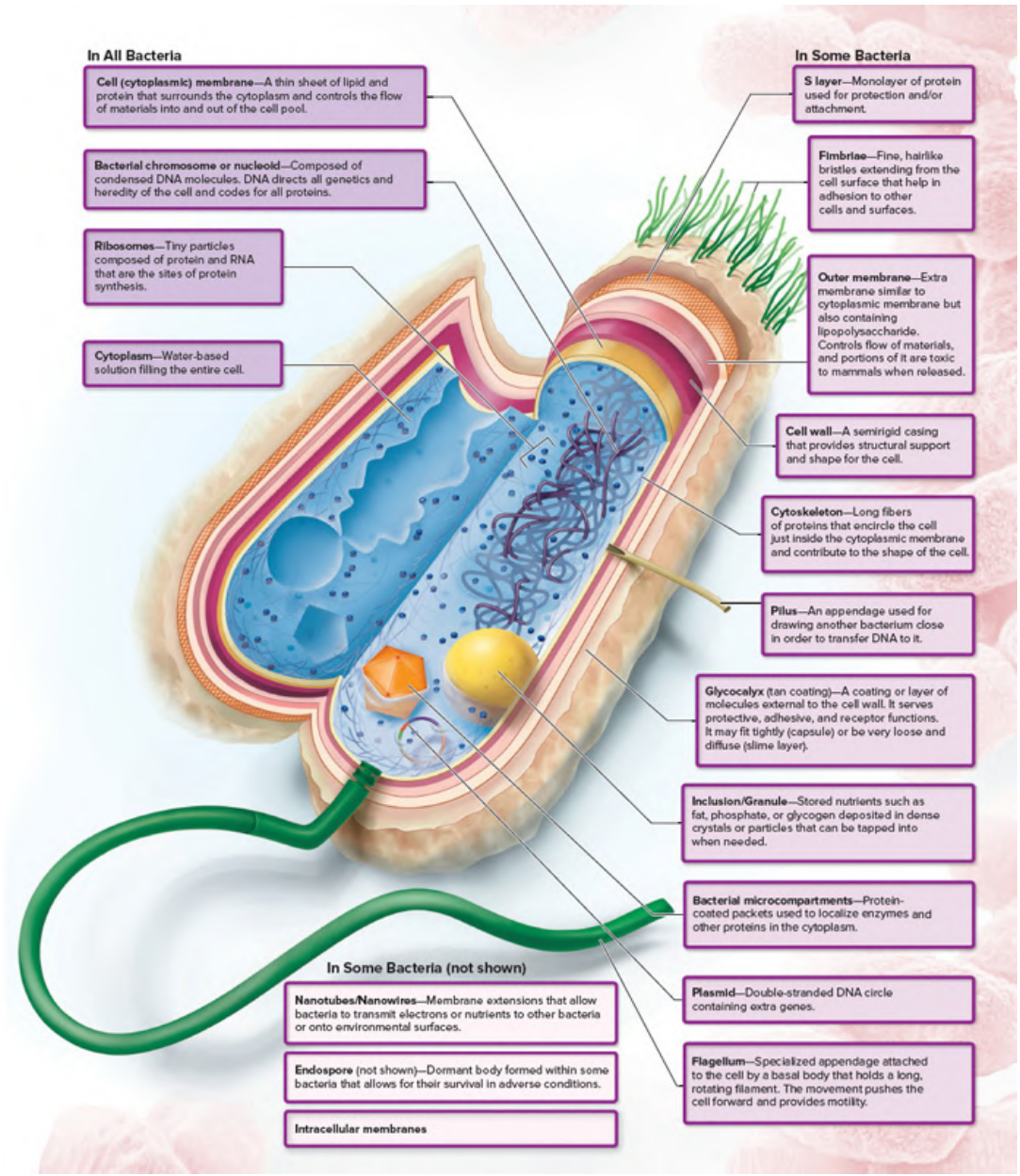


Figure 3.1 **Structure of a bacterial cell**. Cutaway view of a typical rod-shaped bacterium, showing major structural features.

will perform a microscopic dissection of sorts, beginning with the outer cell structures and proceeding to the internal contents.

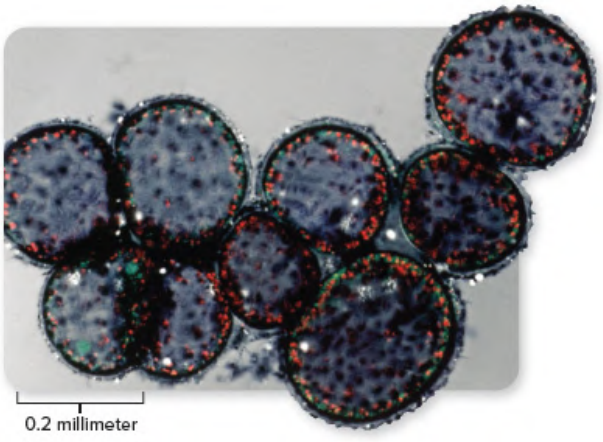


Figure 3.2 ***Thiomargarita namibiensis*.** The bacteria are each about half the width of a common paperclip wire—in other words—quite large!.
©Max Planck Institute/AFP/Newscom

Bacterial Shapes and Arrangements

For the most part, bacteria function as independent single-celled, or unicellular, organisms. Each individual bacterial cell is fully capable of carrying out all necessary life activities, such as reproduction, metabolism, and nutrient processing, unlike the more specialized cells of a multicellular organism. On the other hand, sometimes bacteria *can* act as a group. When bacteria are close to one another in colonies or in biofilms, they communicate with each other through chemicals that cause them to behave differently than if they were living singly. More surprisingly, some bacteria seem to communicate with each other using structures called nanotubes or nanowires, which are appendages that can be many micrometers long and are used for transferring electrons or other substances outside the cell onto metals in the environment. The wires also intertwine with the wires of neighboring bacteria, and can be used for exchanging nutrients.

Bacteria come in all shapes and sizes. They also exist in many different types of groupings. Let's start with size. Bacterial cells have an average size of about 1 µm. Cocci have a circumference of 1 µm, and rods may have a length of 2 µm with a width of 1 µm. But that's just the average. As with everything in nature, there is a lot of variation. One of the largest non-eukaryotes yet discovered is a bacterial species living in ocean sediments near the African country of Namibia. These gigantic cocci are arranged in strands that look like pearls and contain hundreds of golden sulfur granules, inspiring their name, *Thiomargarita namibiensis* ("sulfur pearl of Namibia") **(figure 3.2)**. The size of the individual cells ranges from 100 up to 750 µm (0.1 to 0.75 mm), and many are large enough to see with the naked eye. By way of comparison, if the average bacterium were the size of a mouse, *Thiomargarita* would be as large as a blue whale! On the other end of the spectrum, we have *Mycoplasma* cells, which are generally 0.15 to 0.30 µm, which is right at the limit of resolution with light microscopes.

One of the most important ways to describe bacteria is by their shape and their arrangement. **Table 3.1** summarizes all of these descriptions. Getting to know these now will be a great help for the rest of your studies in this course.

It is somewhat common for cells of a single species to vary in shape and size. This phenomenon, called **pleomorphism**, is due to individual variations in cell wall structure caused by nutritional or slight genetic differences. For example, although the cells of *Corynebacterium*

diphtheriae are generally considered rod-shaped, in culture they display variations such as club-shaped, swollen, curved, filamentous, and coccoid. **Figure 3.3** depicts the pleomorphic *Rickettsia ricketsii*, which causes Rocky Mountain spotted fever.

Bacterial cells can also be categorized according to arrangement, or style of grouping. The main factors influencing the arrangement of a particular cell type are its pattern of division while it is growing and how the cells remain attached afterward. The greatest variety in arrangement occurs in cocci, which can be single, in pairs (diplococci), in **tetrads** (groups of four), in irregular clusters (as in staphylococci and micrococci), or in chains of a few to hundreds of cells (streptococci). An even more complex grouping is a cubical packet of eight, sixteen, or more cells called a **sarcina** (sar′-sih-nah). These different coccal groupings are the result of the division of a coccus in a single plane, in two perpendicular planes,

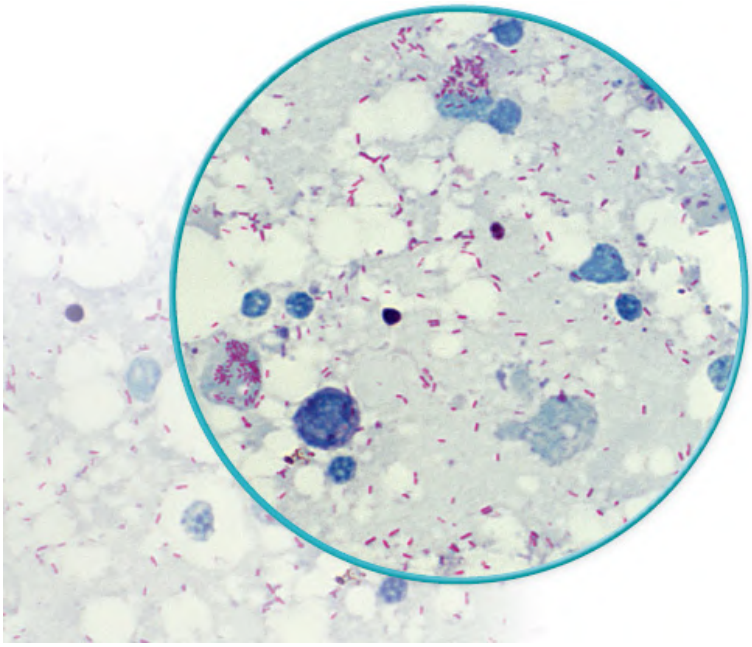


Figure 3.3 **Pleomorphic bacteria**. If you look closely at this micrograph of violet-stained *Rickettsia rickettsii* bacteria, you will see some coccoid cells, rod-shaped cells, and hybrid forms.
Source: CDC/Billie Ruth Bird

Table 3.1 Bacterial Shapes

(a) Coccus	If the cell is spherical or ball-shaped, the bacterium is described as a **coccus** (kok′-us). Cocci (kok′-si) can be perfect spheres, but they also can exist as oval, bean-shaped, or even pointed variants. This is *Staphylococcus* (10,000x).	
(b) Rod/Bacillus	A cell that is cylindrical is termed a rod, or **bacillus** (bah-sil′-lus). There is also a genus named *Bacillus*. Rods are quite varied in their actual form. Depending on the species, they can be blocky, spindle-shaped, round-ended, long and threadlike (filamentous), or even club-shaped or drumstick-shaped. Note: When a rod is short and plump, it is called a **coccobacillus**. This is *Legionella pneumophila*.	

(c) Vibrio	Singly occurring rods that are gently curved are called **vibrio** (vib′-ree-oh). This is *Vibrio vulnificus* (13,000×).	
(d) Spirillum	A bacterium having a slightly curled or spiral-shaped body is called a **spirillum** (spy-ril′-em)—a rigid helix, twisted twice or more along its axis (like a corkscrew). This is *Campylobacter jejuni*.	
(e) Spirochete	Another spiral cell (which contains periplasmic flagella) is the **spirochete**, a more flexible form that resembles a spring. These are spirochetes (7,500×).	
(f) Branching filaments	A few bacteria produce multiple branches off of a basic rod structure, a form called branching **filaments**. This is *Streptomyces* (1,500×).	

Source: CDC/Janice Haney Carr (a); Source: CDC/Janice Haney Carr (b); Source: CDC/Janice Haney Carr (c); Source: USDA/Photo by De Wood. Digital colorization by Chris Pooley (d); ©VEM/Science Source (e); ©Eye of Science/Science Source (f)

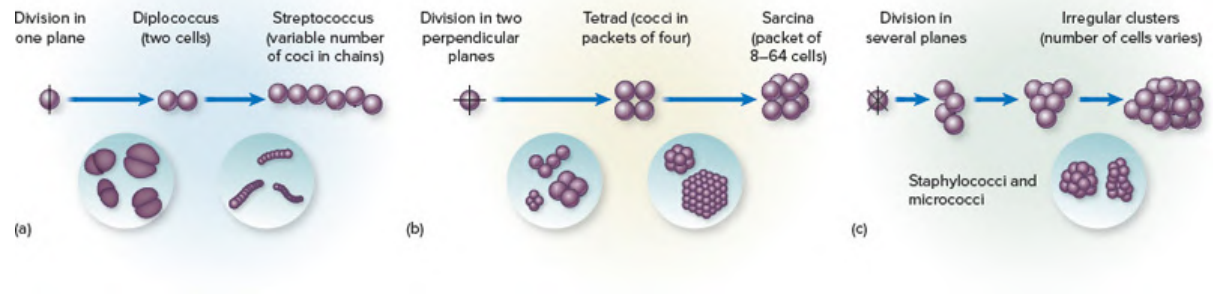


Figure 3.4 Arrangement of cocci resulting from different planes of cell division. **(a)** Division in one plane produces diplococci and streptococci. **(b)** Division in two or three planes at right angles produces tetrads and packets. **(c)** Division in several planes produces irregular clusters.

or in several intersecting planes. After division, the resultant daughter cells remain attached **(figure 3.4)**.

Rods are less varied in arrangement because they divide only in one plane. They occur either as single cells, as a pair of cells with their ends attached (diplobacilli), or as a chain of several cells (streptobacilli). A **palisades** (pal′-ih-saydz) arrangement, typical of the *Corynebacterium* species is formed when the cells of a chain remain partially attached by a small hinge region at the ends. The cells tend to fold (snap) back upon each other, forming a row of cells oriented side by side **(figure 3.5)**. Spirilla are occasionally found in short chains, but spirochetes rarely remain attached after division.

3.1 LEARNING OUTCOMES—Assess Your Progress

1. List the structures all bacteria possess.
2. Identify three structures some but not all bacteria possess.
3. Describe three major shapes of bacteria.
4. Provide at least four terms to describe bacterial arrangements.

3.2 External Structures

Appendages: Cell Extensions

Several different types of accessory structures sprout from the surface of bacteria. These long **appendages** are common but are not present on all species. Appendages can be divided into two major groups: those that provide motility (flagella and axial filaments) and those that provide attachment points or channels (fimbriae, pili, and nanotubes/nanowires).

Flagella—Bacterial Propellers

The bacterial flagellum (flah-jel′-em), an appendage of truly amazing construction, is unique in the biological world. The primary function of flagella is to confer **motility**, or self-propulsion—that is, the capacity of a cell to swim freely through an aqueous habitat. The flagellum has three distinct parts: the filament, the hook (sheath), and the basal body **(figure 3.6)**. The **filament**, a helical structure composed of proteins, is approximately 20 nm in diameter and varies from 1 to 70 μm in length. It is inserted into a curved, tubular hook. The hook is anchored to the cell by the basal body, a stack of rings firmly anchored through the cell wall, to the cytoplasmic membrane and the outer membrane. This arrangement permits the hook with its filament to rotate 360°, rather than undulating back and forth like a whip as was once thought. Although many archaea possess flagella, recent studies have shown that the structure is quite different than the bacterial flagellum. It is called *archaellum* by some scientists.

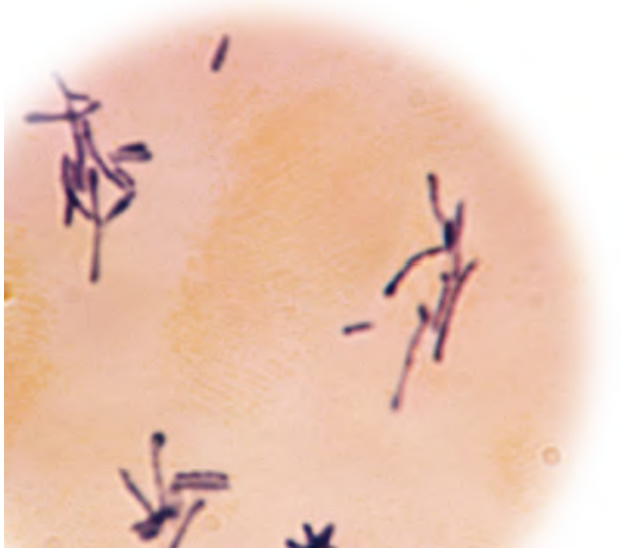


Figure 3.5 ***Corynebacterium*** **cells illustrating the palisades (stacking) tendency.**
©De Agostini/Getty Images

All spirilla, about half of the rods, and a small number of cocci are flagellated. Bacteria can have single or multiple flagella. They can either be found at the ends of a rod-shaped cell or dispersed across a round- or rod-shaped cell. If they are found on the ends of a cell, they are termed *polar*.

1. In a *polar* arrangement, the flagella are attached at one or both ends of the cell. Three subtypes of this pattern are
   - **monotrichous** (mah′-noh-trik′-us), with a single flagellum;
   - **lophotrichous** (lo′-foh-), with small bunches or tufts of flagella emerging from the same site; and
   - **amphitrichous** (am′-fee-), with flagella at both poles of the cell.
2. In a **peritrichous** (per′-ee-) arrangement, flagella are dispersed randomly over the surface of the cell **(figure 3.7)**.

Motility is one piece of information used in the laboratory identification or diagnosis of pathogens. Flagella are hard to visualize via light microscopy, but often it is sufficient to know simply whether a bacterial species is motile. One way to detect motility is to stab a tiny mass of cells into a soft (semisolid) medium in a test tube. Growth spreading rapidly through the entire medium is indicative of motility. Alternatively, cells can be observed microscopically with a hanging drop slide. A truly motile cell will flit, dart, or wobble around the field, making some progress, whereas one that is nonmotile jiggles about in one place but makes no progress.

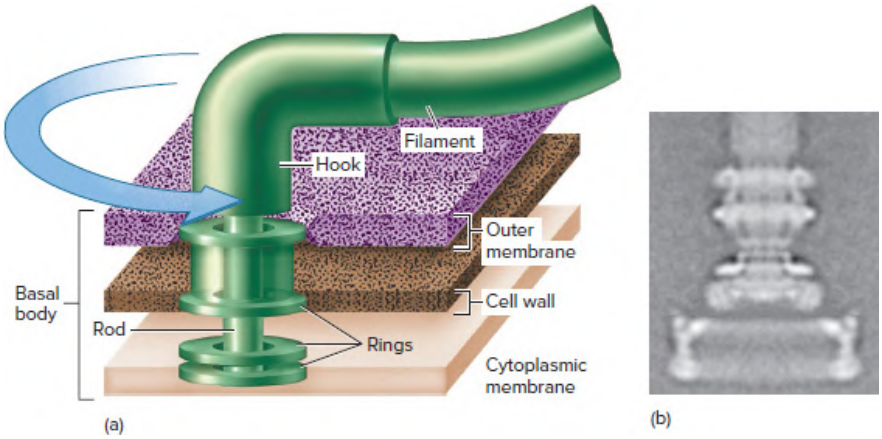


Figure 3.6 **Details of the basal body of a flagellum in a gram-negative cell**. **(a)** The hook, rings, and rod function together as a tiny device that rotates the filament 360°. **(b)** An electron micrograph of the basal body of a bacterial flagellum.

Sarkar MK1, Paul K, Blair D., "Chemotaxis signaling protein CheY binds to the rotor protein FliN to control the direction of flagellar rotation in Escherichia coli," PNAS May 18, 2010 vol. 107 no. 20 9370-9375 (b)

Fine Points of Flagellar Function Flagellated bacteria can perform some rather sophisticated feats. They can detect and move in response to chemical signals—a type of behavior called **chemotaxis** (ke′-moh-tak′-sis). Positive chemotaxis is movement of a cell in the direction of a favorable chemical stimulus (usually a nutrient). Negative chemotaxis is movement away from a potentially harmful compound.

The flagellum is effective in guiding bacteria through the environment primarily because the system for detecting chemicals is linked to the mechanisms that drive the flagellum. There are

clusters of receptors located in the cytoplasmic membrane that bind specific molecules coming from the immediate environment. The attachment of sufficient numbers of these molecules transmits signals to the flagellum and sets it into rotary motion. The actual "fuel" for the flagellum to turn is a gradient of protons (hydrogen ions) that are generated by the metabolism of the bacterium and that bind to and detach from parts of the flagellar motor within the cytoplasmic membrane, causing the filament

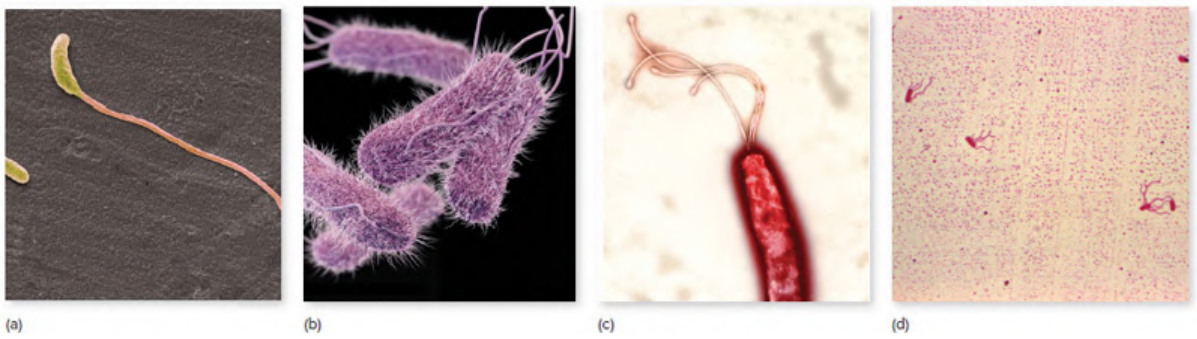


Figure 3.7 **Four types of flagellar arrangements**. **(a)** Monotrichous flagellum on the bacterium *Clostridium phytofermentans*. **(b)** Peritrichous flagella on *Salmonella* (the shorter hairs are fimbriae). **(c)** Lophotrichous flagella on *Helicobacter*. **(d)** Amphitrichous flagella on *Alcaligenes*.

©Science Photo Library/Alamy Stock Photo (a); Source: CDC/Melissa Brower (b); ©Heather Davies/Science Source (c); ©Smith Collection/Gado/Getty Images (d)

to rotate. If several flagella are present, they become aligned and rotate as a group **(figure 3.8)**. As a flagellum rotates counterclockwise, the cell itself swims in a smooth linear direction toward the stimulus. This action is called a *run*. Runs are interrupted at various intervals by *tumbles*, during which the flagellum reverses direction and causes the cell to stop and change its course. Alternation between runs and tumbles generates what is termed a *random walk* form of motility in these bacteria. However, in response to a concentration gradient of an attractant molecule, the bacterium will begin to slow down its tumbles, permitting longer runs and overall progress toward the stimulus **(figure 3.9)**. The movement now becomes a *biased* random walk in which movement is favored (biased) in the direction of the attractant. But what happens when a flagellated bacterium wants to run away from a toxic environment? In this case, the random walk then favors movement away from the concentration of repellent molecules. By delaying tumbles, the bacterium increases the length of its runs, allowing it to redirect itself away from the negative stimulus.

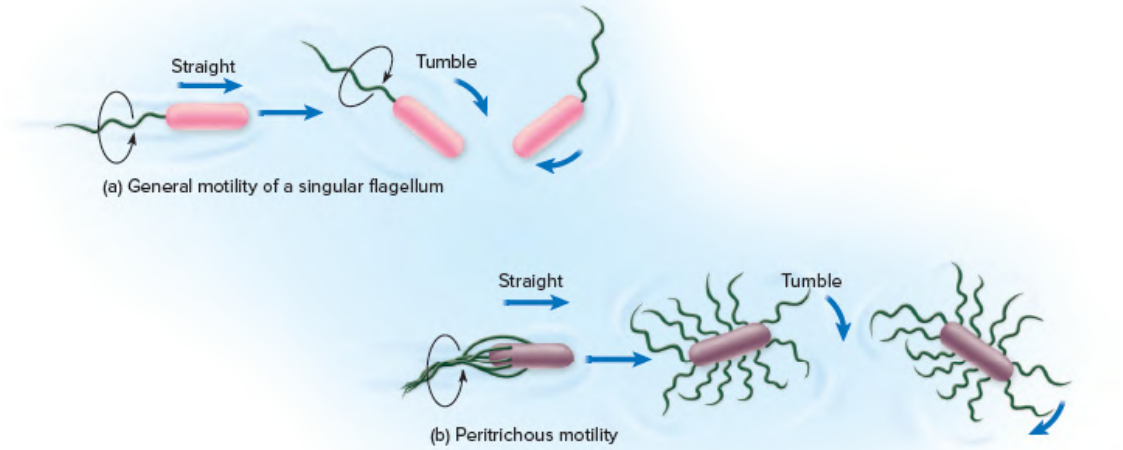


Figure 3.8 **The operation of flagella and the mode of locomotion in bacteria with polar and peritrichous flagella**. **(a)** In general, when a polar flagellum rotates in a counterclockwise direction, the cell swims forward. When the flagellum reverses direction and rotates clockwise, the cell stops and tumbles. **(b)** In peritrichous forms, all flagella sweep toward one end of the cell and rotate as a single group. During tumbles, the flagella lose coordination.

Periplasmic Flagella Corkscrew-shaped bacteria called **spirochetes** (spy′-roh-keets) show an unusual, wriggly mode of locomotion caused by two or more long, coiled threads, the periplasmic flagella or *axial filaments*. A periplasmic flagellum is a type of internal flagellum that is enclosed in the space between the cell wall and the cytoplasmic membrane.

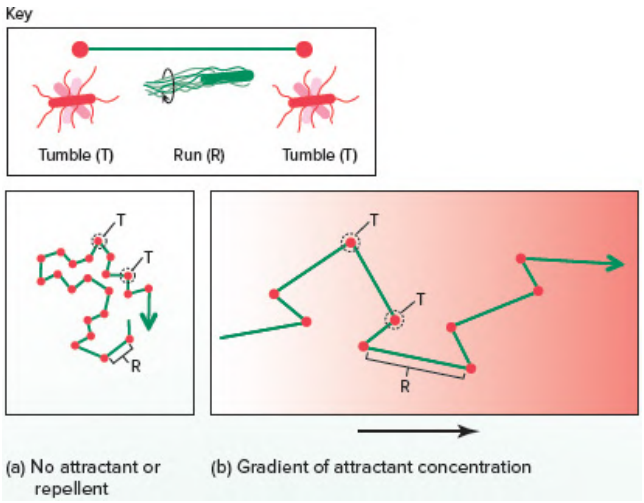


Figure 3.9 **Chemotaxis in bacteria**. **(a)** A bacterium moves via a random series of short runs and tumbles when there is no attractant or repellent. **(b)** The cell spends more time on runs as it gets closer to the attractant.

Appendages for Attachment or Channel Formation

The structures termed **pilus** (pil-us) and **fimbria** (fim′-bree-ah) are both bacterial surface appendages that provide some type of adhesion. **Nanotubes** or nanowires are used as channels for nutrient or energy exchange.

Fimbriae are small, bristlelike fibers sprouting off the surface of many bacterial cells **(figure 3.10)**. Their exact composition varies, but most of them contain protein. Fimbriae have an inherent tendency to stick to each other and to surfaces. They may be partially responsible for the mutual clinging of cells that leads to biofilms and other thick aggregates of cells on the surface of liquids and for the microbial colonization of inanimate solids such as rocks and glass. Some pathogens can colonize and infect host tissues because of a tight adhesion between their fimbriae and epithelial cells. For example, the gonococcus (agent of gonorrhea)

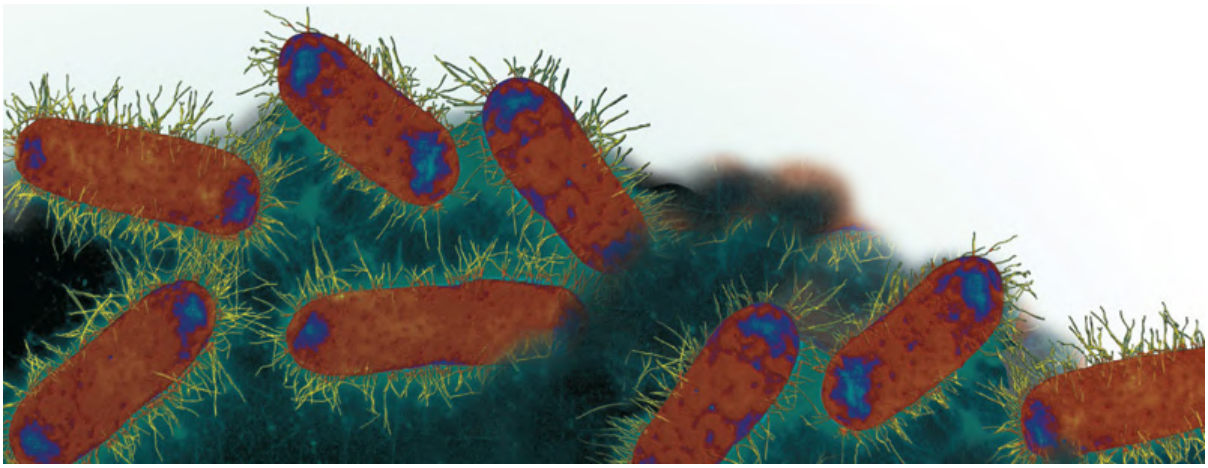


Figure 3.10 **Form and function of bacterial fimbriae**. Several cells of pathogenic *Escherichia coli* covered with numerous stiff fibers called fimbriae (30,000×). Note also the dark blue granules, which are the chromosomes.
©Eye of Science/Science Source

colonizes the genitourinary tract, and *Escherichia coli* colonizes the intestine by this means. Mutant forms of these pathogens that lack fimbriae are unable to cause infections.

A pilus is a long, rigid tubular structure made of a special protein, *pilin*. Conjugation pili are utilized in a "mating" process between cells called **conjugation**, which involves partial transfer of DNA from one cell to another **(figure 3.11)**. A conjugation pilus from the donor cell unites with a recipient cell, thereby providing a cytoplasmic connection for making the transfer. Production of these pili is controlled genetically, and conjugation takes place only between compatible gram-negative cells. Although conjugation does occur in gram-positive bacteria, it does not involve a conjugation pilus. The roles of pili and conjugation are further explored in chapter 8. There is a special type of structure in some bacteria called a type IV pilus. Like the pili described here, it can transfer genetic material. In addition, it can act like fimbriae and assist in attachment, and act like flagella and make a bacterium motile.

Nanotubes (also called nanowires) have only recently been sufficiently understood. They are very thin, long, tubular extensions of the cytoplasmic membrane that bacteria use as channels either to transfer amino acids (i.e., food) among one another or to harvest energy by shuttling electrons from an electron-rich surface in the environment. In this latter scenario, bacteria generate energy in the absence of oxygen. When we study metabolism we will see that cells generate the

necessary energy to grow by consuming nutrients and transferring electrons from the nutrients to oxygen. But many bacteria can generate their energy in the absence of oxygen by sharing electrons with substances in the environment containing iron, such as iron-rich rocks. They use long nanowires to do this, transferring electrons up and down the tubular extensions of the membrane. Scientists call this activity "breathing rock instead of oxygen."

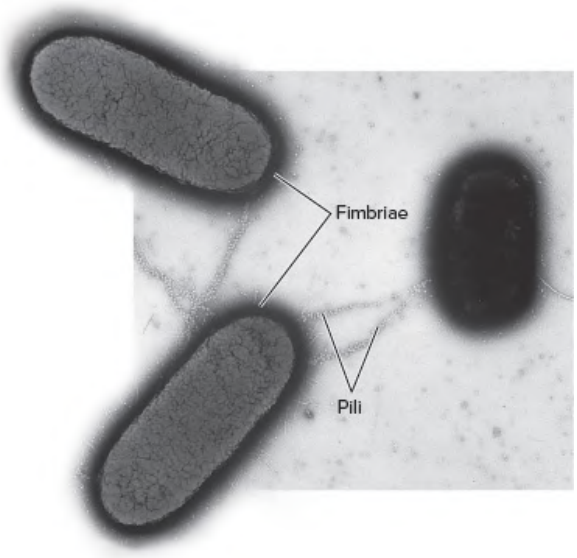


Figure 3.11 **Three bacteria in the process of conjugating**. Clearly evident are the pili forming mutual conjugation bridges between a donor (middle cell) and two recipients (cells on the left side). Fimbriae can also be seen on the two left-hand cells.
©L. Caro/SPL/Science Source

Surface Coatings: The S Layer and the Glycocalyx

The bacterial cell surface is frequently exposed to severe environmental conditions. Bacterial cells protect themselves with either an S layer or a glycocalyx or both. **S layers** are single layers of thousands of copies of a single protein linked together like tiny chain mail. They are often called "the armor" of a bacterial cell **(figure 3.12)**. It took scientists a long time to discover them because bacteria only produce them when they are in a hostile environment. The nonthreatening conditions of growing in a lab in a nutritious broth with no competitors around ensured that bacteria did not produce the layer. We now know that many different species have the ability to produce an S layer, including pathogens such as *Clostridium difficile* and *Bacillus anthracis*. Some bacteria use S layers to aid in attachment as well.

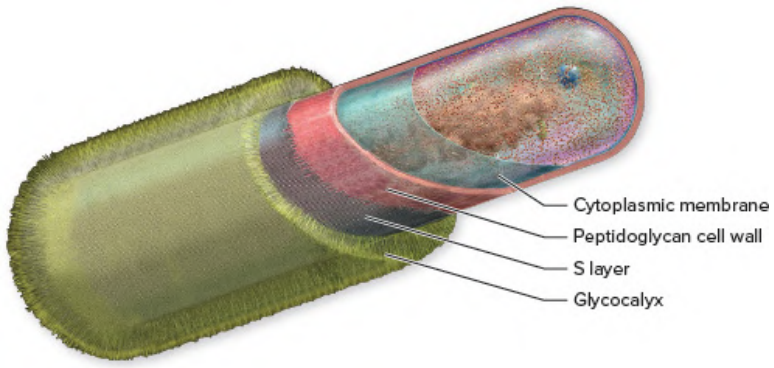


Figure 3.12 **Position of the bacterial S layer, shown in purple**.
©Russell Kightley/Science Source

Medical Moment

Healthcare-Associated Infections

Reducing healthcare-associated infections (HAI) has become an area of intense focus. Defined as an infection that is contracted from the health care environment, such a condition is a costly complication for the patient. However, the harm is preventable. The Centers for Disease Control and Prevention (CDC) has standardized guidelines to prevent device-associated infections. Central venous catheters, endotracheal tubes, and urinary catheters are examples of indwelling devices prone to foster infection. Bacteria may be introduced when the device is inserted in the patient, or when it is accessed during care activities. The plastic may become colonized while dwelling in the patient's body. With health care provider education and the application of risk-reducing protocols, HAI occurrences have been shown to decrease by more than 70%.

Q. Device-associated infections are very common. Can you think of another activity that occurs in hospitals that might be likely to lead to new infections?

Answer in Appendix B.

The glycocalyx develops as a coating of repeating polysaccharide or glycoprotein units. This protects the cell and, in some cases, helps it adhere to its environment. Glycocalyces differ among bacteria in thickness, structure, and chemical composition. Some bacteria are covered with a loose shield called a **slime layer** that evidently protects them from loss of water and nutrients. A glycocalyx is called a **capsule** when it is bound more tightly to the cell than a slime layer is and it is denser and thicker. Capsules are often visible in negatively stained preparations **(figure 3.13*b*)** and produce a prominently sticky (mucoid) character to colonies on agar **(figure 3.13*a*)**.

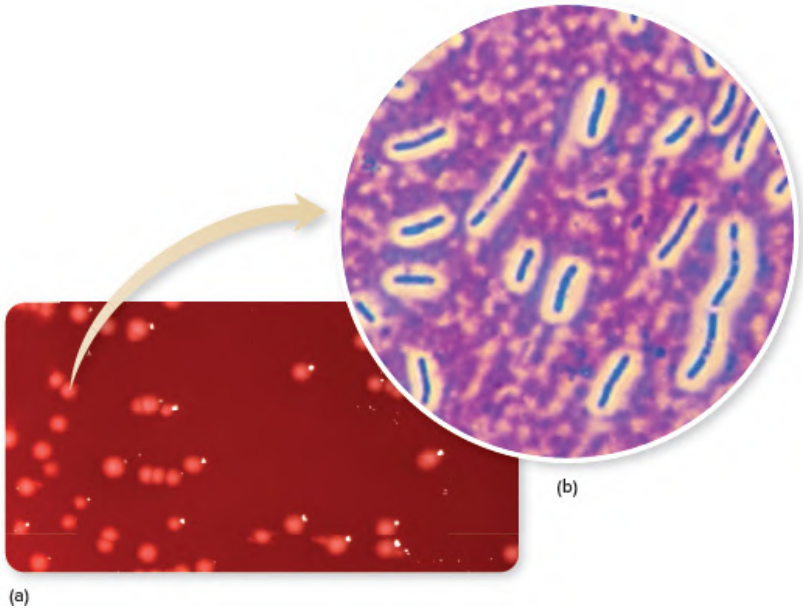


Figure 3.13 **Encapsulated bacteria**. **(a)** Colonies of *Citrobacter* bacteria that are encapsulated. The characteristic of interest here is the relative *glossiness* of the colonies. Bacteria that produce capsules appear glossier on agar. They are called "smooth" by microbiologists, whereas those without capsules are called "rough." **(b)** Special stain of encapsulated bacteria.
Source: CDC (a); ©Michael Abbey/Science Source (b)

Specialized Functions of the Glycocalyx

Capsules are formed by many pathogenic bacteria, such as *Streptococcus pneumoniae* (a cause of pneumonia, an infection of the lung), *Haemophilus influenzae* (one cause of meningitis), and *Bacillus anthracis* (the cause of anthrax). Encapsulated bacterial cells generally have greater disease-causing abilities, because capsules protect the bacteria against white blood cells called phagocytes. Phagocytes are a natural body defense that can engulf and destroy foreign cells through phagocytosis, thus preventing infection. A capsular coating blocks the mechanisms that phagocytes use to attach to and engulf bacteria. By escaping phagocytosis, the bacteria are free to multiply and infect body tissues. Encapsulated bacteria that mutate to nonencapsulated forms usually lose their ability to cause disease.

Glycocalyces can be important in formation of biofilms **(figure 3.14*a*)**. The thick, white plaque that forms on teeth comes in part from the surface slimes produced by certain streptococci in the oral cavity. This slime protects them from being dislodged from the teeth and provides a niche for other oral bacteria that, in time, can lead to dental disease. The glycocalyx of some bacteria is so highly adherent that it has a significant role in the persistent colonization of nonliving materials such as plastic catheters, intrauterine devices, and metal pacemakers that are in common medical use **(figure 3.14*b*)**.

3.2 LEARNING OUTCOMES—Assess Your Progress

5. Describe the structure and function of six different structures found on the exterior of a bacterial cell.
6. Explain how a flagellum works in the presence of an attractant.

3.3 The Cell Envelope: The Wall and Membrane(s)

The majority of bacteria have a chemically complex external covering, termed the *cell envelope*, that lies outside of the cytoplasm. It is composed of two or three basic layers: the cell wall, the cytoplasmic membrane, and, in some bacteria, the outer membrane. Although each envelope layer performs a distinct function, together they act as a single protective unit.

Differences in Cell Envelope Structure

If you made a cross section through a gram-positive cell envelope **(figure 3.15)**, it would resemble an open-faced sandwich: The thick cell wall would represent a tall stack of roast beef on your sandwich. Underneath the meat is the "bread," the thin cytoplasmic membrane. A similar cross section of a gram-negative cell envelope shows a complete sandwich with three layers: an outer membrane, a thin cell wall (less meat on this sandwich), and the cytoplasmic membrane. The cell wall layers in both gram-positive and gram-negative bacteria are made of a unique molecule called peptidoglycan. In gram-positive cells there are several layers of it and in gram-negative cells there is only one layer.

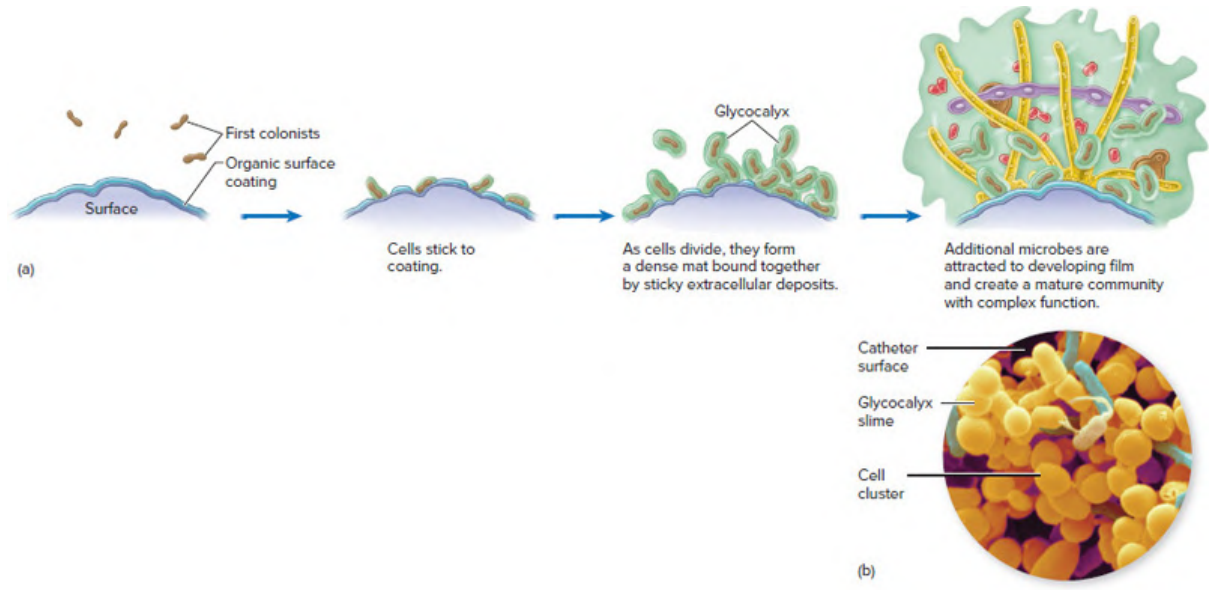


Figure 3.14 **Biofilm formation**. **(a)** The stepwise formation of a biofilm on a surface. **(b)** Scanning electron micrograph of *Staphylococcus aureus* cells attached to a catheter by a slime secretion.
©Scimat/Science Source (b)

Moving from outside to in (see figure 3.1), the outer membrane (if present) lies just under the glycocalyx. Next comes the cell wall. Finally, the innermost layer is always the cytoplasmic membrane. Because only some bacteria have an outer membrane, we discuss the cell wall first.

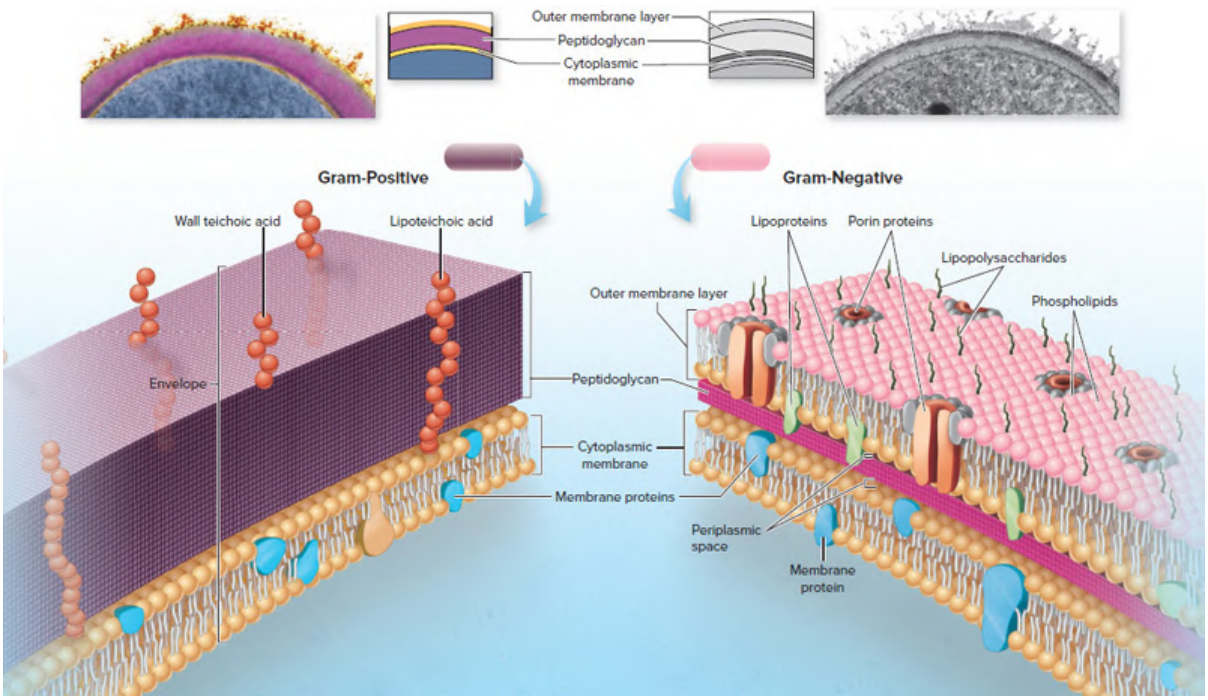


Figure 3.15 **A comparison of the detailed structure of gram-positive and gram-negative cell envelopes**. The images at the top are electron micrographs of actual gram-positive and gram-negative cells.

©Dr. Kari Lounatmaa/Science Source (gram-positive cell.); ©Dennis Kunkel Microscopy, Inc./Medical Images (gram-negative cell)

The Cell Wall

The **cell wall** accounts for a number of important bacterial characteristics. In general, it helps determine the shape of a bacterium, and it also provides the kind of strong structural support necessary to keep a bacterium from bursting or collapsing because of changes in osmotic pressure.

The cell walls of most bacteria gain their relatively rigid quality from a unique macromolecule called peptidoglycan (PG). This compound is composed of a repeating framework of long *glycan* (*sugar*) chains cross-linked by short peptide (protein) fragments to provide a strong but flexible support framework **(figure 3.16)**. The amount and exact composition of peptidoglycan vary among the major bacterial groups.

Bacteria live and grow in environments that have water in them. (Water-based environments are termed "aqueous.") Because of this, they are constantly absorbing excess water by osmosis. Were it not for the strength and rigidity of the peptidoglycan in the cell wall, they would burst from internal pressure. This feature of the cell wall provides us with an obvious target in designing antibacterial drugs. Several types of drugs used to treat infection (penicillin, cephalosporins) are effective because they target the peptide cross-links in the peptidoglycan, causing it to disintegrate. With their cell walls incomplete or missing, such cells have very little protection from **lysis** (ly′-sis), which is the disintegration or rupture of the cell. Our bodies themselves contain a powerful antibacterial substance: Lysozyme, an enzyme contained in tears and saliva, hydrolyzes the bonds in the glycan chains and causes the wall to break down. (Chapter 9 discusses the actions of antimicrobial chemical agents.)

More than a hundred years ago, long before the detailed anatomy of bacteria was even remotely known, a Danish physician named Hans Christian Gram developed a staining technique, the **Gram stain**, that delineates two generally different groups of bacteria. The two major groups shown by this technique are the **gram-positive** bacteria and the **gram-negative** bacteria. The designations *gram-positive* and *gram-negative* are based largely on differences in the peptidoglycan layer of the cell envelope, as you will see next.

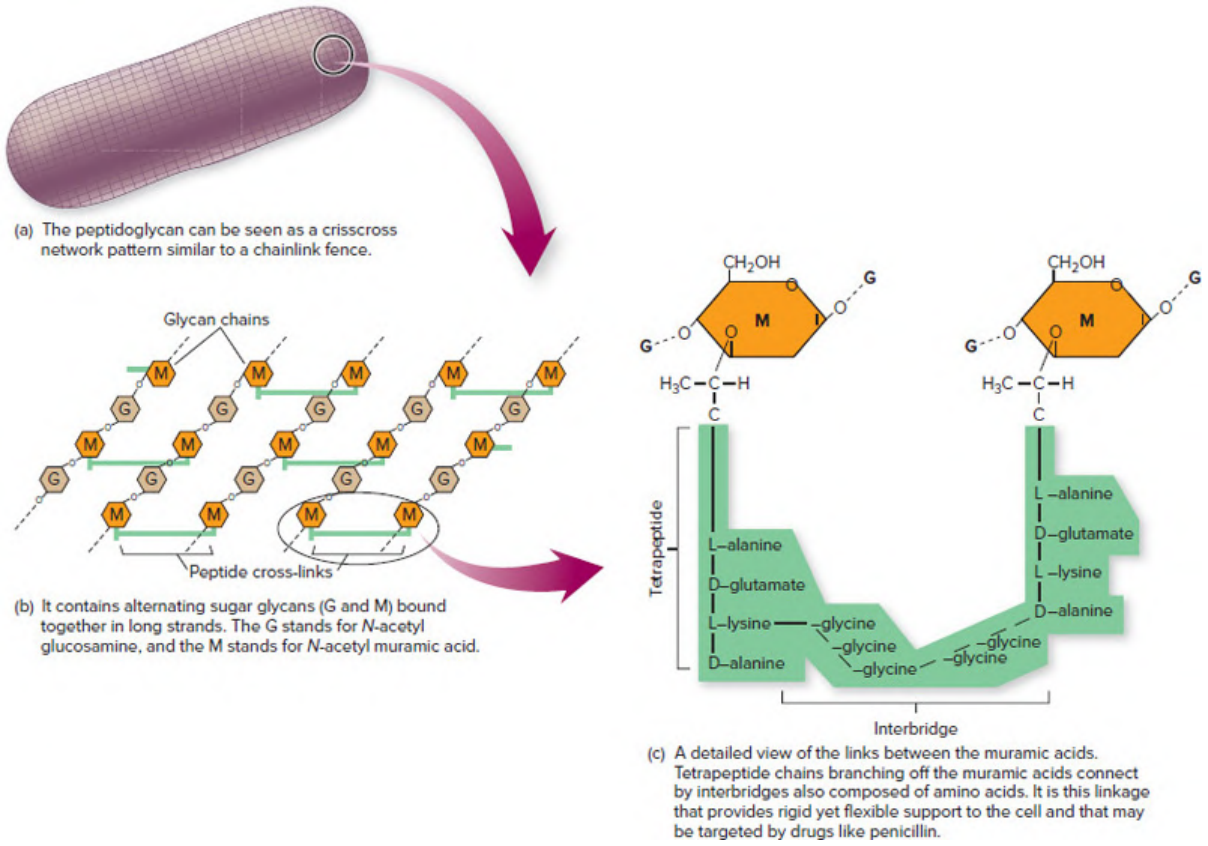


Figure 3.16 **Structure of peptidoglycan in the cell wall.**

The Gram-Positive Cell Wall

The bulk of the gram-positive cell wall is a thick, homogeneous sheath of peptidoglycan ranging from 20 to 80 nm in thickness. It also contains tightly bound acidic polysaccharides, including **teichoic acid** and **lipoteichoic acid** (see figure 3.15). Teichoic acid is a polymer of ribitol or glycerol (alcohols) and phosphate that is embedded in the peptidoglycan matrix. Lipoteichoic acid is similar in structure but is attached to the lipids in the cytoplasmic membrane. These molecules appear to function in cell wall maintenance and enlargement during cell division, and they also contribute to the acidic charge on the cell surface.

The Gram-Negative Cell Wall

The gram-negative cell wall is a single, thin (1–3 nm) sheet of peptidoglycan. Although it acts as a somewhat rigid protective structure as previously described, its thinness makes gram-negative

bacteria more susceptible to lysis.

The Gram Stain

The technique of Hans Christian Gram consisted of timed, sequential applications of crystal violet (the primary dye), Gram's iodine (the mordant), an alcohol rinse (decolorizer), and safranin (a contrasting counterstain). Bacteria that stain purple are called gram-positive, and those that stain red are called gram-negative. The different results in the Gram stain are due to differences in the structure of the cell wall and how it reacts to the series of reagents applied to the cells **(figure 3.17)**.

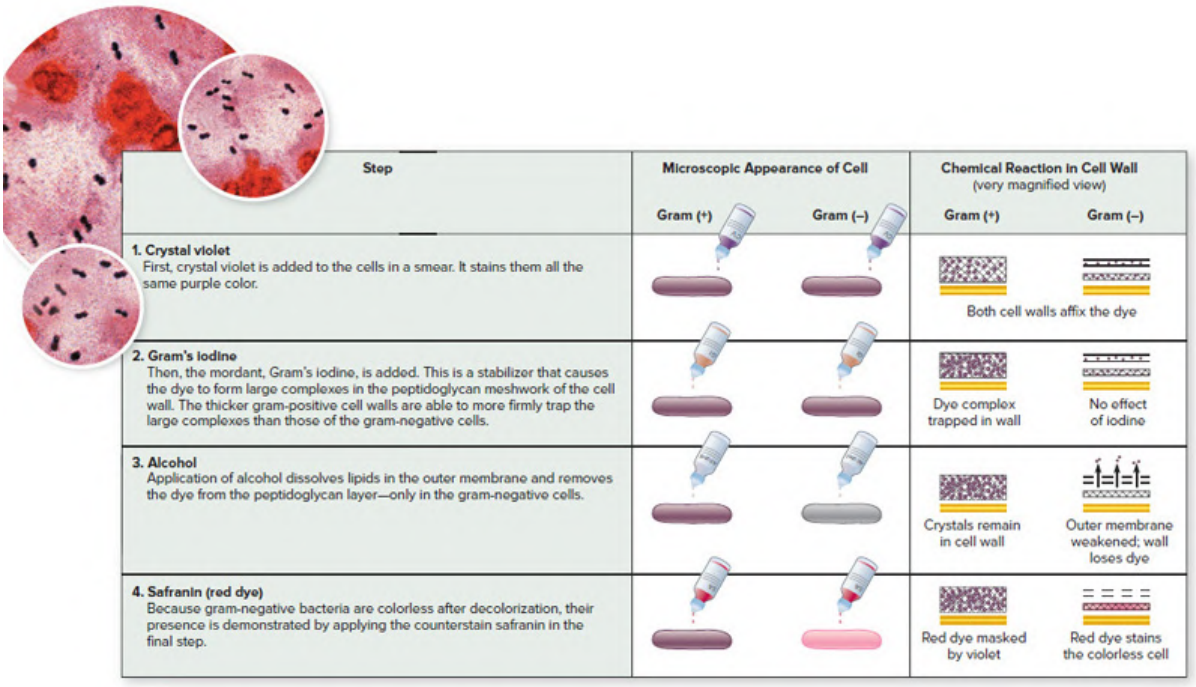


Figure 3.17 **The steps in a Gram stain.**
©McGraw-Hill Education

Medical Moment

Collecting Sputum

The nurse is often responsible for collecting sputum samples for acid-fast staining when a patient with a cough is suspected of having tuberculosis. A sterile container must be provided, and the patient should be instructed that early morning specimens are best, usually collected upon first awakening. This is due to the fact that sputum often "pools" in the bronchi when the patient is sleeping at night; therefore, it is easier to collect a larger sample in the morning after the patient has been lying down all night.

If the patient is unable to produce any sputum, giving him or her an aerosolized dose of saline inhaled by mask may help moisten secretions, making it easier for the patient to produce the sample. Samples are also sometimes collected by suctioning the patient. Doctors may order acid-fast sputum samples for tuberculosis to be collected on three consecutive mornings. This helps to increase the likelihood of identifying the bacteria if they are present.

Q. *Mycobacterium tuberculosis* colonizes the lungs. What technical problem would you anticipate if you were to plate sputum on agar to look for it?

Answer in Appendix B.

This century-old staining method remains the universal basis for bacterial classification and identification. The Gram stain can also be a practical aid in diagnosing infection and in guiding drug treatment. For example, Gram staining a fresh urine or throat specimen can help pinpoint the possible cause of infection, and in some cases it is possible to begin drug therapy on the basis of this stain. Even in this day of elaborate and expensive medical technology, the Gram stain remains an important and unbeatable first tool in diagnosis.

Nontypical Cell Walls

Several bacterial groups have a different cell wall structure than gram-positive or gram-negative bacteria, and some bacteria have no cell wall at all. Although these exceptional forms can stain positive or negative in the Gram stain, examination of their fine structure shows that they do not really fit the descriptions for typical gram-negative or gram-positive cells. For example, the cells of *Mycobacterium* and *Nocardia* contain peptidoglycan and stain gram-positive, but the bulk of their cell wall is composed of unique types of lipids. One of these is a very-long-chain fatty acid called *mycolic acid*, or cord factor, that contributes to the pathogenicity of this group (see chapter 19). The thick, waxy nature bestowed on the cell wall by these lipids also means that these bacteria are highly resistant to certain chemicals and dyes. Such resistance is the basis for the acid-fast stain used to diagnose tuberculosis and leprosy.

The archaea exhibit unusual and chemically distinct cell walls. In some, the walls are composed almost entirely of polysaccharides, and in others, the walls are pure protein. As a group, they all lack the true peptidoglycan structure described previously.

Mycoplasmas and Other Cell-Wall-Deficient Bacteria

Mycoplasmas are bacteria that naturally lack a cell wall. Although other bacteria require an intact cell wall to prevent the bursting of the cell, the mycoplasma cytoplasmic membrane is stabilized by sterols and is resistant to lysis. These extremely tiny, pleomorphic cells are very small bacteria, ranging from 0.1 to 0.5 μm in size. The most important medical species is *Mycoplasma pneumoniae*, which adheres to the epithelial cells in the lung and causes an atypical form of pneumonia in humans (often called "walking pneumonia" because its sufferers can often continue their daily activities, and the illness can often be treated on an outpatient basis) (described in chapter 19).

Some bacteria that ordinarily have a cell wall can lose it during part of their life cycle. These wall-deficient forms are referred to as **L forms** or L-phase variants (for the Lister Institute, where they were discovered). Evidence points to a role for L forms in persistent infections that can be resistant to antibiotic treatment.

The Gram-Negative Outer Membrane

The **outer membrane (OM)** (see figure 3.15) is similar in composition to most membranes, except that it contains specialized types of polysaccharides and proteins. Remember that each membrane is a bilayer of phospholipids. The uppermost layer of the OM is different because some

of the phospholipid molecules are substituted with a macromolecule called *lipopolysaccharide* (LPS) **(figure 3.18)**. The polysaccharide chains extending off the surface function as signaling molecules and receptors. The lipid portion of LPS has been referred to as *endotoxin* because it stimulates fever and shock reactions in gram-negative infections such as meningitis and typhoid fever. The innermost layer of the OM is a phospholipid layer anchored by means of lipoproteins to the peptidoglycan layer below. The outer membrane serves as a sieve by allowing only relatively small molecules to penetrate. Access is provided by special membrane channels formed by *porin proteins* that completely span the outer membrane.

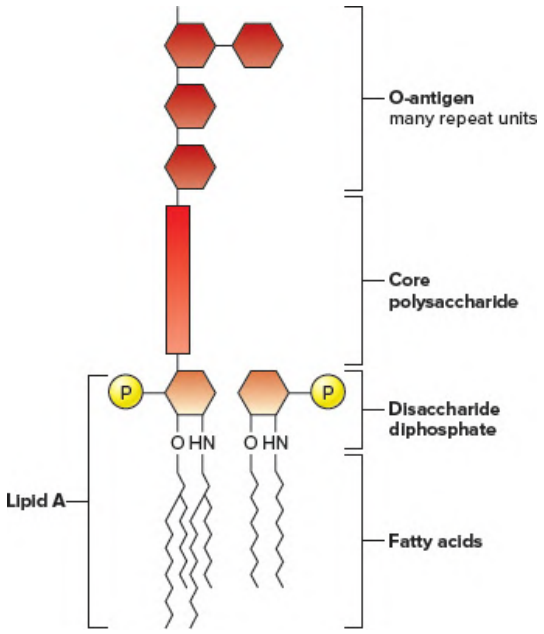


Figure 3.18 **The lipopolysaccharide molecule**.

Bacillus subtilis ©Steven P. Lynch

Cytoplasmic Membrane Structure

Appearing just beneath the cell wall is the cytoplasmic membrane, often also called the cell membrane. We use "cytoplasmic membrane" in this book because the term makes it immediately clear that we are talking about the membrane in direct contact with the cytoplasm. This membrane is a very thin (5–10 nm), flexible sheet molded completely around the cytoplasm. Its general composition is a lipid bilayer with proteins embedded to varying degrees. Bacterial cytoplasmic membranes have this typical structure, containing primarily phospholipids (making up about 30%–40% of the membrane mass) and proteins (contributing 60%–70%). Major exceptions to this description are the membranes of mycoplasmas, which contain high amounts of sterols—rigid lipids that stabilize and reinforce the membrane—and the membranes of archaea, which contain unique branched hydrocarbons rather than fatty acids.

Some environmental bacteria, including photosynthesizers and ammonia oxidizers, contain dense stacks of internal membranes. In many cases, they derive from the cytoplasmic membrane, and they are studded with enzymes or photosynthetic pigments. The inner membranes allow a higher concentration of these enzymes and pigments and also accomplish a compartmentalization that allows for higher energy production.

Functions of the Cytoplasmic Membrane

Because bacteria have none of the eukaryotic organelles, the cytoplasmic membrane provides a site for functions such as energy reactions, nutrient processing, and synthesis. A major action of the cytoplasmic membrane is to regulate *transport*, that is, the passage of nutrients into the cell and the discharge of wastes. Although water and small uncharged molecules can diffuse across the membrane unaided, the membrane is a *selectively permeable* structure with special carrier mechanisms for passage of most molecules (see chapter 6). The glycocalyx and cell wall can bar the passage of large molecules, but they are not the primary transport apparatuses.

The membranes of bacteria are an important site for a number of metabolic activities. Most enzymes of respiration and ATP synthesis reside in the cytoplasmic membrane since bacteria lack mitochondria (see chapter 7).

Practical Considerations of Differences in Cell Envelope Structure

The different envelopes in gram-positive and gram-negative bacteria have practical implications. The outer membrane contributes an extra barrier in gram-negative bacteria that makes them resistant to some antimicrobial chemicals such as dyes and disinfectants. For this reason, they are generally more difficult to inhibit or kill than are gram-positive bacteria. One exception is for alcohol-based compounds, which can dissolve the lipids in the outer membrane and therefore damage the cell. This is why alcohol swabs are often used to cleanse the skin prior to certain medical procedures, such as venipuncture. Treating infections caused by gram-negative bacteria often requires different drugs from gram-positive infections, especially drugs that can cross the outer membrane.

3.3 LEARNING OUTCOMES—Assess Your Progress

7. Differentiate between the two main types of bacterial envelope structure.
8. Discuss why gram-positive cell walls are stronger than gram-negative cell walls.
9. Name a substance in the envelope structure of some bacteria that can cause severe symptoms in humans.

3.4 Bacterial Internal Structure

Contents of the Cell Cytoplasm

The **cytoplasm** is a gelatinous solution encased by the cytoplasmic membrane. Its major component is water (70%–80%), which serves as a solvent for the contents of the cell, which is a complex mixture of nutrients including sugars, amino acids, and salts. Some people distinguish the cytoplasm from the *cytosol*. In this situation, cytoplasm refers to everything inside the cytoplasmic membrane, and cytosol is only the fluid portion. We won't use that distinction.

Bacterial Chromosomes and Plasmids

The hereditary material of most bacteria exists in the form of a single circular strand of DNA designated as the **bacterial chromosome**. Some bacteria have multiple chromosomes. By definition, bacteria do not have a nucleus; that is, their DNA is not enclosed by a nuclear

membrane but instead is aggregated in a dense area of the cell called the **nucleoid**. The chromosome is actually an extremely long molecule of double-stranded DNA that is tightly coiled around special basic protein molecules so as to fit inside the cell. Arranged along its length are genetic units (genes) that carry information required for bacterial maintenance and growth.

Although the chromosome is the minimal genetic requirement for bacterial survival, many bacteria contain other, nonessential pieces of DNA called **plasmids** (refer to figure 3.1). These tiny strands exist as separate double-stranded circles of DNA, although at times they can become integrated into the chromosome. During conjugation, they may be duplicated and passed on to related nearby bacteria. During bacterial reproduction, they are duplicated and passed on to offspring. They are not essential to bacterial growth and metabolism, but they do provide protective traits such as resisting drugs and producing toxins and enzymes (see chapter 8). Because they can be readily manipulated in the laboratory and transferred from one bacterial cell to another, plasmids are an important agent in genetic engineering techniques.

Ribosomes: Sites of Protein Synthesis

A bacterial cell contains thousands of tiny **ribosomes**, which are the site of protein synthesis. When viewed even by very high magnification, ribosomes show up as fine, spherical specks dispersed throughout the cytoplasm that often occur in chains called polysomes. Many are also attached to the cytoplasmic membrane. Chemically, a ribosome is a combination of a special type of RNA called ribosomal RNA, or rRNA (about 60%), and protein (40%). Ribosomes are characterized by their density, designated by "S units." Ribosomes consist of a small subunit and a large subunit **(figure 3.19)**. Both of these are made of a mixture of rRNA and protein. The small subunit has an S value of 30, and the large subunit has an S value of 50. Overall, the bacterial ribosome has a density of 70S. (It is not simply an additive property; that is why the total S value is not a product of the small and large subunits.) The two subunits fit together to form a miniature platform upon which protein synthesis is performed. Note that eukaryotic ribosomes are similar but still different. Because of this, we can design drugs to target bacterial ribosomes that do not harm our own. Eukaryotic ribosomes are designated 80S. Although archaea possess 70S ribosomes, they are more similar in structure to that of 80S eukaryotic ribosomes.

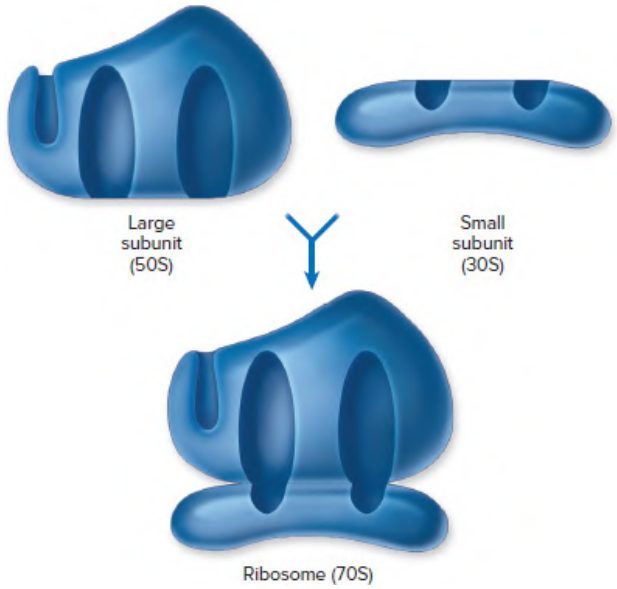


Figure 3.19 **A model of a bacterial ribosome, showing the small (30S) and large (50S) subunits, both separate and joined**.

Inclusion Bodies and Microcompartments

Bacteria manufacture structures referred to as inclusion bodies to respond to their environmental conditions. They can store nutrients in inclusion bodies to respond to periods of low food availability. They can pack gas into inclusion vesicles to provide buoyancy in an aquatic environment. They can even store crystals of iron oxide with magnetic properties in inclusion bodies. These magnetotactic bacteria use the granules to orient themselves in polar and gravitational fields to bring them to environments

with the proper oxygen content. **Figure 3.20** illustrates a bacterium with these iron oxide crystals.

In the early 2000s, new compartments inside bacterial cells were discovered. These were named *bacterial microcompartments* (BMCs). Their outer shells are made of protein, arranged geometrically, and are packed full of enzymes that are designed to work together in biochemical pathways, thereby ensuring that they are in close proximity to one another.

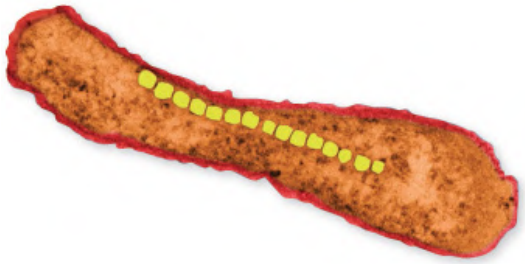


Figure 3.20 **Bacterial inclusion bodies**. The string of yellow objects are particles of iron oxide. This bacterium is magnetotactic—that is, it uses the iron particles to align itself with magnetic fields.
©Dennis Kunkel Microscopy, Inc./Medical Images

The Cytoskeleton

Until recently, scientists thought that the shape of all bacteria was completely determined by the peptidoglycan layer (cell wall). Although this is true of many bacteria, particularly the cocci, other bacteria produce long polymers of proteins that are very similar to structures in eukaryotic cells for the cytoskeleton. (Cytoskeleton means “cell skeleton.”) In eukaryotes, the cytoskeleton is made of three main types of proteins: actin, tubulin, and intermediate filament proteins. Bacteria have proteins that are similar to each of these three, and contain additional types of proteins in their cytoskeletons. These proteins are arranged in helical ribbons around the cell just under the cytoplasmic membrane. These fibers contribute to cell shape, perhaps by influencing the way peptidoglycan is manufactured, and also cell division. Cytoskeletal proteins have also been identified in archaea. Because these proteins are sufficiently different from those in eukaryotic cells, they are a potentially powerful target for future antibiotic development.

NCLEX® PREP

1. Why is an alcohol swab applied to skin as an antiseptic prior to administration of an injection?

a. *Alcohol helps stabilize cytoplasmic cell membranes*.
b. *Alcohol swabs prevent infection by dissolving the bacterial lipid membrane*.
c. *Alcohol inhibits protein synthesis in gram-positive bacteria, the bacteria most commonly present on skin*.
d. *The rubbing action congeals the cytoplasmic membrane*.

Bacterial Endospores

The anatomy of bacteria helps them adjust rather well to challenging habitats. But of all microbial structures, nothing can compare to the bacterial endospore for withstanding hostile conditions and facilitating survival.

Endospores are dormant bodies produced by bacteria such as *Bacillus, Clostridium*, and *Sporosarcina*. These bacteria have a two-phase life cycle—a vegetative cell and an endospore **(figure 3.21)**. The vegetative cell is a metabolically active and growing cell. When environmental conditions become challenging, these bacteria will form endospores in a process called **sporulation**. The endospore exists initially inside the vegetative cell, but eventually the cell disintegrates and the endospore is on its own. Both gram-positive and gram-negative bacteria can form endospores, but the medically relevant ones are all gram-positive. Most bacteria form only one endospore; therefore, this is not a reproductive function for them.

Bacterial endospores are the hardiest of all life forms, capable of withstanding extremes in heat, drying, freezing, radiation, and chemicals that would readily kill vegetative cells. Their survival under such harsh conditions is due to several factors. The heat resistance of endospores is due to their high content of calcium and dipicolinic acid. We know, for instance, that heat destroys cells by inactivating proteins and DNA. This process requires a certain amount of water in the protoplasm. There is a chemical in endospores called calcium dipicolinate that removes that water. This leaves the endospore very dehydrated, making it less vulnerable to the effects of heat. The thick, impervious cortex and endospore coats also protect against radiation and chemicals. Bacterial endospores are capable of surviving indefinitely. Recently, microbiologists unearthed a viable endospore from a 250-million-year-old salt crystal. Initial analysis of this ancient microbe indicates it is a species of *Bacillus*, although one that is genetically different from previously known species.

Endospore Formation: Sporulation

The depletion of nutrients, especially carbon or nitrogen sources, is the stimulus for a vegetative cell to begin endospore formation. Once this stimulus has been received by the vegetative cell, it undergoes a conversion to become a sporulating cell

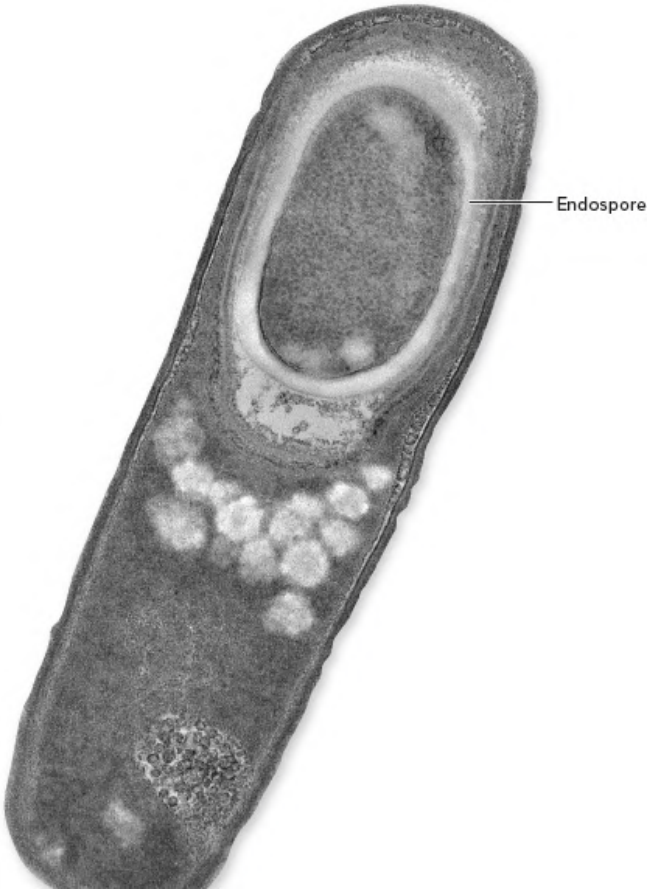


Figure 3.21 **Endospore inside *Bacillus thuringiensis*.** The genus *Bacillus* forms endospores. *B. thuringiensis* additionally forms crystalline bodies (beginning to form under the endospore) that are toxic against insects. ©Science Source

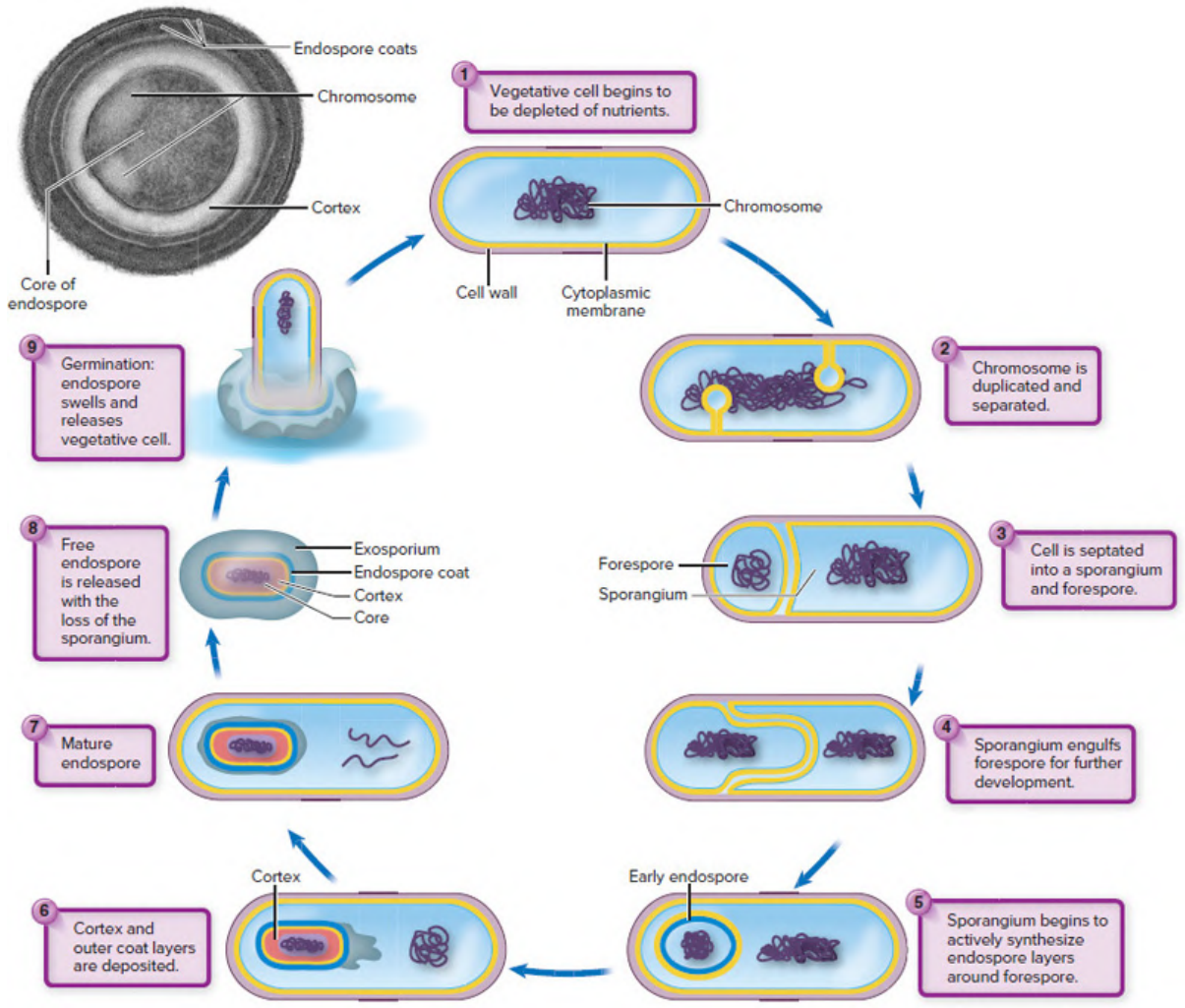


Figure 3.22 **A typical sporulation cycle in *Bacillus* species from the active vegetative cell to release and germination**. The process takes, on average, about 10 hours. Inset is a high magnification (10,000×) cross section of a single endospore showing the dense protective layers that surround the core with its chromosome.
©Science Source

Source: CDC/Laura Rose & Janice Haney Carr

called a **sporangium**. Complete transformation of a vegetative cell into a sporangium and then into an endospore requires 6 to 10 hours in most endospore-forming species. **Figure 3.22** illustrates the major physical and chemical events in this process.

Return to the Vegetative State: Germination

After lying in a state of inactivity for an indefinite time, endospores can be revitalized when favorable conditions arise. Germination—the reversal of dormancy—happens when water and a specific chemical or environmental stimulus (germination agent) are present. Once it begins, it proceeds to completion quite rapidly (1½ hours). Although the specific germination agent varies among species, it is generally a small organic molecule such as an amino acid or an inorganic salt. This agent stimulates

the formation of hydrolytic (digestive) enzymes by the endospore membranes. These enzymes digest the cortex and allow water into the core. As the core rehydrates and takes up nutrients, it begins to grow out of the endospore coats. In time, it reverts to a fully active vegetative cell, resuming the vegetative cycle.

Medical Significance of Bacterial Endospores

Although the majority of endospore-forming bacteria are relatively harmless, several bacterial pathogens are endospore formers. In fact, some aspects of the diseases they cause are related to the persistence and resistance of their spores. *Bacillus anthracis* is the agent of anthrax. Its ability to form endospores makes it an ideal candidate for bioterrorism. The genus *Clostridium* includes even more pathogens, such as *C. tetani*, the cause of tetanus (lockjaw); *C. perfringens*, the cause of gas gangrene; *C. botulinum*, the cause of botulism; and *C. difficile*, the cause of a severe

gastrointestinal disease popularly known as "*C. diff*." (Each of these disease conditions is discussed in the infectious disease chapters, according to the organ systems it affects.)

Because they inhabit the soil and dust, endospores are constant intruders where sterility and cleanliness are important. They resist ordinary cleaning methods that use boiling water, soaps, and disinfectants; and they frequently contaminate cultures and media. Hospitals and clinics must take precautions to guard against the potential harmful effects of endospores, especially those of *C. difficile*. Endospore destruction is a particular concern of the food-canning industry. Several endospore-forming species cause food spoilage or poisoning. Ordinary boiling (100°C) will usually not destroy such endospores, so canning is carried out in pressurized steam at 120°C for 20 to 30 minutes. Such rigorous conditions ensure that the food is sterile and free from viable bacteria.

3.4 LEARNING OUTCOMES—Assess Your Progress

10. Identify seven structures that may be contained in bacterial cytoplasm.
11. Detail the causes and mechanisms of sporogenesis and germination.

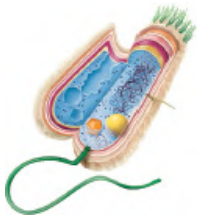
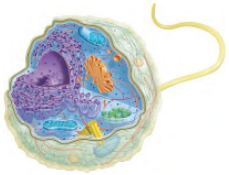
3.5 The Archaea: The Other "Prokaryotes"

The discovery and characterization of novel cells resembling bacteria but with unusual anatomy, physiology, and genetics changed our views of microbial taxonomy and classification (see chapter 1). These single-celled, simple organisms, called archaea, are now considered a third cell type in a separate superkingdom (the domain Archaea). We include them in this chapter because they share many bacterial characteristics. But it has become clear that they are actually more closely related to domain Eukarya than to bacteria. For example, archaea and eukaryotes share a number of ribosomal RNA sequences that are not found in bacteria, and their protein synthesis and ribosomal subunit structures are similar. **Table 3.2** outlines selected points of comparison of the three domains.

Among the ways that the archaea differ significantly from other cell types are that they have entirely unique sequences in their rRNA. They exhibit a novel method of DNA compaction, and they contain unique membrane lipids, cell wall components, and pilin proteins. It is clear that the archaea are the most primitive of all life forms and are most closely related to the first cells that originated on the earth 4 billion years ago. The early earth is thought to have contained a hot, anaerobic "soup" with sulfuric gases and salts in abundance. The modern archaea still live in the remaining habitats on the earth that have these same ancient conditions—the most extreme habitats in nature. It is for this reason that they are often called *extremophiles*, meaning that they "love" extreme conditions in the environment.

Table 3.2 Comparison of Three Cellular Domains

Characteristic	Bacteria	Archaea	Eukarya

Characteristic	Bacteria	Archaea	Eukarya
Chromosomes	Single or a few, circular 	Single, circular	Multiple, linear 
Types of ribosomes	70S	70S but structure is similar to 80S	80S
Contains unique ribosomal RNA signature sequences	+	+	+
Eukarya(-like) protein synthesis	−	+	+
Cell wall made of peptidoglycan	+	−	−
Cytoplasmic membrane lipids	Fatty acids with ester linkages	Long-chain, branched hydrocarbons with ether linkages	Fatty acids with ester linkages
Sterols in membrane	− (some exceptions)	−	+
Nucleus and membrane-bound organelles	No	No	Yes
Flagellum	Bacterial flagellum	Archaellum	Eukaryotic flagellum

Some archaea thrive in extremely high temperatures. Others need extremely high concentrations of salt or acid to survive. Some archaea live on sulfur, reducing it to hydrogen sulfide to get their energy. Members of the group called *methanogens* can convert CO_2 and H_2 into methane gas (CH_4) through unusual and complex pathways. Archaea that are adapted to growth at very low temperatures are called *psychrophilic* (loving cold temperatures). Those growing at very high temperatures are *hyperthermophilic* (loving high temperatures). Hyperthermophiles flourish at temperatures between 80°C and 113°C and find 50°C (which is 122°F) too cold to grow. They live in volcanic waters and soils and submarine vents and are often salt- and acid-tolerant as well.

Archaea are not just environmental microbes. They have been isolated from human tissues such as the colon, the mouth, and the vagina. Recently, an association was found between the degree of severity of periodontal disease and the presence of archaeal RNA sequences in the gingiva, suggesting—but not definitely proving—that archaea may be capable of causing human disease.

3.5 LEARNING OUTCOMES—Assess Your Progress

12. Compare and contrast the major features of archaea, bacteria, and eukaryotes.

Thermophilic archaea and cyanobacteria colonizing a thermal pool in Yellowstone National Park.
©Corbis

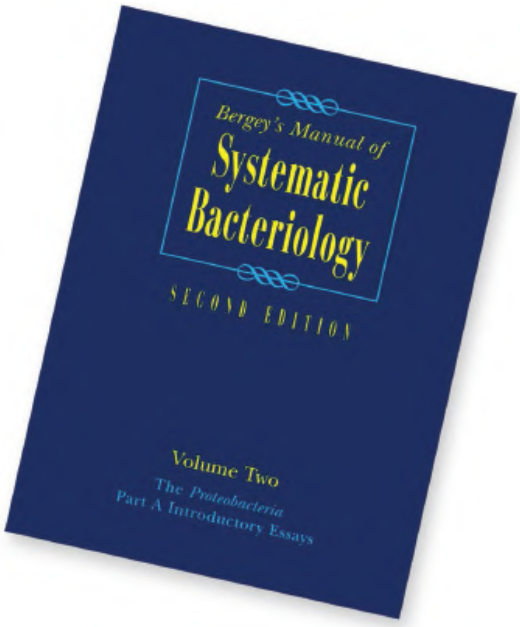
3.6 Classification Systems for Bacteria and Archaea

Scientists use classification systems to differentiate and identify species in medical and applied microbiology. They are also useful in organizing microorganisms and as a means of studying their relationships and origins. Since classification began around 200 years ago, several thousand species of bacteria and archaea have been identified, named, and cataloged.

There are two comprehensive databases that help scientists classify bacteria and archaea. One, called *Bergey's Manual of Systematic Bacteriology*, presents a comprehensive view of bacterial and archaeal relatedness, based largely on rRNA sequencing

information. (We need to remember that all bacteria and archaea classification systems are in a state of constant flux; no system is ever finished.)

A separate database, called *Bergey's Manual of Determinative Bacteriology*, is based entirely on phenotypic characteristics, which are the observable characteristics of an organism such as its shape (seen by microscopy) and its metabolic capabilities (such as what it requires to grow). It is utilitarian in focus, categorizing bacteria by traits commonly tested for in clinical, teaching, and research labs. It is widely used by microbiologists who need to identify bacteria but do not need to know their evolutionary backgrounds. This type of classification is more useful for students of medical microbiology as well.



Courtesy Bergey's Manual Trust

Taxonomic Scheme

Bergey's Manual of Determinative Bacteriology organizes the bacteria and archaea into four major divisions. These divisions are based on the nature of the cell wall. The **Gracilicutes** (gras′-ih-lik′-yoo-teez) have gram-negative cell walls and thus are thin-skinned; the **Firmicutes** have gram-positive cell walls that are thick and strong; the **Tenericutes** (ten′-er-ik′-yoo-teez) lack a cell wall and thus are soft; and the **Mendosicutes** (men-doh-sik′-yoo-teez) are the archaea (also called archaebacteria), primitive cells with unusual cell walls and nutritional habits. The system used in *Bergey's Manual* further organizes bacteria and archaea into subcategories such as classes, orders, and families, but these are not available for all groups.

Species and Subspecies in Bacteria and Archaea

Among most organisms, the species level is a distinct, readily defined, and natural taxonomic category. In animals, for instance, a species is a distinct type of organism that can produce viable offspring only when it mates with others of its own kind. This definition does not work for bacteria and archaea primarily because they do not use a typical mode of sexual reproduction. Also, they can accept genetic information from unrelated forms, and they can alter their genetic makeup by a variety of mechanisms. Thus, it is necessary to hedge a bit when we define a *bacterial species*.

©Purestock/Getty Images

Theoretically, it is a collection of bacterial cells, all of which share an overall similar pattern of traits, in contrast to other groups whose patterns differ significantly. Members of a bacterial species should also share at least 70%–80% of their genes. Although the boundaries that separate two closely related species in a genus are in some cases arbitrary, this definition still serves as a method to separate the bacteria and archaea into various kinds that can be cultured and studied.

Individual members of given species can show variations, as well. Therefore, more categories within species exist, but they are not well defined. Microbiologists use terms like *subspecies, strain*, or *type* to designate bacteria of the same species that have differing characteristics. *Serotype* refers to representatives of a species that stimulate a distinct pattern of antibody (serum) responses in their hosts, because of distinct surface molecules.

3.6 LEARNING OUTCOMES—Assess Your Progress

13. Differentiate between *Bergey's Manual of Systematic Bacteriology* and *Bergey's Manual of Determinative Bacteriology*.
14. Name four divisions ending in *–cutes* and describe their characteristics.
15. Define a *species* in terms of bacteria.

CASE FILE WRAP-UP

The circle contains an electron micrograph of *Clostridium difficile*, the endospore-forming bacterium that causes a common healthcare-associated intestinal infection.
©Dave and Les Jacobs/Blend Images (nurse); Source: CDC/Dr. Gilda Jones (*Clostridium difficile* bacteria)

Clostridium difficile is an endospore-forming bacterium that has gained attention over the last few years as the causative agent of a common (and potentially deadly) healthcare-associated infection. Often called "*C. diff*," this disease is spread by direct contact with an infected individual or the pathogen itself. In the hospital, the bacterium and more often its endospores can be present on bedrails, bedside tables, sinks, and even on surfaces such as stethoscopes and blood pressure cuffs. Endospores are most often the source of infection because they are extremely resistant to many cleaning agents.

Individuals at higher risk of contracting the disease include the elderly, individuals with weakened immune systems, people with intestinal disorders, and people who have recently taken antibiotics. The patient in the opening case file was elderly, had recently had major surgery, and was already battling another infection that was being treated with antibiotics, all risk factors for the development of *C. diff*. The disease can range from a mild infection to a life-threatening illness causing severe diarrhea up to 15 times a day. Note that some people are asymptomatic carriers of this pathogen, which makes controlling the disease that much more difficult, especially in health care settings.

Treatment of *C. diff* involves antibiotic therapy. For mild to moderate disease, metronidazole is used; vancomycin is used to treat severe infections. Probiotics can help to restore normal biota within the intestinal tract, because the overgrowth of *C. difficile* often occurs due to antibiotic-induced loss of these beneficial microbes. Unfortunately, approximately one-fourth of the individuals who recover from *C. diff* will experience a recurrence of the disease at some point—either due to regrowth of the initial pathogen or a new infection. Recurring bouts of *C. diff* often require treatment with different antibiotics. New studies indicate fecal transplants may be a beneficial option in some of these cases, as you will see in later chapters.

A Sticky Situation

The human microbiome generally exists in a peaceful relationship with its host. However, when circumstances change, the microbiome can become more sinister. One way that circumstances can change is when some type of foreign material is implanted in the body. Artificial heart valves, an intrauterine device, and an artificial hip are all susceptible to being colonized by bacteria from the microbiome. Nonhuman surfaces in the body are particularly susceptible to attachment by bacteria. When the microorganisms continue to accumulate, they form a structure called a **biofilm**.

A study published in the *Proceedings of the National Academy of Sciences* in 2013 revealed just how quickly biofilms can clog commonly used medical devices, such as cardiovascular stents. Researchers from Princeton utilized narrow tubes closely resembling those found in certain medical devices. Specific materials were chosen to replicate the surface of the equipment, and the tubes were then exposed to fluid under pressure in order to closely mimic conditions within the human body.

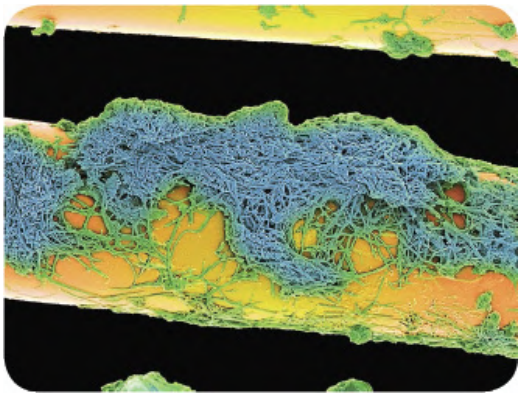
The researchers used microbes that are known to contaminate medical devices and engineered them to produce a pigment that could be observed microscopically. After forcing a stream of these microbes through the experimental tubes for approximately 40 hours, microscopic analysis revealed the formation of a biofilm on the inside walls of the device.

Over the next few hours, the researchers then forced a stream of different microbes into the experimental tubes. Within a short period of time, these cells were noted adhering to the biofilm-coated inner walls of the tubes. Further analysis revealed that the flow within the narrow tubes nudged the trapped cells into threadlike “streamers” that rippled along with the moving fluid.

Initially, the formation of these microbial threads only slightly decreased the rate of fluid flow within the experimental tubes. However, after 55 hours, the streamers began to weave together, creating a net similar to a spider’s web. This newly formed structure spanned the diameter of the narrow tube and trapped even more flowing cells, triggering a total blockage of the experimental tube within an hour.

This experiment revealed an important phenomenon that may explain why devices such as stents often fail. In addition, the researchers were able to identify which bacterial genes are likely involved in biofilm formation within a fluid environment. These data could lead to new strategies that maintain flow through medical devices, which could prevent unnecessary replacement of these devices or, in some cases, even death.

Source: *Proceedings of the National Academy of Sciences*. February 11, 2013.



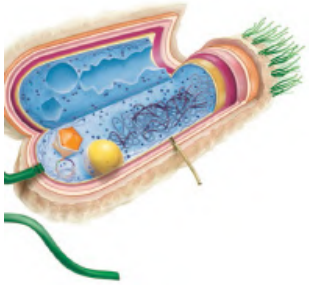
An accumulation of bacteria on a single fiber of a gauze bandage.

©Steve Gschmeissner/Science Source

Chapter Summary

3.1 Form and Function of Bacteria and Archaea

- Bacteria and archaea are distinguished from eukaryotes by (a) the way their DNA is packaged, (b) their cell walls, and (c) their lack of membrane-bound internal structures.
- Bacteria invariably have a cytoplasmic membrane, cytoplasm, ribosomes, a nucleoid, and a cytoskeleton.
- Bacteria may also have a cell wall, a glycocalyx, flagella, an outer membrane, a pilus, fimbriae, nanotubes, plasmids, inclusions, and/or microcompartments.



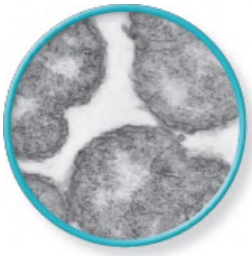
- Most bacteria have one of three general shapes: coccus (round), bacillus (rod), or spiral (spirochete, spirillum). Additional shapes are vibrio and branching filaments.
- Shape and arrangement of cells are key means of describing bacteria. Arrangements of cells are based on the number of planes in which a given species divides.

3.2 External Structures

- The external structures of bacteria include appendages (flagella, fimbriae, pili, nanotubes/nanowires) and the glycocalyx.
- Flagella vary in number and arrangement as well as in the type and rate of motion they produce.
- Archaeal flagella have a different structure than bacterial flagella but the same function: motility.

3.3 The Cell Envelope: The Wall and Membrane(s)

- The cell envelope is the boundary between inside and outside in a bacterial cell. Gram-negative bacteria have an outer membrane, the cell wall, and the cytoplasmic membrane. Gram-positive bacteria have only the cell wall and cytoplasmic membrane.
- In a Gram stain, gram-positive bacteria retain the crystal violet and stain purple. Gram-negative bacteria lose the crystal violet and stain red from the safranin counterstain.
- The outer membrane of gram-negative cells contains lipopolysaccharide (LPS), which is toxic to mammalian hosts.
- The bacterial cytoplasmic membrane is typically composed of phospholipids and proteins, and it performs many metabolic functions as well as transport activities.



Source: CDC/VD, SCSD

3.4 Bacterial Internal Structure

- The cytoplasm of bacterial cells serves as a solvent for materials used in all cell functions.
- The genetic material of bacteria is DNA, arranged on large, circular chromosomes. Additional genes can be carried on plasmids.

- Bacterial ribosomes are dispersed in the cytoplasm and are also embedded in the cytoplasmic membrane.
- Bacteria may store nutrients or other useful substances in their cytoplasm in either inclusions or microcompartments.
- Bacteria manufacture several types of proteins that help determine their cellular shape.
- A few families of bacteria produce dormant bodies called endospores, which are the hardiest of all life forms, surviving for hundreds or thousands of years.

- The genera *Bacillus* and *Clostridium* are endospore formers, and both contain deadly pathogens.

3.5 The Archaea: The Other "Prokaryotes"

- Archaea constitute the third domain of life. They superficially resemble bacteria but are most genetically related to eukaryotes.
- Although they exhibit similar external and internal structure, the unusual biochemistry and genetics of archaea set them apart from bacteria. Many members are adapted to extreme habitats with low or high temperature, salt, pressure, or acid.

3.6 Classification Systems for Bacteria and Archaea

- Bacteria and archaea are formally classified by phylogenetic relationships and phenotypic characteristics.
- Medical identification of pathogens uses an informal system of classification based on Gram stain, morphology, biochemical reactions, and metabolic requirements. It is summarized in *Bergey's Manual of Determinative Bacteriology*.
- A *bacterial species* is loosely defined as a collection of bacterial cells that shares an overall similar pattern of traits different from other groups of bacteria and that shares at least 70%–80% of its genes.
- Variant forms within a species (subspecies) include strains, types, and serotypes.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. Archaea **a**. are most genetically related to bacteria. **b**. contain a nucleus. **c**. cannot cause disease in humans. **d**. lack peptidoglycan in their cell walls.	**2**. Recall from section 1.1 that both bacteria and archaea appear to have evolved from the Last Common Ancestor (LCA). Based on what you know about their habitats and structures, speculate on *why* there are differences between the two.	**3**. Suppose an argument in your city has erupted about whether bacteria can be called species. Write a letter to the editor for your local newspaper arguing one side or the other.
Cell Structure and Function	**4**. Which of the following is present in both gram-positive and gram-negative cell walls? **a**. an outer membrane **b**. peptidoglycan **c**. teichoic acid **d**. lipopolysaccharides	**5**. As a supervisor in the infection control unit, you hire a local microbiologist to analyze samples from your hospital's hot-water tank for microbial contamination. Although she was unable to culture any microbes, she reports that basic microscopic analysis	**6**. Clearly bacteria are very different from eukaryotic cells. Yet they have similar processes and structures. Their DNA is very similar. Their ribosomes are very similar. The way they make

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
		revealed the presence of cells 0.8 µm in diameter that lacked a nucleus. Transmission electron microscopy showed that the cells lacked membrane-bound organelles but did contain ribosomes. Which domain of life do you hypothesize the cells represent? What further analysis would you perform?	proteins is similar. Speculate as to what led to this similarity in such radically different organisms.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Metabolic Pathways	**7**. Bacterial endospores usually function in **a**. reproduction. **b**. metabolism of nutrients. **c**. survival. **d**. storage.	**8**. Creating extremely long tubes (nanotubes) out of existing cell membrane material seems like a lot of hard work. Why would this be justified for an organism in an oxygen-poor environment?	**9**. Bacteria and archaea have a much greater diversity in their metabolic capabilities than do eukaryotes. They can live at 113°C (235°F) and at incredibly high atmospheric pressures. Construct an argument for why these simple cells have developed the ability to do this.

Information Flow and Genetics	**10**. Which structure plays a direct role in the exchange of genetic material between bacterial cells? **a**. flagellum **b**. pilus **c**. capsule **d**. fimbria	**11**. Bacteria have been found to change the structures they produce when they are placed in a situation of high flow rates, especially in a narrow space,	**12**. Bacterial and archaeal chromosomes are not enclosed in a membrane-bound nucleus. You will learn about some advantages of this

		like a tube. Often these new structures allow them to attach themselves or trap other bacteria in a biofilm. Identify some advantages to this strategy from the bacterium's perspective.	arrangement in another chapter, but can you think of some disadvantages?
Microbial Systems	**13**. Nanotubes are extensions of the _____ that can function in _____. **a**. membrane, genetic exchange **b**. pilus, genetic exchange **c**. flagellum, motility **d**. membrane, nutrient transfer	**14**. The results of your patient's wound culture just arrived, and Gram staining revealed the presence of pink, rod-shaped bacterial cells organized in pairs. You quickly realize that this patient could be at risk for developing fever and shock. Explain how the culture results indicated this potential risk.	**15**. We know that bacteria/archaea and their genetics are clearly influenced by their environment since they have developed membranes and structures that allow them to live in a wide variety of conditions. Do you think the environment in which they live has likewise experienced change due to *their* presence? Can you cite any examples?
Impact of Microorganisms	**16**. Find the true statement about biofilms. **a**. They are found only in outdoor	**17**. Suggest more than one reason why bacteria may express glycocalyces in	**18**. Construct arguments agreeing with and refuting this statement:

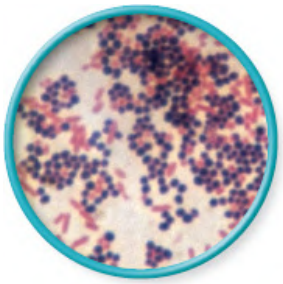
	environments. **b**. They are found only on artificial medical implants. **c**. They consist of many representatives of a single bacterial species. **d**. They complicate the treatment of some infections.	natural circumstances but not in the laboratory.	*Human infections may have originated as accidental encounters between humans and microbes that were actually meant to interact with other parts of the natural environment*.
Scientific Thinking	**19**. Which of the following would be used to identify an unknown bacterial culture that came from a patient in the intensive care unit? **a**. Gray's Anatomy **b**. Bergey's Manual of Determinative Bacteriology **c**. Bergey's Manual of Systematic Bacteriology **d**. The Physician's Desk Reference	**20**. During the cold war between the Soviet Union and the United States, both countries were interested in "weaponizing" *Bacillus anthracis* (anthrax) endospores to deliver via missiles and other weapons. Why did they choose this particular organism?	**21**. During the cold war between the Soviet Union and the United States, both countries were interested in "weaponizing" *Bacillus anthracis* (anthrax) endospores to deliver via missiles and other weapons. Do you believe there is any ethical difference between using infectious agents as weapons compared to traditional weapons? Explain.

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From chapter 2, figure 2.18**. Explain why some cells are pink and others are purple in this image of a Gram-stained bacterial smear.



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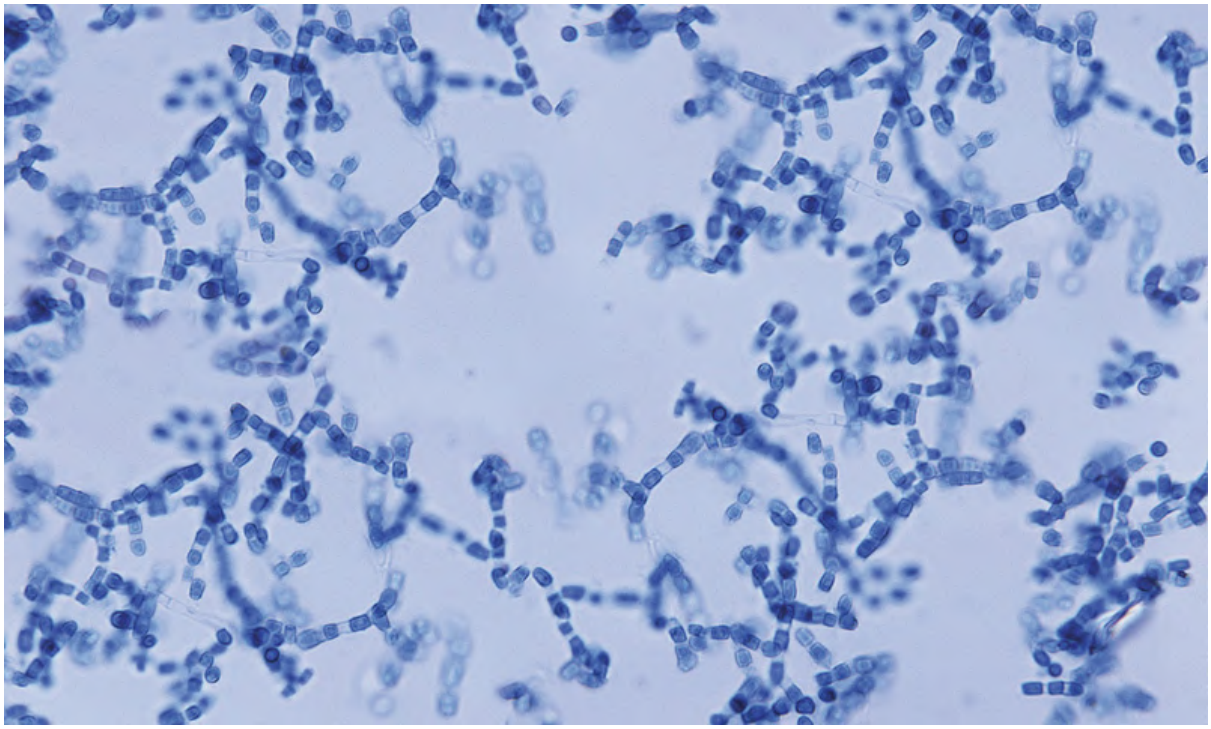
Resources are available through Connect, including interactive learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

4

Eukaryotic Cells and Microorganisms

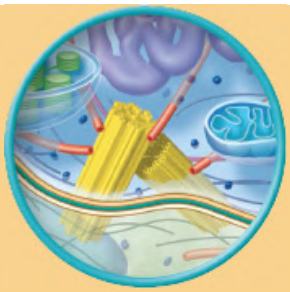


Source: CDC/Dr. Lucille K. George (*Coccidioides immitis* arthroconidia); ©BSIP/Universal Images Group/Getty Images (*Aspergillus flavus*); ©Eye of Science/Science Source (*Trichomonas vaginalis*. Magnification-9,000x); ©Eye of Science/Science Source (liver fluke. Magnification unknown); ©Jade/Blend Images (health care worker)

IN THIS CHAPTER…

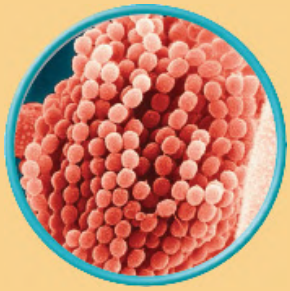
4.1 Overview of the Eukaryotes

1. Relate bacterial, archaeal, and eukaryotic cells to the *Last Common Ancestor*.
2. List the types of eukaryotic microorganisms, and identify which are unicellular and which are multicellular.



4.2 Structures of the Eukaryotic Cell

3. Differentiate among the flagellar structures of bacteria, eukaryotes, and archaea.
4. List similarities and differences between eukaryotic and bacterial cytoplasmic membranes.
5. Describe the main structural components of a nucleus.
6. Diagram how the nucleus, endoplasmic reticulum, and Golgi apparatus act together with vesicles during the transport process.
7. Explain the function of the mitochondrion.
8. Explain the importance of ribosomes, and differentiate between eukaryotic and bacterial types.
9. List and describe the three main fibers of the cytoskeleton.
10. Explain how endosymbiosis contributed to the development of eukaryotic cells.

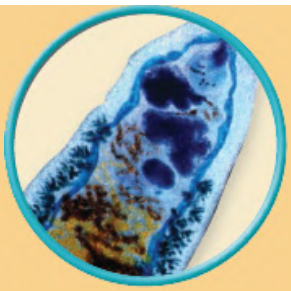


4.3 The Fungi

11. List two detrimental and two beneficial activities of fungi (from the viewpoint of humans).
12. List three general features of fungal anatomy.
13. Differentiate among the terms *heterotroph, saprobe*, and *parasite*.
14. Explain the relationship between fungal hyphae and the production of a mycelium.
15. Describe two ways in which fungal spores arise.

4.4 The Protozoa

16. Describe the protozoan characteristics that illustrate why protozoa are informally placed into a single group.
17. List three means of locomotion exhibited by protozoa.
18. Explain why a cyst stage may be useful to a protozoan.
19. Give an example of a disease caused by each of the four types of protozoa.



4.5 The Helminths

20. List the two major groups of helminths, and provide examples representing each body type.
21. Summarize the stages of a typical helminth life cycle.

CASE FILE

Puzzle in the Valley

Working as a newly graduated radiology technologist in a rural hospital in California, I encountered a case that would prove to be a challenge for everyone involved. The patient was a male migrant farm worker in his mid-30s who presented to the ER with common flulike symptoms: fever, chills, weakness, cough, muscular aches and pains, and headache. He also had a painful red rash on his lower legs.

It was summertime, so influenza was unlikely. The emergency room physician believed that the patient likely had pneumonia, but she found the rash puzzling. She asked

me to obtain a chest X ray. I performed anterior-posterior and lateral views of the chest, which revealed two nodules approximately 2 cm in size in the patient's left upper lobe. The physician stated that the nodules were consistent with pneumonia, but the possibility of cancer could not be ruled out. The patient's age and the fact that he was a nonsmoker, however, made a diagnosis of lung cancer much less likely than pneumonia.

The patient was admitted to the hospital for IV antibiotic treatment. Before the antibiotic therapy was started, a sputum sample was collected and sent to a larger center for culture and sensitivity (C&S) testing. Despite IV fluids, rest, and broad-spectrum antibiotics targeting both gram-positive and gram-negative bacteria, the patient showed no improvement. After receiving the C&S report, it became clear why the intravenous antibiotics were not working: The patient had a fungal infection, not a bacterial infection as first suspected. The physician immediately started the patient on amphotericin B, a potent antifungal medication that would properly treat the patient's case of coccidioidomycosis.

- How might the patient have contracted this infection?
- Why did the initial antibiotic therapy fail to improve the patient's symptoms?

Case File Wrap-Up appears at the end of the chapter.

Table 4.1 Eukaryotic Organisms Studied in Microbiology

Always Unicellular	May Be Unicellular or Multicellular	Always Multicellular
Protozoa	Fungi Algae	Helminths (have unicellular egg or larval forms)

4.1 Overview of the Eukaryotes

Evidence from paleontology indicates that the first eukaryotic cells appeared on the earth approximately 2 to 3 billion years ago. While it used to be thought that eukaryotic cells evolved directly from ancient prokaryotic cells, we now believe that bacteria, archaea, and eukaryotes evolved from a different kind of cell, a precursor to both prokaryotes and eukaryotes that biologists call the *Last Common Ancestor*. This ancestor was neither prokaryotic nor eukaryotic but gave rise to all three current cell types.

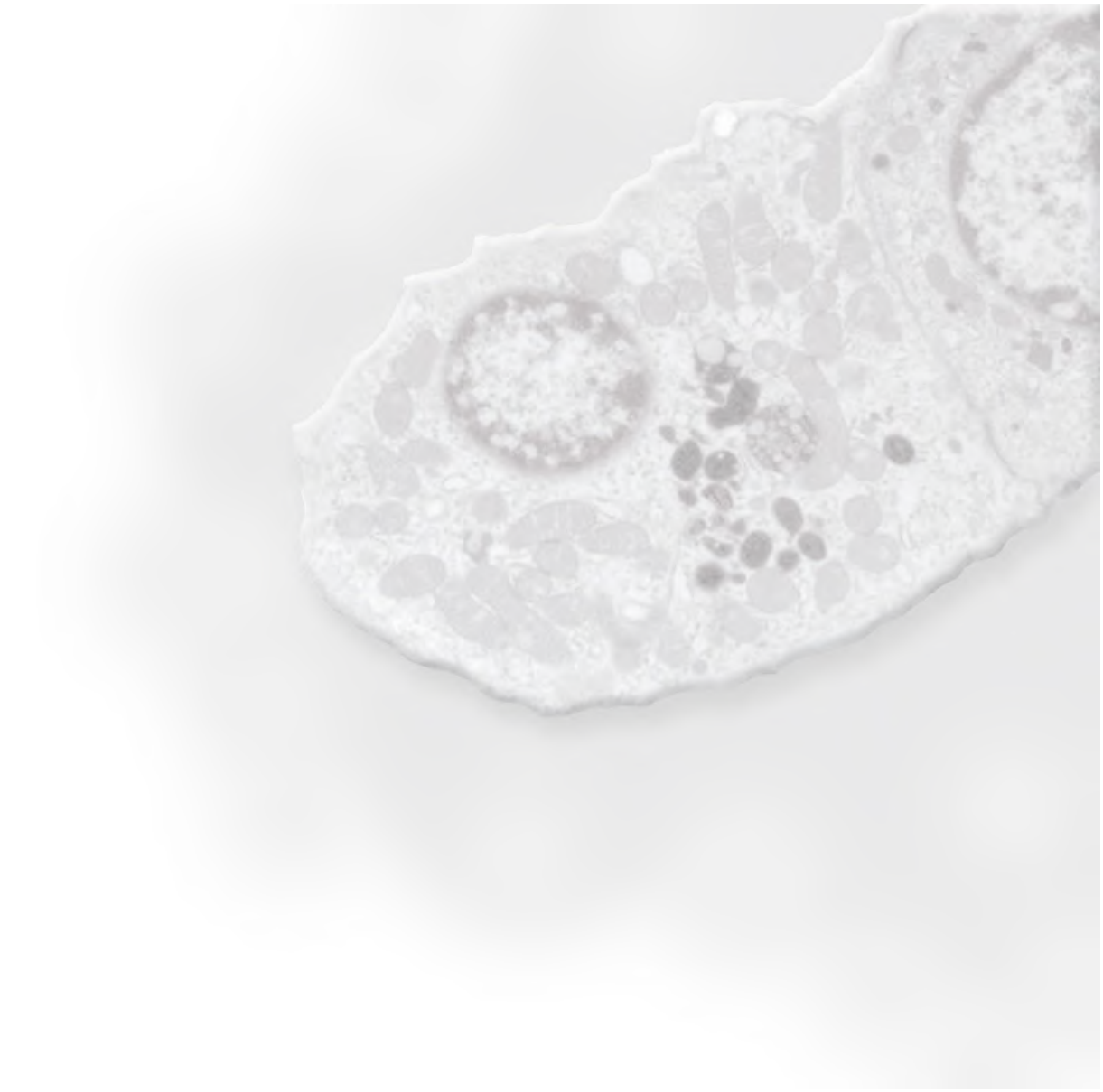
The first primitive eukaryotes were probably single-celled and independent, but, over time, some cells began to aggregate, forming colonies. With further evolution, some of the cells within colonies became *specialized*, or adapted to perform a particular function advantageous to the whole colony, such as movement, feeding, or reproduction. Complex multicellular organisms evolved as individual cells in the organism lost the ability to survive apart from the intact colony.

Only certain eukaryotes are traditionally studied by microbiologists—primarily the protozoa, the microscopic algae and fungi, and helminths **(table 4.1)**. Because the vast

majority of algae do not cause infections of humans, we will discuss only the other three eukaryotic microbes in this chapter.

4.1 LEARNING OUTCOMES—Assess Your Progress

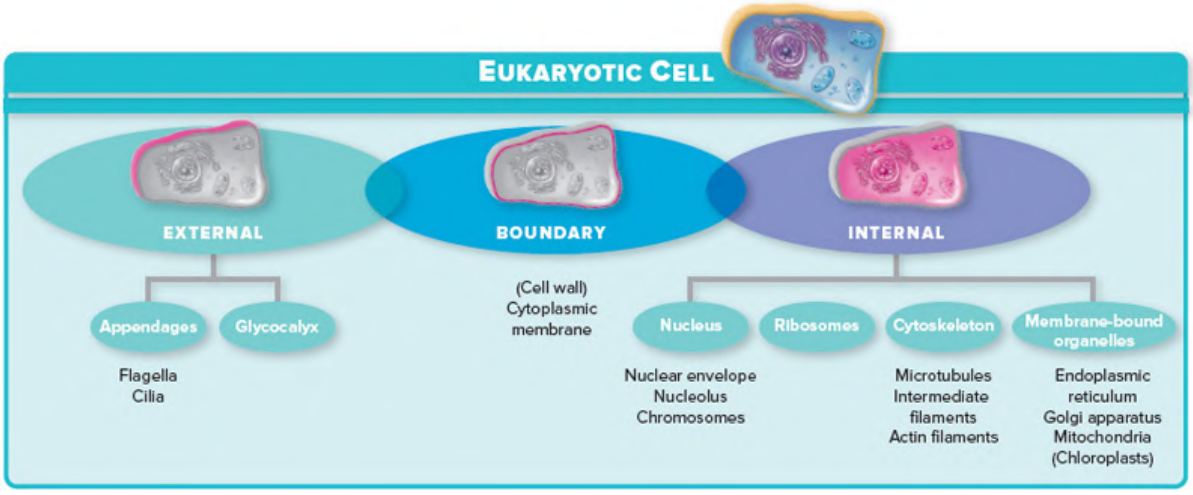
1. Relate bacterial, archaeal, and eukaryotic cells to the *Last Common Ancestor*.
2. List the types of eukaryotic microorganisms, and identify which are unicellular and which are multicellular.



©EM Research Services, Newcastle University (cell)

4.2 Structures of the Eukaryotic Cell

Nearly all eukaryotic microbial cells have a cell membrane, nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, vacuoles, cytoskeleton, cytoplasm, and glycocalyx. Only some types of eukaryotes have a cell wall, locomotor appendages, and chloroplasts **(figure 4.1)**. In the following sections, we cover the microscopic structure and functions of the eukaryotic cell. As with the bacteria, we begin on the outside and proceed inward through the cell.



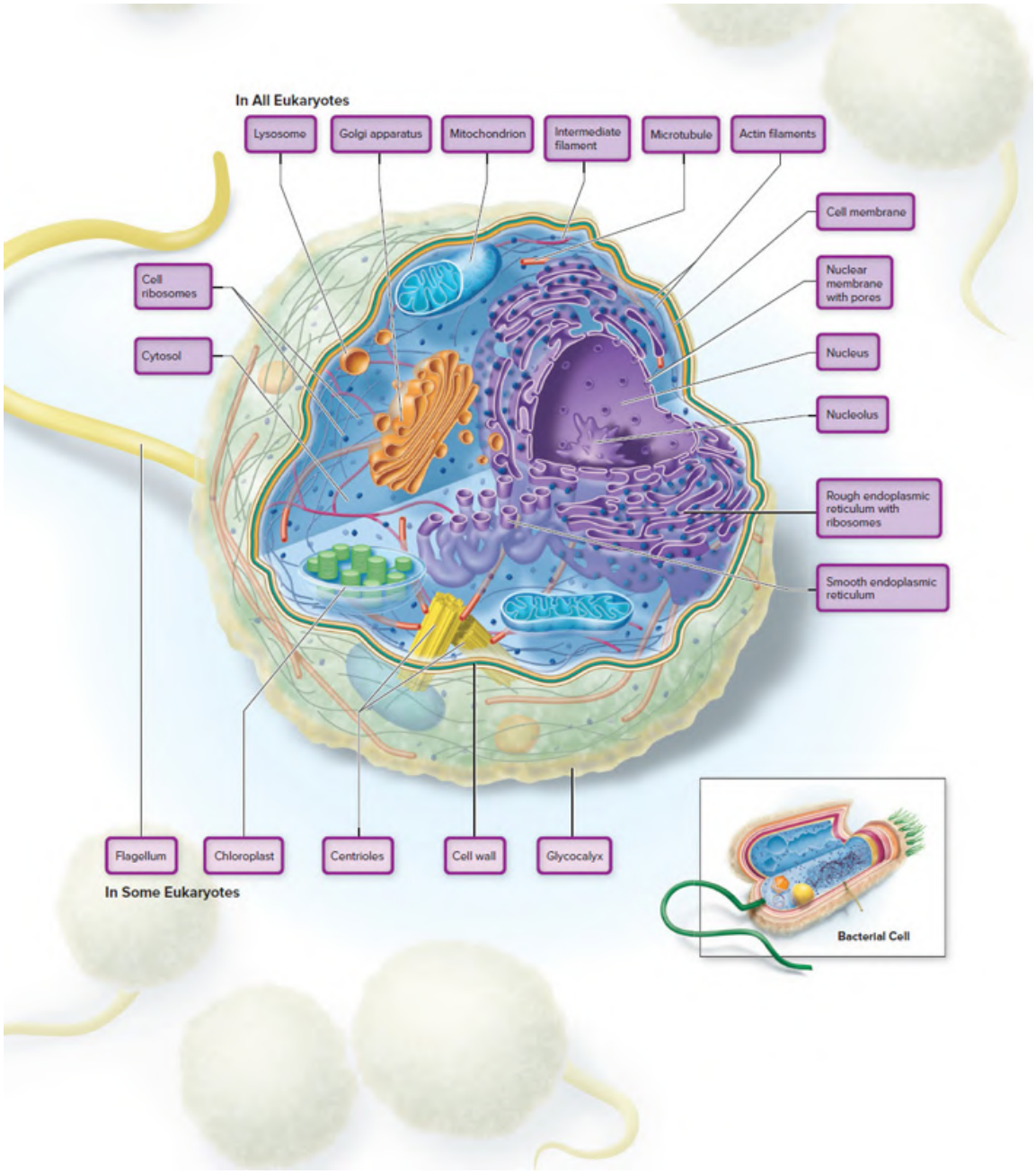


Figure 4.1 **Structure of a eukaryotic cell**. The figure of a bacterial cell from chapter 3 is included here for comparison.

External Structures

Appendages for Moving: Cilia and Flagella

Motility allows microorganisms to locate nutrients and to migrate toward positive stimuli such as sunlight; it also enables them to avoid harmful substances and stimuli. Eukaryotic microbes move by using flagella or cilia. This type of motility is common in protozoa, many algae, and a few fungal and animal cells.

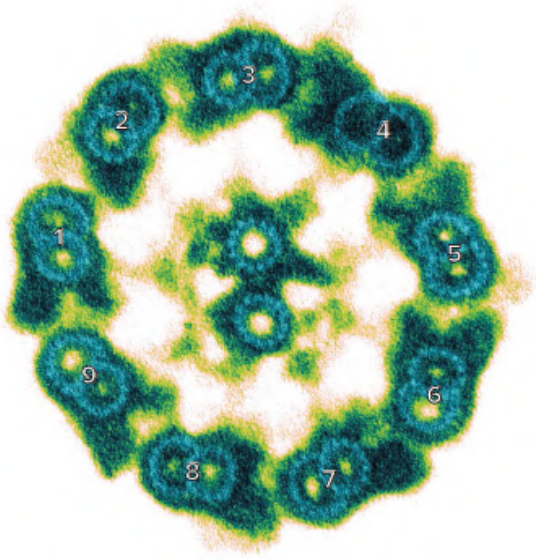


Figure 4.2 Microtubules in flagella. A cross section that reveals the typical 9 + 2 arrangement found in both flagella and cilia.
© Aaron J. Bell/Science Source

Eukaryotic flagella are much different from those of bacteria, even though they have the same name. The eukaryotic flagellum is thicker (by a factor of 10), structurally more complex, and covered by an extension of the cell membrane. A single flagellum is a long, sheathed cylinder containing regularly spaced hollow tubules—microtubules—that extend along its entire length **(figure 4.2)**. A cross section reveals nine pairs of closely attached microtubules surrounding a single central pair. This scheme, called the 9 + 2 arrangement, is found in eukaryotic flagella and cilia (figure 4.2). During locomotion, the adjacent microtubules slide past each other, whipping the flagellum back and forth. Although details of this process are too complex to discuss here, it involves expenditure of energy and a coordinating mechanism in the cell membrane. The placement and number of flagella can be useful in identifying flagellated protozoa and certain algae.

Cilia are very similar in overall architecture to flagella, but they are shorter and more numerous (some cells have several thousand). They are found only on a single group of protozoa and in certain animal cells. In the ciliated protozoa, the cilia occur in rows over the cell surface, where they beat back and forth in regular oar-like strokes. These protozoa are among the fastest of all motile cells. On some cells, cilia also function as feeding and filtering structures.

The Glycocalyx

Many eukaryotic cells have a glycocalyx, an outermost layer that comes into direct contact with the environment (see figure 4.1). This structure, which is sometimes called an extracellular matrix, is usually composed of polysaccharides. It appears as a network of fibers, a slime layer, or a capsule much like the glycocalyx of bacteria and archaea. Because of its

position on the outside of the cell, the glycocalyx contributes to protection, adherence of cells to surfaces, and reception of signals from other cells and from the environment. The nature of the layer beneath the glycocalyx varies among the several eukaryotic groups. Fungi and most algae have a thick, rigid cell wall surrounding a cell membrane, whereas protozoa, a few algae, and all animal cells do not have a cell wall but only a cell membrane.

Boundary Structures

The Cell Wall

Protozoa and helminths do not have cell walls, but fungi do. The cell walls of fungi are rigid and provide structural support and shape, but they are different in chemical composition from bacterial and archaeal cell walls. They have an inner layer of polysaccharide fibers composed of chitin or cellulose, and an outer layer of mixed glycans **(figure 4.3)**.

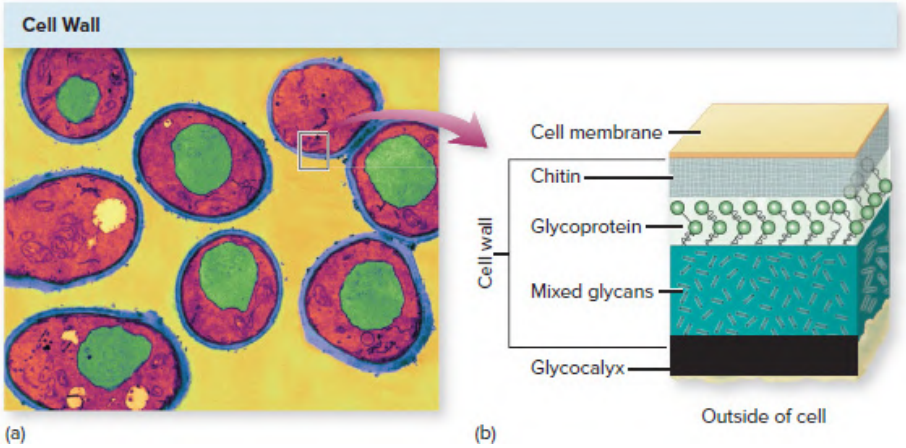


Figure 4.3 **Cross-sectional views of fungal cell walls**. **(a)** An electron micrograph of fungal cells. **(b)** A drawing of the section of the wall inside the square in **(a)**.
©Thomas Deerinck, NCMIR/Science Source

The Cell Membrane

The cell (or cytoplasmic) membrane of eukaryotic cells is a typical bilayer of phospholipids in which protein molecules are embedded. In addition to phospholipids, eukaryotic membranes also contain *sterols* of various kinds. Sterols are a form of lipid, but they are different from phospholipids in both structure and behavior. They are fairly rigid molecules, and this makes eukaryotic membranes more stable than those of non-eukaryotic cells. This strengthening feature is extremely important in those cells that don't have a cell wall. Cytoplasmic membranes of eukaryotes have the same function as those in bacteria and archaea, serving as selectively permeable barriers.

Internal Structures

Unlike bacteria and archaea, eukaryotic cells contain a number of individual membrane-bound organelles that are extensive enough to account for 60% to 80% of their volume. They are

contained in a fluid called the cytosol. The entire contents of the cell (outside of the nucleus) is termed the cytoplasm. The terms cytosol and cytoplasm are sometimes used interchangeably.

The Nucleus

The nucleus is a compact sphere that is the most prominent organelle of eukaryotic cells. It is separated from the cell cytoplasm by an external boundary called a nuclear envelope. The envelope has a unique architecture. It is composed of two parallel membranes—that means two lipid bilayers—separated by a narrow space. It is perforated with small, regularly spaced openings, or pores. The pores are formed by the connection of the inner and outer nuclear membranes **(figure 4.4)**. The nuclear pores are passageways through which macromolecules move from the nucleus to the cytoplasm and vice versa. The nucleus is filled with a matrix called the nucleoplasm and a granular mass, the **nucleolus**, that stains more intensely than the immediate surroundings because of its RNA content. The nucleolus is the site for ribosomal RNA synthesis and a collection area for ribosomal subunits. The subunits are transported through the nuclear pores into the cytoplasm for final assembly into ribosomes.

A prominent feature of the nucleoplasm in stained preparations is a network of dark fibers known as **chromatin**. Chromatin is made of linear DNA, which, of course, is the genetic material of the cell. When it is wound around histone proteins,

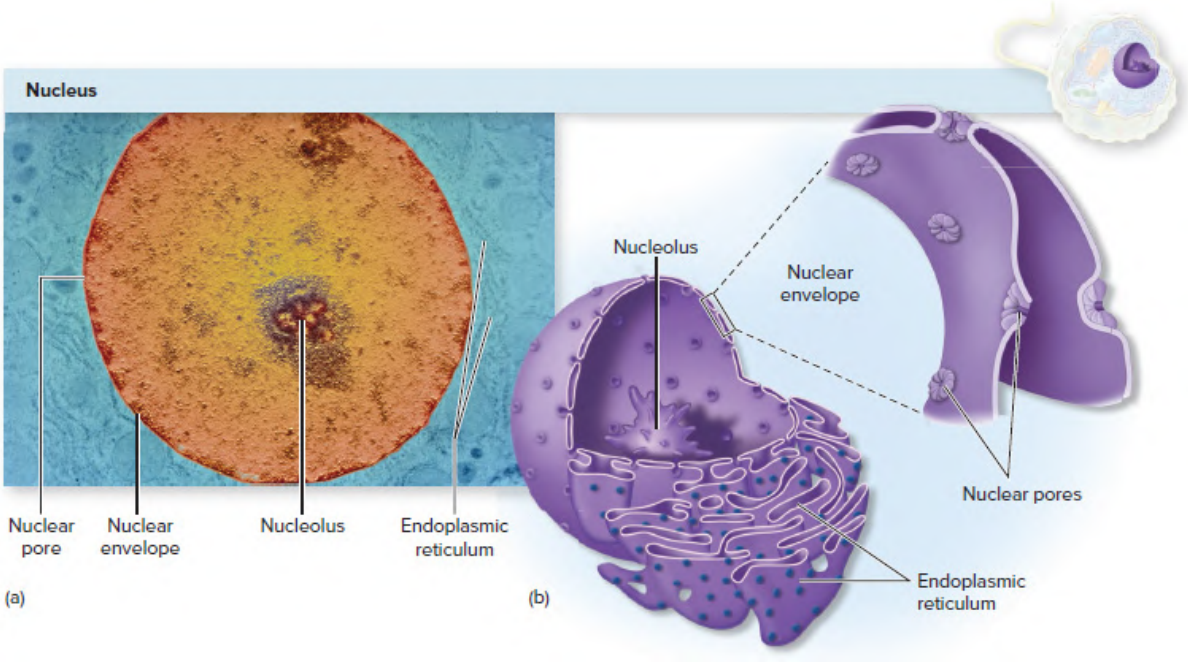


Figure 4.4 **The nucleus. (a)** Electron micrograph section of a nucleus, showing its most prominent features. **(b)** Cutaway three-dimensional view of the relationships of the nuclear envelope and pores.
©D Spector/Photolibrary/Getty Images

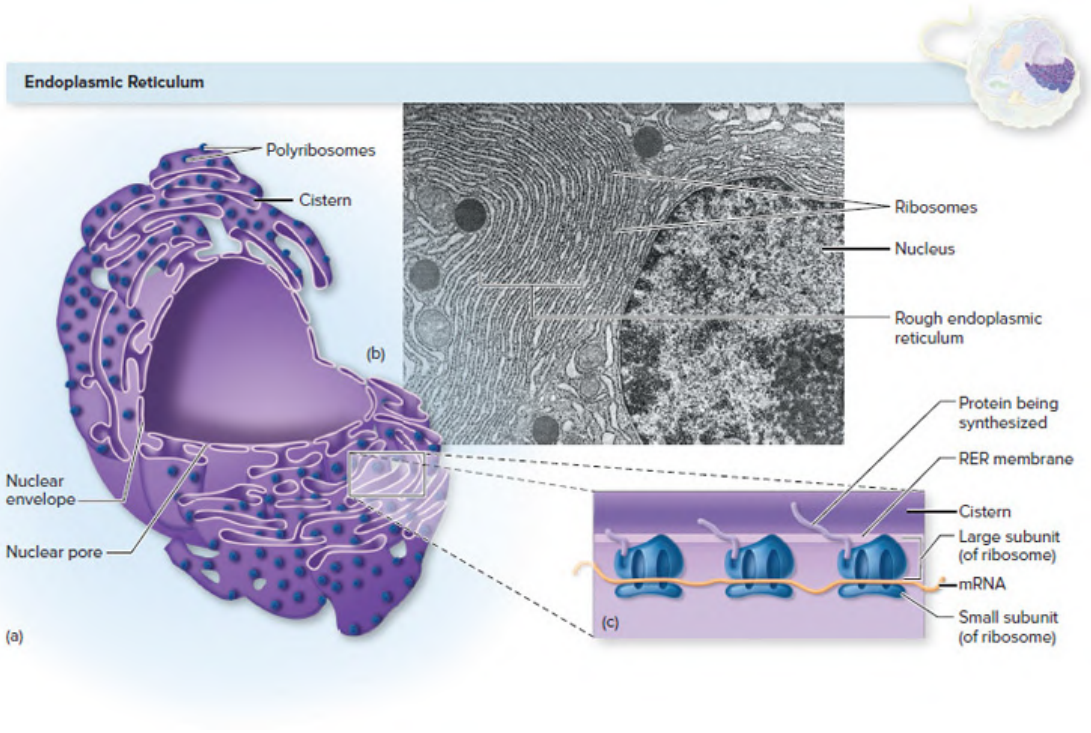


Figure 4.5 **The origin and detailed structure of the rough endoplasmic reticulum (RER). (a)** Schematic view of the origin of the RER from the outer membrane of the nuclear envelope. **(b)** Electron micrograph of the RER. **(c)** Detail of the orientation of a ribosome on the RER membrane.
©Don W. Fawcett/Science Source

chromatin forms structures called chromosomes. Elaborate processes have evolved for transcription and duplication of this genetic material.

Endoplasmic Reticulum

The **endoplasmic reticulum (ER)** is a series of membrane tunnels used in transport and storage. There are two kinds of endoplasmic reticulum: the **rough endoplasmic reticulum (RER)** **(figure 4.5)** and the **smooth endoplasmic reticulum (SER)**. The RER is a continuation of the outer membrane of the nuclear envelope and extends in a continuous network through the cytoplasm, even all the way out to the cell membrane. This architecture permits the spaces in the RER, called cisternae (singular, *cistern*), to transport materials from the nucleus to the cytoplasm and ultimately to the cell's exterior. The RER appears rough because of large numbers of ribosomes attached to its membrane surface. Proteins synthesized on the ribosomes are shunted into the inside space (the lumen) of the RER and held there for later packaging and transport. In contrast to the RER, the SER is a closed tubular network without ribosomes. It functions in nutrient processing and in synthesis and storage of nonprotein macromolecules such as lipids.

Golgi Apparatus

The **Golgi apparatus**, also called the Golgi complex or Golgi body, is the site in the cell in which proteins are modified and then sent to their final destinations. It is an organelle

consisting of a stack of several flattened, disc-shaped sacs called cisternae. These sacs have both membranes and cavities like those of the endoplasmic reticulum, but they do not form a continuous network **(figure 4.6)**. This organelle is always closely associated with the endoplasmic reticulum. At a site where it meets the Golgi apparatus, the endoplasmic reticulum buds off tiny membrane-bound packets of protein called *transitional vesicles* that are picked up by the face of the Golgi apparatus. Once inside the Golgi body, the proteins are often modified by the addition of polysaccharides and lipids. The final action of this apparatus is to pinch off finished

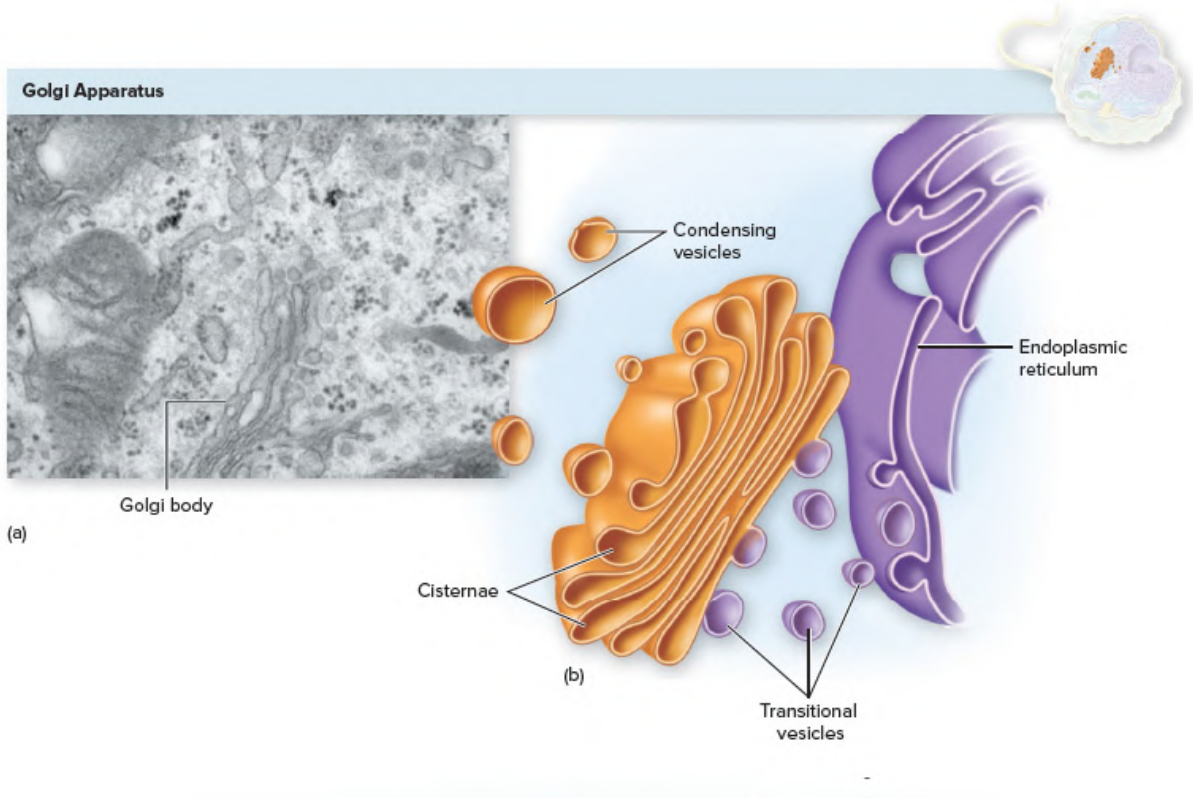


Figure 4.6 Detail of the Golgi body. **(a)** Micrograph showing the Golgi body. **(b)** The Golgi body (gold) receives vesicles from the endoplasmic reticulum and releases other vesicles from its other side.
©EM Research Services, Newcastle University

condensing vesicles that will carry the modified proteins to organelles such as lysosomes or outside the cell as secretory vesicles.

Nucleus, Endoplasmic Reticulum, and Golgi Apparatus: Nature's Assembly Line

As the keeper of the eukaryotic genetic code, the nucleus ultimately governs and regulates all cell activities. But, because the nucleus is located in a specific cellular site, it must direct these activities through a structural and chemical network. This network includes ribosomes, which

originate in the nucleus, and the rough endoplasmic reticulum **(figure 4.7)**. Remember that the RER is continuously connected with the

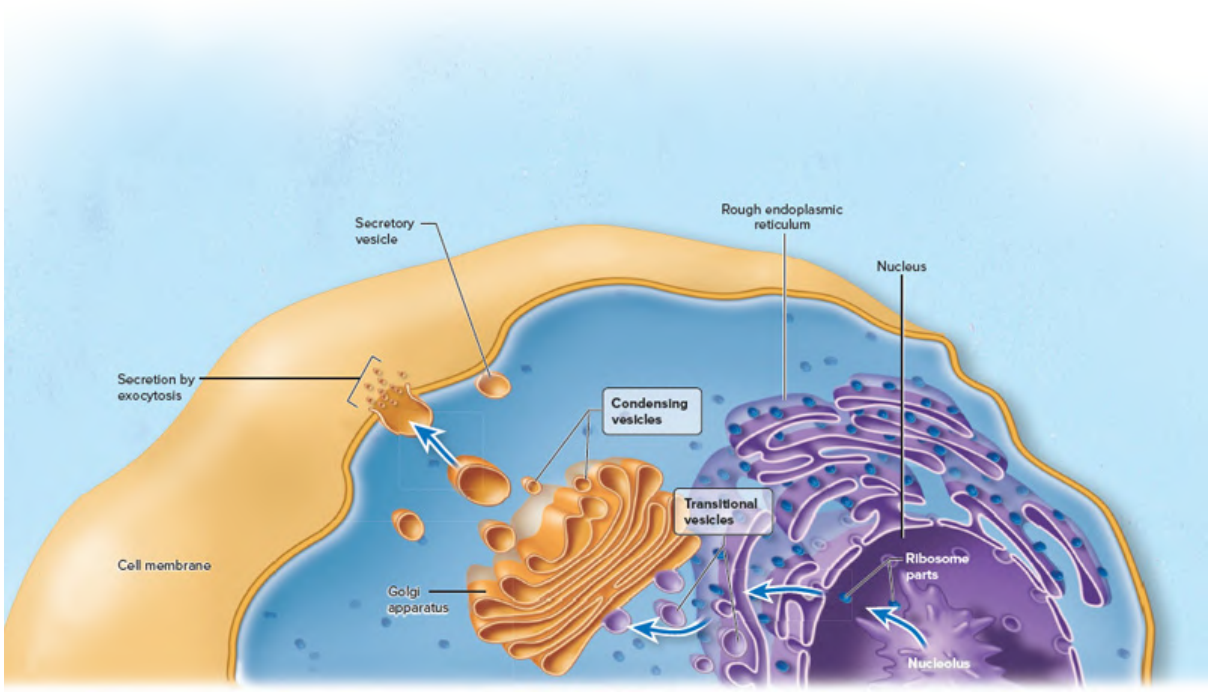
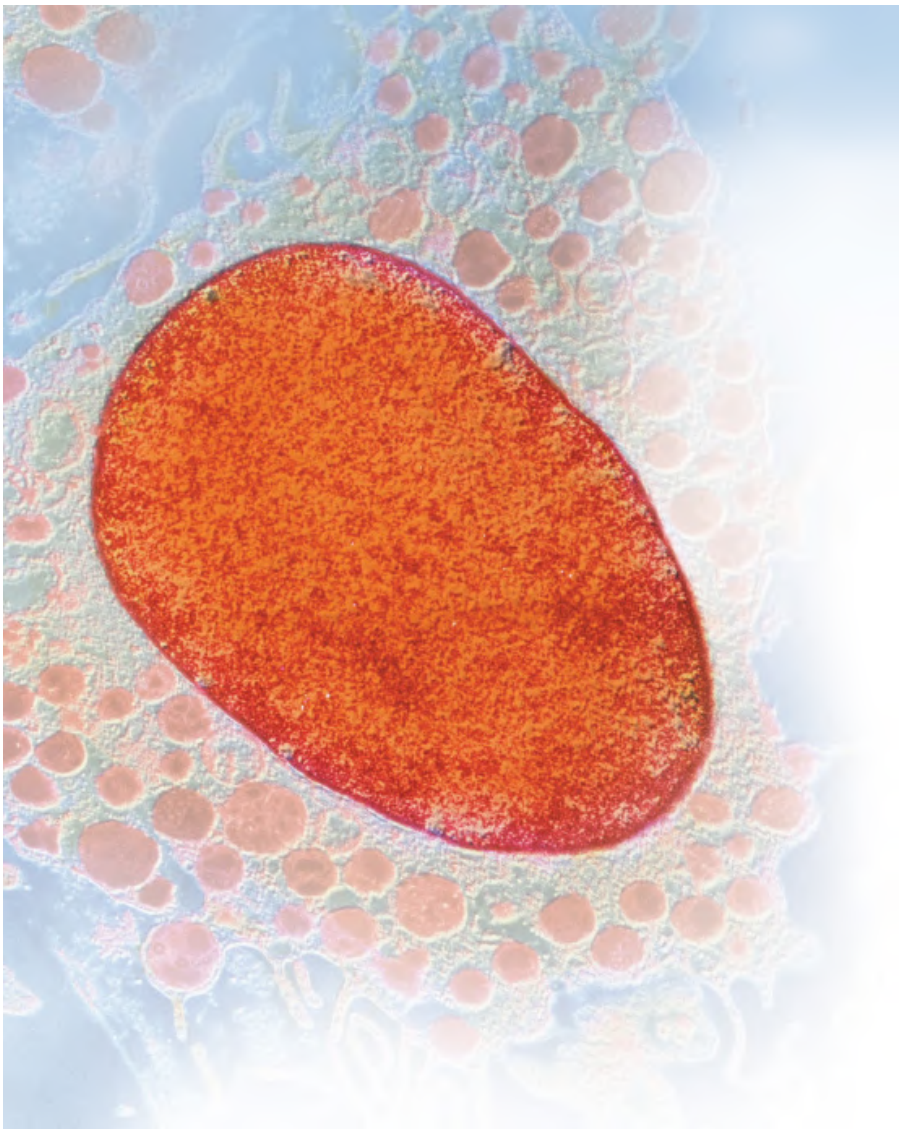


Figure 4.7 **The transport process**. The cooperation of organelles in protein synthesis and transport: Nucleus → RER → Golgi apparatus → vesicles → secretion.



This colorized transmission electron micrograph of a mast cell, a cell type of the immune system, shows the nucleus as a large orange oval.

©Science Photo Library/Getty Images

nuclear envelope, as well as the smooth endoplasmic reticulum and the Golgi apparatus. Initially, a segment of DNA containing the instructions for producing a protein is copied into RNA, and this RNA transcript is passed out through the nuclear pores directly to the ribosomes on the endoplasmic reticulum. Here, specific proteins are synthesized from the RNA code and deposited in the lumen (space) of the endoplasmic reticulum. After being transported to the Golgi apparatus, the protein products are chemically modified and packaged into vesicles that can be used by the cell in a variety of ways. Some of the vesicles contain enzymes to digest food inside the cell; other vesicles are secreted to digest materials outside the cell, and others are important in the enlargement and repair of the cell wall and membrane.

A **lysosome** is a vesicle that buds off of the Golgi apparatus. It contains a variety of enzymes. Lysosomes are involved in digestion of food particles inside the cell and in protection against invading microorganisms. They also participate in digestion and removal of

cell debris in damaged tissue. Another type of vesicle, the peroxisome, contains a wide variety of enzymes. (Peroxisomes do not originate from the Golgi apparatus.) Other types of vesicles include **vacuoles** (vak′-yoo-ohlz), which are membrane-bound sacs containing fluids or solid particles to be digested, excreted, or stored. They are found in phagocytic cells (certain white blood cells and protozoa) in response to food and other substances that have been engulfed. The contents of a food vacuole are digested through the merger of the vacuole with a lysosome. This merged structure is called a phagosome **(figure 4.8)**. Other types of vacuoles are used in storing reserve food such as fats and glycogen. Protozoa living in freshwater habitats use structures called contractile vacuoles to regulate osmotic pressure. These vacuoles systematically expel excess water that has diffused into the cell (described later).

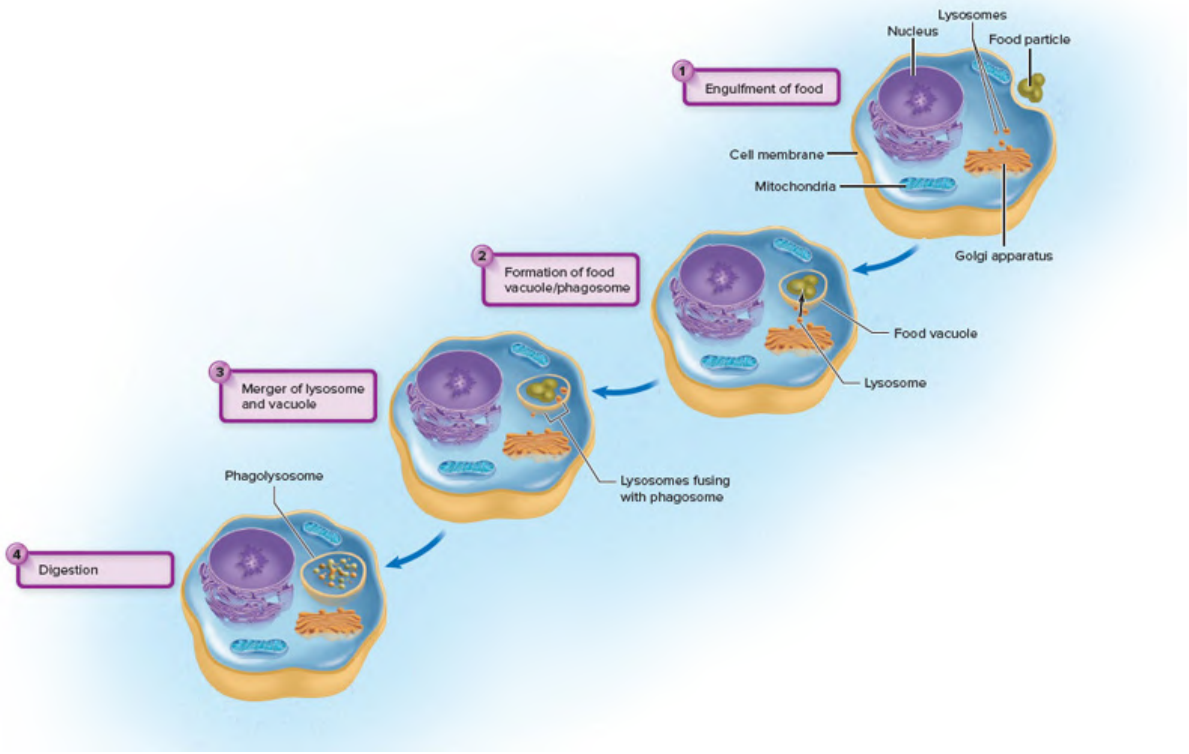


Figure 4.8 **The origin and action of lysosomes in phagocytosis**.

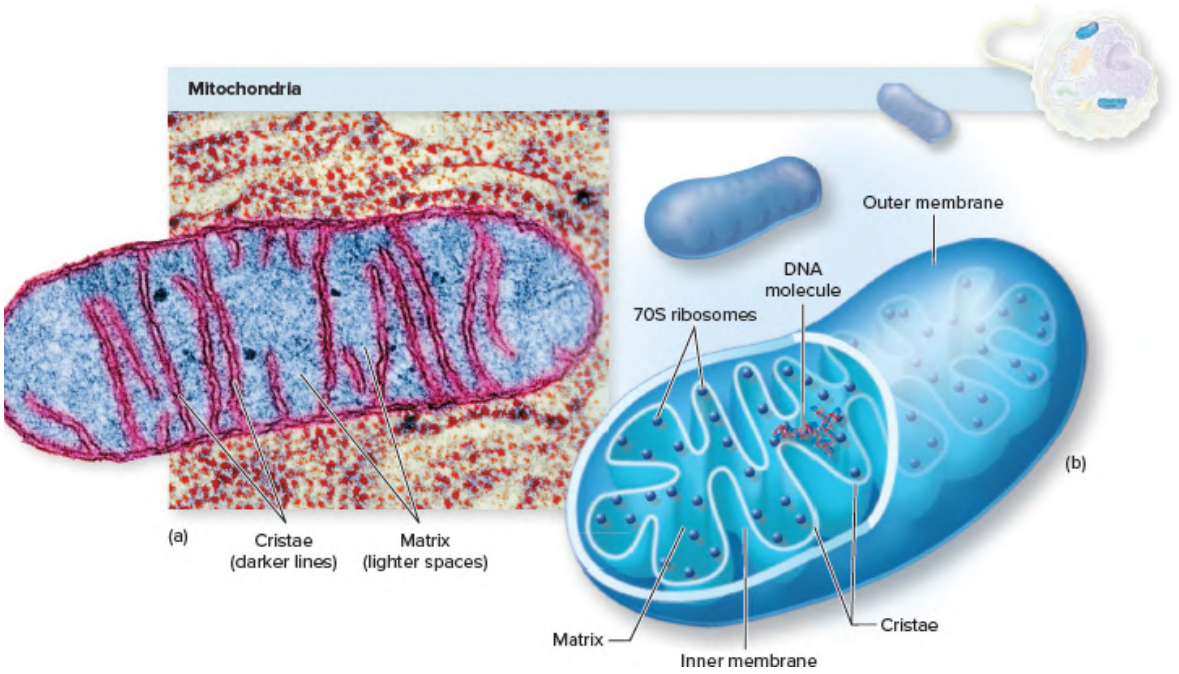


Figure 4.9 **General structure of a mitochondrion**.
©CNRI/Science Photo Library/Getty Images

NCLEX® PREP

1. An RN is educating a student nurse about cellular processes. Which of the following statements by the student demonstrates understanding of the function of mitochondria?
   - **a.** *"Mitochondria transport proteins within the cell."*
   - **b.** *"The genetic code is contained within mitochondria."*
   - **c.** *"Mitochondria generate energy for the cell."*
   - **d.** *"Cell debris is digested and removed by mitochondria."*

Mitochondria

Although the nucleus is the cell's control center, none of the cellular activities it commands could proceed without a constant supply of energy. The bulk of a cell's energy is generated in most eukaryotes by **mitochondria** (my″-toh-kon′-dree-uh). When viewed with light microscopy, mitochondria appear as round or elongated particles scattered throughout the cytoplasm. The internal ultrastructure reveals that a single mitochondrion consists of a smooth, continuous outer membrane that forms the external contour, and an inner, folded membrane nestled neatly within the outer membrane **(figure 4.9)**. The folds on the inner membrane, called **cristae** (kris′-te), may be tubular (like fingers) or folded into shelflike bands.

The cristae membranes hold the enzymes and electron carriers needed in aerobic respiration. This is an oxygen-using process that extracts chemical energy contained in nutrient molecules and stores it in the form of high-energy molecules, or ATP. Mitochondria (along with chloroplasts) are unique among organelles in that they divide independently of the

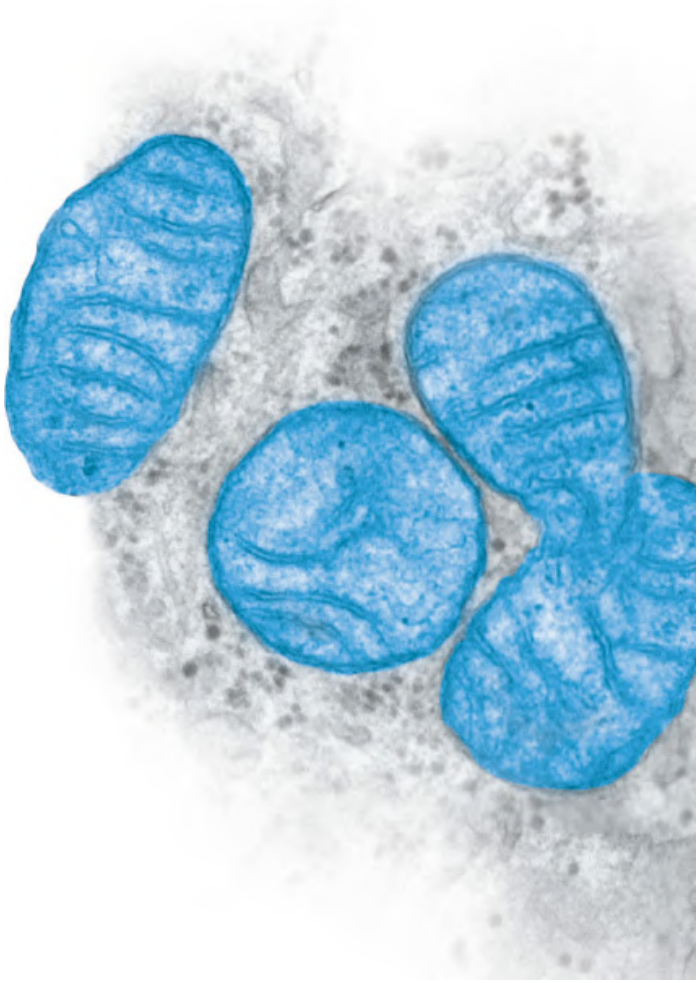
cell, contain circular molecules of DNA, and have bacteria-sized 70S ribosomes. These characteristics have caused scientists to suggest that mitochondria were once bacterial cells that developed into eukaryotic organelles over time. While it was previously thought that all eukaryotic organisms must have mitochondria, scientists have discovered that some protozoa have pared-down versions of mitochondria (called mitosomes) and one species has even been found that contains neither mitochondria nor mitosomes.

Chloroplasts

Chloroplasts are remarkable organelles found in algae and plant cells that are capable of converting the energy of sunlight into chemical energy through photosynthesis. Another important product of the photosynthesis process in chloroplasts is oxygen gas. Although chloroplasts resemble mitochondria, chloroplasts are larger, contain special pigments, and are much more varied in shape.

Ribosomes

In an electron micrograph of a eukaryotic cell, ribosomes are numerous, tiny particles that give a “dotted” appearance to the cytoplasm. Ribosomes are distributed throughout the cell: Some are scattered freely in the cytoplasm and cytoskeleton; others are attached to the rough endoplasmic reticulum as previously described. Still others appear inside the mitochondria and in chloroplasts. Multiple ribosomes are often found arranged in short



Mitochondria
©EM Research Services, Newcastle University

chains called polyribosomes (polysomes). The basic structure of eukaryotic ribosomes is similar to that of bacterial ribosomes, described in chapter 3. Both are composed of large and small subunits of ribonucleoprotein (see figure 4.5). By contrast, however, the eukaryotic ribosome (except in the mitochondrion) is the larger 80S variety that is a combination of 60S and 40S subunits. This difference means that we can use antibiotics that target prokaryotic ribosomes without harming our own eukaryotic ribosomes. As in the bacteria, eukaryotic ribosomes are the staging areas for protein synthesis.

The Cytoskeleton

The cytoplasm of a eukaryotic cell is criss-crossed by a flexible framework called the cytoskeleton. This framework appears to have several functions, such as anchoring organelles, moving RNA and vesicles, and permitting shape changes and movement in some cells **(figure 4.10)**. The three main types of cytoskeletal elements are *actin filaments, intermediate*

filaments, and *microtubules*. **Actin** filaments are long thin protein strands about 7 nm in diameter. They are found throughout the cell but are most highly concentrated just inside the cell membrane. Actin filaments are responsible for cellular movements such as contraction, crawling, pinching during cell division, and formation of cellular extensions. **Microtubules** are long, hollow tubes that maintain the shape of those eukaryotic cells that don't have walls, and they transport substances from one part of a cell to another. The spindle fibers that play an essential role in mitosis are actually microtubules that attach to chromosomes and separate them into daughter cells. As indicated earlier, microtubules are also responsible for the movement of cilia and flagella. **Intermediate filaments** are ropelike structures that are about 10 nm

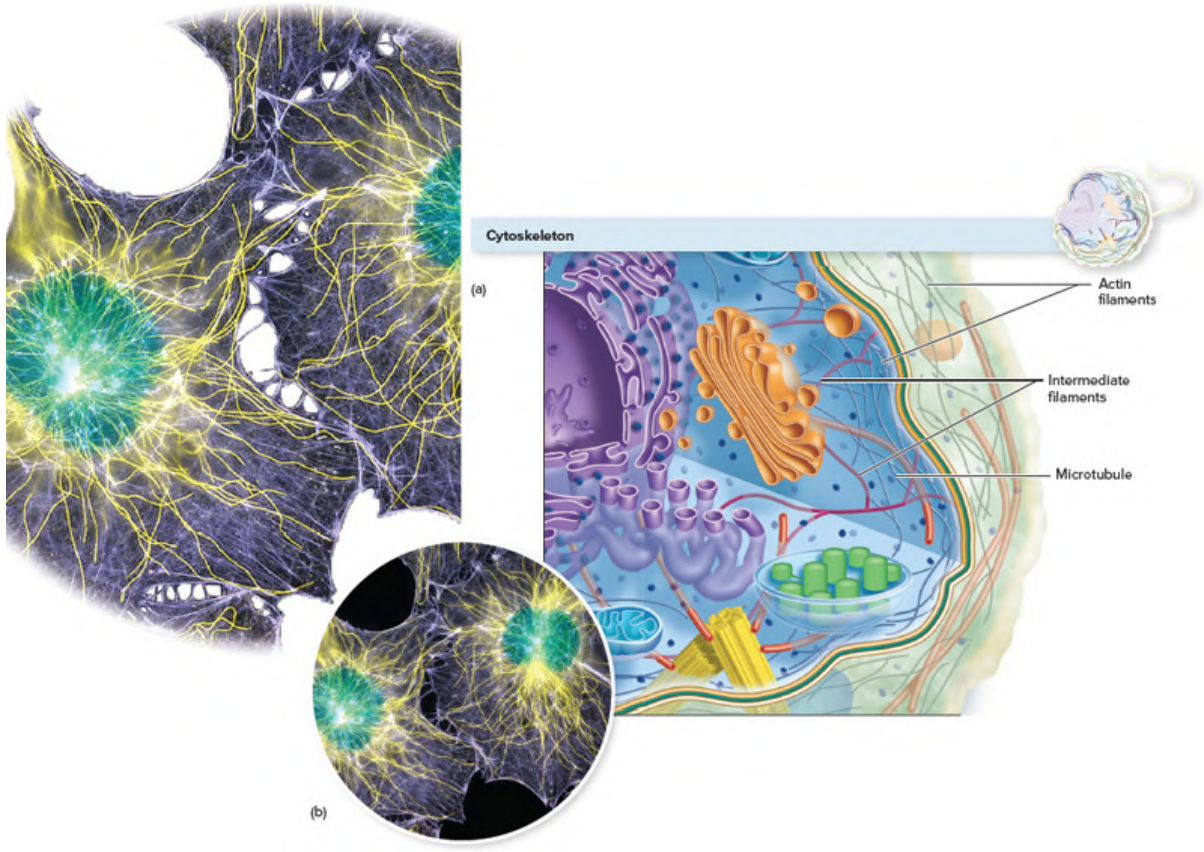
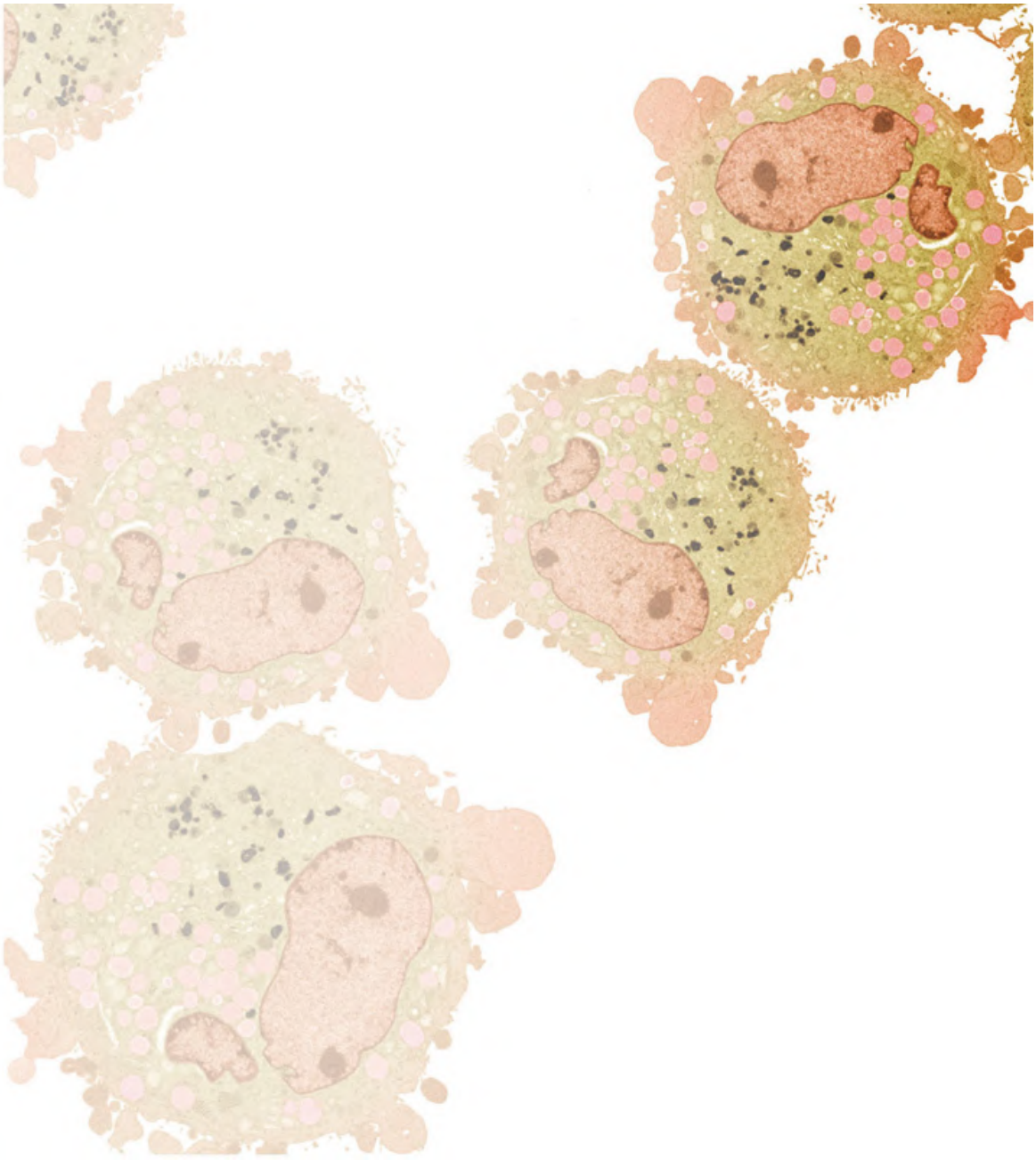


Figure 4.10 **The cytoskeleton**. **(a)** Drawing of microtubules, actin filaments, and intermediate filaments. **(b)** Microtubules are dyed fluorescent yellow in this micrograph.
©Dr. Torsten Wittmann/Science Source

in diameter. (Their name comes from their intermediate size, between actin filaments and microtubules.) Their main role is in structural reinforcement to the cell and to organelles. For example, they support the structure of the nuclear envelope.

Table 4.2 summarizes the differences between eukaryotic and bacterial and archaeal cells. Viruses (discussed in chapter 5) are included as well.



This human epithelial cell has turned cancerous. It has an irregular surface and an enlarged nucleus.
©Steve Gschmeissner/Science Photo Library/Getty Images

Becoming Eukaryotic

Now is a good time to introduce the concept of **endosymbiosis**. As you recall, many scientists believe that both bacterial and eukaryotic cells emerged from an earlier, now-extinct, cell type, called the Last Common Ancestor (LCA). You remember that eukaryotes are thought to have evolved later than bacteria and archaea. It is believed that a bacterial cell parasitized another descendant cell of the LCA (**figure 4.11**) and eventually became a permanent part of

that cell as its mitochondrion. Similarly, photosynthetic bacteria are thought to have become part of the

Table 4.2 Components and Characteristics of Each Organism Type*

Function or Structure	Characteristic	Bacterial/Archaeal Cells	Eukaryotic Cells	Viruses**
Genetics	Nucleic acids	+	+	+
	Chromosomes	+	+	–
	True nucleus	–	+	–
	Nuclear envelope	–	+	–
Reproduction	Mitosis	–	+	–
	Production of sex cells	+/–	+	–
	Binary fission	+	+	–
Biosynthesis	Independent	+	+	–
	Golgi apparatus	–	+	–
	Endoplasmic reticulum	–	+	–
	Ribosomes	+***	+	–
Respiration	Mitochondria	–	+	–
Photosynthesis	Pigments	+/–	+/–	–
	Chloroplasts	–	+/–	–
Motility/locomotor structures	Flagella	+/–***	+/–	–
	Cilia	–	+/–	–
Shape/protection	Membrane	+	+	+/– (called "envelope" when present)
	Cell wall	+***	+/–	– (have capsids instead)
	Glycocalyx	+/–	+/–	–
Complexity of function		+	+	+/–
Size (in general)		0.5-3 μm****	2-100 μm	<0.2 μm

*+ Means most members of the group exhibit this characteristic; – means most lack it; +/– means some members have it and some do not.

**Viruses cannot participate in metabolic or genetic activity outside their host cells.

***The bacterial/archaeal type is functionally similar to the eukaryotic but structurally unique.

****Much smaller and much larger bacteria exist.

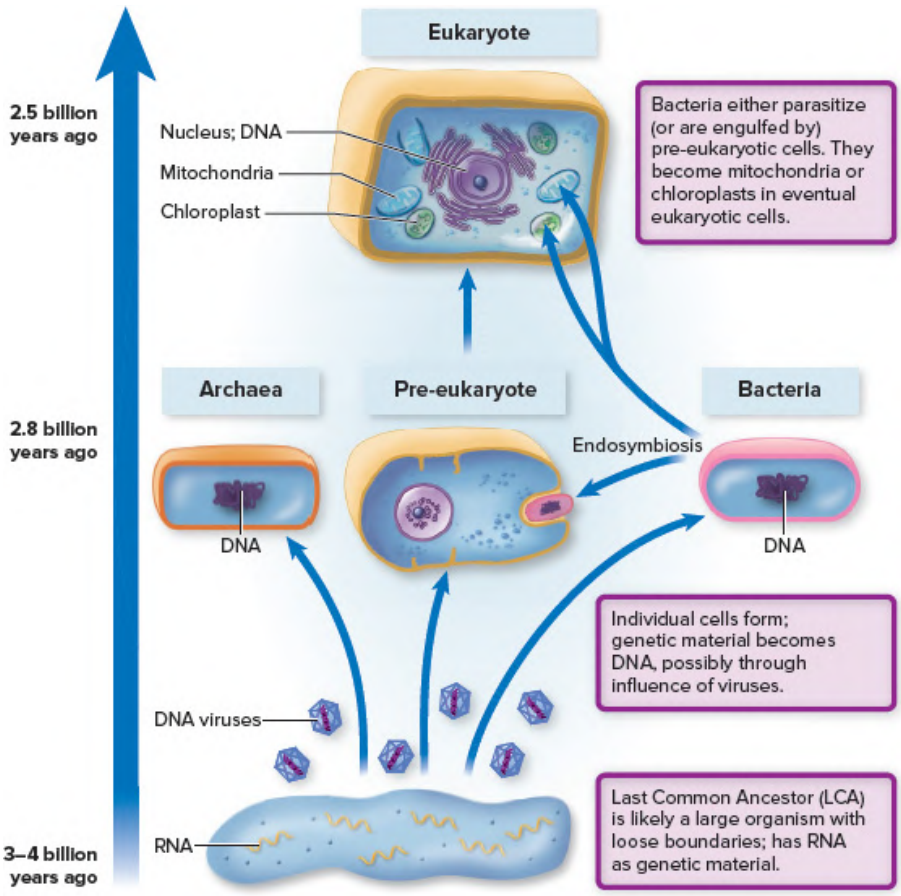


Figure 4.11 **Endosymbiosis**. Many scientists believe that bacteria and archaea–and a pre-eukaryotic cell type–developed from the Last Common Ancestor, possibly under the influence of DNA viruses. Eukaryotes then evolved from an interaction (endosymbiosis) between the pre-eukaryotic cell and bacteria.

precursor eukaryotic cell, eventually becoming the chloroplast in eukaryotic plant cells. That could explain why mitochondria and chloroplasts have their own (circular) DNA, 70S ribosomes, and own two-layer membranes. Figure 4.11 depicts a widely held view of how this happened.

4.2 LEARNING OUTCOMES—Assess Your Progress

3. Differentiate among the flagellar structures of bacteria, eukaryotes, and archaea.
4. List similarities and differences between eukaryotic and bacterial cytoplasmic membranes.
5. Describe the main structural components of a nucleus.
6. Diagram how the nucleus, endoplasmic reticulum, and Golgi apparatus act together with vesicles during the transport process.
7. Explain the function of the mitochondrion.
8. Explain the importance of ribosomes, and differentiate between eukaryotic and bacterial types.
9. List and describe the three main fibers of the cytoskeleton.

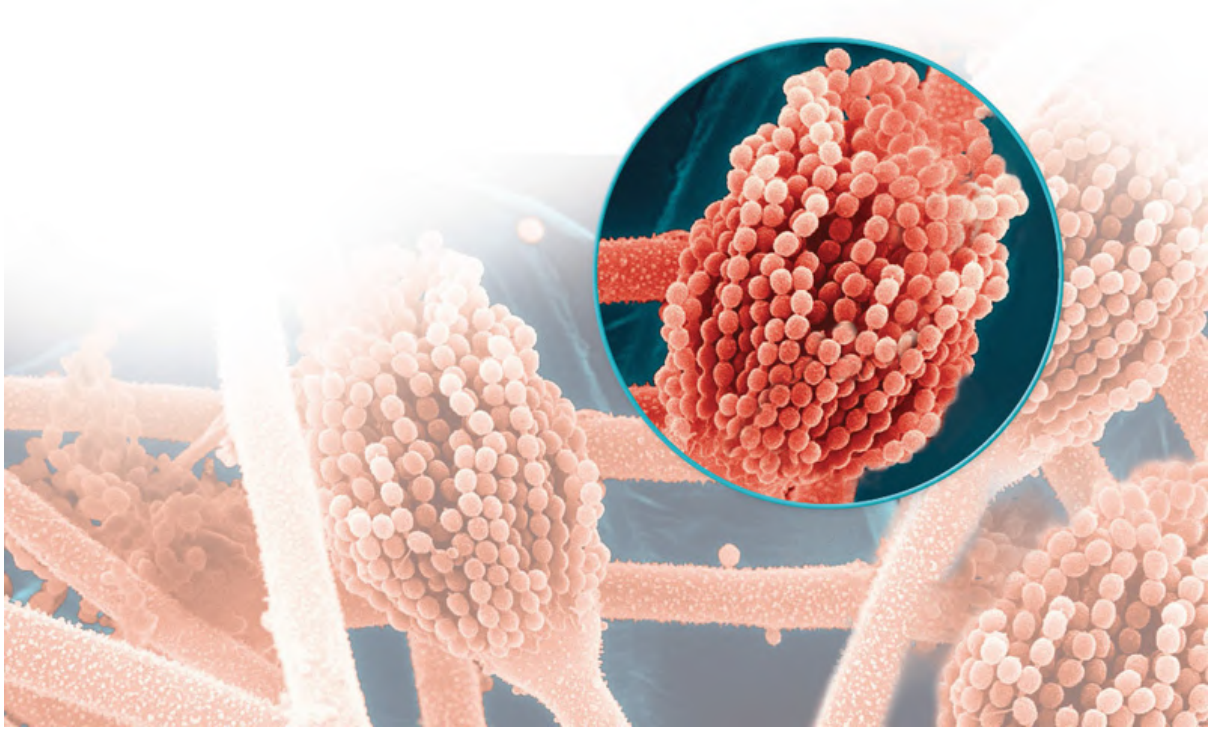
10. Explain how endosymbiosis contributed to the development of eukaryotic cells.

4.3 The Fungi

The kingdom Fungi is large and filled with a great variety and complexity of forms. In medical microbiology we are most concerned with the fungi known as yeasts and molds. Other fungi include mushrooms and puffballs. Although the majority of fungi are either unicellular or colonial (i.e., form colonies), a few complex forms such as mushrooms and puffballs are truly multicellular. Cells of the microscopic fungi exist in two basic forms: yeasts and hyphae. A yeast cell is distinguished by its round to oval shape and by its mode of asexual reproduction. It grows swellings on its surface called buds, which then become separate cells. **Hyphae** (hy′-fee) are long, threadlike cells found in the bodies of fungi of the filamentous type. These are called molds **(figure 4.12)**. Some species form a **pseudohypha**, a chain of yeast cells formed when buds remain attached in a row **(figure 4.13)**. Because of its manner of formation, it is not a true hypha like that of molds. While some fungal cells exist only in a yeast form and others occur primarily as hyphae, a few are classified as **dimorphic**. This means they can take either form, depending on growth conditions, such as changing temperature. This variability in growth form is particularly characteristic of some fungi that cause human disease.

Many fungi make their home on the human body, as part of the normal human microbiome. Yet nearly 300 species of fungi can also cause human disease. The Centers for Disease Control and Prevention currently identifies three types of fungal disease in humans: (1) community-acquired infections caused by environmental pathogens in the general population, (2) hospital-associated infections caused by fungal pathogens in clinical settings, and (3) opportunistic infections caused by low-virulence species infecting already weakened individuals **(table 4.3)**.

Mycoses (the term for fungal infections) vary in the way the pathogen enters the body and the degree of tissue involvement they display. Even so-called harmless species found in the air and dust around us



Aspergillus hyphae and spores
©BSIP/Universal Images Group/Getty Images

may be able to cause infections, especially in individuals who already have AIDS, cancer, or diabetes. Fungi can cause other dangerous medical conditions without establishing an actual infection. Fungal cell walls give off chemical substances that can trigger allergies. The toxins produced by poisonous mushrooms can induce neurological disturbances and even death. The mold *Aspergillus flavus* synthesizes a potentially lethal poison called aflatoxin. The consumption of grain contaminated with this mold has led to increased cases of liver cancer in developing nations.

Fungi pose problems to the agricultural industry. A number of species can be damaging to field plants such as corn and grain. This reduces crop production and can also cause disease in domestic animals that eat the contaminated feed crops. Fungi can also rot fresh produce during shipping and storage. It has been estimated that as much as 40% of the yearly fruit crop is consumed not by humans but by fungi. On the beneficial side, however, fungi play an essential role in decomposing organic matter and returning essential minerals to the soil. They form stable associations with plant roots, forming structures called mycorrhizae, that increase the ability of the roots to absorb water and nutrients. Industry has tapped the biochemical potential of fungi to produce large quantities of antibiotics, alcohol, organic acids, and vitamins. Some fungi are eaten or used to provide flavorings to food. The yeast *Saccharomyces* produces the alcohol in beer and wine and the gas that causes bread to rise. Blue cheese, soy sauce, and cured meats derive their unique flavors from the actions of fungi.

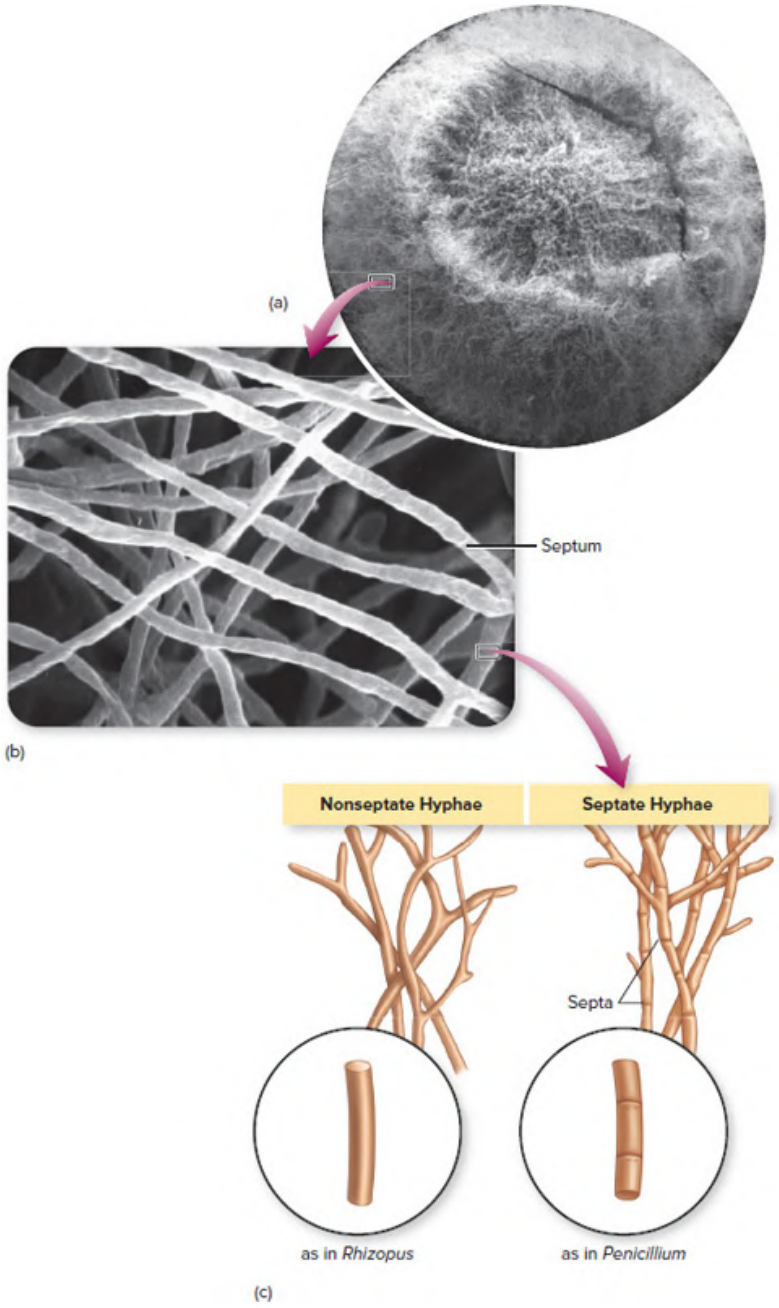


Figure 4.12 ***Diplodia maydis*, a pathogenic fungus of corn plants.** **(a)** Scanning electron micrograph of a single colony showing its filamentous texture (24×). **(b)** Close-up of hyphal structure (1,200×). **(c)** Basic structural types of hyphae.

Courtesy Dr. Judy A. Murphy, Murphy Consultancy Microscopy & Digital Imaging, Stockton, CA

Fungal Nutrition

All fungi are **heterotrophic**. They acquire nutrients from a wide variety of organic materials. (Sources of nutrition are called **substrates**.) Most fungi are **saprobes**, meaning that they obtain these substrates from the remnants of dead plants and animals in soil or aquatic habitats. Fungi can also be **parasites** on the bodies of living animals or plants, although very few fungi absolutely require a living host. In general, the fungus penetrates the substrate and

secretes enzymes that reduce it to small molecules that can be absorbed by the cells. Fungi have enzymes for digesting an incredible array of substances, including feathers, hair, cellulose, petroleum products, wood, and rubber. Fungi are often found in nutritionally poor or adverse environments. Various fungi thrive in substrates with high salt or sugar content, at relatively high temperatures, and even in snow and glaciers.

Morphology of Fungi

The cells of most microscopic fungi grow in loose associations or colonies. The colonies of yeasts are much like those of bacteria in that they have a soft, uniform texture and appearance. The colonies of filamentous fungi are noted for the striking cottony, hairy, or velvety textures that arise from their microscopic organization and morphology. The woven, intertwining mass of hyphae that makes up the body or colony of a mold is called a **mycelium**.

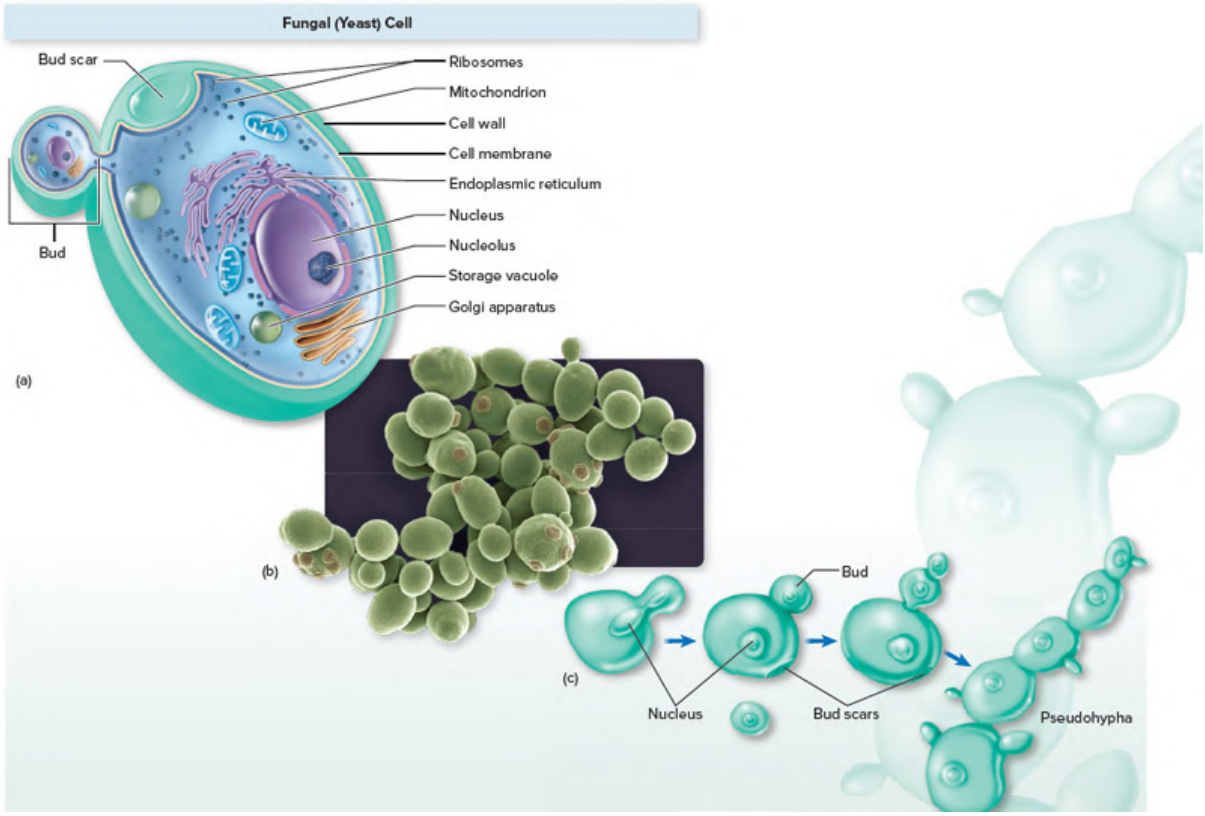


Figure 4.13 **Microscopic morphology of yeasts**. **(a)** General structure of a yeast cell, representing major organelles. Note the presence of a cell wall and lack of locomotor organelles. **(b)** Scanning electron micrograph of the brewer's, or baker's, yeast *Saccharomyces cerevisiae* (21,000x). **(c)** Formation and release of yeast buds and a pseudohypha (a chain of budding yeast cells).

©Science Photo Library/Getty Images

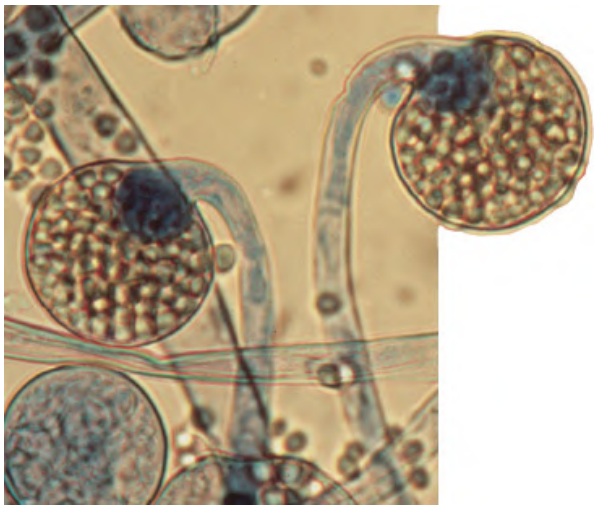
Table 4.3 Three Categories of Fungal Infections of Humans

Source of Fungus	Who Is Affected?	Examples	Site of Symptoms

Source of Fungus	Who Is Affected?	Examples	Site of Symptoms
Community-Acquired Infections			
Environmental species	General population	Histoplasmosis, caused by a fungus that thrives in bird and bat droppings	Affects lungs and respiratory tract
		Coccidioidomycosis (also called Valley fever), caused by a fungus that lives in dust and soil, particularly in the SW United States	Flulike symptoms in respiratory tract and rest of body; includes headaches, night sweats, and muscle and joint pain
Hospital-Associated Infections			
Pathogens in clinical settings	People in hospital or long-term care	Various fungi that contaminate health care facilities	———
Opportunistic Infections			
Environmental species or normal fungal biota	People who have compromised immune systems, or disrupted microbiota	Mucormycosis, caused by a fungus found in soil, leaves, compost, and rotting wood	Affects lungs and sinuses most often; skin can also be the target
		Candidiasis, caused by a yeast that is normal biota on human mucosal surfaces	The mucosal site where the microbe normally lives in small numbers; when the normal balance of microbiota is disrupted the yeast proliferates and causes inflammation

Although hyphae contain the usual eukaryotic organelles, they also have some unique features. In most fungi, the hyphae are septate, meaning they are divided into segments by cross walls called **septa** (singular, *septum;* see figure 4.12*c*). The nature of the septa varies from solid partitions with no communication between the compartments to partial walls with small pores that allow the flow of organelles and nutrients between adjacent compartments. Nonseptate hyphae consist of one long, continuous cell *not* divided into individual compartments by cross walls. With this construction, the cytosol and organelles move freely from one region to another, and each hyphal element can have several nuclei.

Hyphae can also be classified according to their particular function. Two types are vegetative hyphae and reproductive, or fertile, hyphae. Vegetative hyphae (mycelia) are responsible for the visible mass of growth that appears on the surface of a substrate and penetrates it to digest and absorb nutrients. During the development of a fungal colony, the vegetative hyphae can give rise to reproductive hyphae, which branch off a vegetative mycelium. These hyphae are responsible for the production of fungal reproductive bodies called **spores**.



A fungus called *Circinella*
©Kathleen Talaro

Reproductive Strategies and Spore Formation

Fungi have many complex reproductive strategies. Most can propagate by the simple outward growth of existing hyphae or by fragmentation, in which a separated piece of mycelium can generate a whole new colony. But the primary reproductive mode of fungi involves the production of various types of spores. (Do not confuse fungal spores with the more resistant, nonreproductive bacterial spores.) Spores help the fungus disperse throughout the environment. Because of their compactness and relatively light weight, spores are dispersed widely through the environment by air, water, and living things. Upon encountering a favorable substrate, a spore will germinate and produce a new fungus colony in a very short time.

Fungal spores are explicitly responsible for multiplication. Different fungi have such a wide variety of different spores that they are largely classified and identified by their spores and spore-forming structures, but we won't cover this information. Instead, we will focus on the most general subdivision, which is based on the way the spores arise. Asexual spores are the products of mitotic division of a single parent cell, and sexual spores are formed through a process involving the fusing of two parental nuclei followed by meiosis. An important consequence of meiosis and sexual reproduction is that it serves to increase the genetic variation among spores.

Asexual Spore Formation

There are two types of asexual spores, **sporangiospores** and **conidiospores**, also called conidia **(figure 4.14):**

1. Sporangiospores **(figure 4.14*a*)** are formed by successive cleavages within a saclike head called a **sporangium**, which is attached to a stalk, the sporangiophore.
2. Conidiospores, or **conidia**, are free spores not enclosed by a spore-bearing sac. They develop either by the pinching off of the tip of a special fertile hypha or by the

segmentation of a preexisting vegetative hypha. There are many different forms of conidia, illustrated in **figure 4.14*b***.

Medical Moment

Vaginal Candidiasis

Almost every woman will experience a vaginal yeast infection, caused by an overgrowth of *Candida albicans*, at some time in her life. Although uncomfortable due to irritation, itching, and vaginal discharge, the infection is easily treatable with antifungal medication in the form of creams, oral medications, or vaginal suppositories.

The female reproductive system is quite amazing, and a very delicate balance is maintained within this environment. A small amount of *C. albicans* is nearly always present within the vagina, but its growth is limited by the acidic pH of the vaginal canal. Interestingly, the acid-producing bacteria also living within the vagina help to tightly control this pH level. When something disrupts the vaginal pH, *C. albicans* takes the opportunity to proliferate, leading to an overgrowth of this microbe which can become a "yeast infection."

But what causes disruption of the normal vaginal pH? Although pregnancy, diabetes, obesity, and monthly hormonal changes can cause pH levels to fluctuate, by far the most common cause that leads to the development of a yeast infection is antibiotic therapy. This is due to the fact that along with the pathogen it is really trying to target, the drug kills the protective bacteria (normal biota) of the vagina that help to keep yeast in check.

Q. Yeast infection is one common side effect of antibiotic treatment. Diarrhea is another common side effect. Suggest an explanation for this.

Answer in Appendix B.

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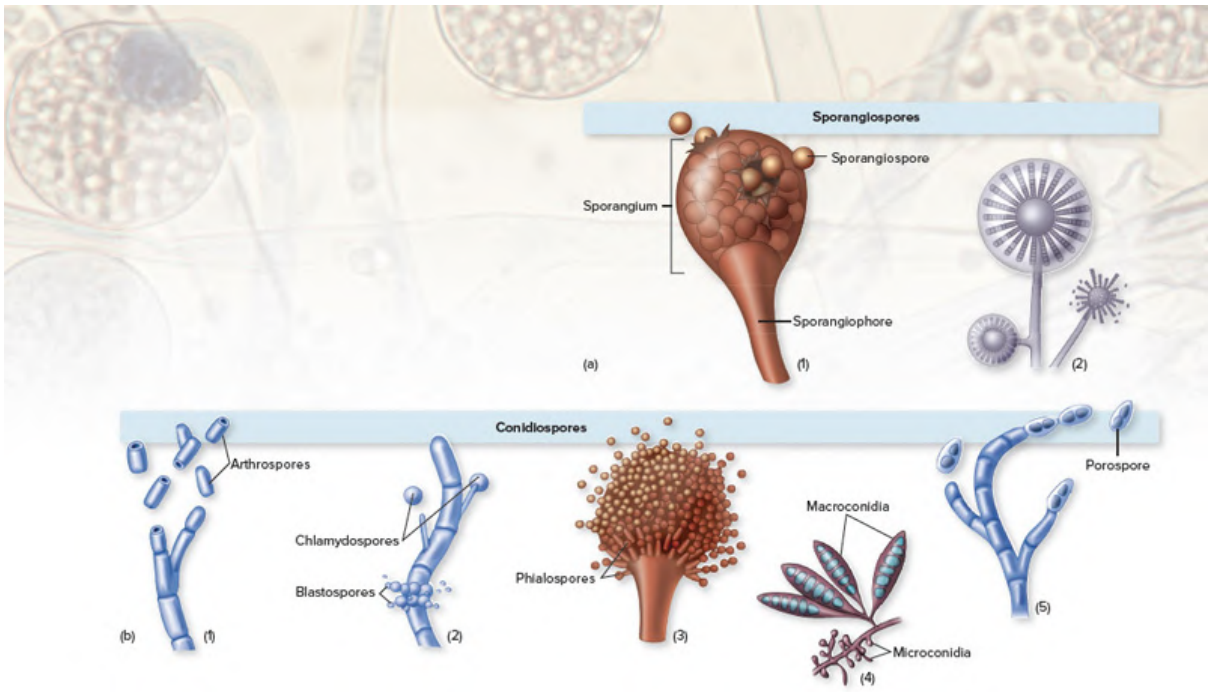

Figure 4.14 **Types of asexual mold spores**. **(a)** Sporangiospores: (1) *Absidia*, (2) *Syncephalastrum*. **(b)** Conidial variations: (1) arthrospores (e.g., *Coccidioides*), (2) chlamydospores and blastospores (e.g., *Candida albicans*), (3) phialospores (e.g., *Aspergillus*), (4) macroconidia and microconidia (e.g., *Microsporum*), and (5) porospores (e.g., *Alternaria*).

Sexual Spore Formation

Fungi are hugely successful at reproduction with their millions of asexual spores. That being the case, why is the production of sexual spores necessary? The answer lies in important variations that occur when fungi of different genetic makeup combine their genetic material. Just as in plants and animals, this mixing of DNA from two parents creates offspring with combinations of genes different from that of either parent. The offspring from such a union can have slight variations in form and function that are potentially advantageous in the adaptation and survival of their species.

The majority of fungi produce sexual spores at some point. The details of this process vary greatly. It could be as simple as the fusion of fertile hyphae of two different strains, or as complicated as a complex union of differentiated male and female structures or the development of special fruiting structures. It may be a surprise to discover that the fleshy part of a mushroom is actually a fruiting body designed to protect and help disseminate its sexual spores.

4.3 LEARNING OUTCOMES—Assess Your Progress

11. List two detrimental and two beneficial activities of fungi (from the viewpoint of humans).
12. List three general features of fungal anatomy.
13. Differentiate among the terms *heterotroph, saprobe*, and *parasite*.

14. Explain the relationship between fungal hyphae and the production of a mycelium.
15. Describe two ways in which fungal spores arise.

Medical Moment

Opportunistic Fungal Infection

Ordinarily harmless organisms that cause disease in a weakened host are described as opportunistic pathogens. One example is mucormycosis, a rare but serious fungal infection. *Mucormyocetes* are fungi commonly found in soil or decaying matter. The fungal conidia may be inhaled from the air or introduced by skin trauma. Most humans are not affected by these spores, but those with impaired immune systems or poorly controlled diabetes are a perfect host for the fungus. In inhaled mucormycosis, hyphae invade the tissues and blood vessels of the sinuses, lungs, eyes, and face. The infection is treated with intravenous antifungal medications. The fungus spreads rapidly, often requiring extensive, repeated surgeries to restore affected facial bones and tissues. If not aggressively treated, the fungal infection may spread into the brain and is frequently fatal.

Q. It is difficult to treat fungal infections without doing some harm to the host. Drugs that treat bacterial infections are less likely to harm the human host. Can you think of why that might be?

Answer in Appendix B.

4.4 The Protozoa

Although their name comes from the Greek for "first animals," protozoa are far from being simple, primitive organisms. The protozoa constitute a very large group (about 12,000 species) of creatures that, although single-celled, have startling properties when it comes to movement, feeding, and behavior. Although most members of this group are harmless, free-living inhabitants of water and soil, a few species are pathogens that are collectively responsible for hundreds of millions of infections of humans each year. Remember that the term *protozoan* is more of a convenience than an accurate taxonomic designation. As we next describe them, you will see why protozoa are categorized together. As it turns out, it is because of their similar physical characteristics rather than their genetic relatedness.

Protozoan Form and Function

Most protozoan cells are single cells containing all the major eukaryotic organelles. Their organelles can be highly specialized for feeding, reproduction, and locomotion. The cytoplasm is usually divided into a clear outer layer called the **ectoplasm** and a more granular inner region called the endoplasm. Ectoplasm is involved in locomotion, feeding, and protection. Endoplasm houses the nucleus, mitochondria, and food and contractile vacuoles. Some protozoa even have organelles that work somewhat like a primitive nervous system to coordinate movement. Protozoa can move through fluids by means of *pseudopods* ("false feet"), flagella, or cilia. Because protozoa lack a cell wall, they have a certain amount of flexibility. Their outer boundary is a cell membrane that regulates the movement of food, wastes, and secretions. Their cell shape can remain constant (as in most ciliates) or can

change constantly (as in amoebas). Certain amoebas encase themselves in hard shells made of calcium carbonate. The size of most protozoan cells falls within the range of 3 to 300 μm. Some notable exceptions are giant amoebas and ciliates that are large enough (3 to 4 mm in length) to be seen swimming in pond water.

Nutritional and Habitat Range

The protozoa we will be interested in are typically heterotrophic and usually require their food in a complex organic form. Free-living species scavenge dead plant or animal debris and even graze on live bacteria and algae. Some species have special feeding structures, such as oral grooves, which carry food particles into a passageway or gullet that packages the captured food into vacuoles for digestion. Some protozoa absorb food directly through the cell membrane. Pathogenic species may live on the fluids of their host, such as plasma and digestive juices, or they can actively feed on tissues.

Although protozoa have adapted to a wide range of habitats, their main limiting factor is the availability of moisture. Their predominant habitats are fresh and marine water, soil, plants, and animals. Even extremes in temperature and pH are not a barrier to their existence. Hardy species are found in hot springs, ice, and habitats with very low or very high pH.

Life Cycles and Reproduction

Protozoa are called **trophozoites** when they are in their motile feeding stage. This is a stage that requires ample food and moisture to remain active. A large number of species are also capable of entering into a dormant, resting stage called a **cyst** when conditions in the environment become unfavorable for growth and feeding. During *encystment*, the trophozoite cell rounds up into a sphere, and its ectoplasm secretes a tough, thick cuticle around the cell membrane **(figure 4.15)**. Because cysts are more resistant than ordinary cells to heat, drying, and chemicals, they can survive

Euglena is a type of flagellated protozoan that can be found in a drop of pond water or even aquarium water. ©Stephen Durr

adverse periods. They can also be dispersed by air currents and may even be an important factor in the spread of diseases such as amoebic dysentery. If provided with moisture and nutrients, a cyst breaks open and releases the active trophozoite.

The life cycles of protozoans vary from simple to complex. Several protozoan groups exist only in the trophozoite state. Many alternate between a trophozoite and a cyst stage, depending on the conditions of the habitat. The life cycle of a parasitic protozoan dictates its mode of transmission to other hosts. For example, the flagellate *Trichomonas vaginalis* causes a common sexually transmitted infection. Because it does not form cysts, it is more delicate and must be transmitted by intimate contact between sexual partners. In contrast, intestinal pathogens such as *Entamoeba histolytica* and *Giardia lamblia* form cysts and are readily transmitted in contaminated water and foods.

All protozoa reproduce by relatively simple, asexual methods, usually mitotic cell division. Several pathogenic species, including the causative agents of malaria and toxoplasmosis, reproduce asexually by multiple rounds of division inside a host cell. Sexual reproduction also occurs during the life cycle of most protozoa. Ciliates participate in conjugation, a form of genetic exchange in which two cells fuse temporarily and exchange micronuclei. This process of sexual recombination yields new and different genetic combinations that can be advantageous in evolution.

An amoeba exhibiting pseudopod formation
©Stephen Durr

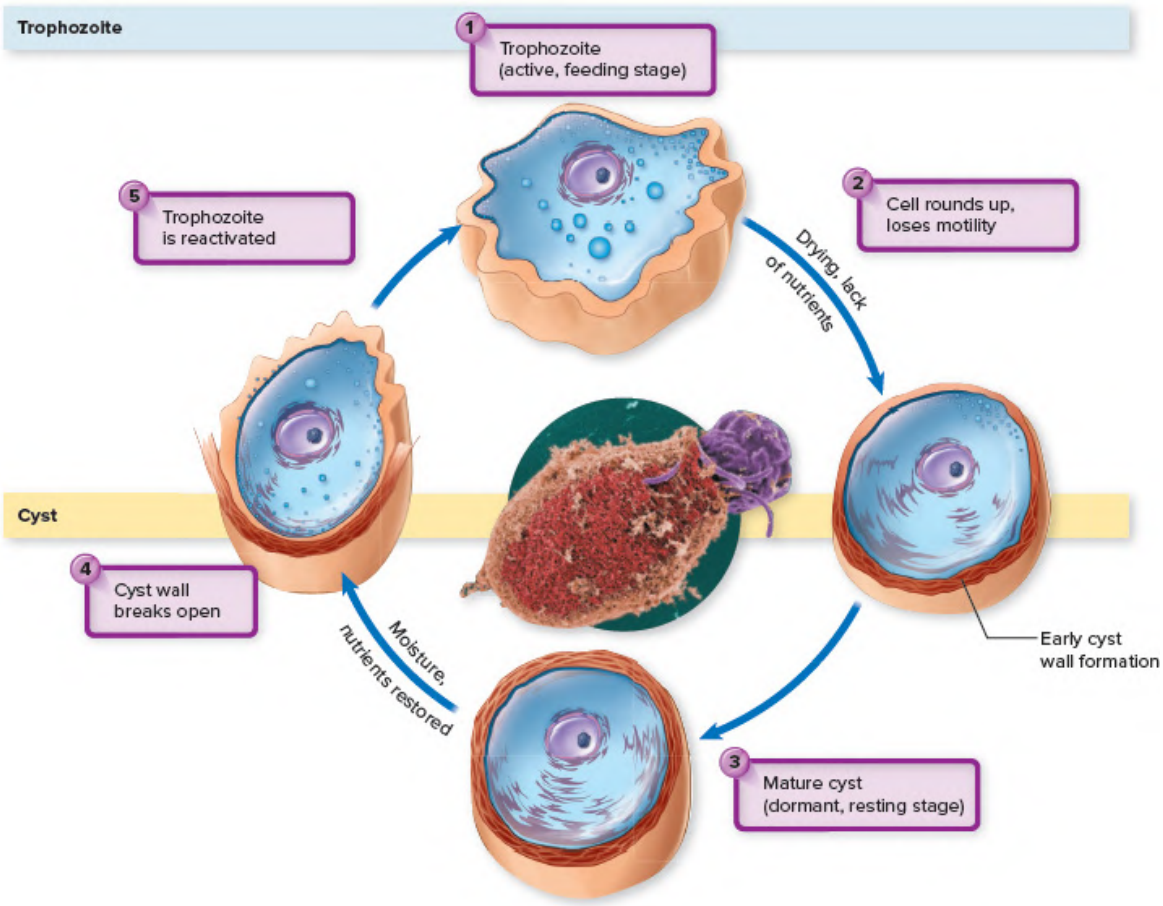

Figure 4.15 **The general life cycle exhibited by many protozoa**. All protozoa have a trophozoite form, but not all produce cysts. The photo in the center shows a *Giardia* trophozoite (purple) emerging from its cyst form (orange). Source: CDC/Dr. Stan Erlandsen

Giardia lamblia
©Dr. Tony Brain/Science Source

Classification of Selected Medically Important Protozoa

As has been stated, taxonomists have problems classifying protozoa. They are very diverse and frequently frustrate attempts to generalize or place them in neat groupings. We will use a common and simple system of four groups, based on their method of motility: Sarcodina

(pseudopods), Ciliophora (cilia), Mastigophora (flagella), and Sporozoa (gliding motility) **(table 4.4)**.

4.4 LEARNING OUTCOMES—Assess Your Progress

16. Describe the protozoan characteristics that illustrate why protozoa are informally placed into a single group.
17. List three means of locomotion exhibited by protozoa.
18. Explain why a cyst stage may be useful to a protozoan.
19. Give an example of a disease caused by each of the four types of protozoa.

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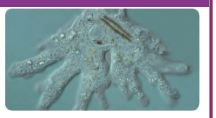
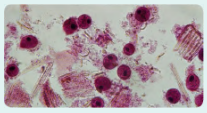
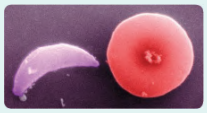
2. A client is diagnosed with *Trichomonas vaginalis*. Which of the following indicates that the client understands the method of transmission for this disease process? The client states that she
a. *ate contaminated food*.
b. *drank contaminated water*.
c. *had intimate contact*.
d. *had too much sun exposure*.

Table 4.4 Major Pathogenic Protozoa

Protozoan	Disease	Reservoir/Source	
Amoeboid Protozoa Using Pseudopods (Sarcodina)			
Entamoeba histolytica	Amoebiasis (intestinal and other symptoms)	Humans, water, and food	
Naegleria, Acanthamoeba	Brain infection	Water	
Ciliated Protozoa (Ciliophora)			
Balantidium coli	Balantidiosis (intestinal and other symptoms)	Pigs, cattle	
Flagellated Protozoa (Mastigophora)			
Giardia lamblia	Giardiasis (intestinal distress)	Animals, water, and food	
Trichomonas vaginalis	Trichomoniasis (vaginal symptoms)	Human	
Trypanosoma brucei, T. cruzi	Trypanosomiasis (intestinal distress and widespread organ damage)	Animals, vector-borne	
Leishmania donovani, L. tropica, L. brasiliensis	Leishmaniasis (either skin lesions or widespread involvement of internal organs)	Animals, vector-borne	
Apicomplexan Protozoa - Nonmotile (Sporozoa)			
Plasmodium vivax, P. falciparum, P. malariae	Malaria (cardiovascular and other symptoms)	Human, vector-borne	
Toxoplasma gondii	Toxoplasmosis (flulike illness or silent infection)	Animals, vector-borne	
Cryptosporidium	Cryptosporidiosis (intestinal and other symptoms)	Water, food	
Cyclospora cayetanensis	Cyclosporiasis (intestinal and other symptoms)	Water, fresh produce	

©Stephen Durr (Amoeba proteus); ©J. R. Factor/Science Source (*Balantidium coli*); ©Eye of Science/Science Source (SEM of *Trichomonas vaginalis*. Magnification-9,000x); ©BSIP/Universal Images Group/Getty Images (SEM of *Toxoplasma gondii*. Magnification not available)

4.5 The Helminths

Tapeworms, flukes, and roundworms are collectively called *helminths*, from the Greek word meaning "worm." Adult specimens are usually large enough to be seen with the naked eye, and they range from the longest tapeworms, measuring up to about 25 m in length, to roundworms less than 1 mm in length. Helminths are included in the study of microbes mainly due to their infective abilities and the fact that they produce microscopic eggs and larvae.

On the basis of body type, the two major groups of pathogenic helminths are the flatworms (phylum Platyhelminthes) and the roundworms (phylum Aschelminthes, also called **nematodes**). Flatworms have a very thin, often segmented body plan **(figure 4.16)**, and roundworms have an elongated, cylindrical, unsegmented body **(figure 4.17)**. The flatworm group is subdivided into the **cestodes**, or tapeworms, named for their long, ribbonlike arrangement; and the **trematodes**, or flukes, characterized by flat, ovoid bodies. Not all flatworms and roundworms are parasites by nature; many live free in soil and water. Because most disease-causing

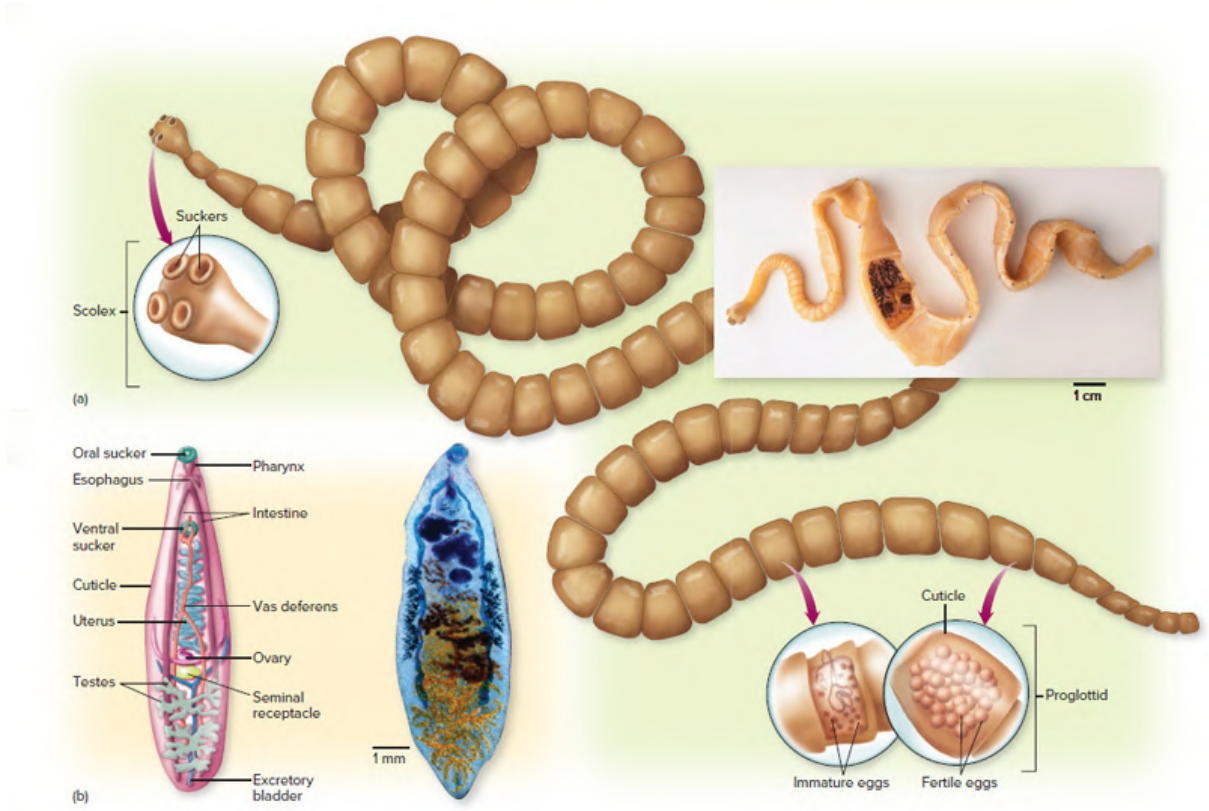


Figure 4.16 **Pathogenic flatworms**. **(a)** A cestode (tapeworm), showing the scolex; long, tapelike body; and magnified views of immature and mature proglottids (body segments). The photo shows an actual tapeworm. **(b)** The structure of a trematode (liver fluke). Note the suckers that attach to host tissue and the dominance of reproductive and digestive organs.

©Geoff Brightling/Dorling Kindersley/Getty Images (a); ©Eye of Science/Science Source (b)

helminths spend part of their lives in the gastrointestinal tract, they are discussed in chapter 20.

General Worm Morphology

All helminths are multicellular animals equipped to some degree with organs and organ systems. In pathogenic helminths, the most developed organs are those of the reproductive tract, with some degree of reduction in the digestive, excretory, nervous, and muscular systems.

Life Cycles and Reproduction

The complete life cycle of helminths includes the fertilized egg (embryo), larval, and adult stages. In the majority of helminths, adults derive nutrients and reproduce sexually in a host's body. In nematodes, the sexes are separate and usually different in appearance. In trematodes, the sexes can be either separate or **hermaphroditic**, meaning that male and female sex organs are in the same individual worm. Cestodes are generally hermaphroditic. Helminths must complete their life cycle by transmitting an infective form, usually an egg or larva, to the body of another host, either of the same or a different species. The host in which larval development occurs is the intermediate (secondary) host, and the host in which adulthood and mating occur is the **definitive (final) host**. A *transport host* is an intermediate host that experiences no parasitic development but is an essential link in the completion of the cycle.

NCLEX® PREP

3. A patient has been diagnosed with a parasitic helminth infection. Which of the following is not a helminth that causes disease?

a. *ringworm*
b. *blood fluke*
c. *pork tapeworm*
d. *pinworm*

Planaria is a flatworm that is often studied in high school and college biology labs.
Source: NHPA/M. I. Walker

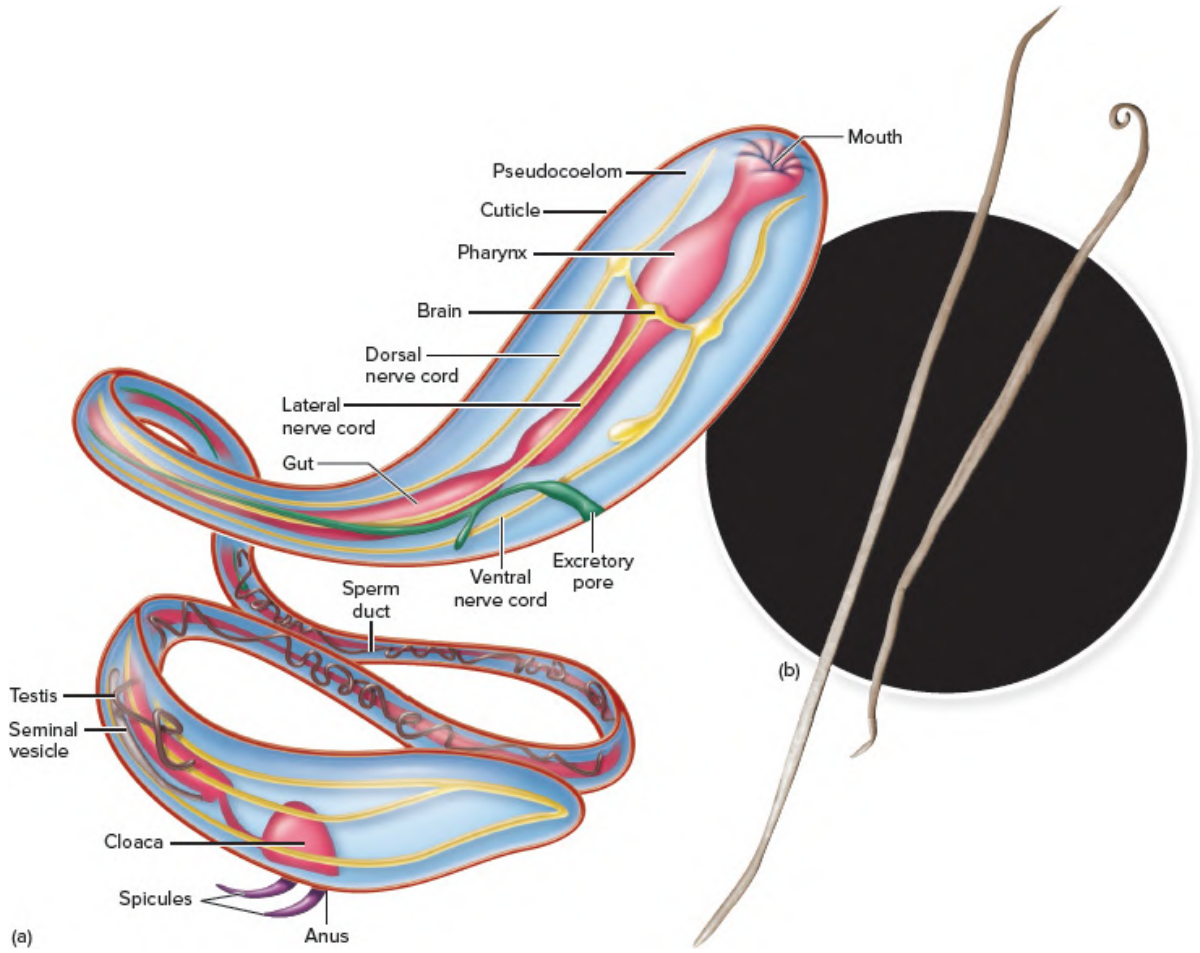


Figure 4.17 **Pathogenic roundworm**. **(a)** A male *Ascaris* nematode (roundworm). **(b)** Female (left) and male (right) *Ascaris* worms.
Source: CDC

Table 4.5 Examples of Helminths and How They Are Transmitted

Common Name	Disease or Worm	Host Requirement	Spread to Humans By
Roundworms			
Nematodes			
Intestinal Nematodes			Ingestion
Infective in egg (embryo) stage			
Ascaris lumbricoides	Ascariasis	Humans	Fecal pollution of soil with eggs
Enterobius vermicularis	Pinworm	Humans	Close contact
Infective in larval stage			
Trichinella spiralis	Trichina worm	Pigs, wild mammals	Consumption of meat containing larvae
Tissue Nematodes			Burrowing of larva into tissue

Common Name	Disease or Worm	Host Requirement	Spread to Humans By
Onchocerca volvulus	River blindness	Humans, black flies	Fly bite
Dracunculus medinensis	Guinea worm	Humans and *Cyclops* (an aquatic invertebrate)	Ingestion of water containing *Cyclops*
Flatworms			
Trematodes			
Schistosoma japonicum	Blood fluke	Humans and snails	Skin penetration of larval stage
Cestodes			
Taenia solium	Pork tapeworm	Humans, swine	Consumption of undercooked or raw pork
Diphyllobothrium latum	Fish tapeworm	Humans, fish	Consumption of undercooked or raw fish

Fertilized eggs are usually released to the environment and are provided with a protective shell and extra food to aid their development into larvae. Even so, most eggs and larvae are vulnerable to heat, cold, drying, and predators and are destroyed or unable to reach a new host. To counteract this, certain worms have adapted a reproductive capacity that borders on the incredible: A single female *Ascaris* can lay 200,000 eggs a day, and a large female can contain over 25 million eggs at varying stages of development! If only a tiny number of these eggs makes it to another host, the parasite will have been successful in completing its life cycle.

In general, humans become infected through ingestion or through penetration of tissues by the worm. The sources of the infective stage may be contaminated food, soil, or water, or other infected animals. Humans are the definitive hosts for many of the parasites listed in **table 4.5**. In about half the diseases, they are also the sole biological reservoir. In other cases, animals or insect vectors serve as reservoirs or are required to complete worm development.

A Helminth Cycle: The Pinworm

To illustrate a helminth cycle in humans, we use the example of a roundworm, *Enterobius vermicularis*, the pinworm or seatworm. This worm causes a very common infestation of the large intestine. Worms range from 2 to 12 mm long and have a tapered, curved cylindrical shape **(figure 4.18)**. The condition they cause, enterobiasis, is usually a simple, uncomplicated infection that does not spread beyond the intestine.

A cycle starts when a person swallows microscopic eggs picked up from another infected person by direct contact or by touching articles that person has touched. The eggs hatch in the intestine and then release larvae that mature into adult worms within about 1 month. Male and female worms mate, and the female migrates out to the anus to deposit eggs, which cause intense itchiness that the infected human relieves by scratching. Herein lies a significant means of dispersal: Scratching contaminates the fingers, which, in turn, transfers eggs to bedclothes

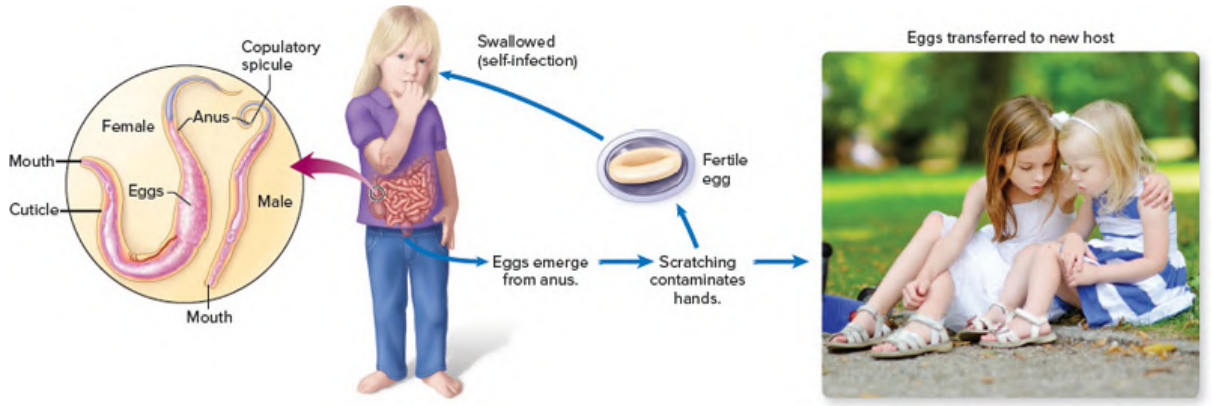


Figure 4.18 **The life cycle of the pinworm, a roundworm**. Eggs are the infective stage and are transmitted by contaminated hands. Children frequently reinfect themselves and also pass the parasite on to others.
©MNStudio/Shutterstock

Medical Moment

Neglected Parasitic Infections

It might come as a surprise to you that one-fourth of the world's population is infected with intestinal roundworms. Or maybe it is easier to stomach because we are confident that protozoan and helminthic infections are relatively rare in the United States.

Think again. The CDC has begun a campaign against what they are calling "neglected parasitic infections (NPIs)" in the United States. Three of them are protozoan infections, and two are caused by helminths. They are:

- Chagas disease–a protozoan disease caused by *Trypanosoma cruzi*
- Neurocysticercosis–caused by the tapeworm *Tania sodium*
- Toxocariasis–caused by worms that travel through tissues and can cause blindness
- Toxoplasmosis–60 million people in the United States are infected by the protozoan causing this disease.
- Trichomoniasis–a protozoan infection of the genital tracts

Neurocysticercosis is the single most common infectious cause of seizures in some areas of the United States. Up to 300,000 people in the United States are infected with the protozoan that causes Chagas disease.

It is time to stop thinking of these infections as "other people's diseases."

Q. There is a protozoan disease that is not considered "neglected." It causes new infections in over 200 million people per year, and millions of dollars are poured into vaccine and treatment research every year. What protozoan disease is this?

Answer in Appendix B.

and other inanimate objects. This person becomes a host and a source of eggs, and can spread them to others in addition to reinfecting him- or herself. Enterobiasis occurs most often among families and in other close living situations. Its distribution is worldwide among all socioeconomic groups, but it seems to attack younger people more frequently than older ones.

Distribution and Importance of Pathogenic Worms

About 50 species of helminths cause disease in humans. The pathogenic helminths are frequently called *parasites*, though we will encounter a more specific definition of this word in chapter 11. They are distributed in all areas of the world that support human life. Some worms are restricted to a given geographic region, and many have a higher incidence in tropical areas. This knowledge must be tempered with the realization that jet-age travel, along with human migration, is gradually changing the patterns of helminth infections, especially of those species that do not require alternate hosts or special climatic conditions for development. The yearly estimate of worldwide cases numbers in the billions, and these are not confined to developing countries. A conservative estimate places 50 million helminth infections in North America alone.

4.5 LEARNING OUTCOMES—Assess Your Progress

20. List the two major groups of helminths, and provide examples representing each body type.

21. Summarize the stages of a typical helminth life cycle.

CASE FILE WRAP-UP

©Jade/Blend Images (health care worker); Source: CDC/Dr. Lucille K. George (*Coccidioides immitis* arthroconidia)

Coccidioidomycosis develops when an individual inhales spores produced by the fungus *Coccidioides immitis*. This disease, which is often called *Valley fever*, is endemic to the desert regions of the southwestern United States, and cases are commonly seen in both South and Central America. Most people who become exposed to the fungus never exhibit any signs or symptoms of illness. Others develop flulike symptoms or pneumonia that may persist for months. Individuals with weakened immune systems tend to experience the most severe forms of Valley fever, and in some cases the disease is fatal.

The patient in the opening case file was a farm worker and likely inhaled spores while working outdoors, because California is one of the states where *Coccidioides immitis* can

be found. His chest X ray revealed the lung nodules typical of this disease (which may be mistaken for cancer), and the patient's blood tested positive for the fungus. Microscopic evidence of the fungus was identified in his sputum sample. Once the patient was started on an antifungal medication, he began to gradually improve and fully recovered from the disease. Due to misinterpretation of the initial symptoms, the medication the patient was initially started on was an antibiotic drug. Although effective against bacteria, antibiotics will not lead to targeted destruction of this fungus.

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The Microbiome

Are Eukaryotic Microorganisms Part of Our Microbiome?

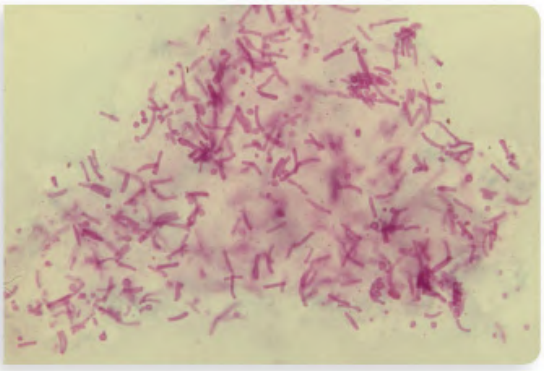
Yes! Most definitely. Let's focus on fungi. Fungi are well known to be normal inhabitants —and sometimes pathogens—for humans. One example is the fungus that causes yeast infections of the gut, mouth, and vagina, called *Candida albicans*.

Fungal infections of the skin (and hair and nails) are quite common as well. Twentynine million people in the United States experience this every year. The Human Microbiome Project has finally made it possible to look at the fungal species that live normally on our skin and other surfaces.

In 2013, a research team from the National Institutes of Health (NIH) documented the fungi they found on human surfaces. They sampled 14 body sites from each of 10 healthy adults. Their DNA analysis identified more than 80 fungal genera. Previously, when these studies were conducted using culture techniques, only 18 different genera were found. They discovered that one species, *Malassezia*, is commonly found as a normal inhabitant of skin on the head and on most of the body (the trunk). *Malassezia* has been recognized before when it causes very superficial skin infections, and it has been associated with the

condition of dandruff. This study tells us that most of the time it is a normal inhabitant of our surfaces.

For some reason, the body part displaying the most diverse fungal population was the heel, containing about 80 fungal genera. Toenails had about 60 genera, and the webs of the toes had about 40. Skin surfaces like the head and trunk displayed just 2 to 10 genera each.



Malassezia furfur at 100x magnification
Source: CDC/Dr. Lucille K. George

Chapter Summary

4.1 Overview of the Eukaryotes

- Eukaryotes are cells with a nucleus and organelles compartmentalized by membranes. They, like bacteria, originated from a primitive cell referred to as the *Last Common Ancestor*. Eukaryotic cell structure enabled eukaryotes to diversify from single cells into a huge variety of complex multicellular forms.

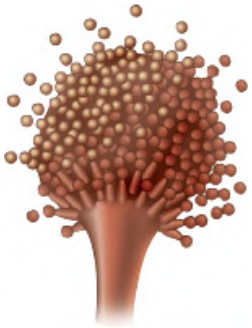
4.2 Structures of the Eukaryotic Cell

- The cell structures common to most eukaryotes are the cell membrane, nucleus, vacuoles, mitochondria, endoplasmic reticulum, Golgi apparatus, and a cytoskeleton. Cell walls, chloroplasts, and locomotor organs are present in some eukaryote groups.
- Single-celled eukaryotes use locomotor organs such as flagella or cilia for moving themselves or their food.
- The glycocalyx is the outermost boundary of most eukaryotic cells. Its functions are protection, adherence, and receiving chemical signals from the environment or from other organisms. The glycocalyx is supported by either a cell wall or a cell membrane.
- The cell membrane of eukaryotes is similar in function to that of bacteria, but it differs in composition, possessing sterols as additional stabilizing agents.
- Fungi have a cell wall that is composed of glycans and chitin or cellulose.
- The genome of eukaryotes is located in the nucleus, a spherical structure surrounded by a double membrane. The nucleus contains the nucleolus, the site of ribosome synthesis. DNA is organized into chromosomes in the nucleus.

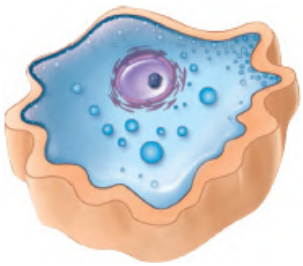
- The endoplasmic reticulum (ER) is an internal network of membranous passageways extending throughout the cell.
- The Golgi apparatus is a packaging center that receives materials from the ER and then forms vesicles around them for storage or for transport to the cell membrane for secretion.
- The mitochondria generate energy in the form of ATP to be used in numerous cellular activities.
- Chloroplasts, membranous packets found in plants and algae, are used in photosynthesis.
- Ribosomes are the sites for protein synthesis present in both eukaryotes and bacteria.
- The cytoskeleton maintains the shape of cells and produces movement of cytoplasm within the cell, movement of chromosomes at cell division, and, in some groups, movement of the cell as a unit.
- Endosymbiotic theory tells us that eukaryotic cells acquired mitochondria and chloroplasts from early bacteria that were parasitizing pre-eukaryotic cells.

4.3 The Fungi

- The fungi are a nonphotosynthetic species with cell walls. They are either saprobes or parasites and may be unicellular, colonial, or multicellular.
- There are two categories of fungi that cause human disease: the primary pathogens, which infect healthy persons, and the opportunistic pathogens, which cause disease only in compromised hosts.



- All fungi are heterotrophic.
- Fungi use both asexual and sexual reproductive strategies.
- Fungi can produce asexual spores called sporangiospores and conidiospores.
- Fungal sexual spores enable the organisms to incorporate genetic variations in form and function.



4.4 The Protozoa

- Protozoa are mostly unicellular eukaryotes that lack specialized tissues.
- Disease-causing protozoa are typically heterotrophic and usually display some form of locomotion. All have a trophozoite form, and many produce a resistant stage, or cyst.

4.5 The Helminths

- The kingdom Animalia has only one group that contains members that are studied in microbiology. These are the helminths, or worms. Pathogenic members include flatworms and roundworms that are able to invade and reproduce in human tissues.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. Mitochondria likely originated from **a**. archaea. **b**. invagination of the cell membrane. **c**. bacteria. **d**. chloroplasts.	**2**. Summarize the endosymbiotic theory and explain how it accounts for major structural similarities and differences between bacterial and eukaryotic cells.	**3**. The best theory of the evolution of the three domains of life (figure 4.11) envisions cellular organisms switching to a DNA vs an RNA lifestyle very early. Create a scenario in which it would be advantageous for the Last Common Ancestor to use RNA, while it would be advantageous for its descendants to use DNA.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Cell Structure and Function	**4**. Yeasts are _____ fungi, and molds are _____ fungi. **a**. macroscopic; microscopic **b**. unicellular; filamentous **c**. motile; nonmotile **d**. water; terrestrial	**5**. Compare and contrast the structure and function of the following among bacteria and eukaryotes. **a**. ribosome **b**. flagellum **c**. glycocalyx	**6**. Using components from all three types of eukaryotic organisms in this chapter, design an ideal organism, and explain your choices.
Metabolic Pathways	**7**. The Golgi apparatus **a**. receives vesicles from the mitochondria. **b**. packages products into transitional vesicles. **c**. modifies proteins. **d**. synthesizes proteins and sterols.	**8**. Considering the role of fungi in nature, speculate on why they have such a wide array of metabolic capabilities.	**9**. Speculate on why fungi evolved to produce products such as alcohol, antibiotics, and vitamins.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Information Flow and Genetics	**10**. Fungi produce which structures for reproduction and multiplication? **a**. endospores **b**. cysts **c**. spores **d**. eggs	**11**. Write a paragraph illustrating the life of a protein, from DNA to mature polypeptide, and the course of its travels within a cell throughout its synthesis.	**12**. Investigate whether there are other organisms, besides fungi, that have both sexual and asexual forms of reproduction and devise a hypothesis about why that might be advantageous.
Microbial Systems	**13**. Which of these organisms has the best potential to survive in extreme environments? **a**. fungi **b**. trophozoite form of protozoa **c**. helminth **d**. yeast	**14**. Why do you think the incidence of opportunistic fungal diseases in humans has increased dramatically in the last 50 years?	**15**. Do you suppose any of these eukaryotic microbes populate biofilms in nature? Defend your answer.

Impact of Microorganisms	**16**. Which of these groups causes the most casualties on an annual basis globally? **a**. helminths	**17**. Provide at least three examples illustrating both beneficial and detrimental aspects of	**18**. Do you suspect that the fact that humans use microbes such as fungi to manufacture massive amounts of metabolic products for our use will change the evolution of these organisms? Defend your answer.

	b. protozoa **c**. fungi **d**. algae	fungi in the modern world today.	
Scientific Thinking	**19**. Which of the following is not useful to determine whether a clinical isolate is a bacterium, fungus, or protozoan? **a**. its size under a light microscope **b**. whether it has a cell wall **c**. whether it can form protective structures under stress **d**. all of the above are reliable	**20**. Why were protozoa originally considered a single group and why is that no longer the case?	**21**. Write a paragraph that would explain the difference between heterotroph, saprobe, and parasite to a middle school class.

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From chapter 2, figure 2.1**. Discuss how the techniques of the Five I's of microbiology would be completed if your patient's infection was due to a protozoan, a eukaryotic microbe.

©Monty Rakusen/Getty Images (scientist in lab); Source: CDC/James Gathany (lab worker holding petri dish); ©Pixtal/age fotostock (kneeling scientist); Source: CDC (*Vibrio cholerae* petri dish); ©McGraw-Hill Education (drawing of microscope); ©McGraw-Hill Education/Lisa Burgess (DNA gel electrophoresis)

connect® Resources are available through Connect, including interactive learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

5

Viral Structure and Multiplication

©Science Photo Library/Alamy Stock Photo (chest anatomy, 3D CT scan); ©Eye of Science/Science Source (TEM - Herpes simplex viruses); Source: Bakonyi T, Lussy H, Weissenböck H, Hornyák A, Nowotny N. Emerging Infectious Diseases, Vol. 11, No. 2, Feb. 2005. (cytopathic effect (CPE) of Vero cells caused by Usutu virus infection); Source: CDC/DR. Al Jenny (Creutzfeldt-Jakob diseased tissue); Source: CDC/Cynthia GoldsmithCDC/Cynthia Goldsmith (scanning electron micrograph of HIV-1 budding from cultured lymphocyte); ©DreamPictures/Pam Ostrow/Blend Images LLC (health care worker)

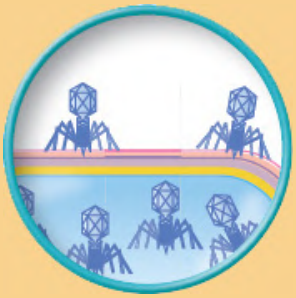
IN THIS CHAPTER…

5.1 The Position of Viruses in the Biological Spectrum

1. Explain what it means when viruses are described as *filterable*.
2. Identify better terms for viruses than *alive* or *dead*.

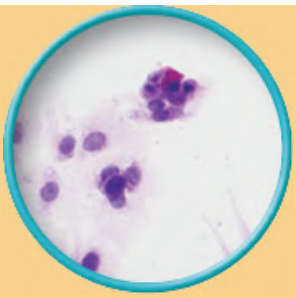
5.2 The General Structure of Viruses

3. Discuss the size of viruses relative to other microorganisms.
4. Describe the function and structure(s) of viral capsids.
5. Distinguish between enveloped and naked viruses.
6. Explain the importance of viral surface proteins, or spikes.
7. Diagram the possible nucleic acid configurations that viruses may possess.



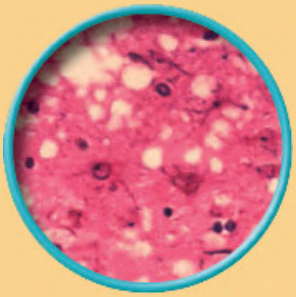
5.3 Modes of Viral Multiplication

8. Diagram the five-step life cycle of animal viruses.
9. Define the term *cytopathic effect* and provide one example.
10. Discuss both persistent and transforming infections.
11. Provide thorough descriptions of both lysogenic and lytic bacteriophage infections.



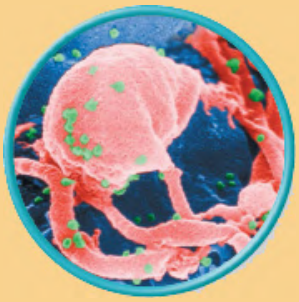
5.4 Techniques in Cultivating and Identifying Animal Viruses

12. List the three principal purposes of cultivating viruses.
13. Describe three ways in which viruses are cultivated.



5.5 Other Noncellular Infectious Agents

14. Name three noncellular infectious agents besides viruses.



5.6 Viruses and Human Health

15. Analyze the relative importance of viruses in human infection and disease.
16. Discuss the primary reason that antiviral drugs are more difficult to design than antibacterial drugs.

CASE FILE

A Case of Croup

I was an LPN working in a pediatrician's office. In my role, I saw both ill and well children and it was important that I could identify serious symptoms that required immediate care. One day, I cared for an 18-month-old female, accompanied by her mother. The patient was being seen for respiratory symptoms, which was a common reason for a visit to our office in fall and winter months.

Immediately upon assessing the girl, I noted that she did not look well. The patient had a hoarse, barky cough. Her mother reported that she had a fever, runny nose, and "squeaky" cry for the past 24 hours, which worsened overnight. The patient was lethargic and lying in her mother's arms. I applied an oxygen saturation probe and found that the child had normal oxygen saturation despite her labored breathing. When I listened to her lungs with my stethoscope, she sounded clear in all lung fields.

I administered an inhaled epinephrine treatment, as ordered by the pediatrician. The patient's breathing improved after the treatment. She became more irritable with a worsening cough as we treated her so I tried to limit my stimulation. Based on the patient's history and symptoms, the pediatrician diagnosed her with croup.

We kept the patient in the office for another hour to observe her response to the respiratory treatment. Her breathing, while still labored, had improved. The mother requested antibiotics to treat the patient's illness. I provided education on the viral causes of croup and that antibiotics are ineffective in treating viral illness.

The patient was sent home with a prescription for a dose of oral steroids. The mother was instructed to fill the prescription immediately and to call if her child's symptoms worsened.

- What clinical signs did the pediatrician use to diagnose the child with croup?
- What can be done to treat viral illnesses?

Case File Wrap-Up appears at the end of the chapter.

5.1 The Position of Viruses in the Biological Spectrum

Viruses are a unique group of biological entities known to infect every type of cell, so that includes bacteria, algae, fungi, protozoa, plants, and animals. Viruses are extremely abundant on our planet. For example, it is documented that seawater can contain 10 million viruses per milliliter, and human feces probably contain 100 times that many. It is estimated that the sum of viruses in the ocean represents 270 million metric tons of organic matter. We are just beginning to understand the impact of these huge numbers of viruses on our environment.

This variegated tulip gets its beautiful colors from a viral infection.
©Anna Yu/Getty Images

For many years, the cause of viral infections such as smallpox and polio was unknown, even though it was clear that the diseases were transmitted from person to person. The French scientist Louis Pasteur was certainly on the right track when he hypothesized that rabies was caused by a "living thing" smaller than bacteria. In 1884 he was able to develop the first vaccine for rabies. Pasteur also proposed the term *virus* (which is Latin for poison) to name this special group of infectious agents.

The first important hints about the unique characteristics of viruses occurred in the 1890s. First, D. Ivanovski and M. Beijerinck showed that a disease in tobacco was caused

by a virus (tobacco mosaic virus). Then, Friedrich Loeffler and Paul Frosch discovered an animal virus that causes foot-and-mouth disease in cattle. These early researchers found that when infectious fluids from the victims were passed through porcelain filters designed to trap bacteria, the fluid that came through the filter remained infectious. This result proved that an infection could be caused by a fluid containing agents smaller than bacteria and thus first introduced the concept of a *filterable virus*.

Over the succeeding decades, a remarkable picture of the physical, chemical, and biological nature of viruses has taken form. Viruses have been shown to be noncellular particles with a definite size, shape, and chemical composition. The development of special techniques meant that they could be cultured in the laboratory. Then, thanks to new genomic techniques, including DNA arrays and "next-generation" nucleic acid sequencing techniques, we developed a much clearer picture of the number and variety of viruses on earth. Studies of the human virome (a part of the human microbiome) and of the world's oceans are showing us that there are vast multitudes of viruses that have roles we cannot even guess about.

The exceptional and curious nature of viruses raises many questions, including the following:

1. Are they organisms; that is, are they alive?
2. What role did viruses play in the evolution of life?
3. What are their distinctive biological characteristics?
4. How can particles so small, simple, and seemingly insignificant be capable of causing disease and death?
5. What is the connection between viruses and cancer?

In this chapter, we address these questions and many others.

The unusual structure and behavior of viruses have led to debates about their connection to the rest of the microbial world. One viewpoint holds that since viruses are unable to multiply independently from the host cell, they are not living things but should instead be called infectious molecules. Another viewpoint proposes that even though viruses do not exhibit most of the life processes of cells, they can *direct* them and thus are more than inert and lifeless molecules. In many ways, this debate doesn't matter for our purposes. Viruses are agents of disease and must be dealt with through control, therapy, and prevention, whether we regard them as living or not. In keeping with all of this, it is best to describe viruses as either *active* or *inactive* (rather than alive or dead).

Recent discoveries suggest that viruses have been absolutely vital in forming cells and other life forms as they are today. By infecting other cells, and sometimes

Table 5.1 Properties of Viruses

- Are obligate intracellular parasites of bacteria, protozoa, fungi, algae, plants, and animals
- Estimated 10^{31} virus particles on earth, approximately 10 times the number of bacteria and archaea combined.

- Are ubiquitous in nature and have had major impact on development of biological life
- Are ultramicroscopic in size, ranging from 20 nm up to 1,000 nm (diameter)
- Are not cells; structure is very compact and economical
- Do not independently fulfill the characteristics of life
- Basic structure consists of protein shell (capsid) surrounding nucleic acid core
- Nucleic acid can be either DNA or RNA, but not both
- Nucleic acid can be double-stranded DNA, single-stranded DNA, single-stranded RNA, or double-stranded RNA
- Molecules on virus surface give them high specificity for attachment to host cell
- Multiply by taking control of host cell's genetic material and regulating the synthesis and assembly of new viruses
- Lack enzymes for most metabolic processes
- Lack machinery for synthesizing proteins

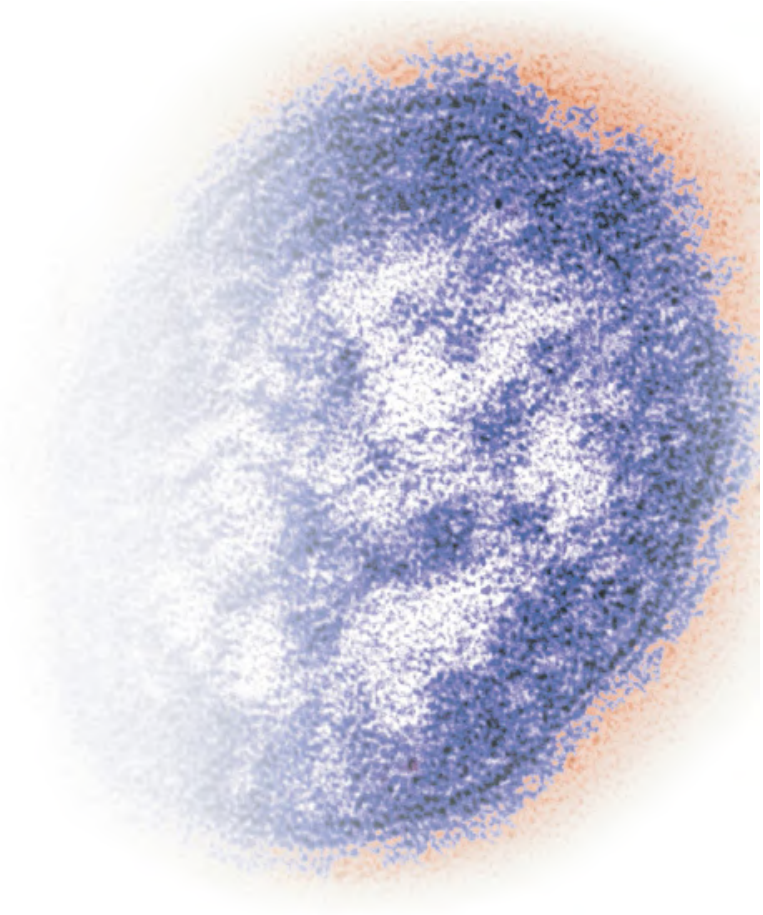
influencing their genetic makeup, they have shaped the way cells, tissues, bacteria, plants, and animals have evolved to their present forms. For example, scientists think that approximately 8% of the human genome consists of sequences that come from viruses that have inserted their genetic material permanently into human DNA. Bacterial DNA also contains 10% to 20% viral sequences. As you learn more about how viruses work, you will see how this could happen.

Viruses are different from their host cells in size, structure, behavior, and physiology. They are *obligate intracellular parasites* that cannot multiply unless they invade a specific host cell and instruct its genetic and metabolic machinery to make and release quantities of new viruses. Other unique properties of viruses are summarized in **table 5.1**.

How Viruses Are Classified and Named

For many years, the animal viruses were classified mainly on the basis of their hosts and the kind of diseases they caused. Newer systems for naming viruses also take into account the actual nature of the virus particles themselves, with only partial emphasis on host and disease. The main criteria currently used to group viruses are structure, chemical composition, and similarities in genetic makeup.

A group called the International Committee on the Taxonomy of Viruses (ICTV) is in charge of classifying and categorizing viruses. Periodically they issue reports and updates that come about as a result of continued research. The most recent report was issued in 2016. The committee listed 8 orders with 38 families, plus another 84 families not yet assigned to any order. These numbers will only continue to grow as we discover more and more about life on earth. Previous to 2000, there had been only a single recognized order of viruses. Usually clinicians use the common vernacular names (for example, poliovirus and rabies virus), and this book will do the same.



Measles virus
Source: CDC/Cynthia Goldsmith

5.1 LEARNING OUTCOMES—Assess Your Progress

1. Explain what it means when viruses are described as *filterable*.
2. Identify better terms for viruses than *alive* or *dead*.

NCLEX® PREP

1. Which statements are accurate regarding properties of viruses? Select all that apply.

- **a.** *Viruses are cell structures that are compact and economical.*
- **b.** *Viruses can be living molecules outside the host cell.*
- **c.** *The basic structure of a virus is composed simply of a protein shell.*
- **d.** *Viruses do not contain enzymes for most metabolic processes.*
- **e.** *Viruses contain either DNA or RNA.*

5.2 The General Structure of Viruses

Size Range

As a group, viruses represent the smallest infectious agents (with some unusual exceptions to be discussed later in this chapter). They are dwarfed by their host cells: More than 2,000 bacterial viruses could fit into an average bacterial cell, and more than 50 million polioviruses could be accommodated by an average human cell. Common animal viruses range in size from the small parvoviruses (around 20 nm [0.02 μm] in diameter) to the herpes simplex virus (around 150 nm) **(figure 5.1)**. Unusual viruses have been discovered recently that are huge, in virus terms. *Pandoravirus* is pictured in figure 5.1, and is about the same size as a coccus-shaped bacterial cell. Some cylindrical viruses are relatively long (800 nm [0.8 μm] in length) but so narrow in diameter (15 nm [0.015 μm]) that even with the high magnification and resolution of an electron microscope they are very difficult to see. Figure 5.1 compares the sizes of several viruses with bacterial and eukaryotic cells and molecules.

Viral architecture is most readily observed using special stains in combination with electron microscopy **(figure 5.2)**.

Viral Components: Capsids, Nucleic Acids, and Envelopes

It is important to realize that viruses bear no real resemblance to cells, and that they do not have any of the protein-synthesizing machinery found in even the simplest cells. Their outer surface is composed of regular, repeating subunits that give rise to their crystalline appearance. The general plan of virus organization is the utmost in simplicity and compactness. Viruses contain only those parts needed to invade and control a host cell: an external coating and a core containing one or more nucleic acid strands of either DNA or RNA, and sometimes one or two enzymes. The box titled “Virus Particle” summarizes viral structure with a diagram similar to the ones we have seen for bacteria and eukaryotes:

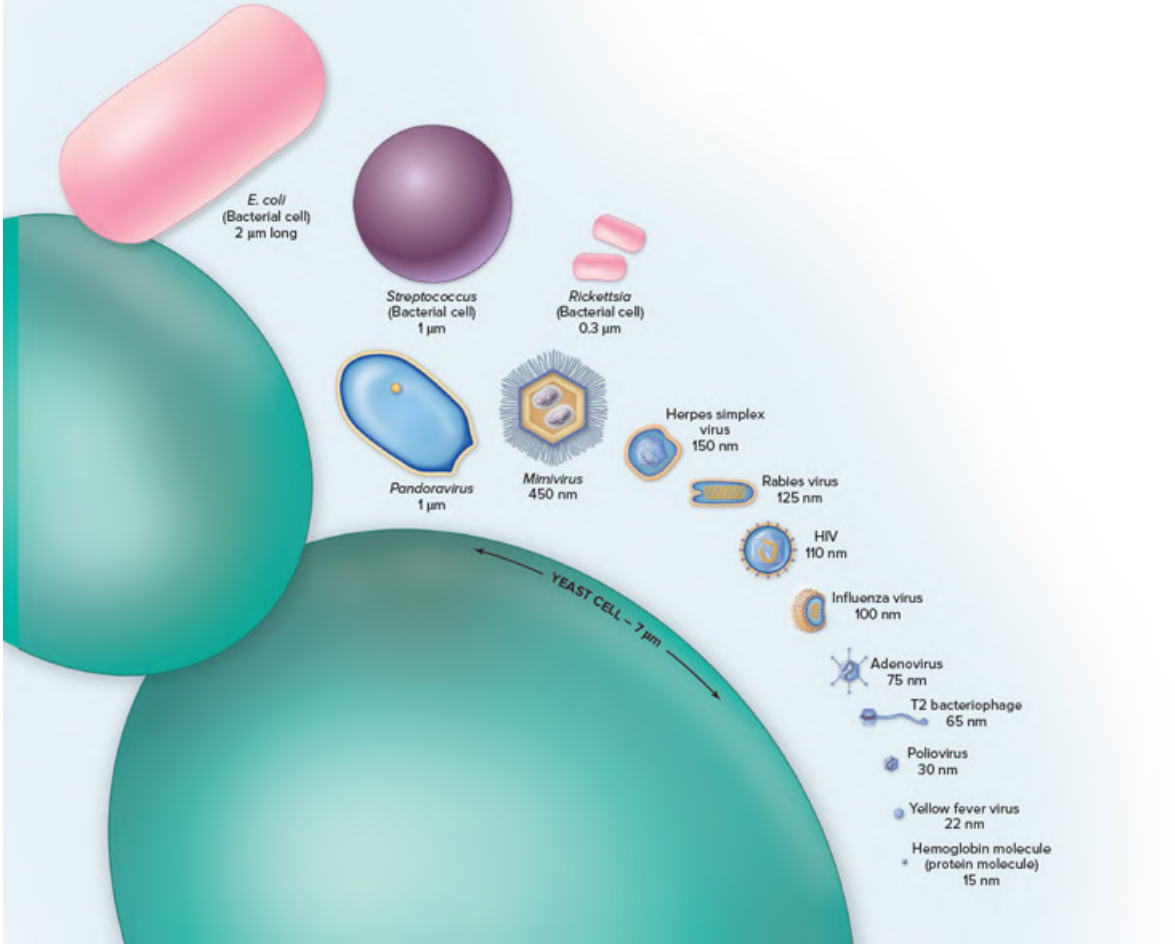
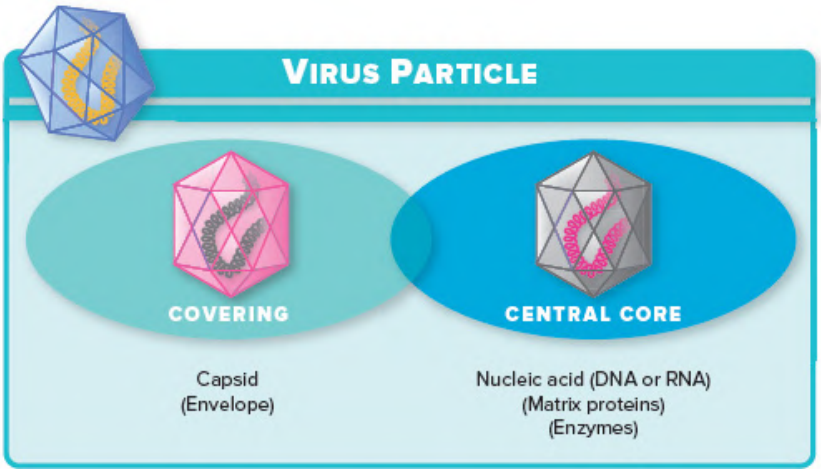


Figure 5.1 Size comparison of viruses with a eukaryotic cell (yeast) and bacteria. A molecule of protein is included to indicate proportion of macromolecules.

Figure 5.2 **Methods of viewing viruses**. **(a)** Negative staining of influenza viruses. **(b)** Positive stain of the Ebola virus. Note the textured capsid. **(c)** Shadowcasting image of a vaccinia virus.
Source: CDCl/Dr. F. A. Murphy (a); ©Phototake (b); ©A.B. Dowsette/SPL/Science Source (c)



All viruses have a protein **capsid**, or shell, that surrounds the nucleic acid in the central core. Together the capsid and the nucleic acid are referred to as the **nucleocapsid** (**figure 5.3**). Many animal viruses also possess an additional covering external to the capsid called an *envelope*, which is usually a modified piece of the host's cell membrane (figure 5.3*b*). Most viruses that infect humans have envelopes. Viruses that consist of only a nucleocapsid are considered *naked viruses* (figure 5.3*a*). Both naked and enveloped viruses possess proteins on their outer surfaces that project from either the nucleocapsid or the envelope. They are the molecules that allow viruses to dock with their host cells and are called *spikes*. As we shall see later, the enveloped viruses differ from the naked viruses in the way that they enter and leave a host cell. A fully formed virus that is able to establish an infection in a host cell is often called a **virion**.

The Viral Capsid and Envelope

When a virus particle is magnified several hundred thousand times, the capsid appears as the most prominent geometric feature. In general, each capsid is constructed from identical subunits called **capsomeres** that are constructed from protein molecules. The capsomeres spontaneously self-assemble into the finished capsid. Depending on how the capsomeres are

shaped and arranged, this assembly results in two different types: helical and icosahedral. Table 5.2 depicts the variations on these two themes.

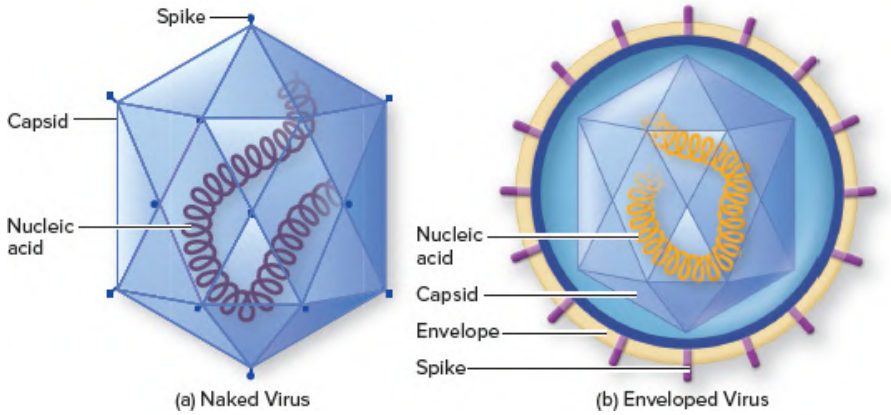
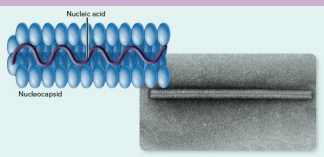
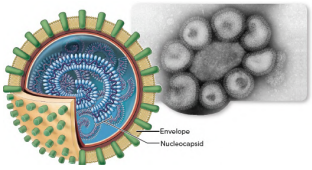
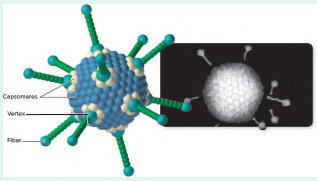
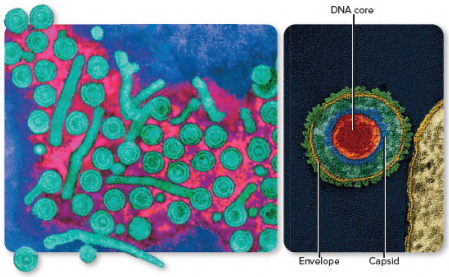
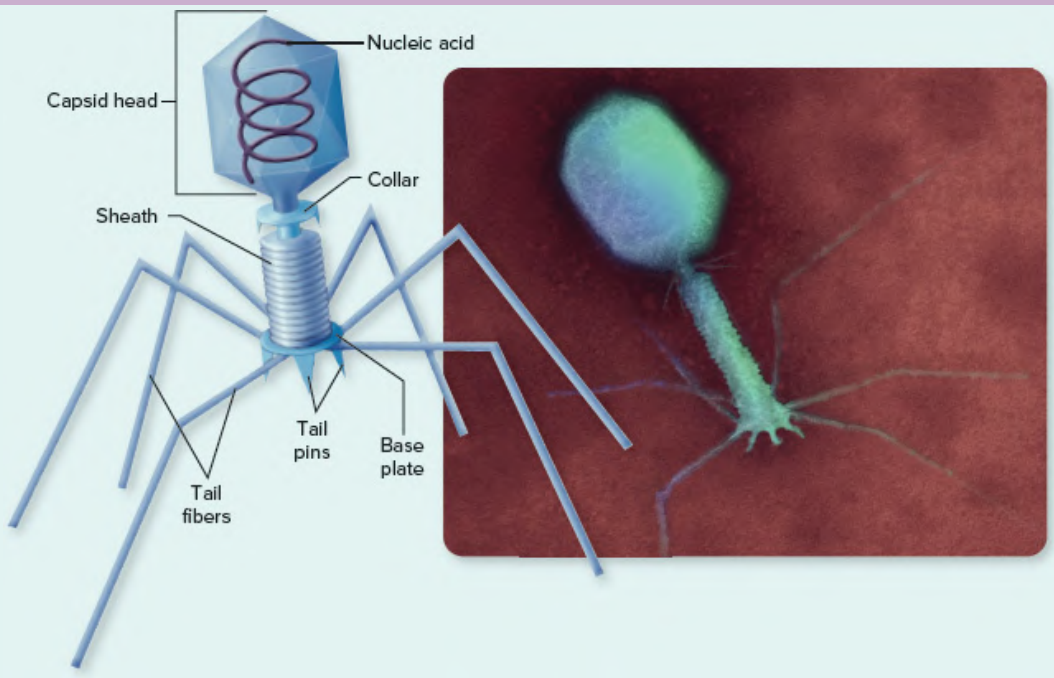


Figure 5.3 **Generalized structure of viruses**. **(a)** The simplest virus is a naked virus (nucleocapsid), consisting of a geometric capsid assembled around a nucleic acid strand or strands. **(b)** An enveloped virus is composed of a nucleocapsid surrounded by a flexible membrane called an envelope.

Table 5.2 Capsid Structure

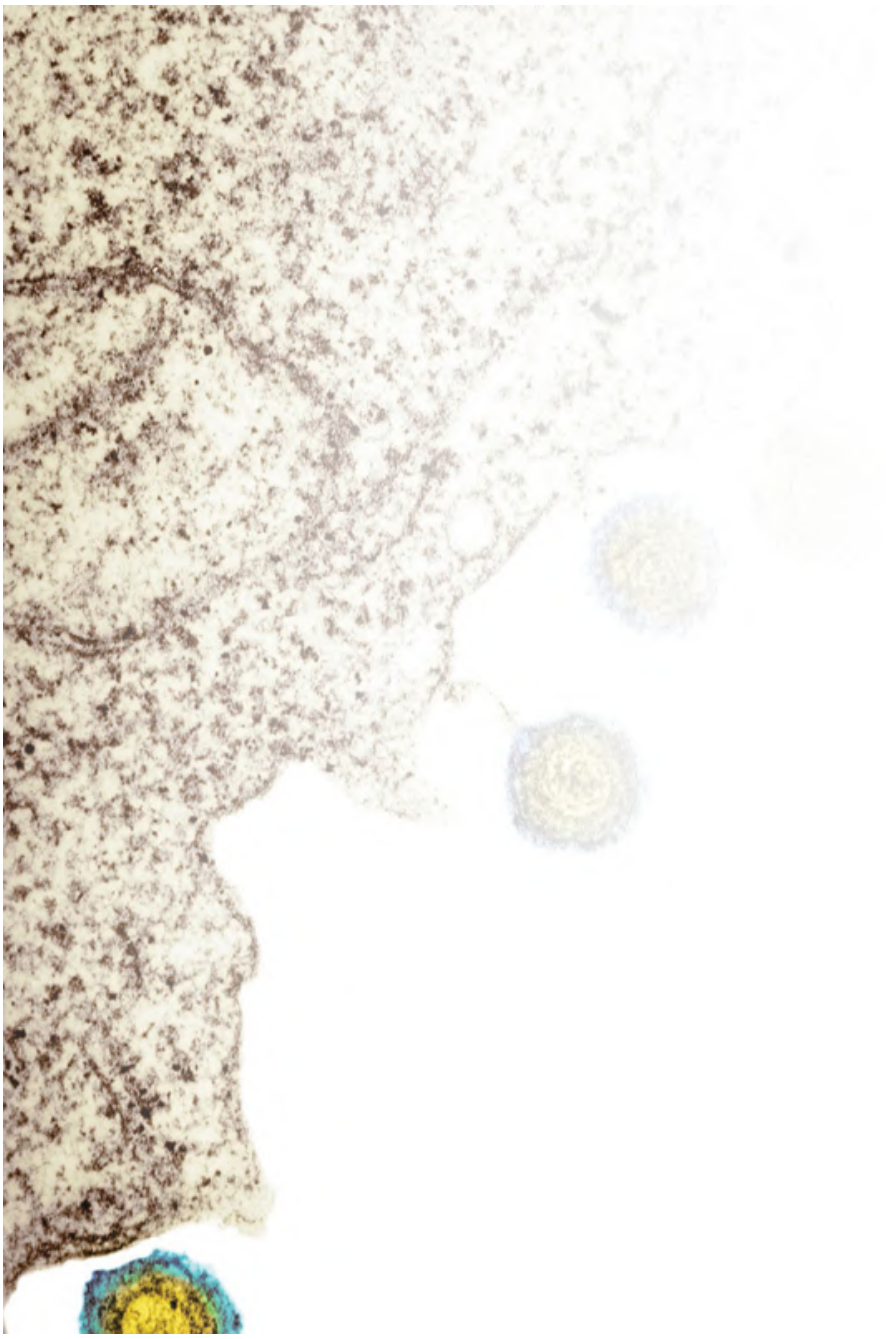
Helical Capsids	The simpler **helical capsids** have rod-shaped capsomeres that bond together to form a series of hollow discs resembling a bracelet. During the formation of the nucleocapsid, these discs link with other discs to form a continuous helix into which the nucleic acid strand is coiled.	
Naked	The nucleocapsids of naked helical viruses are very rigid and tightly wound into a cylinder-shaped package. An example is the *tobacco mosaic virus*, which attacks tobacco leaves (right).	
Enveloped	Enveloped helical nucleocapsids are more flexible and tend to be arranged as a looser helix within the envelope. This type of morphology is found in several enveloped human viruses, including influenza, measles, and rabies.	
Icosahedral Capsids	These capsids form an **icosahedron** (eye″-koh-suh-hee′-drun)—a three-dimensional, 20-sided figure with 12 evenly spaced corners. The arrangements of the capsomeres vary from one virus to another. Some viruses construct the capsid from a single type of capsomere, while others may contain several types of capsomeres. There are major variations in the number of capsomeres; for example, a poliovirus has 32, and an adenovirus has 252 capsomeres.	
Naked	Adenovirus is an example of a naked icosahedral virus. In the photo you can clearly see the spikes, some of which have broken off.	

Enveloped	Two very common viruses, hepatitis B virus (left) and the herpes simplex virus (right), possess enveloped icosahedrons.	
Complex Capsids	Complex capsids, only found in the viruses that infect bacteria, may have multiple types of proteins and take shapes that are not symmetrical. They are never enveloped. The one pictured on the right is a T4 bacteriophage.	

©Science Source (tobacco mosaic virus. TEM Magnification: 260,000x); Source: CDC/Dr. Fred Murphy (negative-stained (TEM) of influenza virus particles. Mag not available.); ©Dr. Linda M. Stannard, University of Cape Town/Science Source (adenovirus); ©Dr. Linda M. Stannard, University of Cape Town/Science Source (hep B virus); ©Eye of Science/Science Source (TEM - Herpes simplex viruses); ©Ami Images/Science Source (complex capsid - T4 bacteriophage. TEM. Mag not available)

When **enveloped viruses** (mostly animal) are released from the host cell, they take with them a bit of the cell's membrane system in the form of an envelope, as described later. Some viruses bud off the cell membrane; others leave via the nuclear envelope or the endoplasmic reticulum. Whichever avenue of escape, the viral envelope differs significantly from the host's membranes. In the envelope, some or all of the regular membrane proteins are replaced with special viral proteins. Some of the envelope proteins attach to the capsid of the virus, and glycoproteins (proteins bound to a carbohydrate) remain exposed on the outside of the envelope. These protruding molecules, called spikes, are essential for the

attachment of viruses to the next host cell. Because the envelope is more flexible than the capsid, enveloped viruses are pleomorphic (of variable shape) and range from spherical to filamentous in shape.



A human herpesvirus (blue and gold) released from its host cell.
©Science Photo Library/Getty Images

Nucleic Acids: At the Core of a Virus

The sum total of the genetic information carried by an organism is called its **genome**. We know that the genetic information of living cells is carried by nucleic acids (DNA, RNA). Viruses, although neither alive nor cells, are no exception to this rule, but there is a significant difference. Unlike cells, which contain both DNA and RNA, viruses contain either DNA or RNA *but not both*.

The number of viral genes is quite small compared with that of a cell. It varies from four genes in hepatitis B virus to hundreds of genes in some herpesviruses. Viruses possess only the genes needed to invade host cells and redirect their activity. By comparison, the bacterium *Escherichia coli* has approximately 4,000 genes, and a human cell has approximately 20,000 genes. These additional genes allow cells to carry out the complex metabolic activity necessary for independent life.

In chapter 1, you learned that DNA usually exists as a double-stranded molecule and that RNA is single-stranded. Viruses are different; they exhibit wide variety in how their RNA or DNA is configured. DNA viruses can have single-stranded (ss) or double-stranded (ds) DNA; the dsDNA can be arranged linearly or in ds circles. RNA viruses can be double-stranded but are more often single-stranded. You will learn in chapter 8 that all proteins are made by translating the nucleic acid code on a single strand of RNA into an amino acid sequence. Single-stranded RNA genomes that are ready for immediate translation into proteins are called *positive-sense* RNA. Other RNA genomes have to be converted into the proper form to be made into proteins, and these are called *negative-sense* RNA. RNA genomes may also be *segmented*, meaning that the individual genes exist on separate pieces of RNA. A special type of RNA virus is called a *retrovirus*. These viruses are distinguished by the fact that they carry their own enzymes to create DNA out of their RNA. **Table 5.3** gives examples of each configuration of viral nucleic acid.

In all cases, these tiny strands of genetic material carry the blueprint for viral structure and functions. In a very real sense, viruses are genetic parasites because they cannot multiply until their nucleic acid has reached the internal habitat of the host cell. At the minimum, they must carry genes for synthesizing the viral capsid and genetic material, for regulating the actions of the host, and for packaging the mature virus.

Medical Moment

Why Antibiotics Are Ineffective Against Viruses

Many people mistakenly believe that they can take an antibiotic to cure a viral infection, such as the common cold. To understand why antibiotics do not work on viral infections, we need to think about what antibiotics do, as well as the properties of viruses that make them unique (see table 5.1).

Most antibiotics target specific functions or processes within bacteria. Antibiotics may inhibit cell wall synthesis, protein synthesis, nucleic acid synthesis, or the synthesis of specific proteins required for the bacteria to survive and reproduce. They may also cause injury to the cytoplasmic membrane.

Viruses lack cytoplasmic membranes, are unable to synthesize proteins, and contain either DNA or RNA (but not both). Viruses can only reproduce by hijacking their host's genetic material to create new viruses. Antibiotics cannot alter functions or processes that do not exist in viruses. This is why antibiotics are not helpful for viral infections.

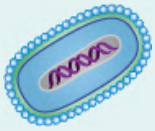
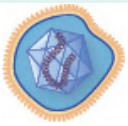
Q. Antibiotics targeting bacteria have proliferated since the mid-1900s. Antiviral medications are few, and were developed only recently. What alternative control strategy did the medical field employ against viral diseases, starting more than 200 years ago?

Answer in Appendix B.

Other Substances in the Virus Particle

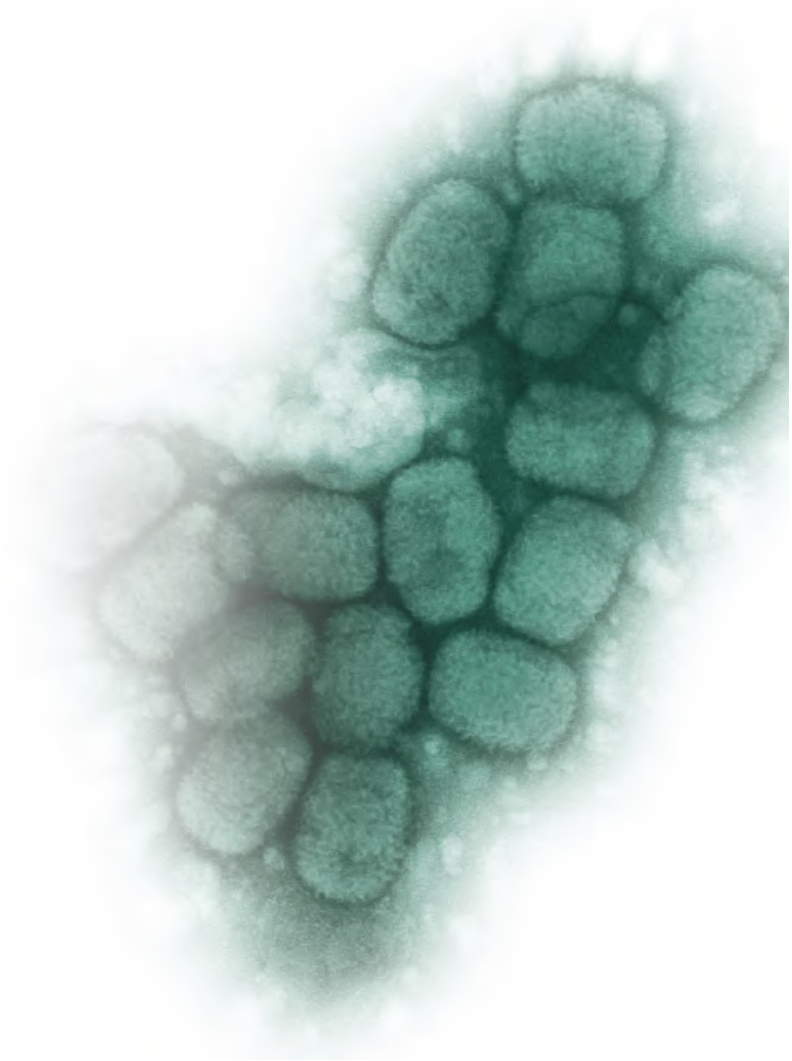
In addition to the protein capsid, the protein and lipid envelopes, and the nucleic acid core, viruses can contain enzymes for specific operations within their host cell. They may come with ready-made enzymes that are required for viral replication. Examples include *polymerases* (pol-im′-ur-ace-uz) that synthesize DNA and RNA, and replicases that copy RNA. Human immunodeficiency virus (HIV) comes equipped with *reverse transcriptase* for synthesizing DNA from RNA. However, viruses completely lack the

Table 5.3 Viral Nucleic Acid

	Diagram	Virus Name	Disease It Causes
DNA Viruses–Examples			
Double-stranded DNA		Variola virus	Smallpox
		Herpes simplex II	Genital herpes
Single-stranded DNA		Parvovirus	Erythema infectiosum (skin condition)
RNA Viruses–Examples			
Single-stranded (+) sense		Poliovirus	Poliomyelitis
Single-stranded (−) sense		Influenza virus	Influenza
Double-stranded RNA		Rotavirus	Gastroenteritis
Single-stranded RNA + reverse transcriptase		HIV	AIDS

genes for synthesis of metabolic enzymes. As we shall see, this deficiency does not represent an obstacle, since viruses have adapted to completely take over their hosts'

metabolic resources. Some viruses can actually carry away substances from their host cell. For instance, arenaviruses pack along host ribosomes, and retroviruses "borrow" the host's tRNA molecules.



Variola virus
Source: CDC/Dr. Fred Murphy

5.2 LEARNING OUTCOMES—Assess Your Progress

3. Discuss the size of viruses relative to other microorganisms.
4. Describe the function and structure(s) of viral capsids.
5. Distinguish between enveloped and naked viruses.
6. Explain the importance of viral surface proteins, or spikes.
7. Diagram the possible nucleic acid configurations that viruses may possess.

5.3 Modes of Viral Multiplication

Viruses are minute parasites that seize control of the synthetic and genetic machinery of cells. The nature of this cycle dictates the way the virus is transmitted and what it does to its host, the responses of the immune defenses, and human measures to control viral infections.

Multiplication Cycles in Animal Viruses

The general phases in the life cycle of animal viruses are **adsorption, penetration** and **uncoating, synthesis, assembly**, and **release** from the host cell. The length of the entire multiplication cycle varies from 8 hours in polioviruses to 36 hours in some herpesviruses. **Table 5.4** walks through the major phases of the viral life cycle, using a + strand RNA virus (of which rubella virus is an example) as a model.

Avian flu viruses (gold) inside host cells (green).
Source: CDC/Cynthia Goldsmith; Jacqueline Katz; Sherif R. Zaki

Notes on the Multiplication Cycle

Adsorption Because a virus can invade its host cell only through making an exact fit with a specific host molecule, the range of hosts it can infect is limited **(figure 5.4)**. This limitation, known as the **host range**, may be highly restricted as in the case of hepatitis B, which infects only liver cells of humans; moderately restrictive like the poliovirus, which infects intestinal and nerve cells of primates (humans, apes, and monkeys); or broad like the rabies virus, which can infect various cells of all mammals. Cells that lack compatible virus

receptors are resistant to adsorption and invasion by that virus. This explains why, for example, human liver cells are not infected by the canine hepatitis virus and dog

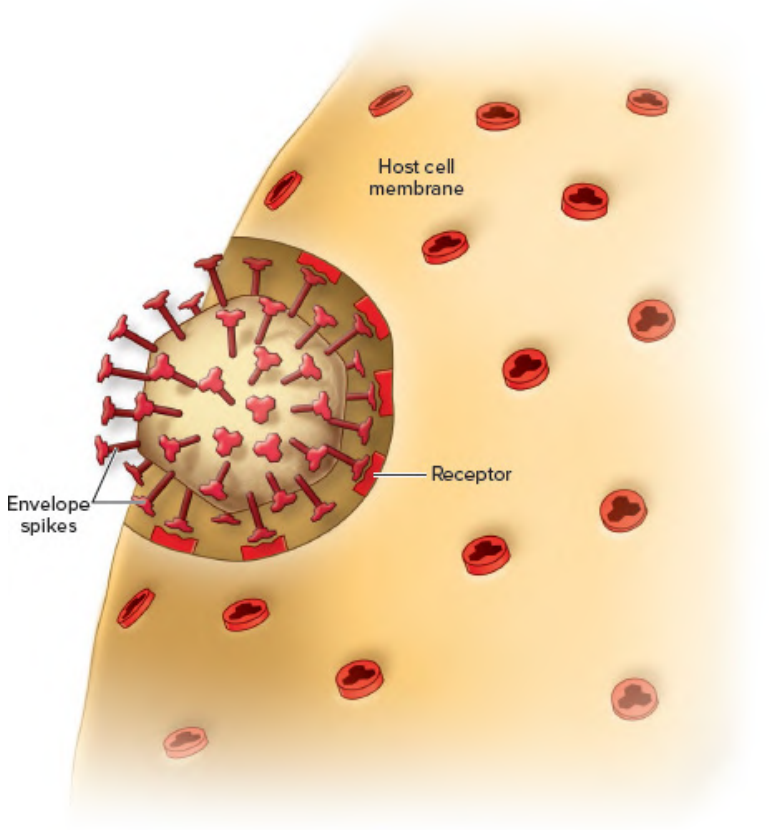
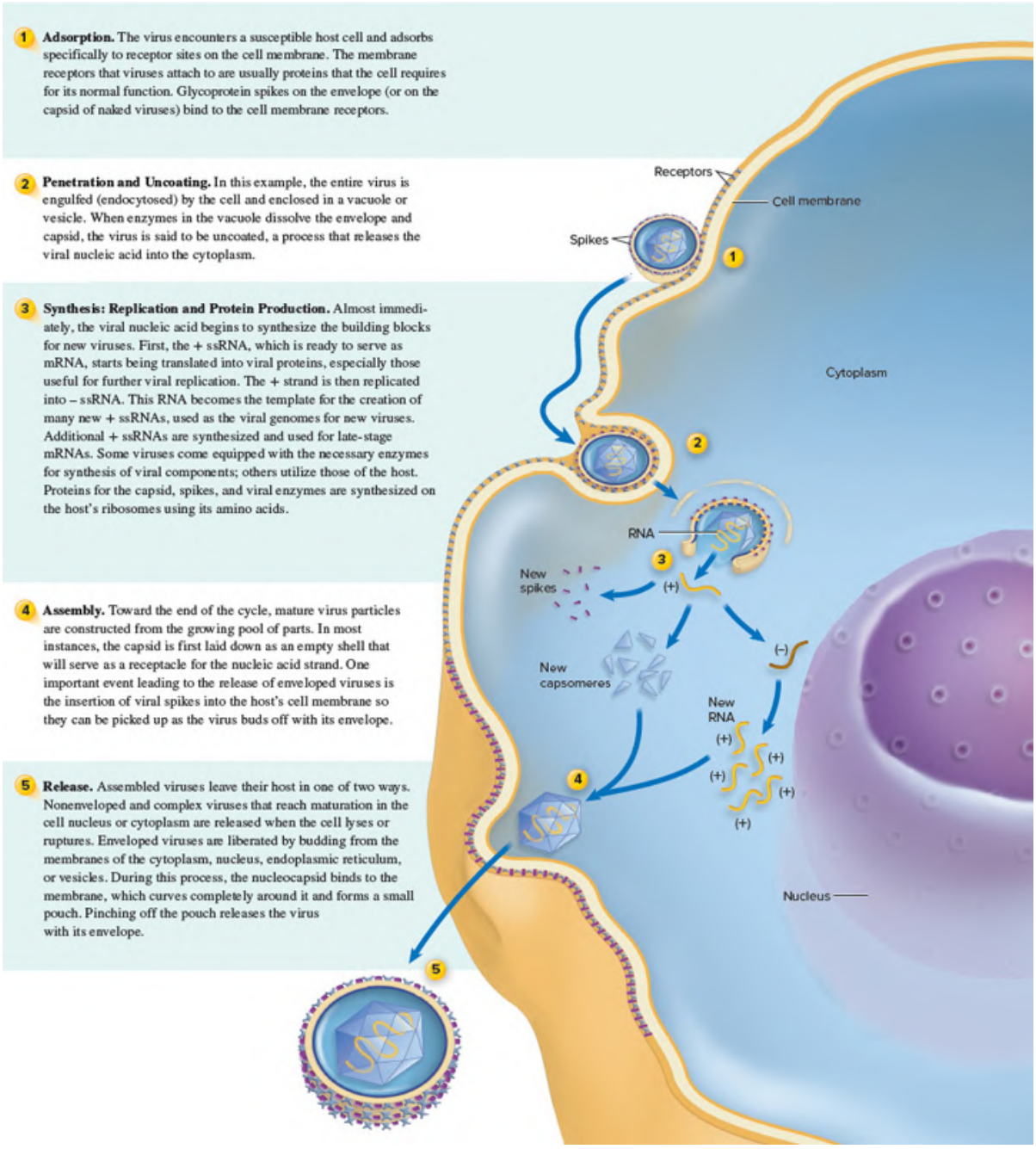


Figure 5.4 **The viral attachment process**. An enveloped coronavirus with spikes. The configuration of the spike has a complementary fit for cell receptors. The process in which the virus lands on the cell and plugs into receptors is termed *docking*.

Table 5.4 Life Cycle of Animal Viruses



liver cells cannot host the human hepatitis A virus. It also explains why viruses usually have tissue specificities called *tropisms* (troh′-pizmz) for certain cells in the body. The hepatitis B virus targets the liver, and the mumps virus targets salivary glands.

Penetration and Uncoating Animal viruses have some impressive mechanisms for entering a host cell. The flexible cell membrane of the host is penetrated either by the whole

virus or by its nucleic acid **(figure 5.5)**. In penetration by **endocytosis** (figure 5.5*a*), the entire virus is engulfed by the cell and enclosed in a vacuole or vesicle. Enzymes inside the vesicle may break down the viral capsid. This leaves the virus in an *uncoated* state, in which the nucleic acid is freed. In other cases, the whole nucleocapsid is released into the cytoplasm, and the virus is not uncoated until later in the process. Another means of entry involves direct fusion of the viral envelope with the host cell membrane (as in influenza and mumps viruses) (figure 5.5*b*). In this form of penetration, the envelope merges directly with the cell membrane, thereby liberating the nucleocapsid into the cell's interior.

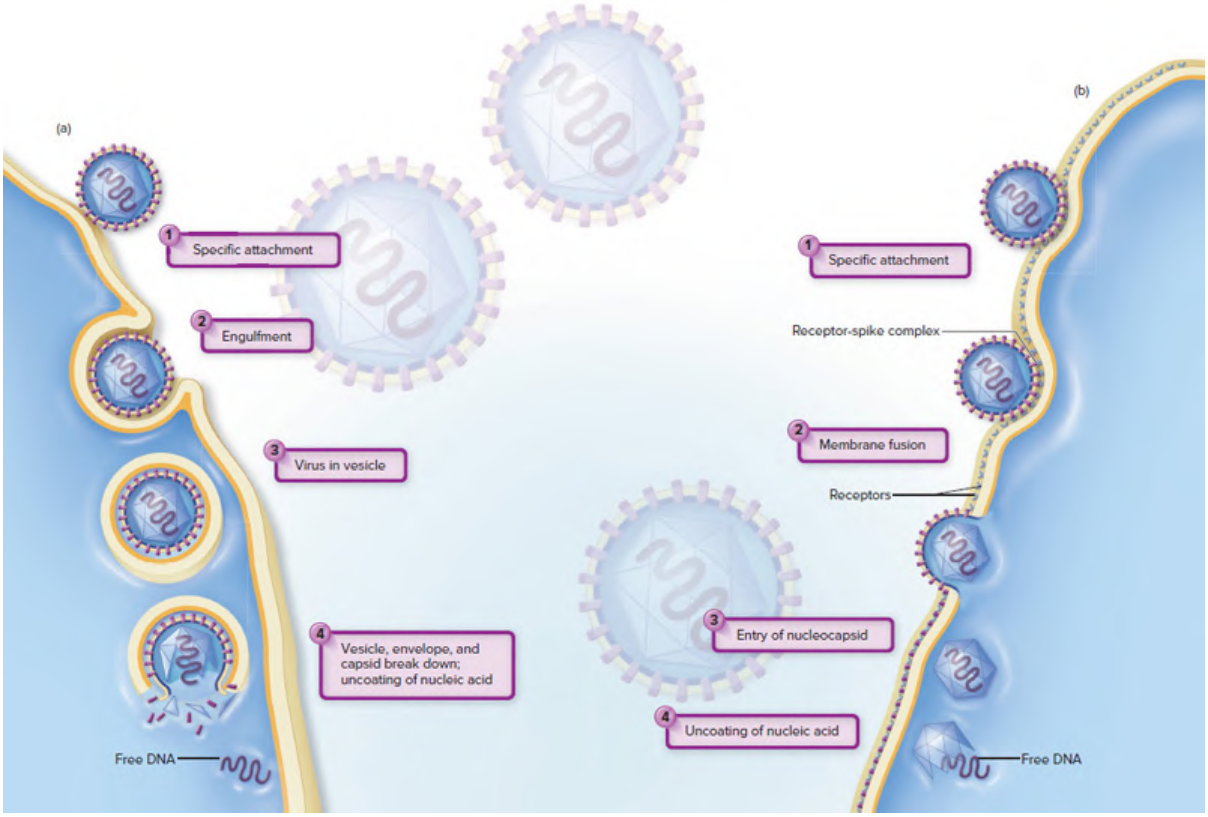


Figure 5.5 **Two principal means by which animal viruses penetrate**. **(a)** Endocytosis (engulfment) and uncoating of a herpesvirus. **(b)** Fusion of the cell membrane with the viral envelope (mumps virus).

Synthesis: Replication and Protein Production Most DNA viruses enter the host cell's nucleus and are replicated and assembled there. With few exceptions (such as retroviruses), RNA viruses are replicated and assembled in the cytoplasm.

In chapter 8 you will learn that cellular organisms make new copies of their new genomes by duplicating their DNA. They also use DNA to make mRNA that directs the creation of proteins. These processes can be very different in viruses.

In the life cycle of dsDNA viruses, the synthesis phase is divided into two parts. During the early phase, viral DNA enters the nucleus, where several genes—usually the ones that make proteins needed to make new viral DNA—are transcribed into a messenger RNA.

That newly synthesized mRNA then moves into the cytoplasm to be translated into viral proteins (enzymes) needed to replicate the viral DNA. This DNA replication occurs in the nucleus. The host cell's own DNA polymerase is often involved, though some viruses (herpes, for example) have their own. During the late phase, other parts of the viral genome are transcribed and translated into proteins required to form the capsid and other structures. The new viral genomes and capsids are assembled, and the mature viruses are released by budding or cell disintegration. In some viruses, the viral DNA becomes silently *integrated* into the host's genome by insertion at a particular site on the host genome. This integration may later lead to the transformation of the host cell into a cancer cell and the production of a tumor.

Assembly As illustrated in table 5.4, this step actually puts together the new viruses using the "parts" manufactured in the synthesis process: new capsids and new nucleic acids.

Release **Figure 5.6** illustrates the mechanics of viral release from host cells. The number of viruses released by infected cells is variable, controlled by factors such as

Technicians who work on certain viruses take special precautions.
Source: CDC/James Gathany

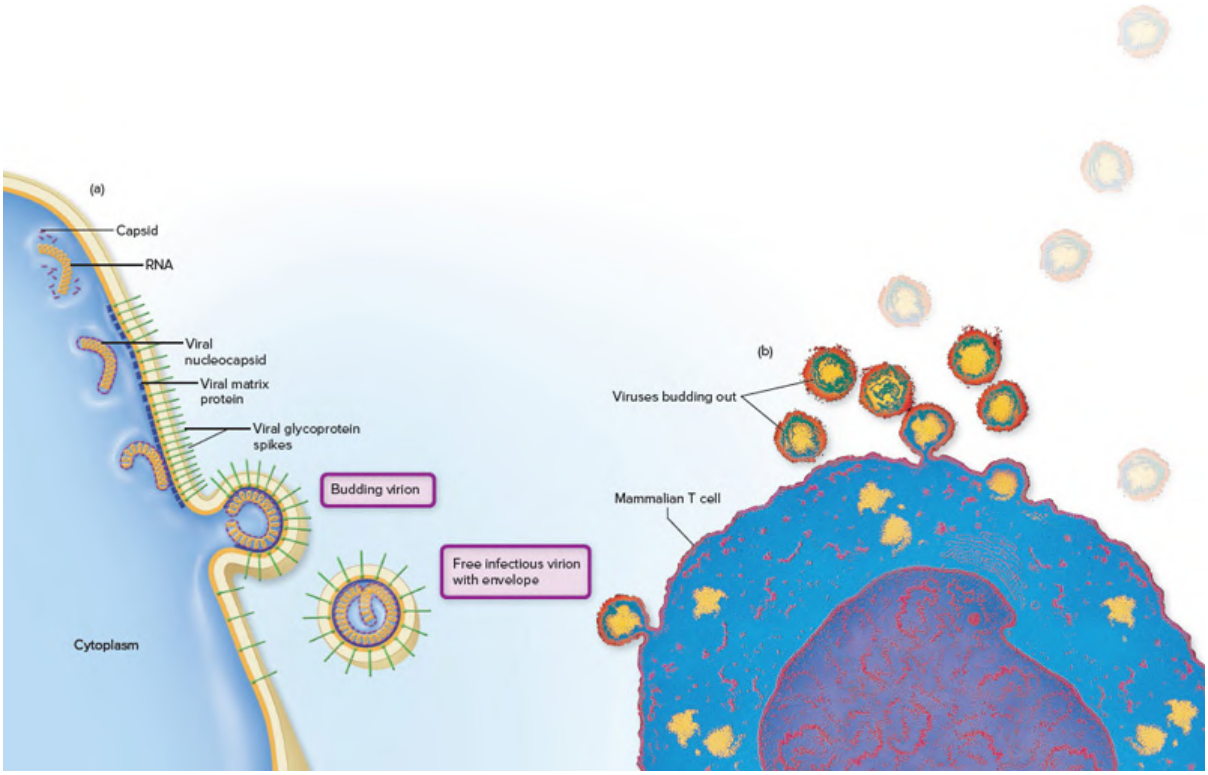


Figure 5.6 Maturation and release of enveloped viruses. **(a)** As the virus is budded off the membrane, it simultaneously picks up an envelope and spikes. **(b)** A micrograph of HIV leaving its host T cell by budding off its surface.

©Chris Bjornberg/Science Source (b)

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2. Genital herpes is an example of an infection that
- **a.** *can be present in a latent state*.
- **b.** *results from the fusion of multiple host cells into single large cells*.
- **c.** *is a provirus*.
- **d.** *causes disease only once*.

the size of the virus and the health of the host cell. About 3,000 to 4,000 virions are released from a single cell infected with poxviruses, whereas a poliovirus-infected cell can release over 100,000 virions. If even a small number of these virions happen to meet another susceptible cell and infect it, the potential for rapid viral proliferation is immense.

Damage to the Host Cell

Cytopathic effects (CPEs) are defined as virus-induced damage to the cell that changes its microscopic appearance. Individual cells can undergo obvious changes in shape or size, or develop intracellular changes **(figure 5.7*a*)**. It is common to find *inclusion bodies*, or

compacted masses of viruses or damaged cell organelles, in the nucleus and cytoplasm (**figure 5.7*b***). Examination of cells and tissues for cytopathic effects can be an important part of the diagnosis of viral infections. One very common CPE is the fusion of multiple damaged host cells into single large cells containing multiple nuclei. These are called **syncytia** (singular, *syncytium*). Sometimes they are called giant cells. They come about as a result of some viruses' ability to fuse membranes. One virus (respiratory syncytial virus) is even named for this effect.

Persistent Infections

Although accumulated damage from a virus infection kills most host cells, some cells maintain a carrier relationship, in which the cell harbors the virus and is not immediately lysed. These so-called *persistent infections* can last from a few weeks to the remainder of the host's life. Viruses can remain latent in the cytoplasm of a host cell, or can incorporate into the DNA of the host. When viral DNA is incorporated into the DNA of the host, it is called a **provirus**. One of the more serious complications occurs with the measles virus. It may remain hidden in brain cells for many years, causing progressive damage and loss of function. Several types of viruses remain in a *chronic latent state*, periodically becoming reactivated. Examples of this are herpes simplex virus (cold sores and genital herpes) and herpes

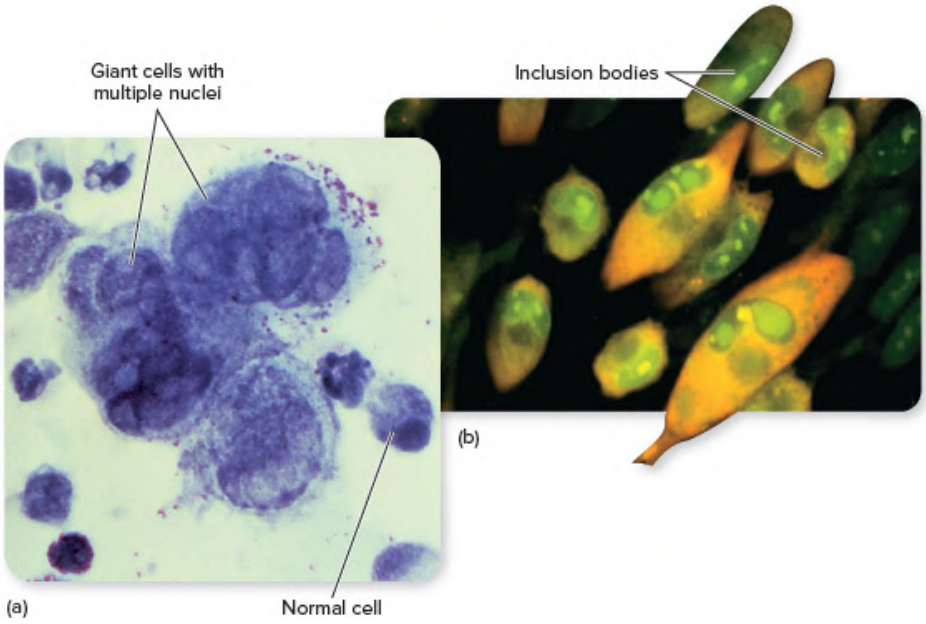


Figure 5.7 **Cytopathic changes in cells and cell cultures infected by viruses**. **(a)** Human epithelial cells infected by herpes simplex virus demonstrate giant cells with multiple nuclei. **(b)** Fluorescent-stained human cells infected with cytomegalovirus. Note the inclusion bodies (arrows). Note also that both viruses disrupt the cohesive junctions between cells, which would ordinarily be arranged side by side in neat patterns.
Source: CDC (a); Courtesy Massimo Battaglia, INeMM CNR, Rome Italy (b)

zoster virus (chickenpox and shingles). Both viruses can go into latency in nerve cells and later emerge under the influence of various stimuli to cause recurrent symptoms.

Viruses and Cancer

Some animal viruses enter a host cell and permanently alter its genetic material, leading to cancer. Experts estimate that about 13% of human cancers are caused by viruses. (The percentage is higher in developing countries.) These viruses are termed *oncogenic*, and their effect on the cell is called **transformation**. Viruses that cause cancer in animals act in several different ways, illustrated in **figure 5.8**. In some cases, the virus carries genes that directly cause the cancer. In other cases, the virus produces proteins that induce a loss of growth regulation in the cell, leading to cancer. Transformed cells have an increased rate of growth. They also have changes in their chromosomes; changes in the cell's surface molecules; and the capacity to divide for an indefinite period, unlike normal animal cells. Mammalian viruses capable of initiating tumors are called **oncoviruses**. Some of these are DNA viruses such as papillomavirus (genital warts are associated with cervical cancer), herpesviruses (one herpesvirus, Epstein-Barr virus, causes Burkitt's lymphoma), and hepatitis B virus (liver cancer). A virus related to HIV, called HTLV-I, is also involved in human cancers. These findings have spurred a great deal of speculation on the possible involvement of viruses in cancers and other diseases such as multiple sclerosis.

Viruses That Infect Bacteria

We now turn to the life cycle of another type of virus called **bacteriophage**. When Frederick Twort and Felix d'Herelle discovered bacterial viruses in 1915, it first appeared that the bacterial host cells were being eaten by some unseen parasite; hence, the name *bacteriophage* was used (*phage* coming from the Greek word for "eating"). These organisms are often referred to as "phages." Most bacteriophages contain double-stranded DNA, although single-stranded DNA and RNA types exist as well. So far as is known, every bacterial species is parasitized by one or more specific bacteriophages. Bacteriophages are of great interest to medical microbiologists because they often make the bacteria they infect more pathogenic for humans (more about this later). Probably the most widely studied bacteriophages are those of the intestinal bacterium *Escherichia coli*—especially the ones known as the T-even phages such as

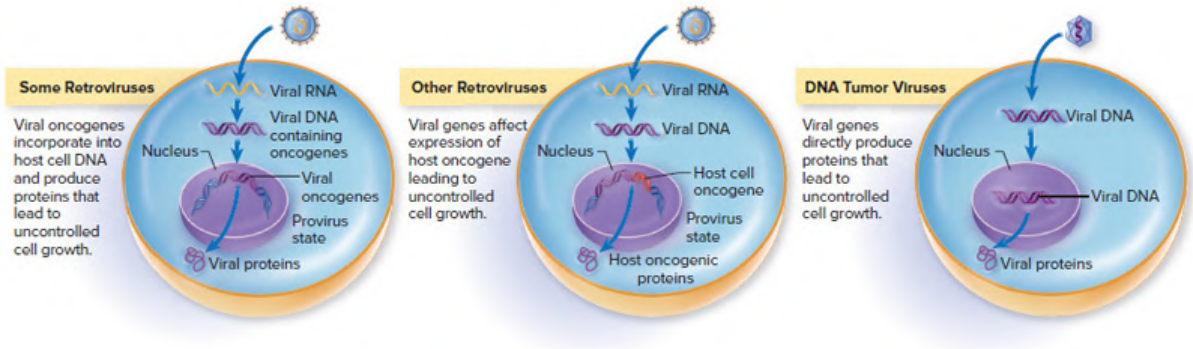


Figure 5.8 **Three mechanisms for viral induction of cancer**.

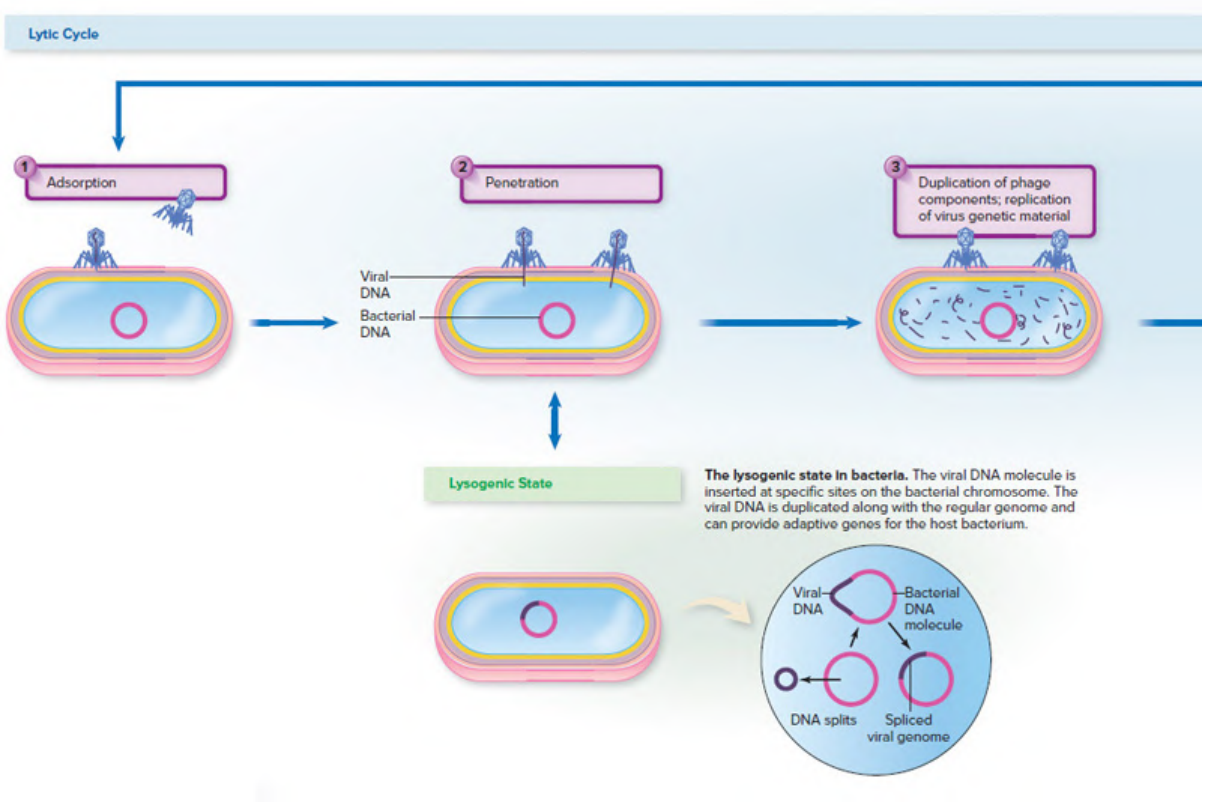


Figure 5.9 **Events in the lytic cycle of T-even bacteriophages**. The lytic cycle (1–6) involves full completion of viral infection through lysis and release of virions. Occasionally the virus enters a reversible state of lysogeny (pictured to the right) and is incorporated into the host's genetic material.

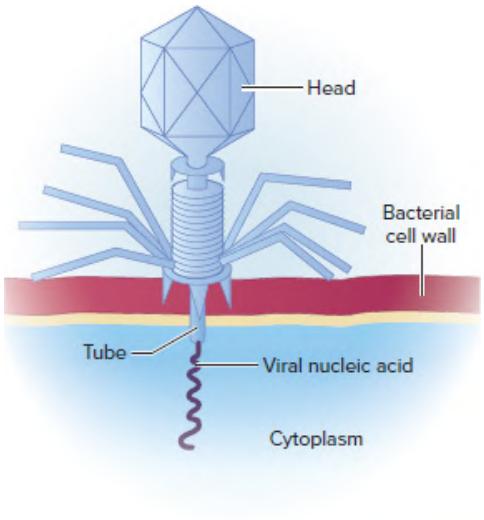
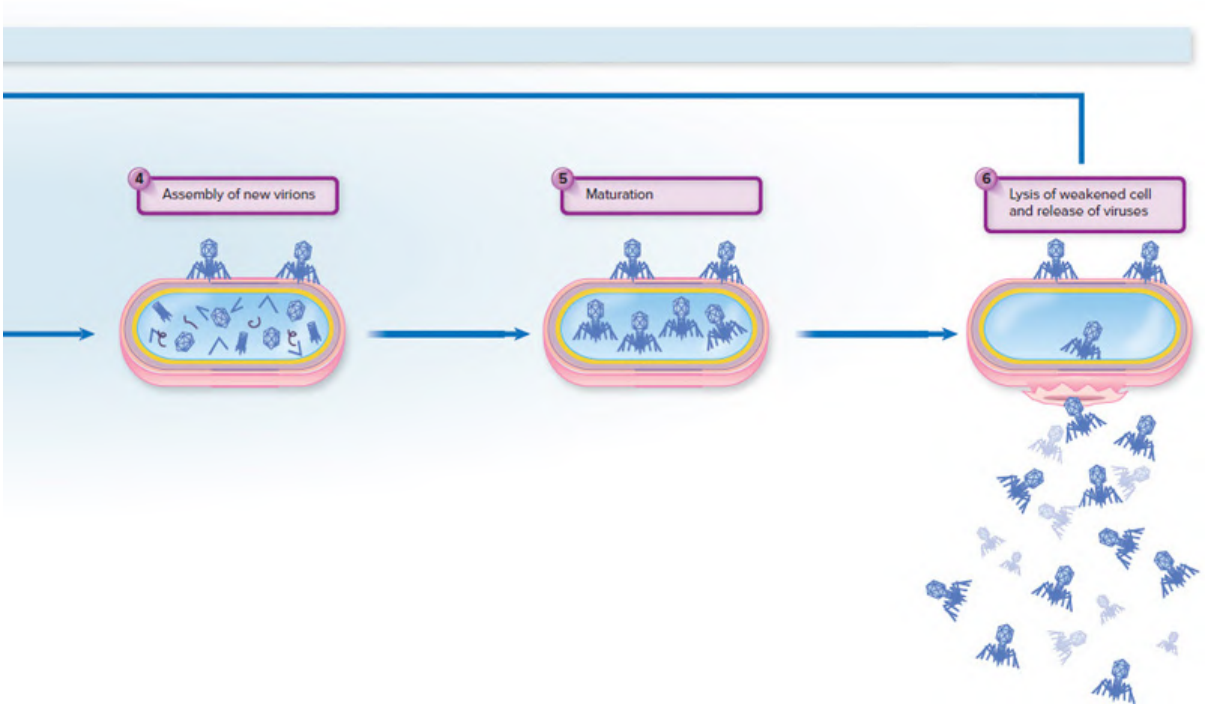


Figure 5.10 **Penetration of a bacterial cell by a T-even bacteriophage**. After adsorption, the phage plate becomes embedded in the cell wall and the sheath contracts, pushing the tube through the cell wall and releasing the nucleic acid into the interior of the cell.

T2 and T4. They have an icosahedral capsid head containing DNA, a central tube (surrounded by a sheath), collar, base plate, tail pins, and fibers, which in combination make an efficient package for infecting a bacterial cell.

T-even bacteriophages go through similar stages as the animal viruses described earlier **(figure 5.9)**. They *adsorb* to host bacteria using specific receptors on the bacterial surface. Although the entire phage does not enter the host cell, the nucleic acid *penetrates* the host after being injected through a rigid tube the phage inserts through the bacterial membrane and wall **(figure 5.10)**. This eliminates the need for *uncoating*. Entry of the nucleic acid causes host cell DNA replication and protein synthesis to stop. Soon the host cell machinery is used for viral *replication* and synthesis of viral proteins. As the host cell produces new phage parts, the parts spontaneously *assemble* into bacteriophages.

An average-size *Escherichia coli* cell can contain up to 200 new phage units at the end of this period. Eventually, the host cell becomes so packed with viruses that it **lyses**—splits open—and releases the mature virions **(figure 5.11)**. This process is hastened by viral enzymes produced late in the infection cycle that digest the cell envelope, thereby weakening it. Upon release, the virulent phages can spread to other susceptible bacterial cells and begin a new cycle of infection.



Bacteriophage infection may result in lysis of the cell, as just described. When this happens, the phage is said to have been in the *lytic* phase or cycle. Alternatively, phages can be less obviously damaging in a cycle called the *lysogenic cycle*.

In 2008, a new type of virus was discovered. These have been named *virophages*. They parasitize other viruses that are infecting the same host cell they infect, using genes from other (usually larger) viruses for their own replication and production. Even though these are parasites of viruses, note that they must be in a host cell, along with their "host" virus.

Lysogeny: The Silent Virus Infection

One category of DNA phages, called **temperate phages**, can participate in a lytic phase or in the very different *lysogenic cycle*. In this cycle they undergo adsorption and penetration into the bacterial host but then do not undergo replication or release immediately. Instead, the viral DNA enters an inactive **prophage** state, reminiscent of the provirus state in animal viruses, during which it is inserted into the bacterial chromosome. This viral DNA will be retained by the bacterial cell and copied during its normal cell division so that the cell's progeny will also have the temperate phage DNA (see figure 5.9). This condition, in which the host chromosome carries bacteriophage DNA, is termed **lysogeny** (ly-soj′-uhn-ee). Because viral particles are not produced, the bacterial cells carrying temperate phages do not lyse, and they appear entirely normal. On occasion, in a process called **induction**, the prophage in a lysogenic cell will be activated and progress directly into viral replication and the lytic cycle. Lysogeny is a less deadly form of parasitism than the full lytic cycle and is thought to be an advancement that allows the virus to spread without killing the host.

Bacteriophages are just now receiving their due as important shapers of biological life. Scientists believe that there are more bacteriophages than all other forms of life in the biosphere combined. As we mentioned in the opening paragraphs of this chapter, viral genes linger in human, animal, plant, and bacterial genomes in huge numbers. As such, viruses can contribute what are essentially permanent traits to the host cells, so much so that it could be said that all organisms are really hybrids of themselves and the viruses that infect them.

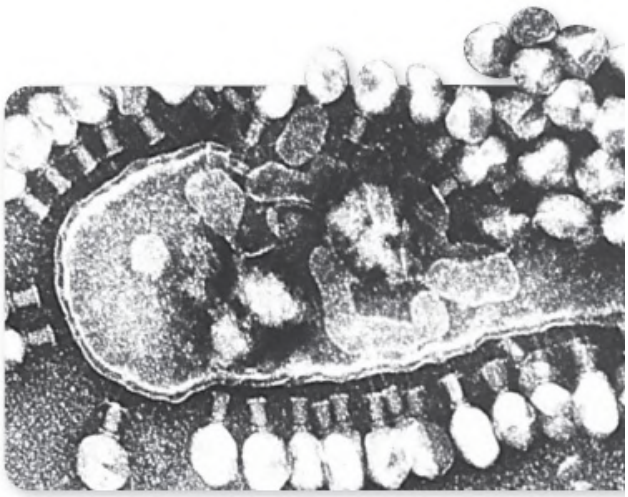


Figure 5.11 **A weakened bacterial cell, crowded with viruses**. The cell has ruptured and released numerous virions that can then attack nearby susceptible host cells. Note the empty heads of "spent" phages lined up around the ruptured wall.

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3. What diagnostic methods would be used to differentiate between bacterial and viral infections in the clinical setting? Select all that apply.
   - **a.** *determination of whether patient is experiencing nausea or vomiting*
   - **b.** *blood cultures*
   - **c.** *rapid strep testing*
   - **d.** *initiation of antibiotic therapy*
   - **e.** *CBC with differential*

The Role of Lysogeny in Human Disease

Many bacteria that infect humans are lysogenized by phages. Sometimes that is very bad news for the human: Occasionally phage genes in the bacterial chromosome cause the production of toxins or enzymes that the bacterium would not otherwise have. When a bacterium acquires a new trait from its temperate phage, it is called **lysogenic conversion**. The phenomenon was first discovered in the 1950s in the bacterium that causes diphtheria, *Corynebacterium diphtheriae*. The diphtheria toxin responsible for the deadly nature of the disease is actually a bacteriophage product. *C. diphtheriae* without the phage are harmless. Other bacteria that are made virulent by their prophages are *Vibrio cholerae*, the agent of cholera, and *Clostridium botulinum*, the cause of botulism.

5.3 LEARNING OUTCOMES—Assess Your Progress

8. Diagram the five-step life cycle of animal viruses.
9. Define the term *cytopathic effect* and provide one example.
10. Discuss both persistent and transforming infections.
11. Provide thorough descriptions of both lysogenic and lytic bacteriophage infections.

5.4 Techniques in Cultivating and Identifying Animal Viruses

In order to study viruses, it is necessary to cultivate them. This presents many problems with organisms that require living cells as their "medium." Scientists have developed methods, which include inoculation of animals and embryonic bird tissues. Methods using living embryos or animals are called *in vivo*. Another strategy is to use cells or tissues that are cultivated in the lab. These are called *in vitro* methods.

The primary purposes of viral cultivation are to

1. isolate and identify viruses in clinical specimens;

2. prepare viruses for vaccines; and
3. do detailed research on viral structure, multiplication cycles, genetics, and effects on host cells.

Using Live Animal Inoculation

Specially bred strains of white mice, rats, hamsters, guinea pigs, and rabbits are the usual choices for animal cultivation of viruses. Invertebrates (insects) or nonhuman primates are occasionally used as well. Because viruses can exhibit host specificity, certain animals can propagate a given virus more readily than others.

Avian flus often originate in parts of Southeast Asia where contact between avians and humans is commonplace.

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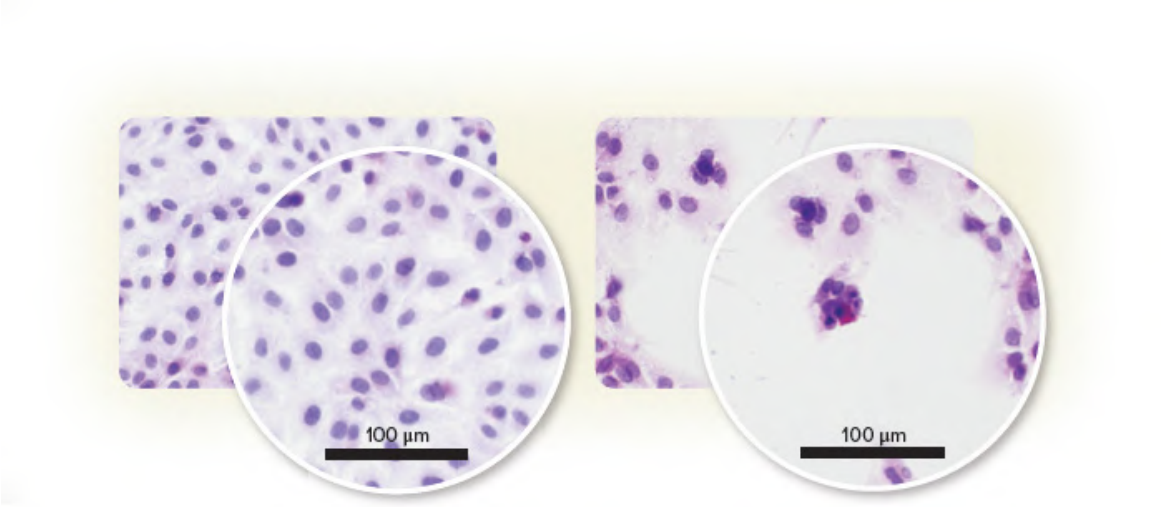

Figure 5.12 Appearance of normal and infected cell culture. Microscopic view of a layer of animal cells before infection with the appropriate virus (left), and after (right).
Source: Bakonyi T, Lussy H, Weissenböck H, Hornyák A, Nowotny N. Emerging Infectious Diseases, Vol. 11, No. 2, Feb. 2005.

Using Bird Embryos

A bird egg containing an embryo provides an intact and self-supporting unit, complete with its own sterile environment and nourishment. Furthermore, it furnishes several embryonic tissues that readily support viral multiplication. Chicken, duck, and turkey eggs are the most common choices for inoculation. The virus must be injected through the egg shell, usually by drilling a hole or making a small window.

Using Cell (Tissue) Culture Techniques

The most important early discovery that led to easier cultivation of viruses in the laboratory was the development of a simple and effective way to grow populations of isolated animal cells in culture. These types of *in vitro* cultivation systems are termed *cell culture*, or *tissue culture*. Animal cell cultures are grown in sterile chambers with special media that contain the correct nutrients required by animal cells to survive. The cultured cells grow in the form of a *monolayer*, a single, confluent sheet of cells that supports viral multiplication and permits close inspection of the culture for signs of infection **(figure 5.12)**.

One way to detect the growth of a virus in culture is to observe degeneration and lysis of infected cells in the monolayer of cells. The areas where virus-infected cells have been destroyed show up as clear, well-defined patches in the cell sheet called **plaques** (figure 5.12). Plaques are essentially the visible manifestation of cytopathic effects (CPEs), discussed earlier. This same technique is used to detect and count bacteriophages, because they also produce plaques when grown in soft agar cultures of their host cells (bacteria). A plaque develops when the viruses released by an infected host cell radiate out to adjacent host cells. As new cells become infected, they die and release more viruses, and so on. As this process continues, the infection spreads gradually and symmetrically from the original

point of infection, causing the macroscopic appearance of round, clear spaces that correspond to areas of dead cells.

Because of ongoing fears of the sudden appearance of pandemic strains of influenza virus in humans, scientists have long tried to find faster and more efficient ways to grow the vaccine strains of influenza virus, which has been grown in chicken eggs since the 1950s. Scientists have succeeded in propagating the viruses in a continuous cell line derived from dog kidney cells. In 2012, for the first time, the FDA approved for general use a cell-culture-based vaccine for the seasonal influenza virus.

5.4 LEARNING OUTCOMES—Assess Your Progress

12. List the three principal purposes of cultivating viruses.

13. Describe three ways in which viruses are cultivated.

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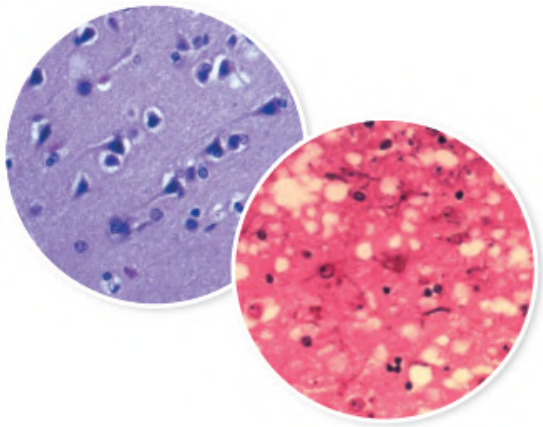
4. Which of the following is a known association between viruses and cancers?

a. *Papillomavirus causes brain cancer*.

b. *Infection with herpesvirus leads to AIDS*.

c. *Hepatitis B is associated with liver cancer*.

d. *Papillomavirus is associated with gastric cancer*.



The damage inflicted on brain tissue by Creutzfeldt-Jakob disease. Diseased tissue (*right*) shows spongelike holes not seen in healthy brains (*left*).

©M. Abbey/Science Source (left); Source: CDC/DR. Al Jenny (right)

5.5 Other Noncellular Infectious Agents

Not all noncellular infectious agents are viruses. One group of unusual forms, even smaller and simpler than viruses, can cause serious diseases in humans and animals. The diseases

are progressive and universally fatal. A common feature of each of these conditions is the deposition of distinct protein fibrils in the brain tissue. Researchers have determined that these fibrils are the agents of the disease and have named them **prions** (pree′-onz).

The first of these diseases discovered in humans was *Creutzfeldt-Jakob* disease. It afflicts the central nervous system of humans and causes gradual degeneration and death. Several animals (sheep, mink, elk) are victims of similar transmissible diseases. Bovine spongiform encephalopathy (BSE), or "mad cow disease," was the subject of fears and a crisis in Europe in the 1980s and 1990s when researchers found evidence that the disease could be acquired by humans who consumed contaminated beef. This was the first incidence of prion disease transmission from animals to humans. In 2015, a new prion disease of humans was recognized. Shy-Drager syndrome (SDS) or multiple system atrophy (MSA) resembles Parkinson's disease. It is characterized by the accumulation of a protein called alpha-synuclein in the brain, and leads to the symptoms throughout the body. Interestingly, both Parkinson's and Alzheimer's display the accumulation of protein fibrils in the brain. Researchers are investigating whether these conditions might also possibly be caused by prion infection.

Medical Moment

Differentiating Between Bacterial and Viral Infections

Viral and bacterial diseases can share many of the same symptoms. How can physicians tell the difference? This is an important question, as treatment for bacterial infections often requires antibiotic therapy, whereas treatment for viral illnesses is often supportive—antibiotic therapy is ineffective against viruses.

Many viral illnesses will cause milder symptoms than their bacterial counterpart. For example, viral meningitis is typically a less serious disease than bacterial meningitis, and viral pharyngitis is likewise a less serious condition than bacterial pharyngitis. Therefore, doctors will take into account how sick the patient is when trying to determine whether a patient is suffering from a viral or bacterial infection. They will also look at duration of symptoms, time of year, and known illnesses circulating in the community.

However, none of the above are hard and fast rules. Doctors become very proficient at recognizing various illnesses, but sometimes even they cannot tell the difference. When in doubt, blood work, cultures, and other diagnostic tests can help them make the diagnosis.

Q. Would a light microscope be useful for the diagnosis of viral infections? Why or why not?

Answer in Appendix B.

The exact mode of prion infection is currently being investigated. The fact that prions are composed primarily of protein (no nucleic acid) has certainly revolutionized our ideas of what can constitute an infectious agent. One of the most compelling questions is just how a prion could be replicated, because all other infectious agents require some nucleic acid.

There are other fascinating viruslike agents in human disease. Satellite viruses are viruslike particles that are dependent on other viruses for replication. One remarkable example is the adeno-associated virus (AAV). It was named that because it was originally thought that it could replicate only in cells infected with an adenovirus. But it can also

infect cells that are infected with other viruses. Another satellite virus, called the delta agent, is a naked circle of RNA that is expressed only in the presence of the hepatitis B virus and can worsen the severity of liver damage.

Plants are also parasitized by viruslike agents called **viroids** that differ from ordinary viruses by being very small (about one-tenth the size of an average virus) and being composed of only naked strands of RNA. They lack a capsid or any other type of coating. Viroids are significant pathogens in several economically important plants, including tomatoes, potatoes, cucumbers, citrus trees, and chrysanthemums.

5.5 LEARNING OUTCOMES—Assess Your Progress

14. Name three noncellular infectious agents besides viruses.

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5. Which of the following diseases is not caused by a noncellular infectious agent?

a. *acquired immunodeficiency syndrome*
b. *tuberculosis*
c. *smallpox*
d. *Creutzfeldt-Jakob disease*
e. *measles*

5.6 Viruses and Human Health

The number of viral infections that occur on a worldwide basis is nearly impossible to measure accurately. Certainly, viruses are extremely common causes of acute infections such as colds, hepatitis, chickenpox, influenza, herpes, and warts. If one also takes into account prominent viral infections found only in certain regions of the world, such as Dengue fever, Rift Valley fever, and yellow fever, the total could easily exceed several billion cases each year. Although most viral infections do not result in death, some, such as rabies, AIDS, and Ebola, have high mortality rates, and others can lead to long-term consequences (polio, neonatal rubella). Current research is focused on the possible connection of viruses to chronic afflictions of unknown cause, such as type 1 diabetes, multiple sclerosis, various cancers, Alzheimer's, and even obesity. Additionally, as mentioned earlier, several cancers have their origins in viral infection.

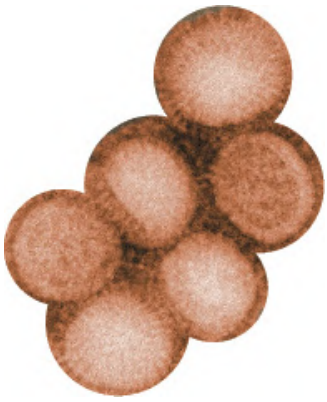
Table 5.5 provides a list of the most common viruses causing diseases in humans.

Table 5.5 Important Human Virus Families, Genera

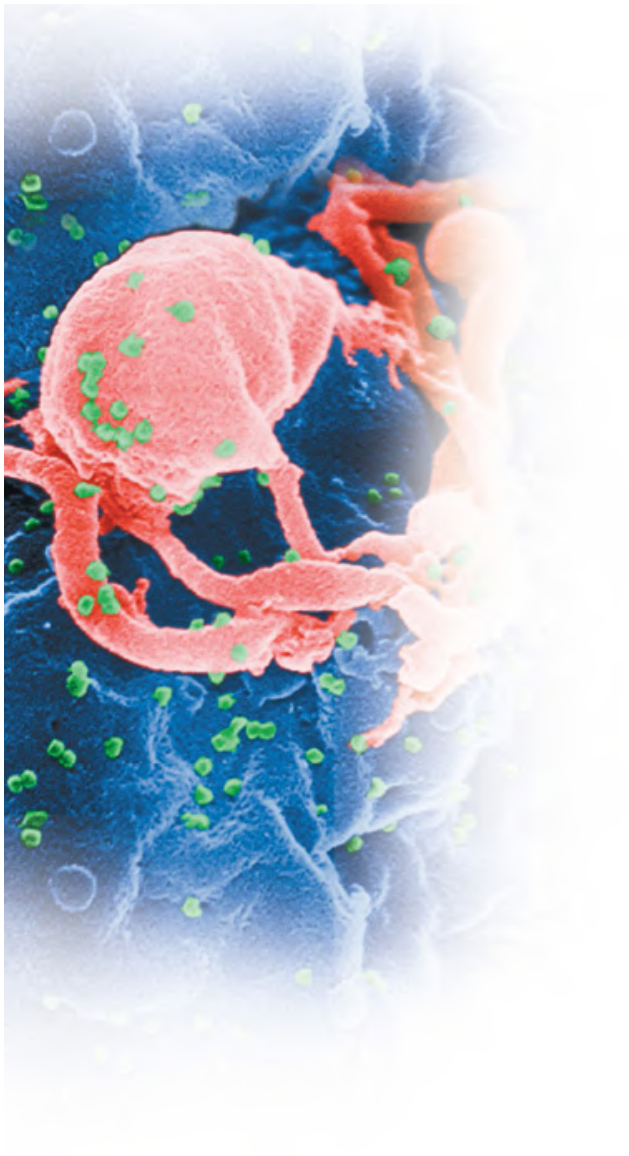
Family	Genus of Virus	Common Name of Genus Members	Name of Disease
DNA Viruses			
Poxviridae	*Orthopoxvirus*	Variola and vaccinia	Smallpox, cowpox

Family	Genus of Virus	Common Name of Genus Members	Name of Disease
Herpesviridae	*Simplexvirus*	Herpes simplex 1 virus (HSV)	Fever blister, cold sores
		Herpes simplex 2 virus (HSV)	Genital herpes
	Varicellovirus	Varicella zoster virus (VZV)	Chickenpox, shingles
	Cytomegalovirus	Human cytomegalovirus (CMV)	CMV infections
Adenoviridae	*Mastadenovirus*	Human adenoviruses	Adenovirus infection
Papovaviridae	*Papillomavirus*	Human papillomavirus (HPV)	Several types of warts
	Polyomavirus	JC virus (JCV)	Progressive multifocal leukoencephalopathy (PML)
Hepadnaviridae	*Orthohepadnavirus*	Hepatitis B virus (HBV or Dane particle)	Serum hepatitis
Parvoviridae	*Erythrovirus*	Parvovirus B19	Erythema infectiosum
RNA Viruses			
Picornaviridae	*Enterovirus*	Poliovirus	Poliomyelitis
		Coxsackie virus	Hand-foot-mouth disease
	Hepatovirus	Hepatitis A virus (HAV)	Short-term hepatitis
	Rhinovirus	Human rhinovirus	Common cold, bronchitis
Caliciviridae	*Norovirus*	Norwalk virus	Viral diarrhea, Norwalk virus syndrome
Togaviridae	*Alphavirus*	Eastern equine encephalitis virus	Eastern equine encephalitis (EEE)
		Western equine encephalitis virus	Western equine encephalitis (WEE)
		St. Louis encephalitis virus	St. Louis encephalitis
	Rubivirus	Rubella virus	Rubella (German measles)
Flaviviridae	*Flavivirus*	Dengue fever virus	Dengue fever
		West Nile fever virus	West Nile fever
		Zika virus	Zika virus disease
Coronaviridae	*Coronavirus*	Infectious bronchitis virus (IBV)	Bronchitis
		Enteric corona virus	Coronavirus enteritis
	Betacoronavirus	SARS virus	Severe acute respiratory syndrome
Filoviridae	*Ebolavirus* *Marburgvirus*	Ebola, Marburg virus	Ebola fever
Orthomyxoviridae	*Influenza A virus*	Influenza virus, type A (Asian, Hong Kong, and swine influenza viruses)	Influenza or "flu"
Paramyxoviridae	*Respirovirus* *Rubulavirus*	Parainfluenza virus, types 1–5	Parainfluenza
		Mumps virus	Mumps
	Morbillivirus	Measles virus	Measles
	Pneumovirus	Respiratory syncytial virus (RSV)	Common cold syndrome
Rhabdoviridae	*Lyssavirus*	Rabies virus	Rabies
Bunyaviridae	*Orthobunyavirus*	Bunyamwera viruses	California encephalitis
	Hantavirus	Sin Nombre virus	Respiratory distress syndrome
	Phlebovirus	Rift Valley fever virus	Rift Valley fever
	Nairovirus	Crimean–Congo hemorrhagic fever virus (CCHF)	Crimean–Congo hemorrhagic fever
Reoviridae	*Coltivirus*	Colorado tick fever virus	Colorado tick fever
	Rotavirus	Human rotavirus	Rotavirus gastroenteritis

Family	Genus of Virus	Common Name of Genus Members	Name of Disease
Retroviridae	*Deltaretrovirus*	Human T-lymphotropic virus I (HTLV-I)	T-cell leukemia
	Lentivirus	HIV (human immunodeficiency viruses 1 and 2)	Acquired immunodeficiency syndrome (AIDS)
Arenaviridae	*Arenavirus*	Lassa virus	Lassa fever



Source: CDC/C. S. Goldsmith and A. Balish



HIV virions (green) being released from the surface of an infected cell (pink).
Source: CDC/Cynthia Goldsmith

Treatment of Animal Viral Infections

The nature of viruses makes it difficult to design effective therapies against them. Because viruses are not bacteria, antibiotics aimed at disrupting bacterial cells do not work on them. Since viruses "borrow" host proteins and functions to propagate themselves, it is difficult to find drugs that will affect the virus without damaging host cells. Almost all antiviral drugs so far licensed have been designed to target one of the steps in the viral life cycle you learned about earlier in this chapter. The integrase inhibitor class of HIV drugs interrupts the ability of HIV genetic information to incorporate into the host cell DNA.

Since antiviral drugs are so much trickier to design than antibacterial drugs, scientists in the 20th century turned to vaccine development to prevent the viral diseases. This is why

nearly all of the vaccines available today are targeted at viral diseases. The 21st century will certainly see advances in antiviral drug and vaccine development.

5.6 LEARNING OUTCOMES—Assess Your Progress

15. Analyze the relative importance of viruses in human infection and disease.
16. Discuss the primary reason that antiviral drugs are more difficult to design than antibacterial drugs.

CASE FILE WRAP-UP

©DreamPictures/Pam Ostrow/Blend Images LLC (health care worker); ©Science Photo Library/Alamy Stock Photo (chest anatomy, 3D CT scan)

In my follow-up call to the family the next day, the patient's symptoms had improved. The mother found that after the steroid dose and rest, her child's breathing and activity level were returning to normal. I instructed her to ensure the fever was managed and the patient had plenty of fluids.

Croup is a respiratory illness, usually caused by a viral agent, which affects children between 6 months and 5 years of age. Numerous viruses that affect the respiratory system can cause croup, most commonly parainfluenza. Swelling occurs in the upper airway and causes the characteristic barky cough, noisy breathing, and hoarse voice. The illness is most commonly diagnosed based on clinical symptoms, and treatment of the viral illness is supportive.

A single dose of steroids is often effective in relieving symptoms of croup by reducing airway swelling. Aerosolized epinephrine is delivered directly to the upper airway by a nebulizer treatment. Inhaled epinephrine works to relax the smooth muscle of the airway and decrease secretions. Some severe cases of croup require hospitalization for a longer steroid course and airway management. Because agitation worsens the symptoms, even hospitalized patients are managed with minimal stimulation.

It is important to rule out other causes of upper airway obstruction, such as a foreign body in the airway. A thorough history of symptoms and events leading up to the illness

help provide clues to the cause. If the patient does not immediately respond to treatment, further workup is done, including chest X ray and blood work.

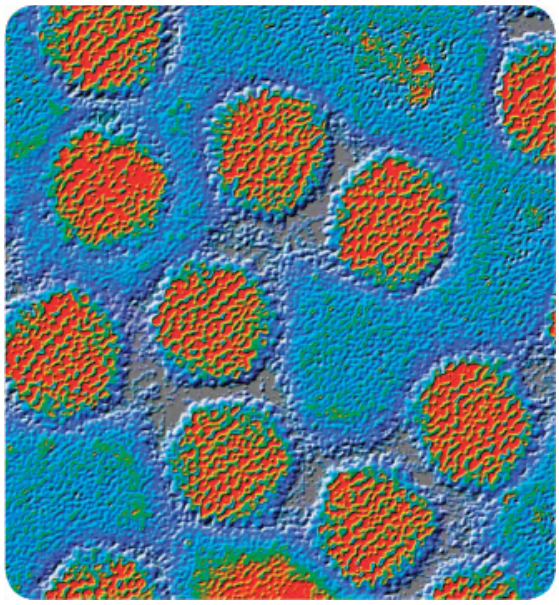
The Microbiome

Are Viruses Part of the Microbiome?

Yes. The sum total of the viruses associated with your body is called the **virome**. As you are learning, viruses must occupy cells as their homes. So the cells of your body are capable of hosting viruses. If they are part of the microbiome, and therefore part of your normal biota, they are likely to be in your cells in a dormant state, in which they are "quiet" inside your cells and not multiplying. And then there are all the viruses, called *bacteriophages*, that are infecting the bacteria that are part of your microbiome. This adds up to perhaps 10^{15} viruses as part of your microbiome. (Written out, that's 1,000,000,000,000,000.) Scientists estimate that there are between 10^{13} and 10^{14} cells of the human body and slightly more bacterial cells in and on the average human. So the viruses win in terms of numbers.

Most people are not aware of this teeming landscape in and on their body. Until rather recently, medical research was concerned only with the viruses that cause diseases —such as rabies, polio, and influenza. But sophisticated molecular techniques have been able to identify the "quiet" viruses—both those that are seemingly always quiet and those that are intermittently quiet but do become pathogenic from time to time. For example, we know that almost every adult is infected with the Epstein-Barr (EB) virus. EB virus is a herpesvirus, and virtually all people are infected with one or more of the herpesviruses. It is estimated that more than 50% of people are infected with the virus causing genital herpes, though the majority—yes, the majority—don't even know it. Most adults are infected with cytomegalovirus (CMV), which does not seem to affect adults, except to perhaps worsen illnesses with other microbes, but it can cause serious birth defects.

Having said all this, the vast majority of viruses in the human body are not pathogenic. We know that the development of the mammalian placenta was influenced by viruses. Recent research even suggests that the presence of bacteriophages in our intestinal mucosa may play a role in protecting us against bacterial infection. Because of their relatively easy movement between hosts, they have probably contributed genes from other organisms in the biosphere that have affected the development of our own physiology in significant ways.



Transmission electron micrograph of the adenovirus. It causes a wide range of human infections, but is so common that it is probably also a part of the human microbiome.

©Science Photo Library/Getty Images

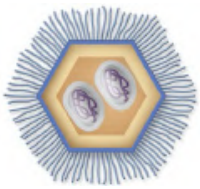
Chapter Summary

5.1 The Position of Viruses in the Biological Spectrum

- Viruses are noncellular entities whose properties have been identified through technological advances in microscopy and tissue culture.
- Viruses are infectious particles that invade every known type of cell. They are not alive, yet they are able to redirect the metabolism of living cells to reproduce virus particles.
- Viruses have a profound influence on the genetic makeup of the biosphere.
- The International Committee on the Taxonomy of Viruses oversees naming and classification of viruses. Viruses are classified into orders, families, and genera.
- Viruses are grouped in various ways. This textbook uses their structure, genetic composition, and host range to categorize them.

5.2 The General Structure of Viruses

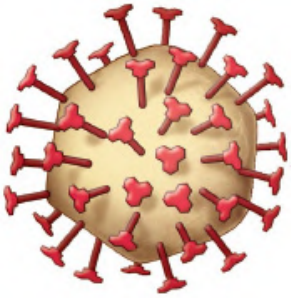
- Virus size range is from 20 to 1,500 nm (diameter). Viruses are composed of an outer protein capsid enclosing either DNA or RNA plus a variety of enzymes. Some viruses also possess an envelope around the capsid.
- Spikes on the surface of the virus capsid or envelope are critical for their attachment to host cells.



5.3 Modes of Viral Multiplication

- Viruses go through a multiplication cycle that generally involves adsorption, penetration (sometimes followed by uncoating), viral synthesis and assembly, and viral release by lysis or budding.
- These events turn the host cell into a factory solely for making and shedding new viruses. This results in the ultimate destruction of the cell.
- Animal viruses can cause acute infections or can persist in host tissues as chronic latent infections that can reactivate periodically throughout the host's life. Some persistent animal viruses can cause cancer.

- Bacteriophages vary significantly from animal viruses in their methods of adsorption, penetration, site of replication, and method of exit from host cells.
- Lysogeny is a condition in which viral DNA is inserted into the bacterial chromosome and remains inactive for an extended period. The viral DNA is replicated with the chromosome every time the bacterium divides.
- Some bacteria express virulence traits that are coded for by the bacteriophage DNA in their chromosomes. This phenomenon is called lysogenic conversion.



5.4 Techniques in Cultivating and Identifying Animal Viruses

- Animal viruses must be studied in some sort of living cell or tissue.
- Cell and tissue cultures are cultures of host cells grown in special sterile chambers using aseptic techniques to exclude unwanted microorganisms.
- Virus growth in cell culture and bacteriophage growth on bacterial lawns are detected by the appearance of plaques.

5.5 Other Noncellular Infectious Agents

- Other noncellular agents of disease are the prions, which are not viruses at all but protein fibers; viroids, extremely small lengths of naked nucleic acid; and satellite viruses, which require the presence of larger viruses to cause disease.

5.6 Viruses and Human Health

- Viruses are easily responsible for several billion infections each year. It is conceivable that many chronic diseases of unknown cause will eventually be connected to viral agents.
- Viral infections are difficult to treat because the drugs that attack viral replication also cause side effects in the host.

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	**A. Bloom's Level 1, 2—Remember and Understand (Choose one.)**	**B. Bloom's Level 3, 4—Apply and Analyze**	**C. Bloom's Level 5, 6—Evaluate and Create**
Evolution	**1**. ___% of human DNA is thought to consist of viral DNA sequences. **a**. 0.08 **b**. 8 **c**. 1 **d**. 45	**2**. Discuss the influence that viruses have on the idea of "species" in bacteria.	**3**. Construct a scenario in which viral latency and lysogeny provide an evolutionary advantage to viruses.
Cell Structure and Function	**4**. The host cells that viruses can infect is determined by the **a**. receptors on the host cells. **b**. DNA in host cells. **c**. proximity of host cells. **d**. concentration of host cells in vicinity.	**5**. If viruses that normally form envelopes were prevented from budding, would they still be infectious? Why or why not?	**6**. Viruses use the host cell cytoplasmic space as their "factories" and their enzymes and other macromolecules as their "tools." Does that make them more sophisticated or less sophisticated than cells? Justify your answer.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Metabolic Pathways	**7**. The general steps in a viral multiplication cycle are **a**. adsorption, penetration, synthesis, assembly, and release. **b**. endocytosis, uncoating, replication, assembly, and budding. **c**. adsorption, uncoating, duplication, assembly, and penetration. **d**. endocytosis, penetration, replication, maturation, and exocytosis.	**8**. Compare and contrast the processes of latency and lysogeny in viruses.	**9**. Pathogenic bacteria lysogenized by phages can cause more serious disease than their counterparts that are not lysogenized. Speculate on whether it is to the bacterium's advantage to cause more serious disease, or to cause less serious disease.

Information Flow and Genetics	**10**. When phage nucleic acid is incorporated into the nucleic acid of its host cell and is replicated when the host DNA is replicated, this is considered	**11**. Describe the cell locations in which RNA and DNA viruses multiply.	**12**. RNA viruses tend to mutate—that is, to accumulate changes in their nucleic acid sequences—more frequently than any other organism. Speculate on

	part of which cycle? **a**. lytic cycle **b**. virulence cycle **c**. lysogenic cycle **d**. cell cycle **e**. multiplication cycle		why that might be and how that can be a positive trait if the virus is a human pathogen.
Microbial Systems	**13**. A virus that undergoes lysogeny is a/an **a**. temperate phage. **b**. intemperate phage. **c**. T-even phage. **d**. animal virus. **e**. DNA virus.	**14**. In figure 4.11, viruses are suggested to have contributed to cellular nucleic acid becoming DNA instead of RNA. What stages in the viral life cycle could have led to this phenomenon?	**15**. Imagine and describe a scenario in which viruses in the human microbiome could exert a positive influence on human health.
Impact of Microorganisms	**16**. Clear patches in cell cultures that indicate sites of virus infection are called **a**. plaques. **b**. pocks. **c**. colonies. **d**. prions.	**17**. Name and discuss one or more medical procedures or treatments that use viruses to benefit human health.	**18**. Construct an argument for whether humans or viruses have had more of an impact on the planet earth as it exists today.
Scientific Thinking	**19**. Since 2000, the number of orders of viruses recognized by the	**20**. Write a paragraph targeted at middle-school students about why	**21**. The earliest drugs developed to treat HIV disease were “anti-reverse-transcriptase”

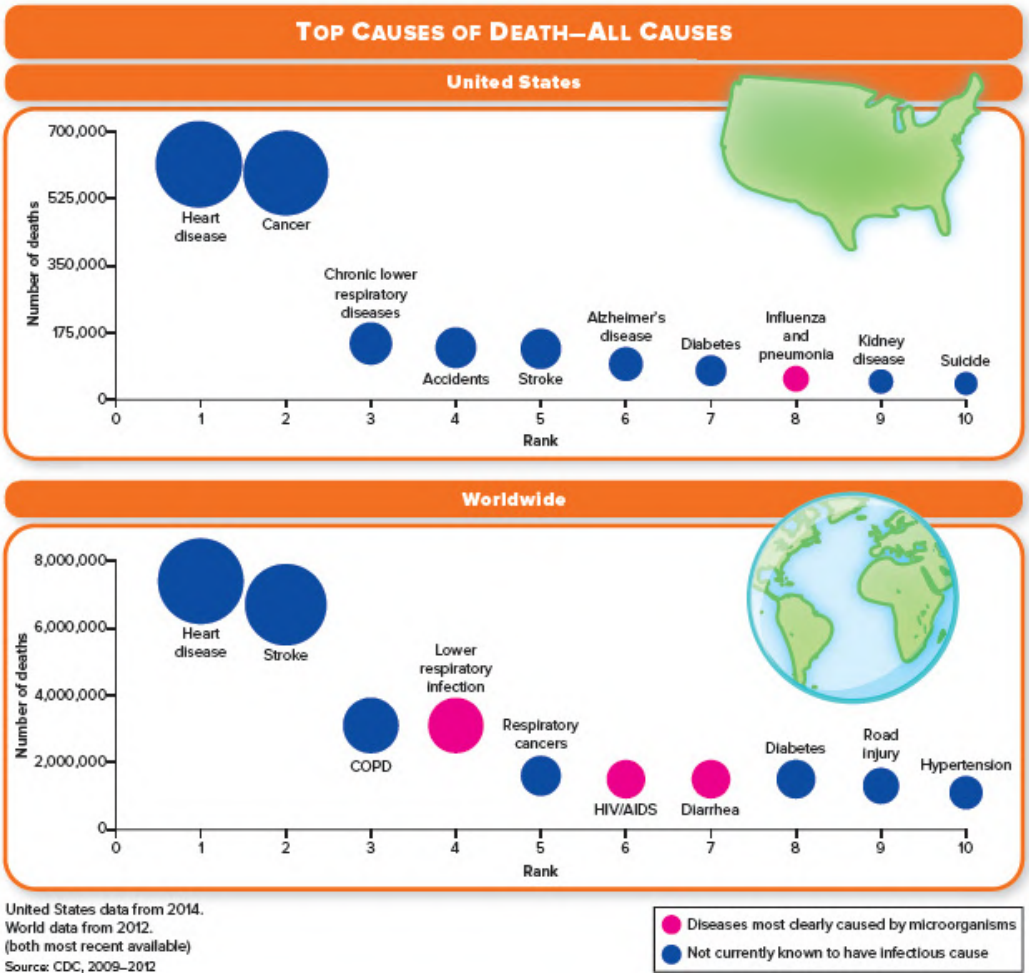
	International Committee on the Taxonomy of Viruses has risen from ___ to ___. **a**. 5, 10 **b**. 20, 50 **c**. 1, 8 **d**. 250, 750	antibiotics do not work on viruses, and why it is difficult to design antiviral agents.	agents. Explain why these would be effective and relatively safe.

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From chapter 1, table 1.1**. This chart from chapter 1 identified diseases most clearly caused by microorganisms. Considering what you have learned in this chapter, are there more deaths caused by microorganisms than might be accounted for by the diseases in pink? Can you make a rough guess of how many total deaths might be caused by viruses?



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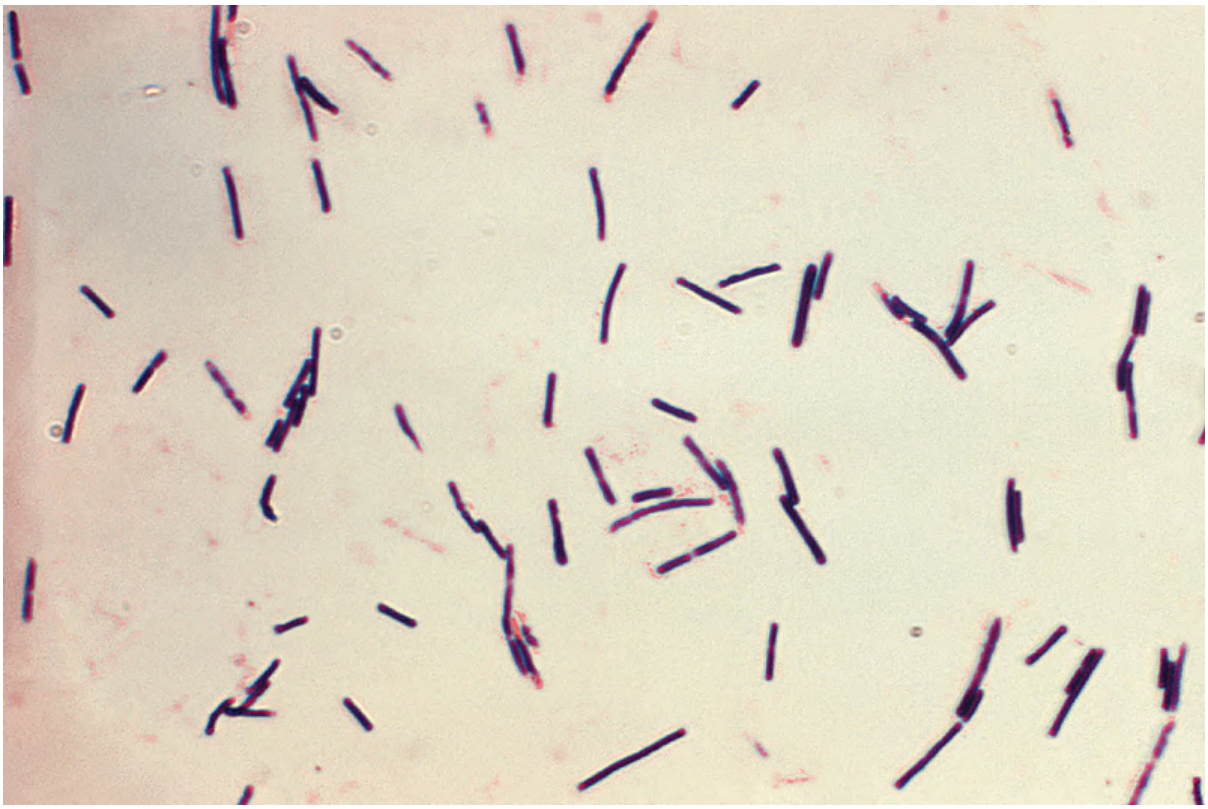
Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

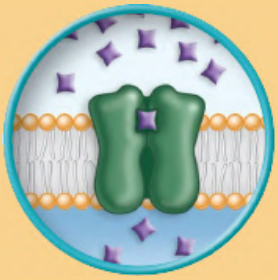
6

Microbial Nutrition and Growth



Source: CDC/Don Stalons (*C. perfringens*); ©Terese M. Barta, Ph.D. (tubes); ©GagliardiImages/Shutterstock (health care worker)

IN THIS CHAPTER…



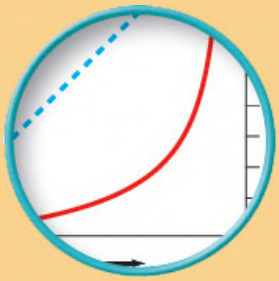
6.1 Microbial Nutrition

1. List the essential nutrients of a bacterial cell.
2. Differentiate between macronutrients and micronutrients.

3. List and define four different terms that describe an organism's sources of carbon and energy.
4. Define *saprobe* and *parasite*, and explain why these terms can be an oversimplification.
5. Compare and contrast the processes of diffusion and osmosis.
6. Identify the effects of isotonic, hypotonic, and hypertonic conditions on a cell.
7. Name two types of passive transport and one type of active transport.

6.2 Environmental Factors That Influence Microbes

8. List and define five terms used to express the temperature-related growth capabilities of microbes.
9. Summarize three ways in which microorganisms function in the presence of differing oxygen conditions.
10. Identify three important environmental factors (other than temperature and oxygen) with which organisms must cope.
11. List and describe the five types of associations microbes can have with their hosts.
12. Discuss characteristics of biofilms that differentiate them from planktonic bacteria.



6.3 The Study of Bacterial Growth

13. Summarize the steps of cell division used by most bacteria.
14. Define *doubling time*, and describe how it leads to exponential growth.
15. Compare and contrast the four phases of growth in a bacterial growth curve.
16. Identify one culture-based and one non-culture-based method used for analyzing bacterial growth.

CASE FILE

Wound Care

I was an RN working in a large city hospital on a medical floor. A lot of our patients had diabetes and were suffering various complications of the disease, particularly diabetic wounds caused by poor circulation. Wound care was a large part of my job. After 2 years on the unit, I decided to pursue wound care certification. Once I became a wound care specialist, I continued to work in the same hospital and saw patients with complicated and/or chronic wounds.

Mr. Jones was one of the first patients I consulted about after I became certified. He was an elderly gentleman who had lost his sight due to diabetes. When I met Mr. Jones, he had a chronic wound on his lower leg that had been present for months. The wound was circumferential, taking up half of his lower leg. It was also grossly infected. Mr. Jones had been admitted to the hospital for antibiotics to treat his infection. It was clear that if the antibiotics failed to improve his wound, Mr. Jones was in danger of losing his leg.

Within a day of admission, we realized that antibiotic therapy alone was not going to be enough. Mr. Jones developed signs of gas gangrene. Wound cultures were positive for *Clostridium perfringens*, which produces toxins that destroy muscle tissue and results in sepsis and death if untreated.

Mr. Jones was taken immediately to surgery where his wound was debrided, meaning that dead or devitalized tissue was removed. Following surgery, he was given large doses of penicillin in an effort to stop the spread of the infection.

The next day, Mr. Jones was started on daily hyperbaric oxygen therapy, with sessions lasting for 45 minutes. Slowly, his wound began to improve. The wound was debrided twice more under anesthesia, and the patient remained on antibiotics until wound cultures came back free of *C. perfringens*. Although the wound took several months to heal, Mr. Jones kept his leg.

- What is hyperbaric oxygen therapy, and why is it used to treat wounds infected with *C. perfringens*?
- Is *C. perfringens* an aerobe or an anaerobe?

Case File Wrap-Up appears at the end of the chapter.

6.1 Microbial Nutrition

With respect to nutrition, microbes are not really so different from humans. All organisms require a constant influx of certain substances from their habitat. Even bacteria living in mud on a diet of inorganic sulfur, or protozoa digesting wood in a termite's intestine, have some basic nutritional needs. In general, all living things require a source of elements such as carbon, hydrogen, oxygen, phosphorus, potassium, nitrogen, sulfur, calcium, iron, sodium, chlorine, and magnesium. But the ultimate source of a particular element, its chemical form, and how much of it the microbe needs are all points of variation between different types of organisms.

Any substance that must be provided to an organism is called an **essential nutrient**. Two categories of essential nutrients are **macronutrients** and **micronutrients**. Macronutrients are required in relatively large quantities and play principal roles in cell structure and metabolism. Examples of macronutrients are carbon, hydrogen, and oxygen. Micronutrients, or **trace elements**, such as manganese, zinc, and nickel, are present in much smaller amounts and are involved in enzyme function and maintenance of protein structure.

Another way to categorize nutrients is according to their carbon content. An inorganic nutrient is an atom or simple molecule that contains a combination of atoms other than carbon and hydrogen. The natural reservoirs of inorganic compounds are mineral deposits in the crust of the earth, bodies of water, and the atmosphere. Examples include metals and their salts (magnesium sulfate, ferric nitrate, sodium phosphate), gases (oxygen, carbon dioxide), and water. In contrast, the molecules of organic nutrients contain carbon and hydrogen atoms and are usually the products of living things. They range from the simplest organic molecule, methane (CH_4), to large polymers (carbohydrates, lipids, proteins, and nucleic acids). The source of nutrients is extremely varied: Some microbes obtain their nutrients entirely from inorganic sources, and others require a combination of organic and inorganic sources.

The pristine waters of this beautiful coral reef depend on keeping microbial nutrients very low so that harmful bacteria are not able to outcompete phytoplankton or cause coral diseases.

©Michael Aw/Photodisc/Getty Images

Chemical Analysis of Microbial Cytoplasm

Table 6.1 lists the major contents of the bacterium *Escherichia coli*. Some of these components are absorbed in a ready-to-use form, and others must be synthesized by the cell from simple nutrients. The important features of cell composition can be summarized as follows:

- Water is the most abundant of all the components (70%).
- Proteins are the next most prevalent chemical.
- About 97% of the dry cell weight is composed of organic compounds.
- About 96% of the dry cell weight is composed of just six elements (represented by CHONPS and shown later in table 6.3).
- Chemical elements are needed in the overall scheme of cell growth, but most of them are available to the cell as compounds and not as pure elements.
- A cell as "simple" as *E. coli* contains on the order of 5,000 different compounds, yet it needs to absorb only a few types of nutrients to synthesize this great diversity.

What Microbes Eat

The earth's limitless habitats and microbial adaptations make for an elaborate menu of microbial nutritional schemes. Fortunately, most organisms show consistent trends and can be described by a few general categories **(table 6.2)** and a few selected terms. To keep these straight you should always remember that the main determinants of a microbe's nutritional type are its source of carbon and its source of energy.

We'll start with an organism's *carbon source:* In this regard, microbes are either **heterotrophs** or **autotrophs**. A heterotroph is an organism that must obtain its carbon

©McGraw-Hill Education/Barry Barker, photographer

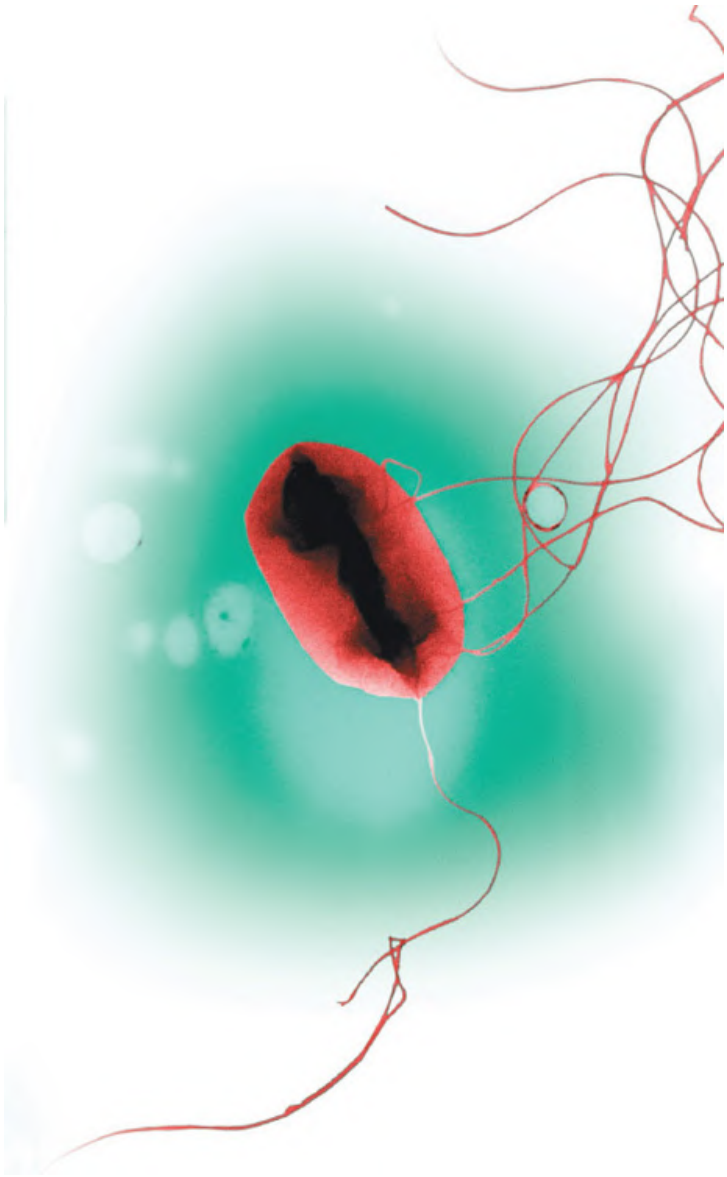
Table 6.1 The Chemical Composition of an *Escherichia coli* Cell

	% Dry Weight		% Dry Weight
Organic Compounds		**Elements**	
Proteins	50	Carbon (C)	50
Nucleic Acids		Oxygen (O)	20
RNA	20	Nitrogen (N)	14
DNA	3	Hydrogen (H)	8
Carbohydrates	10	Phosphorus (P)	3

	% Dry Weight		% Dry Weight
Lipids	10	Sulfur (S)	1
Miscellaneous	4	Potassium (K)	1
Inorganic Compounds		Sodium (Na)	1
Water	(—)	Calcium (Ca)	0.5
All others	3	Magnesium (Mg)	0.5
		Chlorine (Cl)	0.5
		Iron (Fe)	0.2
		Trace metals	0.3

in an organic form. An autotroph ("self-feeder") is an organism that uses inorganic CO_2 as its carbon source. Because autotrophs have the special capacity to convert CO_2 into organic compounds, they are not nutritionally dependent on other living things.

The next way that microbes are categorized is via their *energy source*. They are either **phototrophs** or **chemotrophs**. Microbes that photosynthesize (create energy from sunlight) are phototrophs, and those that get their energy from chemical compounds are chemotrophs.



Escherichia coli
Source: CDC/Peggy S. Hayes & Elizabeth H. White, M.S.

Table 6.2 Nutritional Categories of Microbes by Energy and Carbon Source

Category	Energy Source	Carbon Source	Example
Autotroph			
Photoautotroph	Sunlight	CO_2	Photosynthetic organisms, such as algae, plants, cyanobacteria
Chemoautotroph			
Chemoorganic autotrophs	Organic compounds	CO_2	Methanogens
Chemolithoautotrophs	Inorganic compounds (minerals)	CO_2	*Thiobacillus*, "rock-eating" bacteria
Heterotroph			
Photoheterotroph	Sunlight	Organic	Purple and green photosynthetic bacteria

Category	Energy Source	Carbon Source	Example
Chemoheterotroph	Metabolic conversion of the nutrients from other organisms	Organic	Protozoa, fungi, many bacteria, animals
Saprobe	Metabolizing the organic matter of dead organisms	Organic	Fungi, bacteria (decomposers)
Parasite	Utilizing the tissues, fluids of a live host	Organic	Various parasites and pathogens; can be bacteria, fungi, protozoa, animals

The terms for carbon and energy source are often merged into a single word for convenience. The categories described here are meant to describe only the major nutritional groups and do not include unusual exceptions. **Figure 6.1** illustrates two examples.

Autotrophs and Their Energy Sources

Autotrophs—that is, organisms that get their carbon from CO_2—derive energy from one of two possible nonliving sources: sunlight and chemical reactions involving simple chemicals. When they get their energy from sunlight they are called photoautotrophs, and when they get it from chemicals they are called chemoautotrophs. **Photoautotrophs** are photosynthetic—that is, they capture the energy of light rays and transform it into chemical energy that can be used in cell metabolism. Because photosynthetic organisms (algae, plants, some bacteria) produce organic molecules that can be used by themselves and by heterotrophs, they form the basis for most food webs.

Chemoautotrophs are of two types: One of these is the group called chemoorganic autotrophs. These use organic compounds for energy and inorganic compounds as a carbon source. The second type of chemoautotroph is a group called **lithoautotrophs**, which require neither sunlight nor organic nutrients, relying totally on inorganic minerals. These bacteria derive energy in diverse and rather amazing ways. In very simple terms, they remove electrons from inorganic substrates—such as hydrogen gas, hydrogen sulfide, sulfur, or iron—and combine them with carbon dioxide and hydrogen.

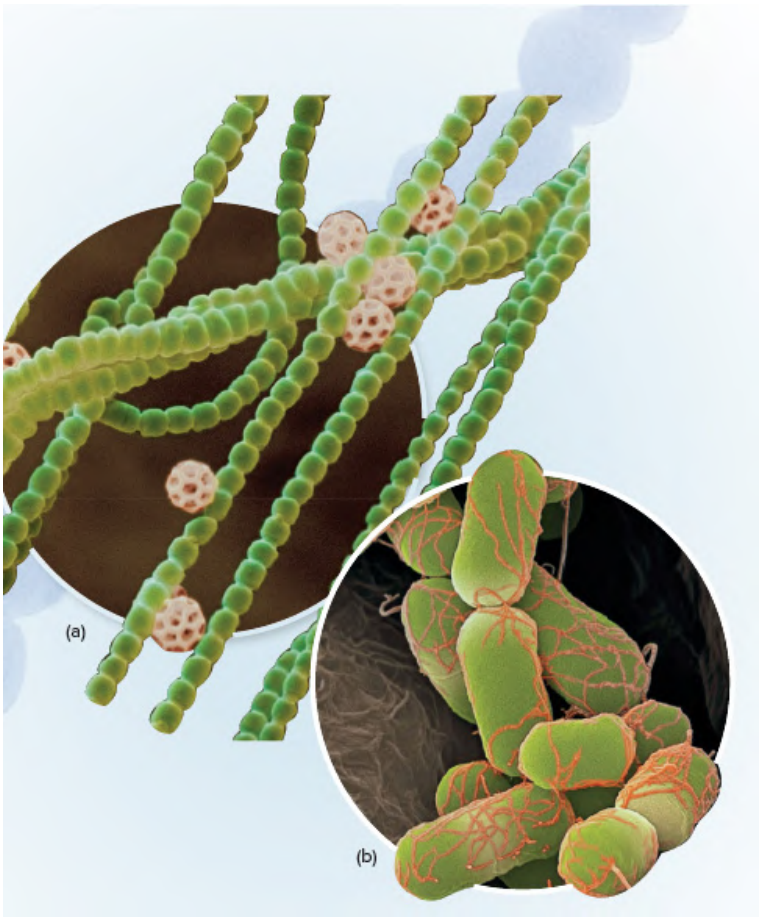


Figure 6.1 **A photoautotroph and a chemoheterotroph**. **(a)** *Cyanobacterium*, in green, a photosynthetic autotroph. **(b)** *Escherichia coli*, a chemoheterotroph.
(a) ©Steve Gschmeissner/Getty Images; (b) ©Martin Oeggerli/Science Source

Heterotrophs and Their Energy Sources

The majority of heterotrophic microorganisms are **chemoheterotrophs** that derive both carbon and energy from organic compounds. Processing these organic molecules by cellular respiration or fermentation releases energy in the form of ATP. Chemoheterotrophic microorganisms belong to one of two main categories that differ in how they obtain their organic nutrients: Saprobes are free-living microorganisms that feed primarily on organic detritus from dead organisms, and parasites ordinarily derive nutrients from the cells or tissues of a living host.

Saprobes occupy a niche as decomposers of plant litter, animal matter, and dead microbes. If not for the work of these decomposers, the earth would gradually fill up with organic material, and the nutrients it contains would not be recycled.

Parasites live in or on the body of a host, which they harm to some degree. Because many parasites cause damage to tissues (disease) or even death, they are also called *pathogens*. Parasites range from viruses to helminths (worms), and they can live on the body (ectoparasites), in the organs and tissues (endoparasites), or even within cells (intracellular parasites, the most extreme type). *Obligate parasites* (for example, the leprosy bacillus and the syphilis spirochete) are unable to grow outside of a living host. Parasites that have less strict requirements can be cultured artificially if provided with the correct nutrients and environmental conditions. Bacteria such as

Streptococcus pyogenes (the cause of strep throat) and *Staphylococcus aureus* can grow on artificial media.

The binary "saprobe" versus "parasite" distinction is a bit of a simplification, which we will address in a later section. For now, just note that the vast majority of microbes causing human disease are chemoheterotrophs.

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1. A saprobe derives its energy from
- **a.** *sunlight*.
- **b.** *conversion of nutrients from other organisms*.
- **c.** *utilizing the tissues/fluids of a living host*.
- **d.** *metabolizing the organic matter of dead organisms*.

NCLEX® PREP

2. Mineral ions used in microbial nutrition include
- **a.** *sodium*.
- **b.** *potassium*.
- **c.** *calcium*.
- **d.** *magnesium*.
- **e.** *all of these*.

Essential Nutrients

Chemicals that are necessary for particular organisms, which they cannot manufacture by themselves, are called *essential*. For microbes, the essential nutrients are carbon, hydrogen, oxygen, nitrogen, phosphate, and sulfur. Biologists remember these elements with the acronym CHONPS **(table 6.3)**.

Table 6.3 Essential Nutrients—Where They Can Be Obtained

Carbon	Among the common organic molecules that can satisfy this requirement are proteins, carbohydrates, lipids, and nucleic acids. In most cases, these molecules provide several other nutrients as well.
Hydrogen	Hydrogen is a major element in all organic and several inorganic compounds, including water (H_2O), salts ($Ca[OH]_2$), and certain naturally occurring gases (H_2S, CH_4, and H_2). These gases are both used and produced by microbes. Hydrogen helps cells maintain their pH, is useful for forming hydrogen bonds between molecules, and also serves as a source of free energy in respiration.
Oxygen	Because oxygen is a major component of organic compounds such as carbohydrates, lipids, nucleic acids, and proteins, it plays an important role in the structural and enzymatic functions of the cell. Oxygen is likewise a common component of inorganic salts such as sulfates, phosphates, nitrates, and water. Free gaseous oxygen (O_2) makes up 20% of the atmosphere.
Nitrogen	The main reservoir of nitrogen is nitrogen gas (N_2), which makes up 79% of the earth's atmosphere. This element is indispensable to the structure of proteins, DNA, RNA, and ATP. Such compounds are the primary nitrogen source for heterotrophs, but to be useful, they must first be degraded into their basic building blocks (proteins into amino acids; nucleic acids into nucleotides). Some bacteria and algae utilize inorganic nitrogenous nutrients (NO_3^-, NO_2^-, or NH_3). A small number of bacteria and archaea can transform N_2 into compounds usable by other organisms

	through the process of nitrogen fixation. Regardless of the initial form in which the inorganic nitrogen enters the cell, it must first be converted to NH_3, the only form that can be directly combined with carbon to synthesize amino acids and other compounds.
Phosphate	The main inorganic source of phosphorus is phosphate (PO_4^{3-}), derived from phosphoric acid (H_3PO_4) and found in rocks and oceanic mineral deposits. Phosphate is a key component of nucleic acids and is therefore essential to the genetics of cells and viruses. Because it is also found in ATP, it serves in cellular energy transfers. Other phosphate-containing compounds are phospholipids in cytoplasmic membranes and coenzymes such as NAD^+.
Sulfur	Sulfur is widely distributed throughout the environment in mineral form. Rocks and sediments (such as gypsum) can contain sulfate (SO_4^{2-}), sulfides (FeS), hydrogen sulfide gas (H_2S), and elemental sulfur (S). Sulfur is an essential component of some vitamins (vitamin B_1) and the amino acids methionine and cysteine; the latter help determine shape and structural stability of proteins by forming unique linkages called disulfide bonds.

Other Important Nutrients

Mineral ions are also important components in microbial metabolism. Potassium is essential to protein synthesis and membrane function. Sodium is important for certain types of cell transport. Calcium is a stabilizer of the cell wall and endospores of bacteria. Magnesium is a component of chlorophyll and a stabilizer of membranes and ribosomes. Iron is an important component of the cytochrome proteins of cell respiration. Zinc is an essential regulatory element for eukaryotic genetics. It is a major component of "zinc fingers"—binding factors that help enzymes adhere to specific sites on DNA. Copper, cobalt, nickel, molybdenum, manganese, silicon, iodine, and boron are needed in small amounts by some microbes but not others. On the other hand, in chapter 9 you will see that metals can also be very toxic to microbes. The concentration of metal ions can even influence the diseases microbes cause. For example, the bacteria that cause gonorrhea and meningitis grow more rapidly in the presence of iron.

How Microbes Eat: Transport Mechanisms

A microorganism's habitat provides necessary nutrients—some abundant, others scarce. But they must still be taken into the cell. Survival also requires that cells transport waste materials out of the cell and into the environment. Whatever the direction, transport occurs across the cytoplasmic membrane, the structure specialized for this role. This is true even in organisms with cell walls (bacteria, algae, and fungi), because the cell wall is usually too nonselective to screen the entrance or exit of molecules.

The driving force of transport is atomic and molecular movement—the natural tendency of atoms and molecules to be in constant random motion. This phenomenon of molecular movement, in which atoms or molecules move in a gradient from an area of higher density or concentration to an area of lower density or concentration, is **diffusion**.

Medical Moment

Osmosis and IV Fluids

The provision of intravenous (IV) solutions is a very common practice in medicine. Keeping in mind that the osmotic movement of water occurs as the body attempts to create a balance between the different solute concentrations that exist on either side of a semipermeable membrane, let's look at different types of IV solutions commonly used in medicine.

Isotonic solutions have a tonicity that is the same as the body's plasma. When isotonic solutions are administered, there will be very little movement, if any, between the body tissues and the blood vessels.

Hypertonic solutions have a tonicity that is higher than the body plasma. Administering hypertonic solutions will cause water to shift from the extravascular spaces into the bloodstream to increase the intravascular volume. This is how the body attempts to dilute the higher concentration of electrolytes in the IV fluid.

Hypotonic solutions have a tonicity that is lower than the body plasma, causing water to shift from the intravascular to the extravascular space, and eventually into the cells of the tissues. In this case, the body moves water from the intravascular space to the cells in order to dilute the electrolytes in the cells.

Q. In this situation, what structure is acting as the semipermeable membrane?

Answer in Appendix B.

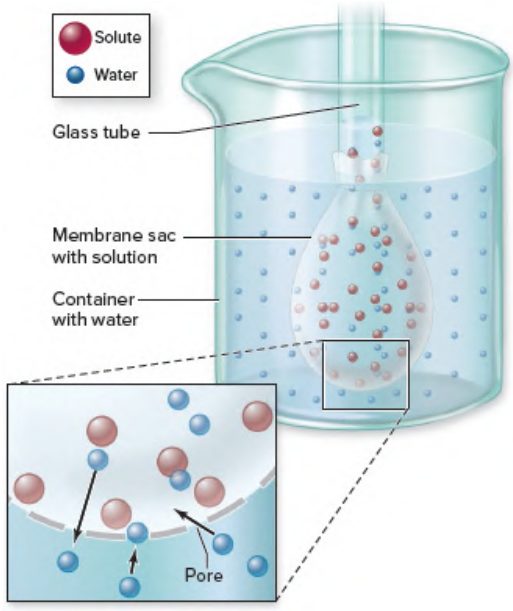

Figure 6.2 Model system to demonstrate osmosis. Here we have a solution enclosed in a sack-shaped membrane and attached to a hollow tube. The membrane is permeable to water (solvent) but not to solute. The sack is immersed in a container of pure water. In the inset, you see that the net direction of water diffusion is into the sac.

The Movement of Water: Osmosis

Diffusion of water through a selectively permeable membrane, a process called **osmosis**, is a physical phenomenon that is easily demonstrated in the laboratory with nonliving materials. It provides a model of how cells deal with various solute concentrations in aqueous solutions. In an osmotic system, the membrane is *selectively*, or *differentially, permeable*, having passageways that allow free diffusion of water but that block certain other dissolved molecules **(figure 6.2)**. When this membrane is placed between solutions of differing concentrations and the solute cannot pass through the membrane, then under the laws of diffusion, water will diffuse at a faster rate from the side that has more water to the side that has less water. As long as the concentrations of the solutions differ, one side will experience a net loss of water and the other a net gain of water, until equilibrium is reached and the rate of diffusion is equalized.

Osmosis in living systems is similar to the model shown in **figure 6.3**. Living membranes generally block the entrance and exit of larger molecules and permit free diffusion of water. Because most cells are surrounded by some free water, the amount of water entering or leaving

has a far-reaching impact on cellular activities and survival. This osmotic relationship between cells and their environment is determined by the relative concentrations of the solutions on either side of the cytoplasmic membrane (figure 6.3). Such systems can be compared using the terms *isotonic, hypotonic*, and *hypertonic*. (The root *-tonic* means "tension." *Iso-* means "the same," *hypo-* means "less," and *hyper-* means "more" or "greater.")

Under **isotonic** conditions, the environment is equal in solute concentration to the cell's internal environment, and because diffusion of water proceeds at the same rate in both directions, there is no net change in cell volume. Isotonic solutions are generally the most stable environments for cells, because they are already in an osmotic steady state with the cell. Microorganisms living in host tissues are most likely to be living in isotonic habitats.

Under **hypotonic** conditions, the solute concentration of the external environment is lower than that of the cell's internal environment. Pure water provides the most hypotonic environment for cells because it has no solute. The net direction of osmosis is from the hypotonic solution into the cell, and cells without walls swell and can burst.

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3. A physician has ordered hypotonic parenteral therapy for a postoperative client over a 24-hour period. Based on this order, what is the *priority* nursing action?

a. *Assess the patient's labs and verify the order with the pharmacy prior to hanging the ordered fluid.*
b. *Contact the physician and request an order for hypertonic solution instead.*
c. *Place the client on intake and output measures.*
d. *Begin intravenous hypotonic fluids immediately.*

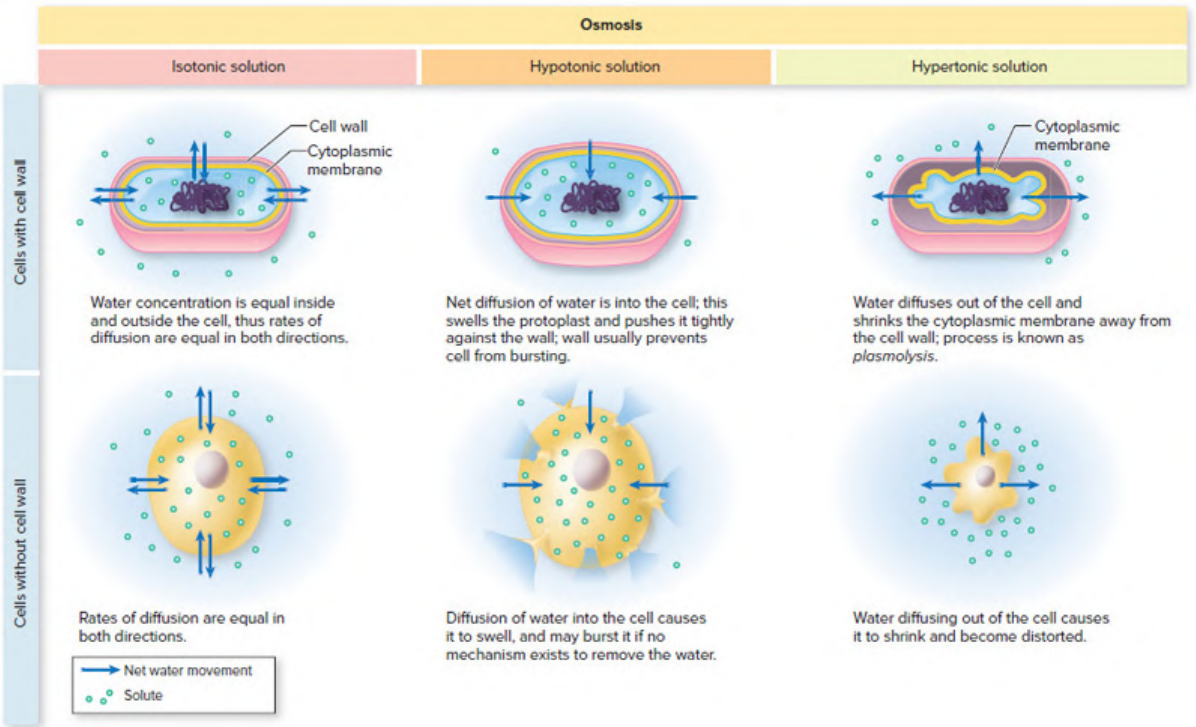


Figure 6.3 **Cell responses to solutions of differing osmotic content**. Note that, unlike in figure 6.2, there is no tube into which the extra fluid can rise.

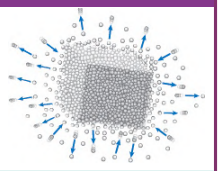
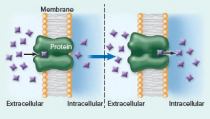
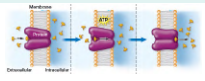
A slightly hypotonic environment can be quite favorable for bacterial cells. The constant slight tendency for water to flow into the cell keeps the cytoplasmic membrane fully extended and the cytoplasm full. This is the optimum condition for the many processes occurring in and on the membrane. Slight hypotonicity is tolerated quite well by most bacteria because of their rigid cell walls.

Hypertonic conditions are also out of balance with the tonicity of the cell's cytoplasm, but in this case, the environment has a higher solute concentration than the cytoplasm. Because a hypertonic environment will force water to diffuse out of a cell, it is said to have high *osmotic pressure*, or potential. Microbes in hypertonic solutions often cannot grow or metabolize because their membranes are so wrinkled up that many cell processes are impossible. This is the principle behind using concentrated salt and sugar solutions as preservatives for food, such as in salted hams.

Movement of Solutes

Simple diffusion is limited to small nonpolar molecules like oxygen or lipid-soluble molecules that may pass through the membranes. But it is imperative that a cell be able to move polar molecules and ions across the plasma membrane as well, and this is impossible via simple diffusion. So microbes have developed multiple mechanisms to move substances across membranes. We look at these (and simple diffusion) in **table 6.4**.

Table 6.4 Transport Processes in Cells

	Examples	Description	Energy Requirements	
Passive	Simple diffusion	A fundamental property of atoms and molecules that exist in a state of random motion	None. Substances move on a gradient from higher concentration to lower concentration.	
	Facilitated diffusion	Molecule binds to a specific receptor in membrane and is carried to other side. Molecule-specific. Goes both directions. Rate of transport is limited by the number of binding sites on transport proteins.	None. Substances move on a gradient from higher concentration to lower concentration.	
Active	Carrier-mediated active transport	Atoms or molecules are pumped into or out of the cell by specialized receptors.	Driven by ATP or the proton motive force	

As you see in table 6.4, very often energy is required to move molecules into or out of cells. In that case, the process is more accurately called transport as opposed to diffusion, and is seen as "active." Features inherent in **active transport** systems are

1. the transport of nutrients against the diffusion gradient or in the same direction as the natural gradient but at a rate faster than by diffusion alone;
2. the presence of specific membrane proteins (permeases and pumps); and

3. the expenditure of energy.

Examples of substances transported actively are monosaccharides, amino acids, organic acids, phosphates, and metal ions.

Endocytosis: Eating and Drinking by Cells

Some eukaryotic cells transport large molecules, particles, liquids, or even other cells across the cell membrane. Because the cell usually expends energy to carry out this transport, it is also a form of active transport. The substances transported do not pass physically through the membrane but are carried into the cell by endocytosis. First the cell encloses the substance in its membrane, simultaneously forming a vacuole and engulfing it. Amoebas and certain white blood cells ingest whole cells or large solid

matter by a type of endocytosis called **phagocytosis**. Liquids, such as oils or molecules in solution, enter the cell through a type of endocytosis called **pinocytosis**.

6.1 LEARNING OUTCOMES—Assess Your Progress

1. List the essential nutrients of a bacterial cell.
2. Differentiate between macronutrients and micronutrients.
3. List and define four different terms that describe an organism's sources of carbon and energy.
4. Define *saprobe* and *parasite*, and explain why these terms can be an oversimplification.
5. Compare and contrast the processes of diffusion and osmosis.
6. Identify the effects of isotonic, hypotonic, and hypertonic conditions on a cell.
7. Name two types of passive transport and one type of active transport.

6.2 Environmental Factors That Influence Microbes

Microbes are exposed to a wide variety of environmental factors in addition to nutrients. These include such factors as heat, cold, gases, pH, radiation, osmotic and hydrostatic pressures, and even the effects of other microbes. For most microbes, environmental factors fundamentally affect the function of metabolic enzymes. Thus, survival in a changing environment is largely a matter of whether the enzyme systems of microorganisms can adapt to alterations in their habitat.

Temperature

Microbial cells are unable to control their own temperature and therefore take on the ambient temperature of their natural habitats. Their survival is dependent on adapting to whatever temperature variations are encountered in that habitat. The range of temperatures for the growth of a given microbial species can be expressed as three *cardinal temperatures*. The **minimum temperature** is the lowest temperature that permits a microbe's continued growth and metabolism; below this temperature, its activities stop. The **maximum temperature** is the highest temperature at which growth and metabolism can proceed. If the temperature rises slightly above maximum, growth will stop, but if it continues to rise beyond that point, the enzymes and nucleic

acids will eventually become permanently inactivated (otherwise known as denaturation), and the cell will die. This is why heat works so well as an agent in microbial control. The **optimum temperature** covers a small range, intermediate between the minimum and maximum, which promotes the fastest rate of growth and metabolism (rarely is the optimum a single point).

Depending on their natural habitats, some microbes have a narrow cardinal range, others a broad one. Some strict parasites will not grow if the temperature varies more than a few degrees below or above the host's body temperature. For instance, the typhus bacterium multiplies only in the range of 32°C to 38°C, and rhinoviruses (one cause of the common cold) multiply most successfully in tissues that are slightly below normal body temperature (33°C to 35°C). Other organisms are not so limited. Strains of *Staphylococcus aureus* grow within the range of 6°C to 46°C, and the intestinal bacterium *Enterococcus faecalis* grows within the range of 0°C to 44°C.

Another way to express temperature adaptation is to describe whether an organism grows optimally in a cold, moderate, or hot temperature range. The terms used for these ecological groups are *psychrophile, mesophile*, and *thermophile* **(figure 6.4)**, respectively.

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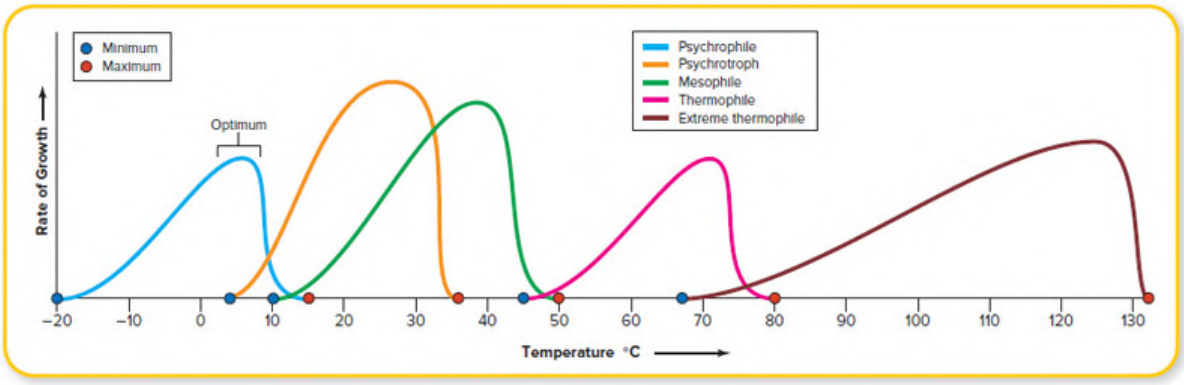


Figure 6.4 **Ecological groups by temperature range**. Psychrophiles can grow at or below 0°C and have an optimum below 15°C. Psychrotrophs have an optimum of from 15°C to 30°C. As a group, mesophiles can grow between 10°C and 50°C, but their optima usually fall between 20°C and 40°C. Generally speaking, thermophiles require temperatures above 45°C and grow optimally between this temperature and 80°C. Extreme thermophiles have optima above 80°C. Note that the ranges can overlap to an extent.

A **psychrophile** (sy′-kroh-fyl)—the blue line in figure 6.4—is a microorganism that has an optimum temperature below 15°C and is still capable of growth at 0°C. It is obligate with respect to cold and generally cannot grow above 20°C. Unlike most laboratory cultures, storage in the refrigerator causes them to grow, rather than inhibiting them. As one might predict, the habitats of psychrophilic bacteria, fungi, and algae are lakes and rivers, snowfields **(figure 6.5)**, polar ice, and the deep ocean. Rarely, if ever, are they pathogenic. True psychrophiles must be distinguished from the less extreme *psychrotrophs* (the gold line in figure 6.4) that grow slowly in cold but have an optimum temperature between 15°C and 30°C.

The majority of medically significant microorganisms are **mesophiles** (mez′-oh-fylz; the green line in figure 6.4), organisms that grow at intermediate temperatures. The optimum growth temperatures (optima) of most mesophiles fall into the range of 20°C to 40°C. Organisms in this group inhabit animals and plants as well as soil and water in temperate, subtropical, and tropical regions. Most human pathogens have optima somewhere between 30°C and 40°C (human body temperature is 37°C). Some mesophilic bacteria, such as *Staphylococcus aureus*, grow optimally at body temperature but are also facultatively psychrotrophic, meaning they can survive and multiply slowly at refrigerator temperatures, causing concern for food storage. (**Facultative** is a term used in biology that designates an organism as capable of growing under differing sets of conditions. We will see the term again when we discuss oxygen requirements.) *Listeria monocytogenes* is a human pathogen that is truly psychrotrophic, meaning its optimum growth is between 30°C and 40°C. But it will grow slowly at temperatures as low as 1°C, and often grows in ice cream and refrigerated meat. *Thermoduric* microbes, which can survive short exposure to high temperatures but are normally mesophiles, are common contaminants of heated or pasteurized foods. Examples include heat-resistant endospore formers such as *Bacillus* and *Clostridium*.

A **thermophile** (thur′-moh-fyl; the pink line in figure 6.4) is a microbe that grows optimally at temperatures greater than 45°C. Such heat-loving microbes live in soil and water associated with volcanic activity, in compost piles, and in habitats directly exposed to the sun. Thermophiles vary in heat requirements, with a general range of growth of 45°C to 80°C. Most eukaryotic forms cannot survive above 60°C, but a few thermophilic bacteria, called extreme thermophiles (the

brown line in figure 6.4), grow between 80°C and 121°C. Strict thermophiles are so heat tolerant that researchers may use an autoclave to isolate them in culture, killling all the possible contaminants with high heat and leaving them to grow alone.

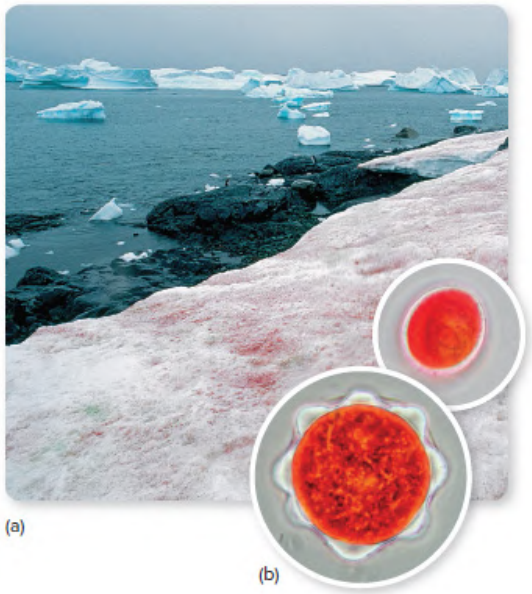


Figure 6.5 **Red snow**. **(a)** An early summer snowbank provides a perfect habitat for psychrophilic photosynthetic organisms like *Chlamydomonas nivalis*. **(b)** Microscopic view of this snow alga (actually classified as a green alga, although a red pigment dominates at this stage of its life cycle).
(a) ©Francois Gohier/Science Source; (b) Courtesy Nozomu Takeuchi

Gases

The atmospheric gases that most influence microbial growth are O_2 and CO_2. Of these, oxygen gas has the greatest impact on microbial growth. Not only is it an important respiratory gas, but it is also a powerful oxidizing agent that exists in many toxic forms. In general, microbes fall into one of three categories:

- those that use oxygen and can detoxify it,
- those that can neither use oxygen nor detoxify it, and
- those that do not use oxygen but can detoxify it.

How Microbes Process Oxygen

As oxygen is acted on by cellular enzymes, it is transformed into several toxic products. *Singlet oxygen* (O) is an extremely reactive molecule. Notably, it is one of the substances produced by phagocytes to kill invading bacteria. The buildup of singlet oxygen and the oxidation of membrane lipids and other molecules can damage and destroy a cell. The highly reactive *superoxide ion* (O_2^-), *hydrogen peroxide* (H_2O_2), and *hydroxyl radicals* (OH^-) are other destructive metabolic by-products of oxygen. To protect themselves against damage, most cells have developed enzymes that go about the business of scavenging and neutralizing these

chemicals. The complete conversion of superoxide ion into harmless oxygen requires a two-step process and at least two enzymes:

Step 1. $2O_2^- + 2H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2$ (hydrogen peroxide) + O_2

Step 2. $2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$

In this series of reactions (essential for aerobic organisms), the superoxide ion is first converted to hydrogen peroxide and normal oxygen by the action of an enzyme called superoxide dismutase. Because hydrogen peroxide is also toxic to cells (after all, it is used as a disinfectant and antiseptic), it must be degraded by the enzyme catalase into water and oxygen. With this system, toxic superoxide ion is completely broken down into harmless water and oxygen. If a microbe is not capable of dealing with toxic oxygen by these or similar mechanisms, it is forced to live in habitats free of oxygen.

Because oxygen requirements of microorganisms differ so dramatically and are so important clinically, microbes are grouped into several general categories **(table 6.5). Figure 6.6** depicts the growth of different microbes in tubes of fluid thioglycollate. The location of growth indicates the oxygen requirements of the microbes.

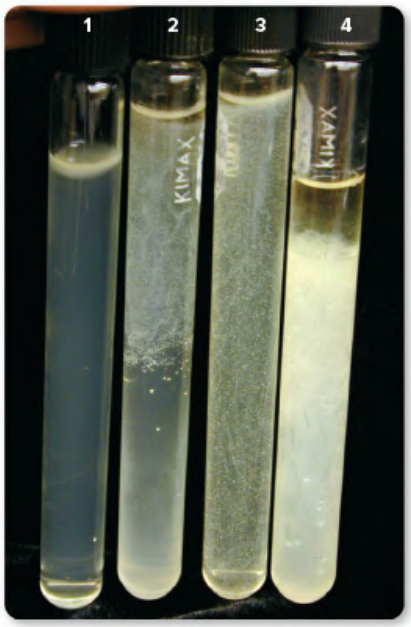


Figure 6.6 Four tubes showing three different patterns of oxygen utilization. These tubes use thioglycollate, which reduces oxygen to water, to restrict oxygen diffusion through the agar. So, whereas there is an oxygen-rich layer at the top of the agar, the oxygen concentration rapidly decreases deeper in the agar. In tube 1, the obligately aerobic *Pseudomonas aeruginosa* grows only at the very top of the agar. Tubes 2 and 3 contain two different examples of facultatively anaerobic bacteria. Many facultatives, though able to grow both aerobically and anaerobically, grow more efficiently in the aerobic mode. This is more obvious in tube 2 (*Staphylococcus aureus*) and less obvious in tube 3 (*Escherichia coli*). Tube 4 contains *Clostridium butyricum*, an obligate anaerobe.
©Terese M. Barta, Ph.D.

Carbon Dioxide

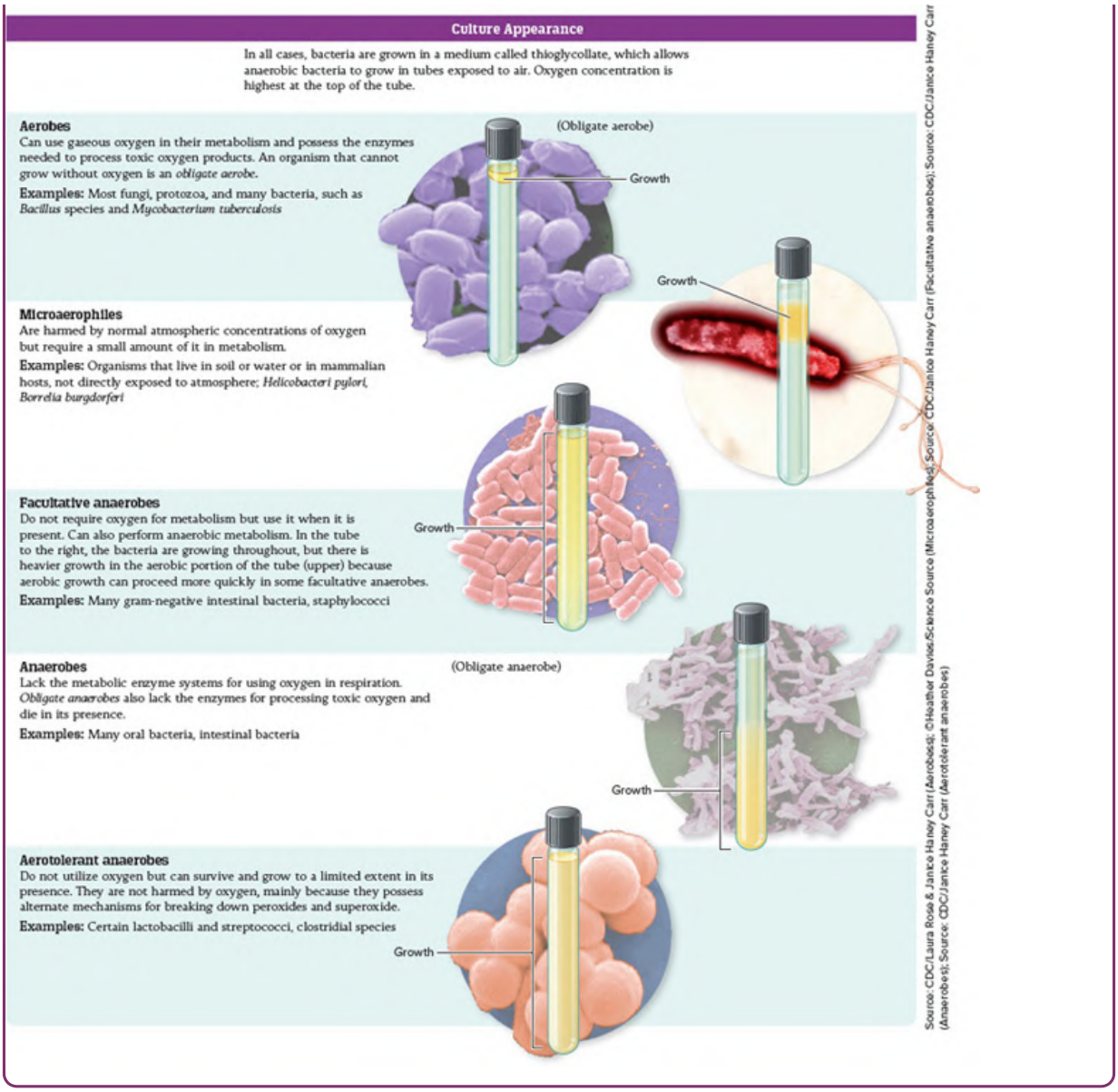
Although all microbes require some carbon dioxide in their metabolism, *capnophiles* grow best at a higher CO_2 tension than is normally present in the atmosphere. Some notable capnophiles are *Neisseria* (gonorrhea, meningitis), *Brucella* (undulant fever), and *Streptococcus pneumoniae*. Growing these from clinical specimens requires providing a higher CO_2 tension than normal.

pH

The term **pH** is defined as the degree of acidity or alkalinity (basicity) of a solution. It is expressed by the pH scale, a series of numbers ranging from 0 to 14. The pH of fresh pure water (7.0) is neutral, neither acidic nor basic. As the pH value decreases toward 0, the acidity increases, and as the pH increases toward 14, the alkalinity increases. The majority of organisms are *neutrophiles* and live or grow in habitats between pH 6 and 8 because strong acids and bases can be highly damaging to enzymes and other cellular substances.

Communities of extreme thermophilic microorganisms live around hot deep-sea ocean vents.
Source: NOAA Vents Program

Table 6.5 Oxygen Usage and Tolerance Patterns in Microbes



A few microorganisms live at pH extremes. Obligate *acidophiles* include *Euglena mutabilis*, an alga that grows in acid pools between 0 and 1.0 pH, and *Thermoplasma*, an archaea that lacks a cell wall and lives in hot coal piles at a pH of 1 to 2, and will die if exposed to pH 7. *Picrophilus* thrives at a pH of 0.7, and can grow at a pH of 0. Because many molds and yeasts tolerate moderate acid, they are the most common spoilage agents of pickled (acidic) foods. *Alkalinophiles*, such as *Natronomonas* species, live in hot pools and soils that contain high levels of basic minerals (up to pH 12.0).

Some neutrophiles can grow in environments outside of their pH range due to compounds they produce during metabolism. *Helicobacter*, which grows in the stomach, is an example of this. Bacteria that decompose urine create alkaline conditions, because ammonium (NH_4^+) can be produced when urea (a component of urine) is digested. (Ammonium raises the pH of solutions.)

Metabolism of urea is one way that *Proteus* spp. can neutralize the acidity of the urine to colonize and infect the urinary system.

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4. Which statements are true with respect to environmental factors' influence on microbes? Select all that apply.
   a. *The optimum temperature range for microbes reflects the highest temperature at which growth will occur.*
   b. *Microbial cells often adapt to the temperature of their habitat, modifying their metabolic processes to allow for survival.*
   c. *Certain microbes require a higher carbon dioxide concentration for growth than that found in our atmosphere.*
   d. *All microbes live and/or grow within a narrow pH range of 6 to 8.*
   e. *The majority of microbes live in hypotonic and/or isotonic solutions.*

Osmotic Pressure

Although most microbes exist under hypotonic or isotonic conditions, a few, called **osmophiles**, live in habitats with a high solute concentration. One common type of osmophile prefers high concentrations of salt; these organisms are called **halophiles** (hay′-loh-fylz). Obligate halophiles such as *Halobacterium* and *Halococcus* inhabit salt lakes, ponds, and other hypersaline habitats. They grow optimally in solutions of 25% NaCl but require at least 9% NaCl (combined with other salts) for growth. These archaea have significant modifications in their cell walls and membranes and will lyse in hypotonic habitats. Facultative halophiles are remarkably resistant to salt, even though they do not normally reside in high-salt environments. For example, *Staphylococcus aureus* can grow on NaCl media ranging from 0.1% up to 20%.

Radiation and Hydrostatic/Atmospheric Pressure

Radiation

Some microbes (phototrophs) can use visible light rays as an energy source, but nonphotosynthetic microbes tend to be damaged by the toxic oxygen products produced by contact with light. Some microbial species produce yellow carotenoid pigments to protect against the damaging effects of light by absorbing and dismantling toxic oxygen. Other types of radiation that can damage microbes are ultraviolet and ionizing rays (X rays and cosmic rays). In chapter 9, you will see just how these types of energy are applied in microbial control.

Pressure

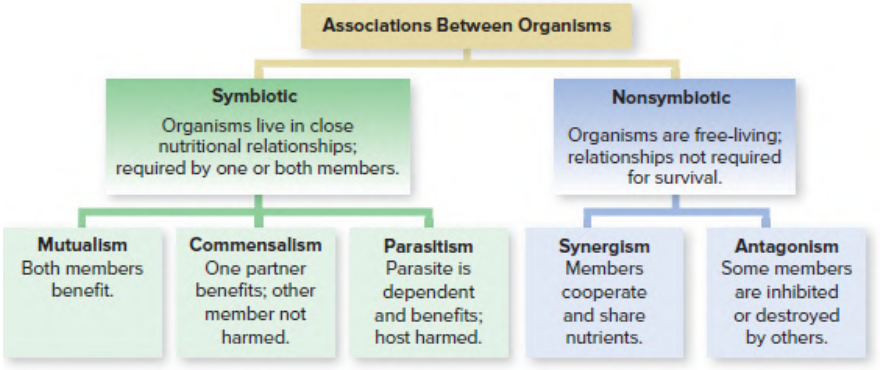
The ocean depths subject organisms to increasing hydrostatic pressure. Deep-sea microbes called **barophiles** (referring to barometric pressure) exist under pressures that range from a few times to over 1,000 times the pressure of the atmosphere. These bacteria are so strictly adapted to high pressures that they will rupture when exposed to normal atmospheric pressure.

Other Organisms

Up to now, we have considered the importance of nonliving environmental influences on the growth of microorganisms. Another profound influence comes from other organisms that share (or sometimes *are*) their habitats. In all but the rarest instances, microbes live in shared habitats,

which give rise to complex and fascinating associations. Some associations are between similar or dissimilar types of microbes; others involve multicellular organisms such as animals or plants. Interactions can have beneficial, harmful, or no particular effects on the organisms involved. They can be obligatory or nonobligatory to the members; and they often involve nutritional interactions. This outline provides an overview of the major types of microbial associations:

Cucumbers placed in a hypertonic solution turn into smaller, denser pickles.
©Photodisc/Alamy Stock Photo



Strong Partnerships: Symbioses

A general term used to denote a situation in which two organisms live together in a close partnership is **symbiosis**, and the members are termed *symbionts*. In everyday language, the term "symbiosis" is almost always used in a positive sense, as in, "Those roommates have a symbiotic relationship." In biology, symbiotic relationships can be either positive or negative. The term simply denotes a close partnership. Three main types of symbiosis occur:

- **Mutualism** exists when organisms live in an obligatory and mutually beneficial relationship. Many microbes that live in or on humans fall in this category. The microbes receive necessary nutrients, and the host gets a variety of benefits, ranging from protection from pathogenic microbes to the healthy development of the immune system that is only possible when a robust resident microbiome is present.
- In a relationship known as **commensalism**, the member called the commensal receives benefits, while its partner is neither harmed nor benefited. Most "normal biota" microbes used to be considered commensals, with the assumption that they provided neither harm nor benefit to their hosts. We now know that the normal biota contribute greatly to the health of their human host, so they are more accurately classified as mutualists.
- **Parasitism** is a relationship in which the host organism provides the parasitic microbe with nutrients and a habitat, and the host suffers from the relationship. Microbes that make humans sick fall in this category.

There is an important distinction to be made here about the use of the word *parasite* in this context, as opposed to its use in describing nutrient lifestyles earlier in this chapter. There, we put all organisms into the categories of "saprobe" or "parasite," meaning living independently either off of dead material or a live host, respectively. Scientists have come to learn that not all organisms that require a living host necessarily *harm* the host. So a more nuanced view of the nutritional requirement categories might be "saprobe" and "nonsaprobe"—with the "nonsaprobe" category having both parasitic and commensal members.

Associations but Not Partnerships: Nonsymbiotic Interactions

Even when organisms are not engaged in symbiotic relationships, they are interacting. Relationships between free-living species can either have negative or positive results. **Antagonism** is an association between free-living species that arises when members of a community compete. In this interaction, one microbe secretes chemical substances into the surrounding environment that inhibit or

destroy another microbe in the same habitat. The first microbe may gain a competitive advantage by increasing the space and nutrients available to it. Interactions of this type are common in the soil, where mixed communities often compete for space and food. *Antibiosis*—the production of inhibitory compounds such as antibiotics—is actually a form of antagonism. Hundreds of naturally occurring antibiotics have been isolated from bacteria and fungi and used as drugs to control diseases.

Synergism is an interrelationship between two or more free-living organisms that benefits them but is not necessary for their survival. Together, the participants cooperate to produce a result that none of them could do alone. Gum disease, dental caries, and some bloodstream infections involve mixed infections by bacteria interacting synergistically.

Biofilms: The Epitome of Synergy Biofilms are communities of bacteria and/or other microbes that are attached to a surface and to each other, forming a multilayer conglomerate of cells and intracellular material. Usually there is a "pioneer" colonizer, a bacterium that initially attaches to a surface, such as a tooth or the lung tissue **(figure 6.7)**. Other microbes then attach either to those bacteria or to the complex sugar and protein substance that inevitably is secreted by microbial colonizers of surfaces. In many cases, once the cells are attached, they are stimulated to release chemicals that accumulate as the cell population grows. By this means, they can monitor the size of their own population. This is a process called **quorum sensing**. Bacteria can use quorum sensing to interact with other members of the same species, as well as members of other species that are close by. Eventually large complex communities are formed, which have different physical and biological characteristics in different locations of the community. The bottom of a biofilm may have very different pH and oxygen conditions than the surface of a biofilm, for example. It is now clearly established that microbes in a biofilm, as opposed to those in a planktonic (free-floating) state, behave and respond very differently to their environments. Different genes are even activated in the two situations. At any rate, a single biofilm is actually a partnership among multiple microbial inhabitants and thus cannot be eradicated by traditional methods targeting individual infections. This kind of synergism has led to the necessity of rethinking treatment of a great many different conditions.

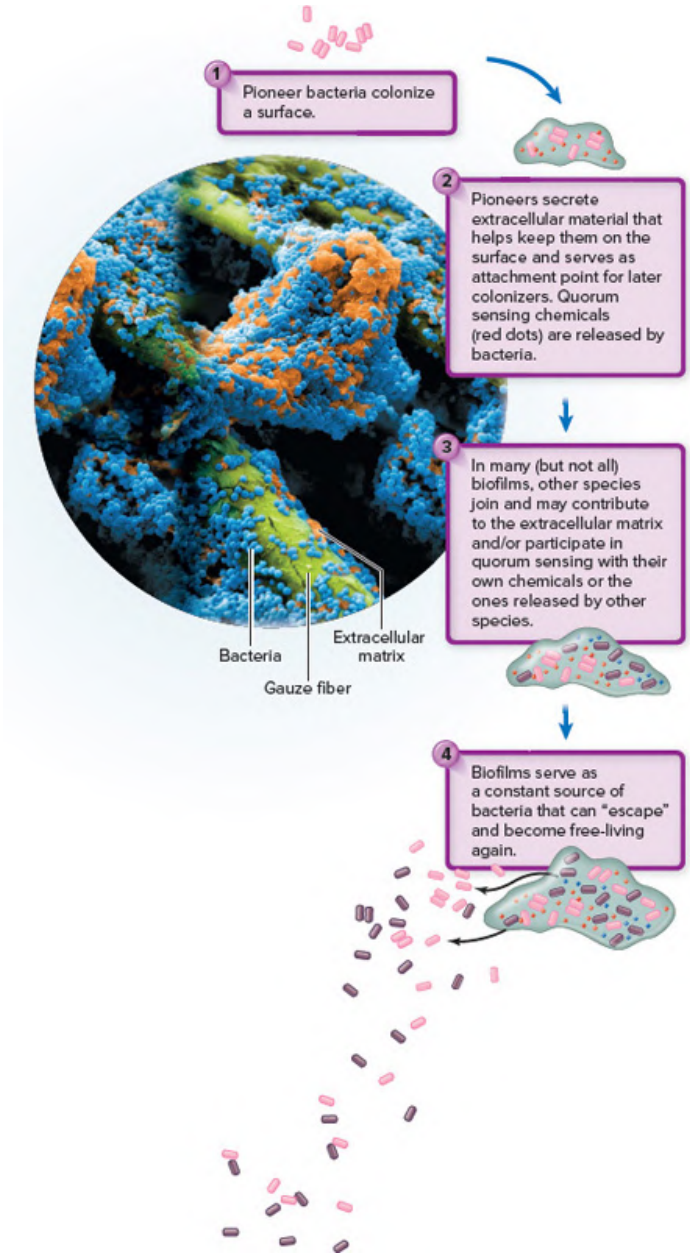


Figure 6.7 Steps in the formation of a biofilm. The photograph is an SEM of a biofilm formed on a gauze bandage. The blue bacteria are methicillin-resistant *Staphylococcus aureus* (MRSA). The orange substance is the extracellular matrix; and the green tubes are fibers of the gauze. This is probably a single-species biofilm.
Courtesy Ellen Swogger and Garth James, Center for Biofilm Engineering, Montana State University

6.2 LEARNING OUTCOMES—Assess Your Progress

8. List and define five terms used to express the temperature-related growth capabilities of microbes.
9. Summarize three ways in which microorganisms function in the presence of differing oxygen conditions.
10. Identify three important environmental factors (other than temperature and oxygen) with which organisms must cope.

11. List and describe the five types of associations microbes can have with their hosts.
12. Discuss characteristics of biofilms that differentiate them from planktonic bacteria.

6.3 The Study of Bacterial Growth

Microorganisms can "grow" in many different ways. In chapter 5 you learned how eukaryotic microbes grow. Here we focus on bacterial growth. While bacteria can be found that grow through budding and hyphal formation (similar to fungi), the majority of bacteria grow by a process called **binary fission**.

Binary Fission

Binary fission refers to the fact that one cell becomes two. During binary fission, the parent cell enlarges, duplicates its chromosome, and then starts to pull its cell envelope together in the center of the cell using a band of protein that is made of substances that resemble actin and tubulin—the protein component of microtubules in eukaryotic cells. The cell wall eventually forms a complete central septum. This process divides the cell into two daughter cells, and is then repeated at intervals by each new daughter cell in turn. With each successive round of division, the population increases. The stages in this continuous process are shown in greater detail in **figure 6.8**.

The Rate of Population Growth

The time required for a complete fission cycle—from one parent cell to two new daughter cells—is called the **generation**, or **doubling, time**. The term *generation* has a similar meaning as it does in humans. It is the period between an individual's birth and the time it produces offspring. In bacteria, each new fission cycle, or generation, increases the population by a factor of 2, or doubles it. Thus, the initial parent stage consists of 1 cell, the first generation consists of 2 cells, the second 4, the third 8, then 16, 32, 64, and so on. As long as the environment remains favorable, this doubling effect can continue at a constant rate. With the passing of each generation, the population will double, over and over again.

The length of the generation time is a measure of the growth rate of an organism. Compared with the growth rates of most other living things, bacteria are notoriously rapid. The average generation time is 30 to 60 minutes under optimum conditions. The shortest generation times can be 10 to 12 minutes, although some bacteria have generation times of days. For example, *Mycobacterium leprae*, the cause of Hansen's disease, has a generation time of 10 to 30 days—as long as that of some animals. Environmental bacteria commonly have generation times measured in months. Most pathogens have relatively short doubling times. *Salmonella enteritidis* and *Staphylococcus aureus*, bacteria that cause foodborne illness, double in 20 to 30 minutes, which is why leaving food at room temperature even for a short period has caused many cases of foodborne disease. In a few hours, a population of these bacteria can easily grow from a small number of cells to several million.

Figure 6.9 shows several quantitative characteristics of growth: The cell population size can be represented by the number 2 with an exponent (2^1, 2^2, 2^3, 2^4); the exponent increases by one in each generation; and the number of the exponent is also the number of the generation. This

growth pattern is termed **exponential**. Because these populations often contain very large numbers of cells, it is useful to express them by means of exponents or logarithms. The data from a growing bacterial population are graphed by plotting the number of cells as a function of time. Plotting the logarithm number (or exponent) over time provides a straight line indicative of exponential growth. Plotting the data arithmetically gives a constantly curved slope. In general, logarithmic graphs are preferred

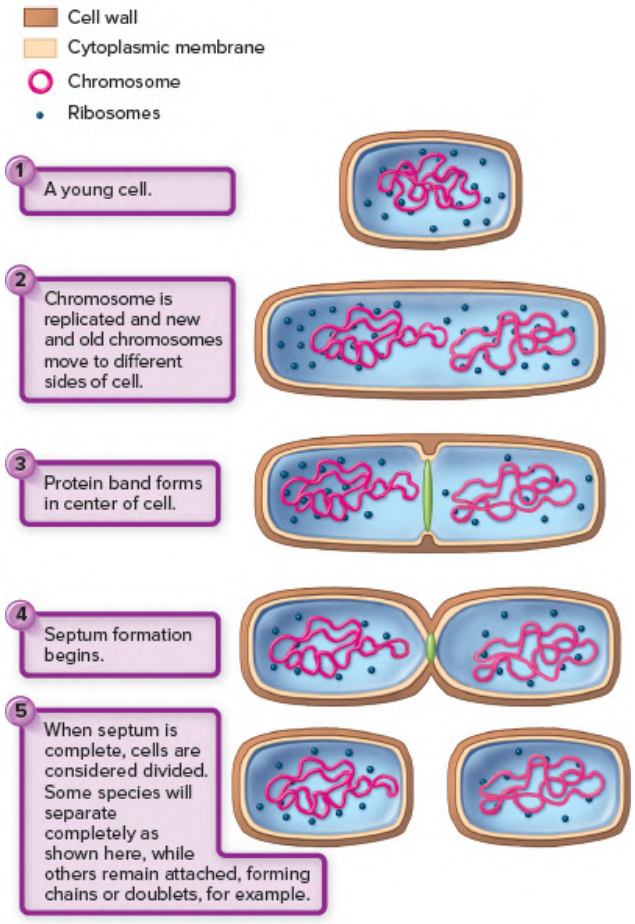


Figure 6.8 **Steps in binary fission of a rod-shaped bacterium**.

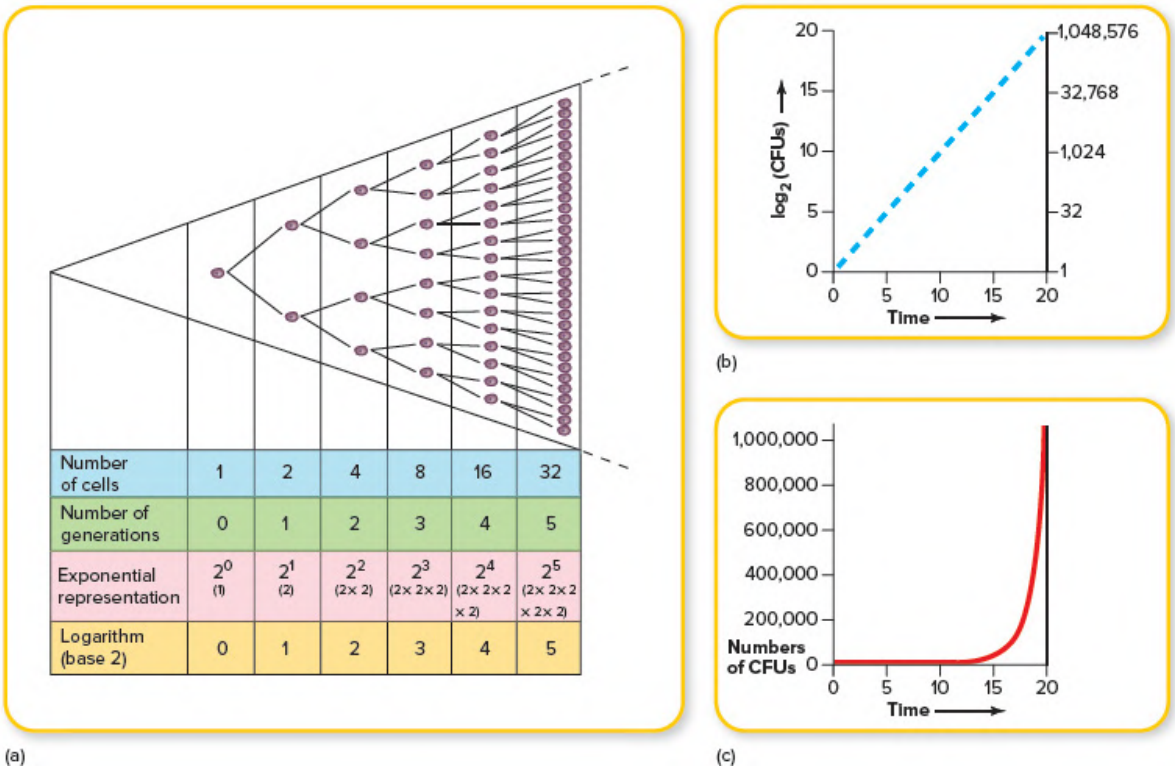


Figure 6.9 **The mathematics of population growth**. **(a)** Starting with a single cell, if each product of reproduction goes on to divide by binary fission, the population doubles with each new cell division or generation. This process can be represented by logarithms using exponents or by simple numbers. **(b)** Plotting the logarithm of the cells produces a straight line indicative of exponential growth, whereas **(c)** plotting the cell numbers arithmetically gives a curved slope.

because an accurate cell number is easier to read, especially during early growth phases.

Predicting the number of cells that will arise during a long growth period (yielding millions of cells) is based on a relatively simple concept. One could use the method of addition (2 + 2 = 4; 4 + 4 = 8; 8 + 8 = 16; 16 + 16 = 32, and so on) or a method of multiplication (for example, $2^5 = 2 \times 2 \times 2 \times 2 \times 2$), but it is easy to see that for 20 or 30 generations, this calculation could be very tedious. An easier way to calculate the size of a population over successive generations is to use this equation:

$$N_t = (N)2^n$$

Here, N_t is the total number of cells in the population (the "*t*" denotes "at some point in time *t*"). N is the starting number, the exponent n denotes the generation number, and 2^n represents the number of cells in that generation. If we know any two of the values, the other values can be calculated. We will use the example of *Staphylococcus aureus* to calculate how many cells (N_t) will be present in an egg salad sandwich after it sits in a warm car for 4 hours. We will assume that N is 10 (number of cells deposited in the sandwich while it was being prepared). To derive n, we need to divide 4 hours (240 minutes) by the

Clusters of *Staphylococcus aureus* bacteria that have divided by binary fission
Source: CDC/Janice Haney Carr

generation time (we will use 20 minutes). This calculation comes out to 12, so 2^n is equal to 2^{12}. Using a calculator, we find that 2^{12} is 4,096.

$$\text{Final number } (N_t) = 10 \times 4{,}096$$
$$= 40{,}960 \text{ bacterial cells in the sandwich}$$

This same equation, with modifications, is used to determine the generation time, a more complex calculation that requires knowing the number of cells at the beginning and end of a growth period. Such data are obtained through actual testing by a method discussed in the following section.

The Population Growth Curve

In reality, a population of bacteria does not maintain its potential growth rate and does not double endlessly, because in closed systems (called batch cultures) numerous factors prevent the cells from continuously dividing at their maximum rate. Laboratory studies indicate that a population typically displays a predictable pattern, or **growth curve**, over time. The method traditionally used to observe the population growth pattern is a *standard* (or *viable*) *plate count* technique, in which the total number of live cells is counted over a given time period. Briefly, this method involves the following:

1. placing a tiny number of cells into a sterile broth,
2. incubating this culture over a period of several hours,
3. sampling the broth at regular intervals during incubation,

4. plating each sample onto solid media (agar), and
5. counting the number of colonies present on each agar plate after incubation.

Figure 6.10 illustrates this process.

Evaluating these agar plates involves a common and important principle in microbiology: One colony on the plate represents one cell or colony-forming unit (CFU) from the original sample. (The term "colony-forming unit" is used sometimes, acknowledging

"Strips" of *Streptococcus* bacteria that have divided by binary fission. When mature cells stay attached like this, it means they stop their binary fission before completely separating.
Source: CDC

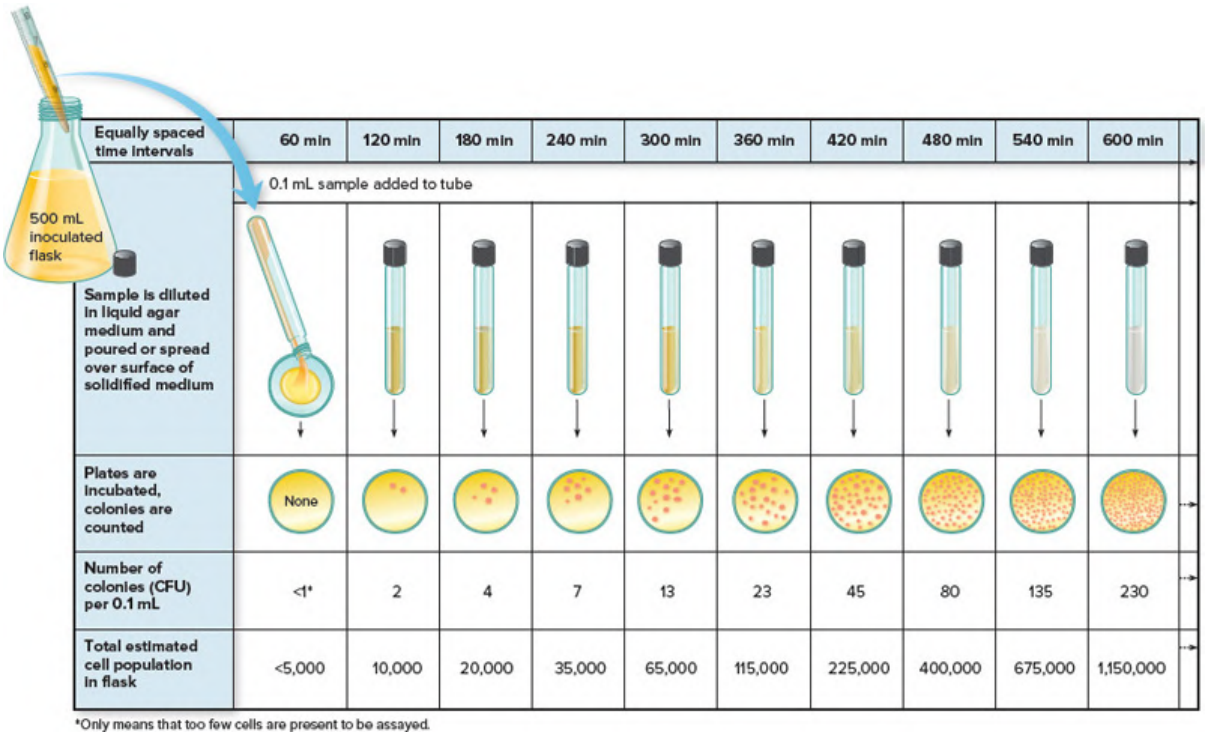


Figure 6.10 **Steps in a viable plate count: batch culture method**. The calculation is number of colonies × 10 (to convert the 0.1 mL sample to a 1.0 mL standard) × total volume of flask (500 mL).

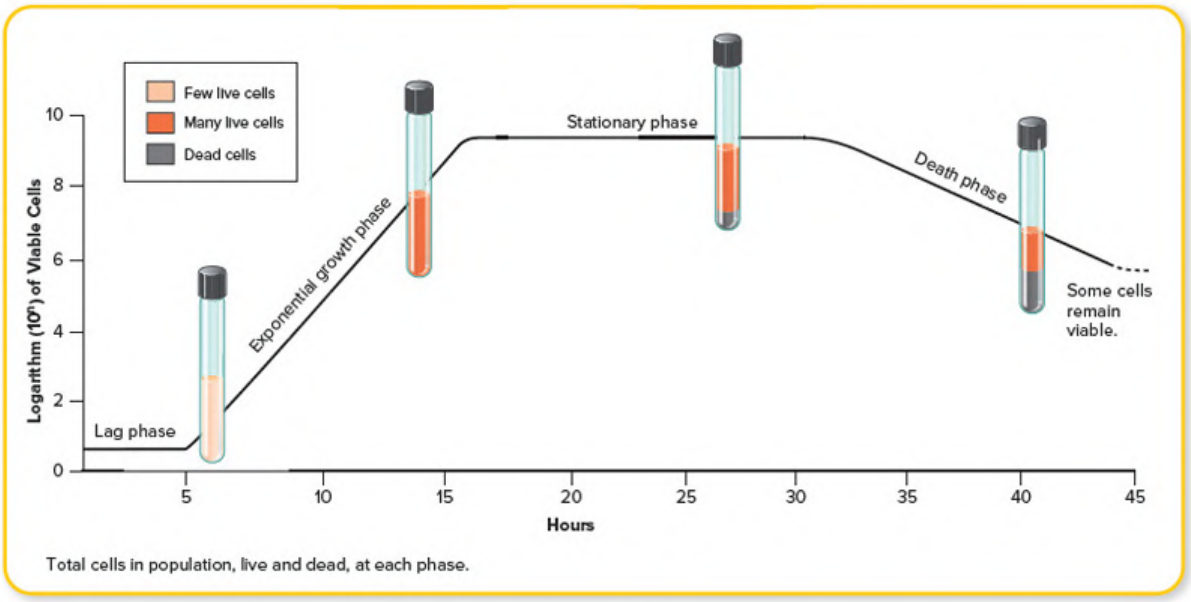


Figure 6.11 **The growth curve in a bacterial culture**. On this graph, the number of viable cells expressed as a logarithm (log) is plotted against time. See text for discussion of the various phases. Note that with a generation time of 35 minutes, the population has risen from 10 (10^1) cells to over 1,000,000,000 (10^9) cells in only 16 hours.

the fact that a group of cells—such as a chain or a pair of connected bacteria—may be the one "unit" placed by itself on that area of the agar.) Multiplication of the number of colonies in a single sample by the container's volume gives a fair estimate of the total population size (number of cells) at any given point. The growth curve is determined by graphing the number for each sample in sequence for the whole incubation period.

Because of the scarcity of cells in the early stages of growth, very early samples can give a zero reading even if there are viable cells in the culture. Also, the sampling itself can remove enough viable cells to alter the tabulations, but since the purpose is to compare relative trends in growth, these factors do not significantly change the overall pattern.

Stages in the Normal Growth Curve

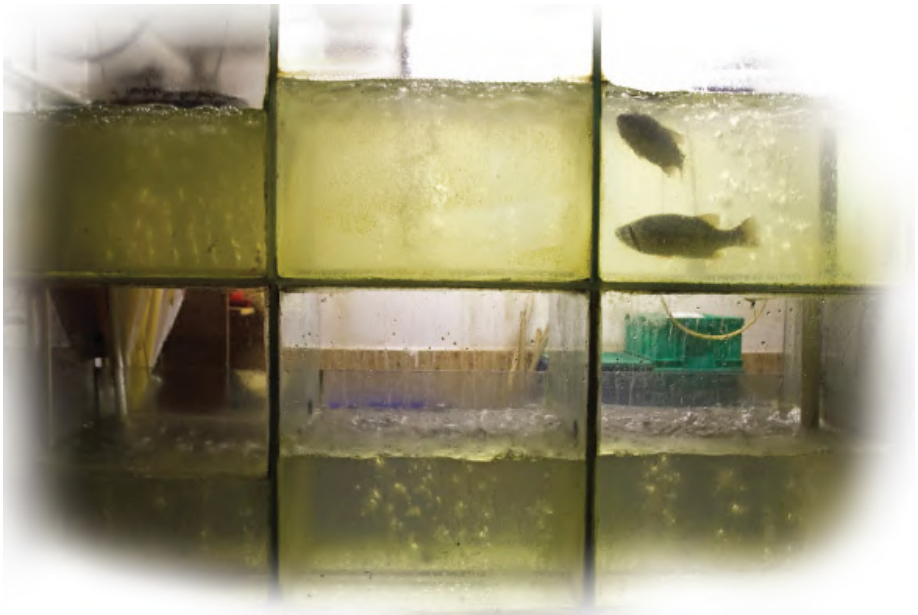
The system of batch culturing just described is *closed*, meaning that nutrients and space are finite and there is no mechanism for the removal of waste products. Data from an entire growth period typically produce a curve with a series of phases termed the *lag phase*, the *exponential growth (log) phase*, the *stationary phase*, and the *death phase* **(figure 6.11)**.

The **lag phase** is a relatively "flat" period on the graph when the population appears not to be growing or is growing at less than the exponential rate. Growth lags primarily because

1. the newly inoculated cells require a period of adjustment, enlargement, and synthesis;
2. the cells are not yet multiplying at their maximum rate; and
3. the population of cells is so sparse or dilute that the sampling misses them.

The length of the lag period varies somewhat from one population to another. It is important to note that even though the population of cells is not increasing (growing), individual cells are metabolically active as they increase their contents and prepare to divide.

The bacteria in cold yogurt are probably in stationary phase. Once inside a warm body, the bacteria can reenter lag and then exponential phases and increase their numbers in the gut.
©McGraw-Hill Education



The cloudiness in fish tanks is due in large part to the growth of bacteria in the water.
©WIN-Initiative/Getty Images

The cells reach the maximum rate of cell division during the **exponential growth (logarithmic or log) phase**, a period during which the curve increases geometrically. This phase will continue as long as cells have adequate nutrients and the environment is favorable.

At the **stationary growth phase**, the population enters a period during which the rates of cell birth and cell death are more or less equal. At this time, the division rate is slowing down (making

it easier for cell death to catch up with the rate of new cell formation). The decline in the growth rate is caused by depleted nutrients and oxygen plus excretion of organic acids and other biochemical pollutants into the growth medium, due to the increased density of cells.

As the limiting factors intensify, cells begin to die at an exponential rate (literally perishing in their own wastes), and they are unable to multiply. The curve now dips downward as the **death phase** begins. The speed with which death occurs depends on the relative resistance of the species and how toxic the conditions are, but it is usually slower than the exponential growth phase. It is now clear that many cells in a culture stay alive, but more or less dormant, for long periods of time. They are so dormant that, although they are alive, they won't grow on culture medium and therefore are missed in colony counts. The name for this state is the **viable nonculturable (VNC)** state.

Practical Importance of the Growth Curve

The tendency for populations to exhibit phases of rapid growth, slow growth, and death has important implications in controlling microbes in the environment, in managing microbial diseases, in industrial microbiology, and more. Antimicrobial agents such as heat and disinfectants rapidly accelerate the death phase in all populations, but microbes in the exponential growth phase are more vulnerable to these agents than are those that have entered the stationary phase. In general, actively growing cells are more vulnerable to growth inhibition and destruction.

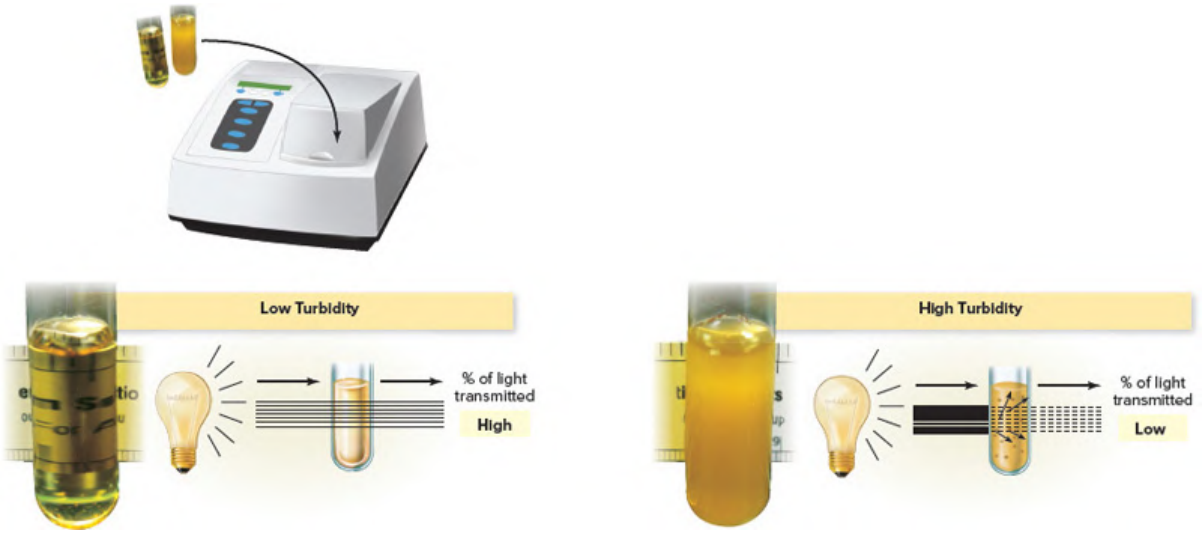
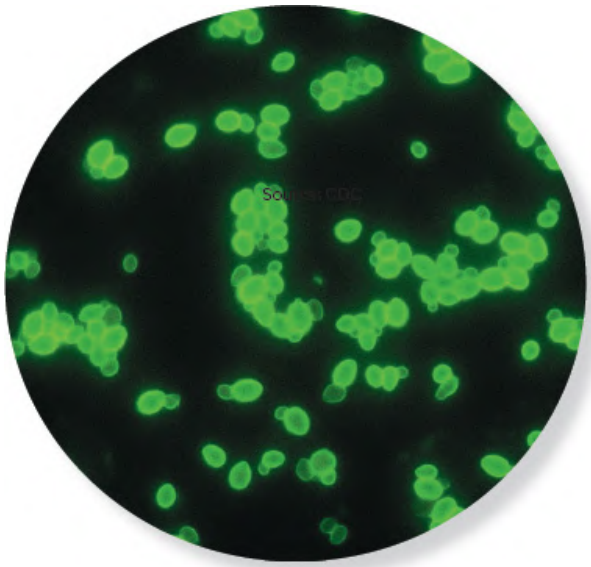


Figure 6.12 **Turbidity measurements as indicators of growth**. Holding a broth to the light is one method of checking for gross differences in cloudiness (turbidity). The broth on the left is transparent, indicating little or no growth; the broth on the right is cloudy and opaque, indicating heavy growth. The eye is not sensitive enough to pick up fine degrees in turbidity; more sensitive measurements can be made with a spectrophotometer. On the left you will see that a tube with no growth will allow light to easily pass. Therefore more light will reach the photodetector and give a higher transmittance value. In a tube with growth (on the right), the cells scatter the light, resulting in less light reaching the photodetector and, therefore, giving a lower transmittance value.

©Kathleen Talaro

In recent years, scientists have begun to consider experiments with microbes that are growing at a slower rate than the "optimal" rate. They point out that in most natural situations (even in the human body), microbes are in competition for substances that are important for their growth. To

understand what happens in the natural state, these scientists argue, we should see what happens with them when we apply a little stress to them, in the form of competitors or lower amounts of nutrients or other growth factors.



These fluorescently labeled yeast cells can be counted using flow cytometry.
Source: CDC

Analyzing Population Size Without Culturing

Turbidity

Microbiologists have developed several alternative ways of analyzing bacterial growth without actually having to grow them. One of the simplest methods for estimating the size of a population is through turbidometry. This technique relies on the simple observation that a tube of clear nutrient solution becomes cloudy, or **turbid**, as microbes grow in it. In general, the greater the turbidity, the larger the population size, which can be measured by means of sensitive instruments **(figure 6.12)**.

Counting

If you know how many cells are present in a specified amount of fluid, you can multiply that to determine the number in the total volume of the fluid. It is possible to conduct a **direct cell count** microscopically **(figure 6.13)**. This technique, very similar to that used in blood cell counts, employs a special microscope slide (cytometer) calibrated to accept a tiny sample that is spread over a premeasured grid. One inherent inaccuracy in this method, as well as in spectrophotometry, is that no distinction can be made between dead and live cells, both of which are included in the count.

Counting can be automated by sensitive devices such as the *Coulter counter*, which electronically scans a fluid as it passes through a tiny pipette. As each cell flows by, it is detected and registered on an electronic sensor **(figure 6.14)**. A *flow cytometer* works on a similar principle, but in addition to counting, it can measure cell size and even differentiate between live and dead cells. When used in conjunction with fluorescent dyes and antibodies to tag cells, it has

been used to differentiate between gram-positive and gram-negative bacteria. It has been adapted for use as a rapid method to identify pathogens in patient specimens and to differentiate blood cells. More sophisticated forms of the flow cytometer can also sort cells of different types into separate compartments of a collecting device. Although flow

Medical Moment

MRSA: PCR Over Culture

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a term for bacterial infection caused by an antibiotic-resistant strain of *S. aureus*. Many people are exposed in the community, becoming carriers without even knowing they are infected. This has driven hospitals to proactively identify carriers.

In many facilities, patients are routinely screened for MRSA upon admission. This surveillance testing allows for appropriate antibiotic selection and prevents spread to other patients. For patients with known MRSA, contact isolation (gown and gloves for patient contact) is necessary.

The MRSA carrier state is typically diagnosed using PCR testing by nasal swab, rather than culture. Polymerase chain reaction (PCR) testing allows for more rapid identification of MRSA by detecting the bacterial DNA in the sample. Conventional culture can take 3 days to return results, providing opportunity for transmission to other patients during the time results are pending.

To collect the PCR sample, the patient's nostrils are swabbed. The cotton swab is then sent to the lab for testing. Studies have shown PCR accuracy to be comparable to culture in detecting the presence of MRSA.

Q. Two contact precautions are mentioned in the story. Can you name some other precautions that should be considered?

Answer in Appendix B.

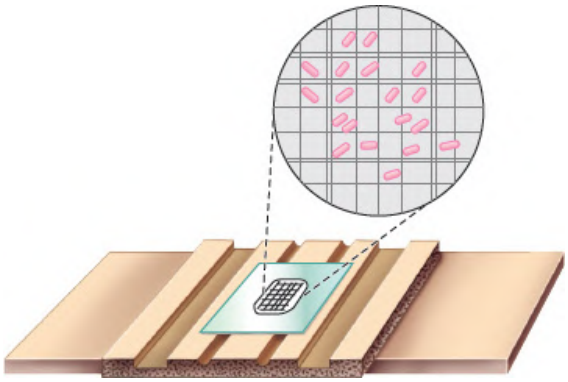


Figure 6.13 Direct microscopic count of bacteria. A small sample is placed on the grid under a cover glass. Individual cells, both living and dead, are counted. This number can be used to calculate the total cell count of a sample.

cytometry can be used to count bacteria in natural samples without the need for culturing them, it requires fluorescent labeling of the cells you are interested in detecting, which is not always possible.

Genetic Probing

A variation of the **polymerase chain reaction (PCR)**, called real-time PCR, allows scientists to quantify bacteria and other microorganisms that are present in environmental or tissue samples

without isolating them and without culturing them. We will learn more about these methods in later sections.

6.3 LEARNING OUTCOMES—Assess Your Progress

13. Summarize the steps of cell division used by most bacteria.
14. Define *doubling time*, and describe how it leads to exponential growth.
15. Compare and contrast the four phases of growth in a bacterial growth curve.
16. Identify one culture-based and one non-culture-based method used for analyzing bacterial growth.

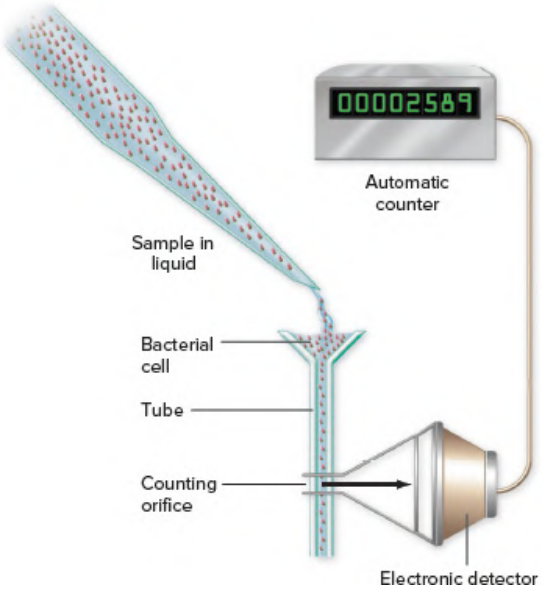


Figure 6.14 **Coulter counter**. As cells pass through this device, they trigger an electronic sensor that tallies their numbers.

CASE FILE WRAP-UP

The bacteria pictured are Clostridium perfringens.
©GagliardiImages/Shutterstock (health care worker); Source: CDC/Don Stalons (*C. perfringens*)

C. perfringens is commonly found in soil but is also found in the normal biota of the intestines. It produces a toxin known as alpha toxin, which can enter into muscle tissue through a wound. The toxin destroys tissue and produces gas, hence the term *gas gangrene*. Massive infection often leads to sepsis and death.

Treatment of gas gangrene includes wound debridement and penicillin; however, penicillin alone is unable to penetrate infected muscle tissue deeply enough to kill all of the bacteria; thus, penicillin is used in combination with surgical treatment. Amputation is often required to definitively treat *C. perfringens*.

Hyperbaric oxygen therapy (HBOT) has been used to successfully treat wound infections, including infections caused by *C. perfringens*. Anaerobic bacteria such as *C. perfringens* are susceptible to increased concentrations of oxygen. With HBOT, large quantities of oxygen-free radicals are generated, which renders bacteria vulnerable to oxidative death. HBOT has also been found to inhibit the release of alpha toxins. Lastly, HBOT enhances the effects of antibiotics. HBOT in cases of *C. perfringens* has resulted in avoidance of amputation, increased patient survival, and shorter hospital stays.

Hyperbaric chamber

©ERproductions Ltd/Blend Images/Getty Images

The Microbiome

The Great Oxidation Event and Earth's Microbiome

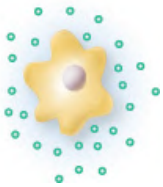
There has been a lot of debate about when oxygen-producing microbes appeared on the earth. Until recently, scientists have estimated that only anaerobic cells existed until about 2 or 3 billion years ago. It was not until what is called the *great oxidation event* (GOE) occurred that life, as we know it, was possible on the earth. What is the GOE, and why is it significant?

Until that time, atmospheric oxygen in the form of O_2 was very low–probably less than 1/10,000 of present levels. Previous to the GOE, the atmosphere contained significant amounts of methane, a greenhouse gas, which kept the earth's temperatures at high levels. The production of oxygen was a significant event in the evolution of life on earth. Until O_2 first accumulated in the atmosphere, the earth was too hot, too full of methane, and too acidic for life to exist.

Scientists led by geomicrobiologist Kirt Konhauser at the University of Alberta have studied oxidation of iron pyrite (FeS_2, also known as fool's gold) into iron oxide. This process released acid that dissolved rocks into chromium and other metals in ancient sea beds. Their data show that chromium levels in the sea beds increased significantly about 2.48 billion years ago, about 100 million years earlier than was previously thought. These **acidophilic** microbes thrived in the extremely low pH of the ocean waters of ancient earth. Konhauser has found evidence of these same bacterial life forms living off of pyrite in the highly acidic wastewater of mining sites (pictured). These organisms are the most ancient oxygen producers known and may be the driving force behind the atmosphere in which we live today.

Source: NOAA Restoration Center & Damage Assessment and Restoration Program

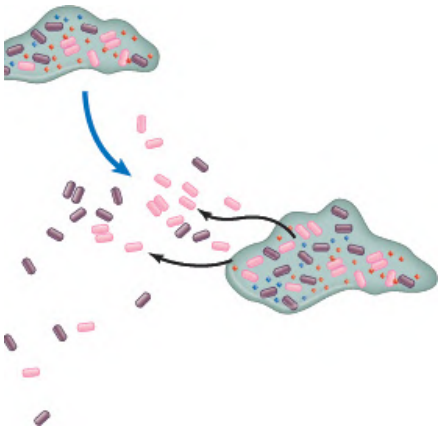
Chapter Summary



6.1 Microbial Nutrition

- Nutrition is a process by which all living organisms obtain substances from their environment to convert to metabolic uses.
- Nutrients are categorized by the amount required (macronutrients or micronutrients), by chemical structure (organic or inorganic), and by their importance to the organism's survival (essential or nonessential).

- Microorganisms are classified both by the chemical form of their nutrients and the energy sources they utilize.
- Although the chemical form of nutrients varies widely, all organisms require six elements—carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur—to survive, grow, and reproduce.
- Nutrients are transported into microorganisms by two kinds of processes: active transport that expends energy and passive transport that does not need energy input.



6.2 Environmental Factors That Influence Microbes

- The environmental factors that control microbial growth are temperature; gases; pH; osmotic, hydrostatic, and atmospheric pressure; radiation; and other organisms in their habitats.
- Environmental factors control microbial growth mainly by their influence on microbial enzymes.
- Three cardinal temperatures for a microorganism describe its temperature range and the temperature at which it grows best. These are the minimum temperature, the maximum temperature, and the optimum temperature.
- Microorganisms are classified by their temperature requirements as psychrophiles, psychrotrophs, mesophiles, thermophiles, or extreme thermophiles.
- Most eukaryotic microorganisms are aerobic, whereas bacteria vary widely in their oxygen requirements from obligately aerobic to anaerobic.
- Microorganisms live in symbiotic or nonsymbiotic association with other species that range from mutualism to parasitism and antagonism.
- Biofilms are examples of complex synergistic communities of microbes that behave differently than free-living microorganisms.

6.3 The Study of Bacterial Growth

- The splitting of a parent bacterial cell to form a pair of similar-size daughter cells is known as binary fission.
- Microbial growth refers both to increase in cell size and increase in number of cells in a population.
- The generation time is a measure of the growth rate of a microbial population. It varies in length according to environmental conditions and species of microbe.
- Microbial cultures in a nutrient-limited batch environment exhibit four distinct stages of growth: the lag phase, the exponential growth (log) phase, the stationary phase, and the death phase.
- Microbial cell populations show distinct phases of growth in response to changing nutrient and waste conditions.
- Population growth can be quantified by counting colony numbers, measuring the turbidity of a solution, and doing direct cell counts.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. Which descriptors are likely to have applied to the earliest microbes on the planet? **a**. chemoautotrophic **b**. thermophilic **c**. chemoheterotrophic **d**. two of the above	**2**. Can you think of any advantages gained when eukaryotes and multicellular organisms evolved to use sexual reproduction instead of binary fission? Discuss.	**3**. Speculate about how earth's atmosphere came to contain oxygen, and the role early bacteria and archaea played.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Cell Structure and Function	**4**. Which of the following is true of passive transport? **a**. It requires a gradient. **b**. It uses the cell wall. **c**. It includes endocytosis. **d**. It only moves water.	**5**. Compare the effects of a hypertonic, hypotonic, and isotonic solution on an amoeba versus a bacterium.	**6**. Usually scientists looking for life on other planets try to determine first if the planet had or has evidence of water. Develop a rationale for this based on cellular chemistry.
Metabolic Pathways	**7**. An organism that can synthesize all its required organic components from CO_2 using energy from the sun is a **a**. photoautotroph. **b**. photoheterotroph. **c**. chemoautotroph. **d**. chemoheterotroph.	**8**. Provide evidence in support of or refuting this statement: Microbial life can exist in the complete absence of sunlight and organic compounds.	**9**. Develop an explanation for why biofilm bacteria use quorum sensing to regulate the size of their community.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Information Flow and Genetics	**10**. Most bacteria increase their numbers by sexual reproduction. hyphae formation. binary fission. endocytosis.	**11**. Looking at figure 6.3, explain how a cell in a hypertonic solution might have trouble dividing.	**12**. In binary fission, the parent chromosome is duplicated so that the two daughter cells each have the same copy of the original chromosome. If this is the case, speculate on what happened to allow bacteria to become diversified into so many types?

Microbial Systems	**13**. A cell exposed to a hypertonic environment will _____ by osmosis. **a**. gain water **b**. lose water **c**. neither gain nor lose water **d**. burst	**14**. Considering figure 6.6, discuss whether tube 2 or tube 3 is more likely to grow in diverse niches or conditions. Defend your answer.	**15**. Bacteria and archaea are ubiquitous on the planet. Would it be to our advantage (if it were possible) to eliminate all of them, in an effort to protect the human population from current and emerging diseases? Why or why not?
Impact of Microorganisms	**16**. A pathogen would most accurately be described as a **a**. parasite.	**17**. How can you explain the fact that an unopened carton of milk in	**18**. Describe a scenario in which it would be to a pathogen's advantage to have a

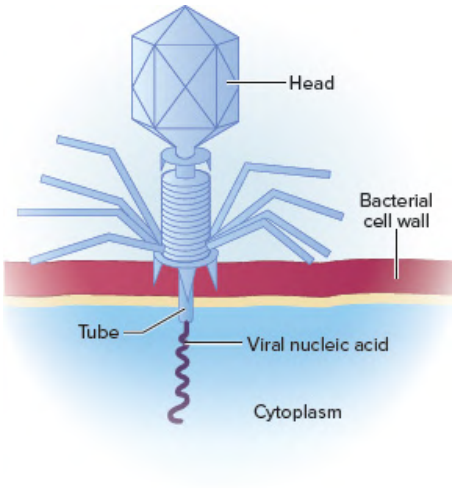
	b. commensal. **c**. symbiont. **d**. saprobe.	the refrigerator will eventually spoil even though it has never been opened?	very long generation time.
Scientific Thinking	**19**. In a viable count, each _____ represents a _____ from the sample population. **a**. CFU, colony **b**. colony, CFU **c**. hour, generation **d**. cell, generation	**20**. If an egg salad sandwich sitting in a car on a warm day contains 40,960 bacteria after 4 hours, how many bacteria will it contain after another generation time for the bacteria?	**21**. Scientists now believe that most bacteria in nature exist in biofilms. Can you suggest some technical reasons that this was not understood for the first 150 years of microbiology?

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From chapter 5, figure 5.10**. What type of symbiotic relationship is pictured here?

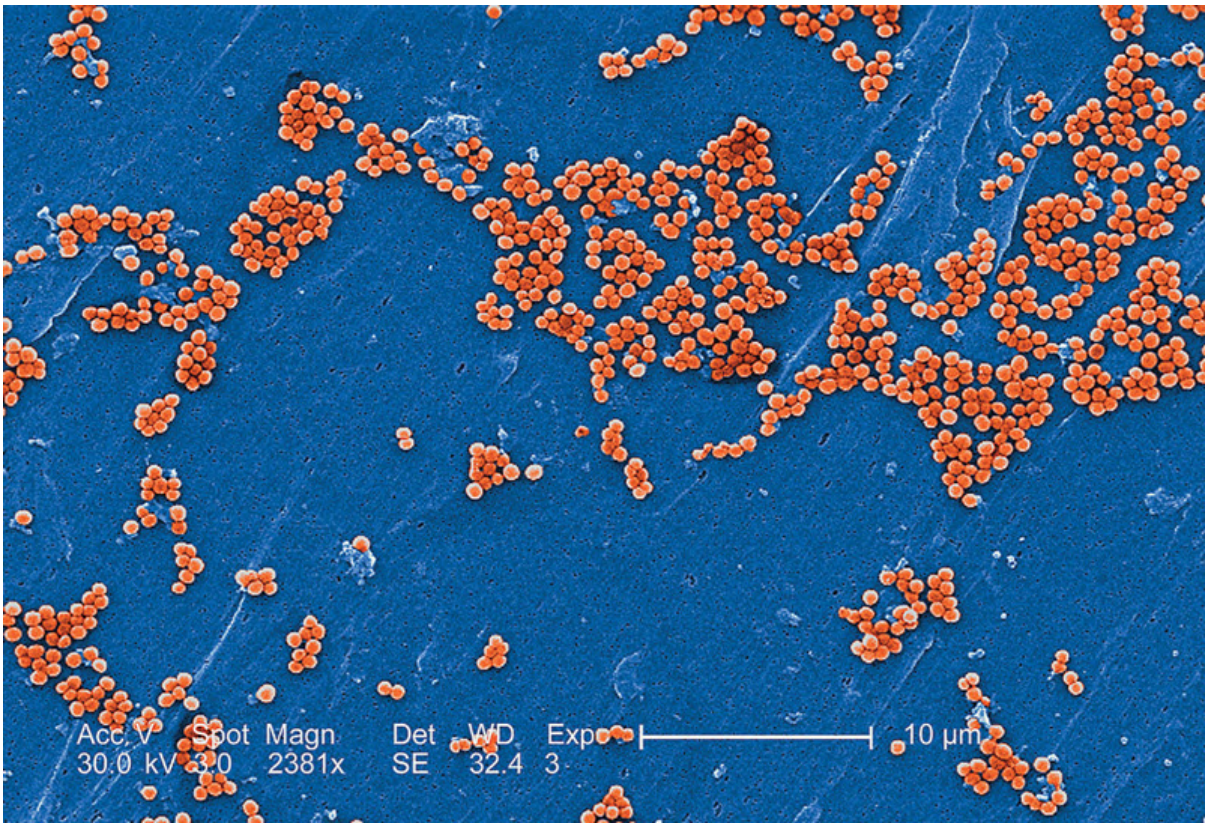


Resources are available through Connect, including interactive learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

7

Microbial Metabolism

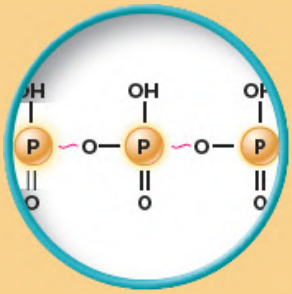


Source: CDC/Janice Haney Carr (*Staphylococcus aureus* bacteria); ©McGraw-Hill Education (health care worker)

IN THIS CHAPTER…

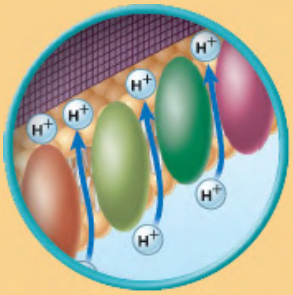
7.1 Metabolism and the Role of Enzymes

1. Describe the relationship among metabolism, catabolism, and anabolism.
2. Fully describe the structure and function of enzymes.
3. Differentiate between constitutive and regulated enzymes.
4. Diagram four major patterns of metabolic pathways.
5. Describe how enzymes are controlled.



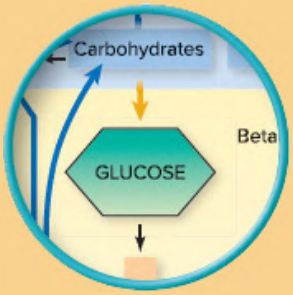
7.2 The Pursuit and Utilization of Energy

6. Name the chemical in which energy is stored in cells.
7. Create a general diagram of a redox reaction.
8. Identify electron carriers used by cells.



7.3 Catabolism

9. Name the three main catabolic pathways and the estimated ATP yield for each.
10. Construct a paragraph summarizing glycolysis.
11. Describe the Krebs cycle, with emphasis on what goes into it and what comes out of it.
12. Discuss the significance of the electron transport system, and compare the process between bacteria and eukaryotes.
13. State two ways in which anaerobic respiration differs from aerobic respiration.
14. Summarize the steps of microbial fermentation, and list three useful products it can create.
15. Describe how noncarbohydrate compounds are catabolized.



7.4 Anabolism and the Crossing Pathways of Metabolism

16. Provide an overview of the anabolic stages of metabolism.
17. Define *amphibolism*.

CASE FILE

Surviving Sepsis

I was working as a nurse in an emergency department. A medical transport team brought in a patient from a long-term care facility. The elderly man was lethargic and required a lot of stimulation before he could respond to my questions. The attendant who accompanied the patient reported he had become drowsier in the past 2 days, causing concern for his caregivers. That morning, his blood pressure was low so the patient was transported to the hospital for further assessment.

In the emergency department, his blood pressure and oxygen saturation levels were low. The patient had a high fever. His pulses were weak and heart rate was rapid. I applied an oxygen mask to the patient with 10 liters of flow. The provider ordered intravenous (IV) fluids to be given quickly as a bolus. Another nurse obtained IV access and started the fluids. I called the pharmacy to notify them that we suspected the patient had septicemia and needed STAT antibiotics.

Next, I prepared to draw labs from the patient. The provider requested a full laboratory workup, including blood and urine cultures. The provider also requested a serum lactate level. Knowing this required special precautions for collection, I obtained supplies for a free-flowing sample and prepared to send it immediately to the laboratory for processing. I obtained blood cultures immediately before the antibiotic doses arrived.

- Why was a serum lactate test ordered for this patient?
- How is sepsis treated?

Case File Wrap-Up appears at the end of the chapter.

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1. Which of the following is/are considered to be an anabolic reaction? Select all that apply.

a. *Krebs cycle*
b. *polypeptide synthesis*
c. *cell assembly*
d. *glycolysis*

7.1 Metabolism and the Role of Enzymes

Metabolism, from the Greek term *metaballein*, meaning "change," pertains to all chemical reactions and physical workings of the cell. Although metabolism entails thousands of different reactions, most of them fall into one of two general categories. **Anabolism**, sometimes also called *biosynthesis*, is any process that results in synthesis of cell molecules and structures. It is a building and bond-making process that forms larger macromolecules from smaller ones, and it usually requires the input of energy. **Catabolism** is the opposite of anabolism. Catabolic reactions break the bonds of larger molecules into smaller molecules and often release energy. In a cell, linking anabolism to catabolism ensures the efficient completion of many thousands of processes. Another fundamental fact about metabolism is that electrons are critical to the process.

In summary, a cell creates and spends energy by transferring electrons from an external source to internal carriers that eventually shuttle it into a series of proteins that create energy. Electron flow is the key. Along the way, metabolism accomplishes the following **(figure 7.1):**

1. assembles smaller molecules into larger macromolecules needed for the cell; in this process, ATP (energy) is utilized to form bonds (anabolism);
2. degrades macromolecules into smaller molecules, a process that yields energy (catabolism); and
3. creates and spends energy in the form of ATP (adenosine triphosphate).

Enzymes: Catalyzing the Chemical Reactions of Life

The chemical reactions of life cannot proceed without a special class of macromolecules called *enzymes*. Enzymes are a remarkable example of **catalysts**, chemicals that increase the rate of a chemical reaction without becoming part of the products or being consumed in the

reaction. It is easy to think that an enzyme creates a reaction, but that is not true. The major characteristics of enzymes are summarized in **table 7.1**.

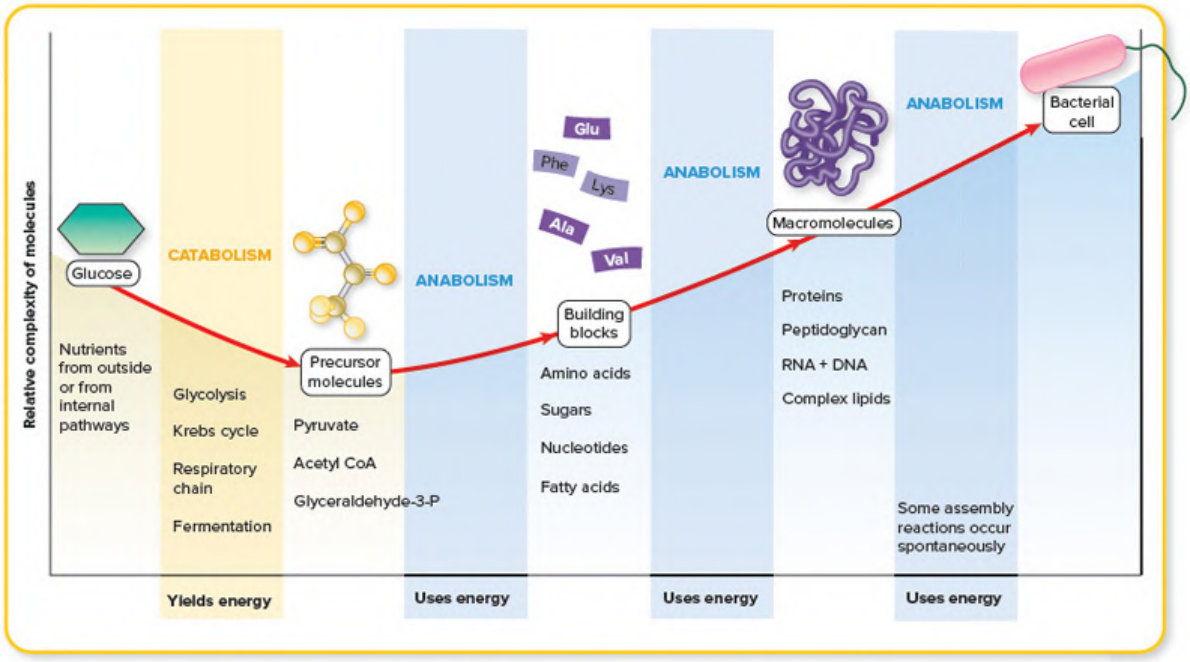

Figure 7.1 Simplified model of metabolism. Cellular reactions fall into two major categories. Catabolism (yellow) involves the breakdown of complex organic molecules to extract energy and form simpler end products. Anabolism (blue) uses the energy to synthesize necessary macromolecules and cell structures from precursors.

Table 7.1 Checklist of Enzyme Characteristics

- Most are made of protein; may require cofactors
- Act as organic catalysts to speed up the rate of cellular reactions
- Have unique characteristics such as shape, specificity, and function
- Enable metabolic reactions to proceed at a speed compatible with life
- Have an active site for target substrates
- Are much larger in size than their substrates
- Associate closely with substrates but do not become integrated into the reaction products
- Are not used up or permanently changed by the reaction
- Can be recycled, thus function in extremely low concentrations
- Are greatly affected by temperature and pH
- Can be regulated by feedback and genetic mechanisms

How Do Enzymes Work?

An enzyme speeds up the rate of a metabolic reaction, but just how does it do this? During a chemical reaction, reactants are converted to products either by bond formation or by bond breakage. A certain amount of energy is required to initiate every such reaction, which limits the rate at which reactions can happen. While the rate could be sped up by increased heat or

other means, biological cells use enzymes to vastly increase the speed of important reactions.

At the molecular level, an enzyme promotes a reaction by serving as a physical site upon which the molecules involved in the reaction, called substrates, can be positioned. The enzyme is almost always much larger in size than its substrate, and it presents a unique active site that matches only that particular substrate. Although an enzyme binds to the substrate and participates directly in changes to the substrate, it does not become a part of the products, is not used up by the reaction, and can function over and over again. Enzyme speeds, defined as the number of substrate molecules converted per enzyme per second, are very rapid. Speeds range from a thousand for lactate dehydrogenase to several million for catalase.

Enzyme Structure

Most enzymes are proteins—although there is a special class that are made of RNA—and they are classified as simple or conjugated. Simple enzymes consist of protein alone, whereas conjugated enzymes **(figure 7.2)** contain protein plus nonprotein molecules. A conjugated enzyme, sometimes referred to as a **holoenzyme**, is a combination of a protein and one or more **cofactors**. In this situation, the actual protein portion is called the **apoenzyme**. Cofactors are either organic molecules, called **coenzymes**, or inorganic elements (metal ions). For example, catalase, an enzyme that we studied in chapter 6, breaks down hydrogen peroxide and requires iron as a metallic cofactor.

Enzyme-Substrate Interactions

For a reaction to take place, a temporary enzyme-substrate union must occur at the active site **(figure 7.3)**. The fit is so specific that it is often described as a "lock-and-key" fit in which the substrate is inserted into the active site's pocket.

The bonds formed between the substrate and enzyme are weak and easily reversible. Once the enzyme-substrate complex has formed, the designated reaction(s) occur on the substrate, often with the aid of a cofactor, and a product is formed and released. The enzyme can then attach to another substrate molecule and repeat this action. Although enzymess can potentially catalyze reactions in both directions, most examples in this chapter depict them working in one direction only.

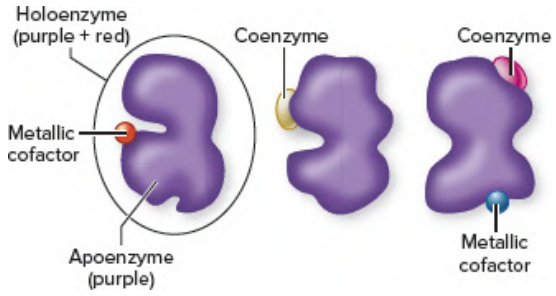

Figure 7.2 Conjugated enzyme structure. Conjugated enzymes have an apoenzyme (polypeptide or protein) component and one or more cofactors.

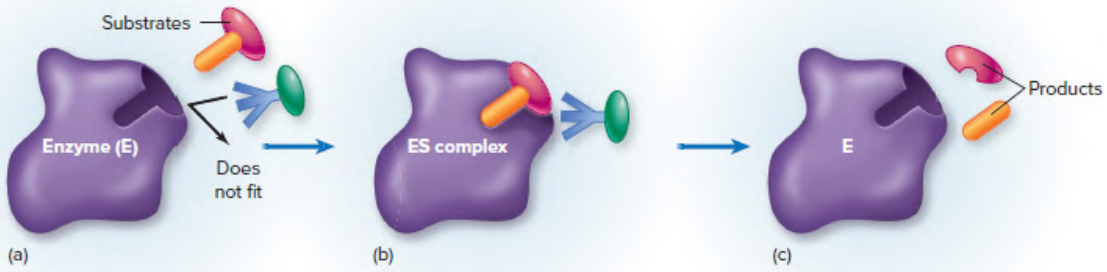

Figure 7.3 **Enzyme-substrate reactions**. **(a)** When the enzyme and substrate come together, the substrate (S) must show the correct fit and position with respect to the enzyme (E). **(b)** When the ES complex is formed, it enters a transition state. During this temporary but tight interlocking union, the enzyme participates directly in breaking or making bonds. **(c)** Once the reaction is complete, the enzyme releases the products.

molecule and repeat this action. Although enzymes can potentially catalyze reactions in both directions, most examples in this chapter depict them working in one direction only.

Cofactors: Supporting the Work of Enzymes

In chapter 6, you learned that microorganisms require specific metal ions called trace elements and also certain organic growth factors. In many cases, the need for these substances arises from their roles as cofactors for enzymes. The metallic cofactors, including iron, copper, magnesium, manganese, zinc, cobalt, selenium, and many others, have precise functions between the enzyme and its substrate. In general, metals activate enzymes, help bring the active site and substrate close together, and participate directly in chemical reactions with the enzyme-substrate complex.

Coenzymes are a type of cofactor. They are organic compounds that work in conjunction with an apoenzyme to perform the alteration of a substrate. The general function of a coenzyme is to remove a chemical group from one substrate molecule and add it to another substrate, thereby serving as a transient carrier of this group. In a later section of this chapter, we shall see that coenzymes carry and transfer hydrogen atoms, electrons, carbon dioxide, and amino groups. Many coenzymes are derived from **vitamins**, which explains why vitamins are important to nutrition and may be required as growth factors for living things. Vitamin deficiencies can prevent the complete holoenzyme from forming. Consequently, both the chemical reaction and the structure or function dependent upon that reaction are compromised.

Naming Enzymes

Enzymes are classified and named according to characteristics such as site of action, type of action, and substrate.

In general, an enzyme name is composed of two parts: a prefix or stem word derived from a certain characteristic—usually the substrate acted upon, the type of reaction catalyzed, or both—followed by the ending *-ase*.

Each enzyme is also assigned a common name that indicates the specific reaction it catalyzes. With this system, an enzyme that digests a carbohydrate substrate is a *carbohydrase;* a specific carbohydrase, *amylase*, acts on starch (amylose is a major component of starch). The enzyme *maltase* digests the sugar maltose. An enzyme that breaks peptide bonds of a protein is a *proteinase, protease*, or *peptidase*. Some fats and other lipids are digested by *lipases*. DNA is broken down by *deoxyribonuclease*, generally shortened to *DNase*. A *synthetase* or *polymerase* bonds together many small molecules into large molecules.

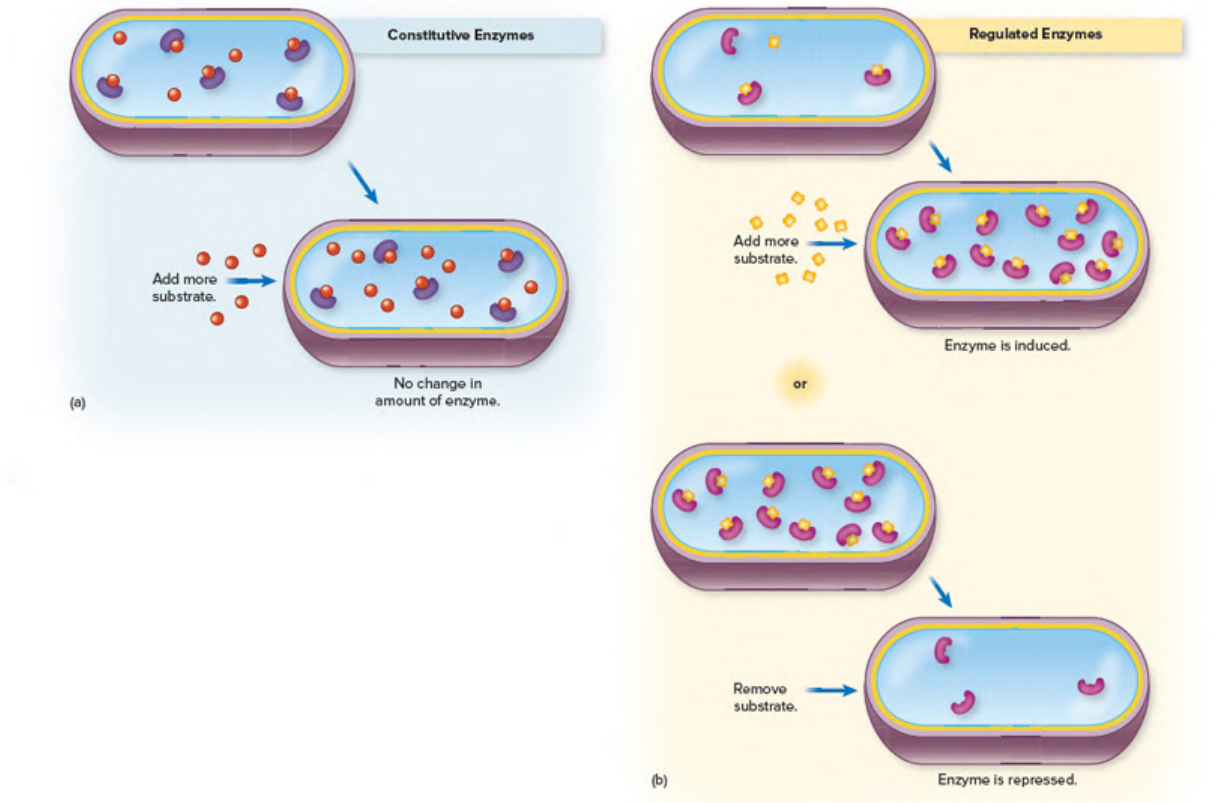

Figure 7.4 **Constitutive and regulated enzymes**. **(a)** Constitutive enzymes are present in constant amounts in a cell. The addition of more substrate does not increase the numbers of these enzymes. **(b)** The concentration of regulated enzymes in a cell increases or decreases in response to substrate levels.

Regulation of Enzyme Action

Enzymes are not all produced in equal amounts or at equal rates. Some, called **constitutive enzymes** (**figure 7.4*a***), are always present and in relatively constant amounts, regardless of the amount of substrate. The enzymes involved in utilizing glucose, for example, are very

important in metabolism and thus are constitutive. Other enzymes are **regulated enzymes** (**figure 7.4*b***), the production of which is either turned on (induced) or turned off (repressed) in response to changes in concentration of the substrate.

The activity of an enzyme is highly influenced by the cell's environment. In general, enzymes operate only under the natural temperature, pH, and osmotic pressure of an organism's habitat. When enzymes are subjected to changes in these normal conditions, they tend to be chemically unstable, or **labile**. Low temperatures inhibit enzyme reactions, and high temperatures denature the apoenzyme. **Denaturation** is a process by which the weak bonds that collectively maintain the native shape of the apoenzyme are broken. This disruption causes extreme distortion of the enzyme's shape and prevents the substrate from attaching to the active site. Such nonfunctional enzymes block metabolic reactions and thereby can lead to cell death. Low or high pH or certain chemicals (heavy metals, alcohol) are also denaturing agents.

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2. An enzyme that catalyzes starch is called _____, whereas an enzyme that digests fat is termed _____.

a. *carbohydrase; lipase*
b. *maltase; lipase*
c. *carbohydrase; peptidase*
d. *maltase; peptidase*

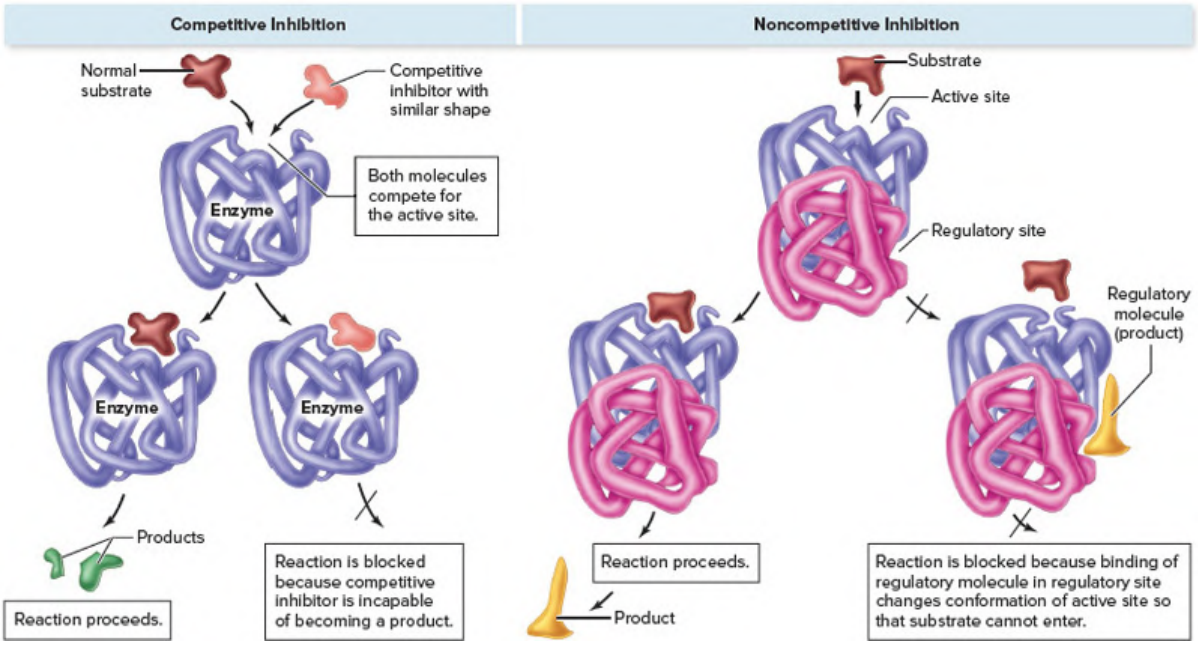

Figure 7.5 **Examples of two common control mechanisms for enzymes.**

Many laundry detergents contain enzymes that target proteins, lipids, and carbohydrates, which are the chemical constituents of stains on clothing.

©McGraw-Hill Education/Jill Braaten, photographer

Direct Controls on the Action of Enzymes

The bacterial cell has many ways of directly influencing the activity of its enzymes. It can inhibit enzyme activity by supplying a molecule that resembles the enzyme's normal substrate. The "mimic" can then occupy the enzyme's active site, preventing the actual substrate from binding there. Because the mimic cannot actually be acted on by the enzyme or function in the way the product would have, the enzyme is effectively shut down. This form of inhibition is called **competitive inhibition**, because the mimic is competing with the substrate for the binding site **(figure 7.5)**. (In chapter 10, you will see that some antibiotics use the same strategy of competing with enzymatic active sites to shut down metabolic processes.)

Another form of inhibition can occur with special types of enzymes that have two binding sites—the active site and another area called the regulatory site (figure 7.5). These enzymes are regulated by the binding of molecules other than the substrate in their regulatory sites. Often the regulatory molecule is the product of the enzymatic reaction itself. This provides a negative feedback mechanism that can slow down enzymatic activity once a certain concentration of product is produced. This is **noncompetitive inhibition**, because the regulator molecule does not bind in the same site as the substrate.

Controls on Enzyme Synthesis

Controlling enzymes by controlling their synthesis is another effective mechanism, because enzymes do not last indefinitely. Some wear out, some are deliberately degraded, and others are diluted with each cell division. For catalysis to continue, enzymes eventually must be replaced. This cycle works into the scheme of the cell, where replacement of enzymes can be regulated according to cell demand. The mechanisms of this

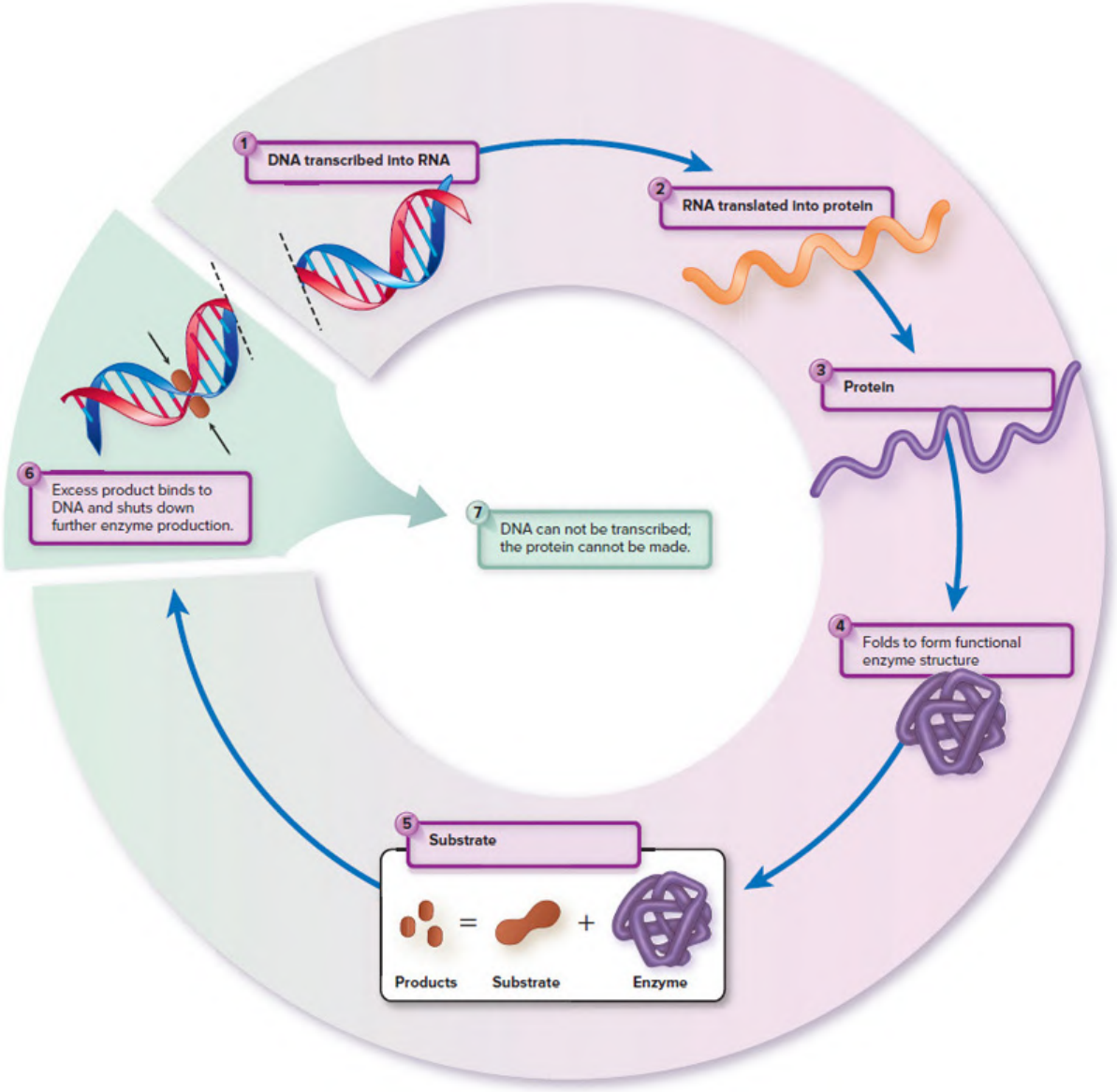

Figure 7.6 **One type of genetic control of enzyme synthesis: enzyme repression**. (1)–(5), The enzyme is synthesized continuously via uninhibited transcription and translation until enough product has been made. (6)–(7), Excess product reacts with a site on DNA that regulates the enzyme's synthesis, thereby inhibiting further enzyme production.

system are genetic in nature; that is, they require regulation of DNA and the protein synthesis machinery—topics we shall encounter once again in chapter 8.

Enzyme repression is a means to stop further synthesis of an enzyme somewhere along its pathway. As the level of the end product from a given enzymatic reaction has built to excess, the genetic apparatus responsible for replacing these enzymes is automatically suppressed **(figure 7.6)**. The response time is longer than for feedback inhibition, but its effects are more enduring.

The inverse of enzyme repression is **enzyme induction**. In this process, enzymes appear (are induced) only when suitable substrates are present—that is, the synthesis of an enzyme is induced by its substrate. Both mechanisms are important genetic control systems in bacteria.

A classic model of enzyme induction occurs in the response of *Escherichia coli* to certain sugars. For example, if a particular strain of *E. coli* is inoculated into a medium whose principal carbon source is lactose, it will produce the enzyme lactase to hydrolyze the disaccharide into its component parts, glucose and galactose. If the bacterium is subsequently inoculated into a medium containing only sucrose as a carbon source, it will cease synthesizing lactase and begin synthesizing sucrase. This response enables the organism to utilize a variety of nutrients, and it also prevents a microbe from wasting energy making enzymes for which no substrates are present.

Enzymes isolated from microbes are used by denim manufacturers to make the fabric softer and impart different colors to it.

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Metabolic Pathways

Metabolic reactions rarely consist of a single action or step. More often, they occur in a multistep series or pathway, with each step catalyzed by an enzyme. The product of one reaction is often the reactant (substrate) for the next, forming a linear chain of reactions. In

addition, many pathways have branches that provide alternate methods for nutrient processing. Other pathways take a cyclic form, in which the starting molecule is regenerated to initiate another turn of the cycle **(figure 7.7)**. On top of that, pathways generally do not stand alone; they are interconnected and merge at many sites.

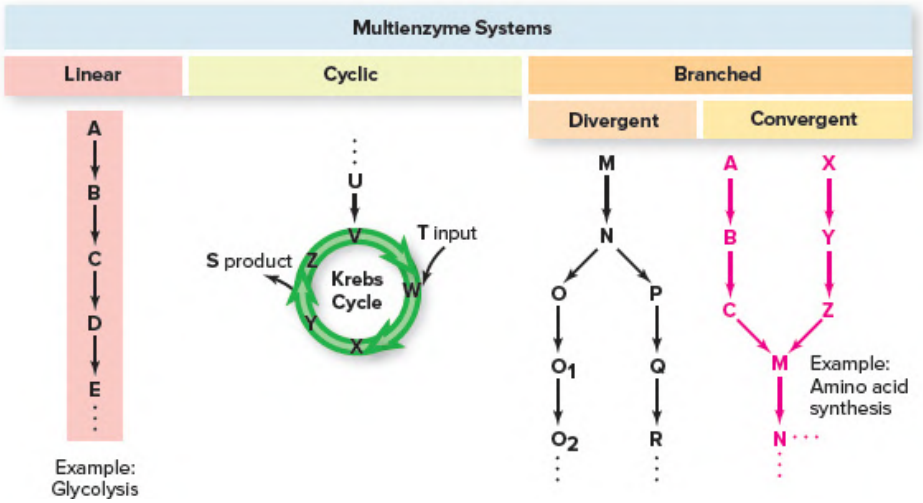

Figure 7.7 **Patterns of metabolism**. In general, metabolic pathways consist of a linked series of individual chemical reactions that produce intermediary metabolites and lead to a final product. These pathways occur in several patterns, including linear, cyclic, and branched. Anabolic pathways involved in biosynthesis result in a more complex molecule, each step adding on a functional group, whereas catabolic pathways involve the dismantling of molecules and can generate energy. Virtually every reaction in a series—represented by an arrow—involves a specific enzyme.

7.1 LEARNING OUTCOMES—Assess Your Progress

1. Describe the relationship among metabolism, catabolism, and anabolism.
2. Fully describe the structure and function of enzymes.
3. Differentiate between constitutive and regulated enzymes.
4. Diagram four major patterns of metabolic pathways.
5. Describe how enzymes are controlled.

7.2 The Pursuit and Utilization of Energy

In order to carry out the work of all of their metabolic processes, cells require constant input of some form of usable energy. The energy can come directly from sunlight (in photosynthesizers), or from free electrons (in electricity-harvesting bacteria). In most bacteria we examine in this book, the energy comes from organic substances (like sugars) when their bonds are broken, releasing and transferring electrons. The energy is mostly stored in ATP.

Energy in Cells

Cells manage energy in the form of chemical reactions that change molecules. This often involves activities such as the making or breaking of bonds and the transfer of electrons. Not all cellular reactions are equal with respect to energy. Some release energy, and others require it to proceed. For example, a reaction that proceeds as follows:

$$X + Y \xrightarrow{\text{Enzyme}} Z + \text{Energy}$$

releases energy as it goes forward. This type of reaction is an **exergonic** (ex-er-gon'-ik) **reaction**. Energy of this type is available for doing cellular work. Energy transactions such as the following:

$$\text{Energy} + A + B \xrightarrow{\text{Enzyme}} C$$

are called **endergonic** (en-der-gon'-ik) **reactions**, because they require the input of energy. In cells, exergonic and endergonic reactions are often coupled, so that released energy is immediately put to use.

Summaries of metabolism may make it seem that cells "create" energy from nutrients, but they do not. What they actually do is extract chemical energy already present in nutrient fuels and apply that energy toward useful work in the cell, much like a gasoline engine releases energy as it burns fuel. The engine does not actually produce energy, but it converts some of the potential energy to do work.

At the simplest level, cells possess specialized enzyme systems that trap the energy present in the bonds of nutrients as they are progressively broken. During exergonic reactions, energy released by bonds is stored in certain high-energy phosphate bonds, such as in ATP. The ability of ATP to temporarily store and release the energy of chemical bonds provides the fuel for endergonic cell reactions. Before discussing ATP, we examine the process behind electron transfer: redox reactions.

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3. A reaction that requires energy as it goes forward is termed
- **a.** *exergonic*.
- **b.** *endergonic*.
- **c.** *oxidative*.
- **d.** *reductive*.

Plant cells, such as in this energy-rich sugar cane, create ATP from photosynthesis.
©Digital Vision/PunchStock

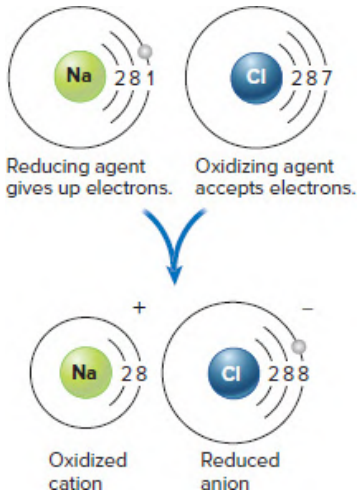


Figure 7.8 **Redox pairs**.

Oxidation and Reduction

Some atoms and compounds readily give or receive electrons and participate in oxidation (the loss of electrons) or reduction (the gain of electrons). The compound that loses the electrons is **oxidized**, and the compound that receives the electrons is **reduced (figure 7.8)**. Such **oxidation-reduction (redox)** reactions are common in the cell and indispensable to the required energy transformations. Important components of cellular redox reactions are oxidoreductases, which remove electrons from one substrate and add them to another. The enzymes carry coenzymes that are vitally important to the transfer of electrons. These coenzymes are nicotinamide adenine dinucleotide (NAD) **(figure 7.9)** and flavin adenine dinucleotide (FAD). As coenzymes, they sit in a groove on the enzymes and accept and donate the electrons as the enzymes do their work. (Take note: Even if by now your eyes are glazing over at all the terms and details, this paragraph is a valuable one! If you remember the statements in this paragraph, then the rest of metabolism will be much easier to understand. A handy mnemonic device for remembering this is OIL RIG: Oxidation is Losing, Reduction is Gaining.)

Redox reactions always occur in pairs, with an electron donor and an electron acceptor, which constitute a *redox pair*. Oxidation-reduction reactions salvage electrons along with the energy they contain.

This changes the energy balance, leaving the just-reduced compound with less energy than the now oxidized one. The energy now present in the electron acceptor can be captured to **phosphorylate** (add an inorganic phosphate) to ADP or to some other compound. This process stores the energy in a high-energy molecule (ATP, for example). In many cases, the cell does not handle electrons as separate entities but rather as parts of an atom such as hydrogen, which contains a proton and an electron. For simplicity's sake, we will continue to use the term *electron transfer*, but keep in mind that hydrogens are often involved in the transfer process. The removal of hydrogens from a compound during a redox reaction is called dehydrogenation. The job of handling these protons and electrons falls to one or more

carriers (the NAD and FAD mentioned earlier), which function as short-term repositories for the electrons until they can be transferred.

Electron Carriers: Molecular Shuttles

The electron carriers NAD and FAD resemble shuttles that are alternately loaded and unloaded, repeatedly accepting and releasing electrons and hydrogens to facilitate the

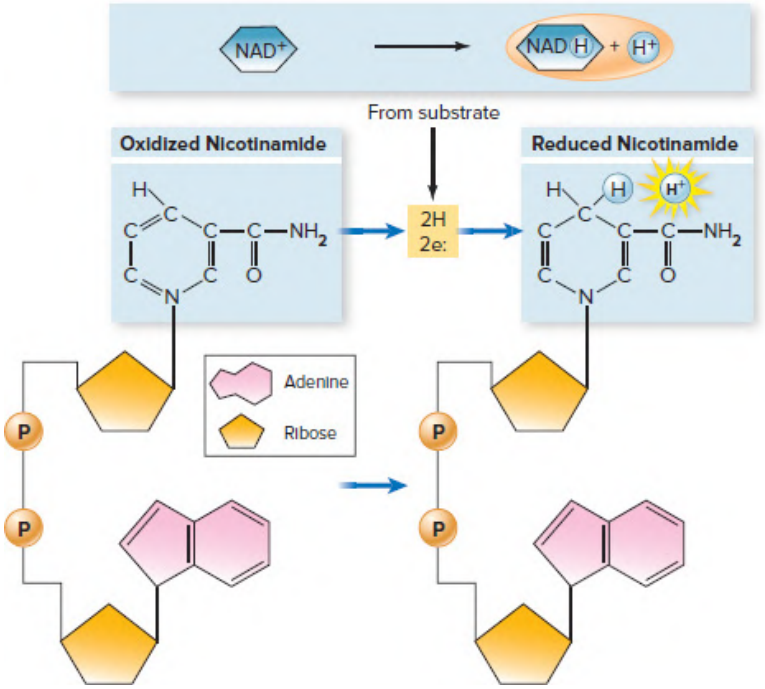


Figure 7.9 **Details of NAD reduction**. The coenzyme NAD contains the vitamin nicotinamide (niacin) and the purine adenine attached to double ribose phosphate molecules (a dinucleotide). The principal site of action is on the nicotinamide (boxed areas). Hydrogens and electrons donated by a substrate interact with a carbon on the top of the ring. One hydrogen bonds there, carrying two electrons, and the other hydrogen is carried in solution as H^+ (a proton).

transfer of redox energy. In catabolic pathways, electrons are extracted and carried through a series of redox reactions until the final electron acceptor at the end of a particular pathway is reached. In aerobic metabolism, this acceptor is molecular oxygen; in anaerobic metabolism, it is some other inorganic or organic compound.

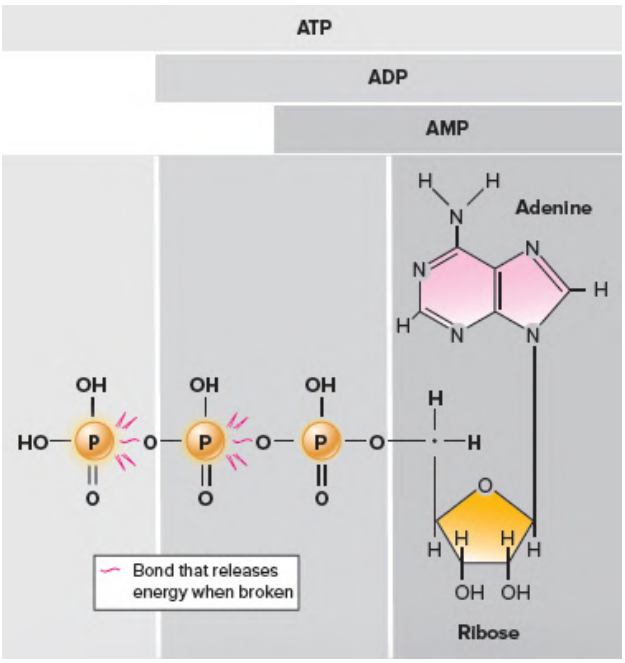


Figure 7.10 **The structure of adenosine triphosphate (ATP)**. Removing the left-most phosphate group yields ADP; removing the next one yields AMP.

Adenosine Triphosphate: Metabolic Money

Let's look more closely at the powerhouse molecule, adenosine triphosphate. ATP has also been described as metabolic money because it can be earned, banked, saved, spent, and exchanged. As an energy repository, ATP provides a connection between energy-yielding catabolism and the other cellular activities that require energy. Some clues to its energy-storing properties lie in its unique molecular structure.

The Molecular Structure of ATP

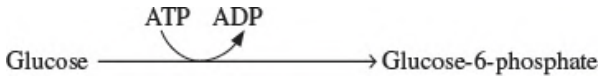
ATP is a three-part molecule consisting of a nitrogen base (adenine) linked to a 5-carbon sugar (ribose), with a chain of three phosphate groups bonded to the ribose **(figure 7.10)**. The high energy of ATP comes from the orientation of the phosphate groups, which are relatively bulky and carry negative charges. The proximity of these repelling electrostatic charges imposes a strain—especially between the last two phosphate groups. The strain on the phosphate bonds accounts for the energetic quality of ATP because removal of the terminal phosphates releases free energy.

Breaking the bonds between the two outermost phosphates of ATP yields adenosine diphosphate (ADP), which can then be further converted to adenosine monophosphate (AMP). AMP derivatives help form the backbone of RNA and are also a major component of certain coenzymes (NAD, FAD, and coenzyme A).

The Metabolic Role of ATP

ATP is the primary energy currency of the cell, and when its energy is used in a chemical reaction, it must then be replaced. Therefore, ATP utilization and replenishment make up an

ongoing cycle. Often, the energy released during ATP hydrolysis drives biosynthesis by providing an activating phosphate to an individual substrate before it is enzymatically linked to another molecule. ATP is also used to prepare molecules for catabolism, such as when a 6-carbon sugar is phosphorylated during the early stages of glycolysis:



When ATP is utilized by the removal of the terminal phosphate to release energy plus ADP, ATP then needs to be re-created. The reversal of this process—that is, adding the terminal phosphate to ADP—will replenish ATP, but it requires an input of energy:

In heterotrophs, the energy infusion that regenerates a high-energy phosphate comes from certain steps of catabolic pathways, in which nutrients such as carbohydrates are degraded and yield energy. ATP is formed when substrates or electron carriers provide a high-energy phosphate that becomes bonded to ADP.

7.2 LEARNING OUTCOMES—Assess Your Progress

6. Name the chemical in which energy is stored in cells.
7. Create a general diagram of a redox reaction.
8. Identify electron carriers used by cells.

ATP is used for energy in all cells, including human cells.
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7.3 Catabolism

Now you have an understanding of all the tools a cell needs to *metabolize*. Metabolism uses *enzymes* to drive reactions that break down (*catabolize*) organic molecules to materials (*precursor molecules*) that cells can then use to build (*anabolize*) larger, more complex molecules that they need. This process was presented symbolically in figure 7.1. Another very important point about metabolism is that *reducing power* (the electrons available when NAD and FAD are in their reduced forms—NADH and $FADH_2$) and *energy* (stored in the bonds of ATP) are needed in large quantities for the anabolic parts of metabolism (the blue bars in that figure). They are produced during the catabolic part of metabolism (the yellow bar).

Metabolism starts with "nutrients" from the environment, usually discarded molecules from other organisms. Cells have to get the nutrients inside; to do this, they use the transport

mechanisms discussed in chapter 6. Some of these require energy, which is available from catabolism already occurring in the cell. In the next step, intracellular nutrients have to be broken down to the appropriate precursor molecules. These catabolic pathways are discussed next.

Getting Materials and Energy

Nutrient processing is extremely varied, especially in bacteria, yet in most cases it is based on three basic catabolic pathways. Frequently, the nutrient is glucose. In previous discussions, microorganisms were categorized according to their requirement for oxygen gas. This designation is related directly to these nutrient processing pathways. **Figure 7.11** provides an overview of the three major pathways for producing the needed precursors and energy (i.e., catabolism).

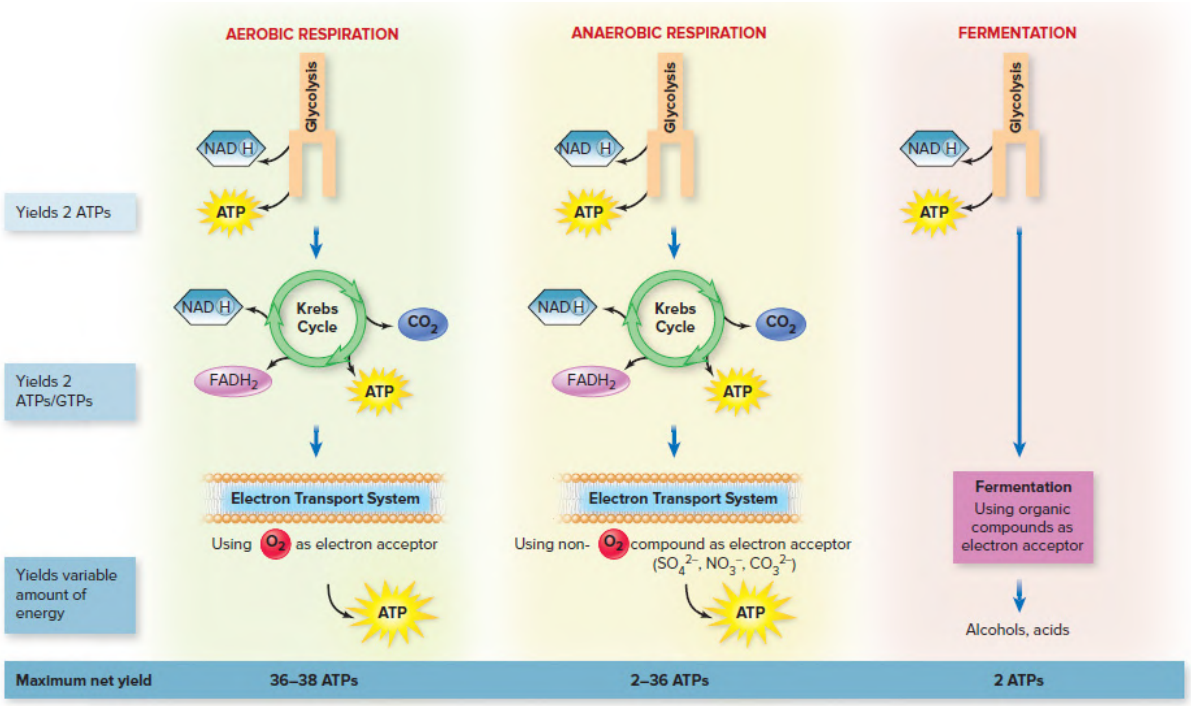

Figure 7.11 **Overview of the three main pathways of catabolism.**

Aerobic respiration is a series of reactions (glycolysis, the Krebs cycle, and the respiratory chain) that converts glucose to CO_2 and allows the cell to recover significant amounts of energy. Aerobic respiration relies on free oxygen as the final acceptor for electrons and hydrogens and produces a relatively large amount of ATP. Aerobic respiration is characteristic of many bacteria, fungi, protozoa, and animals.

Anaerobic respiration is a metabolic strategy used by many microorganisms, some strictly anaerobic and others who are able to metabolize with or without oxygen. This system involves the same three pathways as aerobic respiration, but it does not use oxygen

as the terminal electron acceptor; instead, NO_3^-, SO_4^{2-}, CO_3^{3-}, and other oxidized compounds are utilized.

Fermentation is an adaptation used by facultative and aerotolerant anaerobes to incompletely oxidize (ferment) glucose. In this case, oxygen is not required, organic compounds are the terminal electron acceptors, and a relatively small amount of ATP is produced.

Glycolysis

All three of the metabolic pathways begin with **glycolysis**, which turns glucose into two copies of pyruvic acid—a chemical uniquely capable of yielding energy in the pathways that follow. **Table 7.2** illustrates glycolysis.

Pyruvic Acid—Central to All Three Metabolic Strategies

In strictly aerobic organisms and some anaerobes, pyruvic acid enters the Krebs cycle for further processing and energy release. Facultative anaerobes can use fermentation, in which pyruvic acid is re-reduced into acids or other products.

After Pyruvic Acid Aerobic and Anaerobic Respiration Strategies

Bacterial respiration, whether done aerobically or anaerobically, utilizes the Krebs cycle and the electron transport system to harvest the energy and products needed to build cell parts.

Table 7.2 Glycolysis

Energy Lost or Gained	Overview	Details
Uses 2 ATPs	Glucose (C–C–C–C–C–C) → Fructose-1, 6-diphosphate (C–C–C–C–C–C)	Three reactions alter and rearrange the 6C glucose molecule into 6-C fructose-1,6-diphosphate.
	(C–C–C) (C–C–C)	One reaction breaks fructose-1,6-diphosphate into two 3-carbon molecules.
Yields 4 ATPs and 2 NADHs	Pyruvate (C–C–C) Pyruvate (C–C–C)	Five reactions convert each 3-carbon molecule into the 3C pyruvate.
Total Energy Yield: 2 ATPs and 2 NADHs	Pyruvate is a molecule that is uniquely suited for chemical reactions that will produce reducing power (which will eventually produce ATP).	

Microbes often obtain nutrients from dead plants and organisms.
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The Krebs cycle operates similarly in aerobic and anaerobic respiration and is covered next.

The Krebs Cycle—A Carbon and Energy Wheel

As you have seen, the cleaving and oxidation of glucose during glycolysis yields a comparatively small amount of energy and gives off pyruvic acid. Pyruvic acid is still energy-rich, containing a number of extractable hydrogens and electrons to power ATP

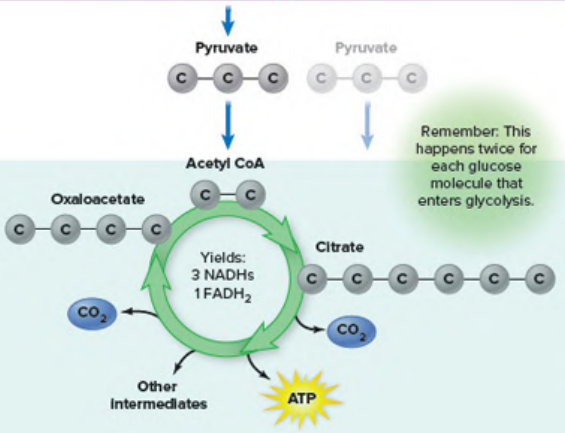
synthesis. The hydrogens and electrons are only made available during the work of the second and third phases of respiration, in which pyruvic acid's hydrogens are transferred to oxygen. In the following section, we examine the second phase of catabolism, which takes place in the cytoplasm of bacteria and in the mitochondrial matrix in eukaryotes. **Table 7.3** summarizes the Krebs cycle.

The **Krebs cycle** (also known as the tricarboxylic acid [TCA] cycle) serves to transfer the energy stored in acetyl CoA to NAD^+ and FAD by reducing them (transferring electrons to them). Thus, the main products of the Krebs cycle are these reduced molecules (as well as 2 ATPs for each glucose molecule). The reduced coenzymes NADH and $FADH_2$ are vital to the energy production that will occur in electron transport. Along the way, the 2-carbon acetyl CoA joins with a 4-carbon compound, oxaloacetic acid, and then participates in seven additional chemical transformations while "spinning off" the NADH and $FADH_2$. That's why we sometimes call the Krebs cycle the "carbon and energy wheel."

The Respiratory Chain: Electron Transport

We now come to the energy chain, which is the final "processing mill" for electrons and hydrogen ions and the major generator of ATP. It is the final step in both aerobic and anaerobic respiration. Overall, the electron transport system (ETS) consists of a chain of special redox carriers that receives electrons from reduced carriers (NADH, $FADH_2$) generated by glycolysis and the Krebs cycle and passes them in

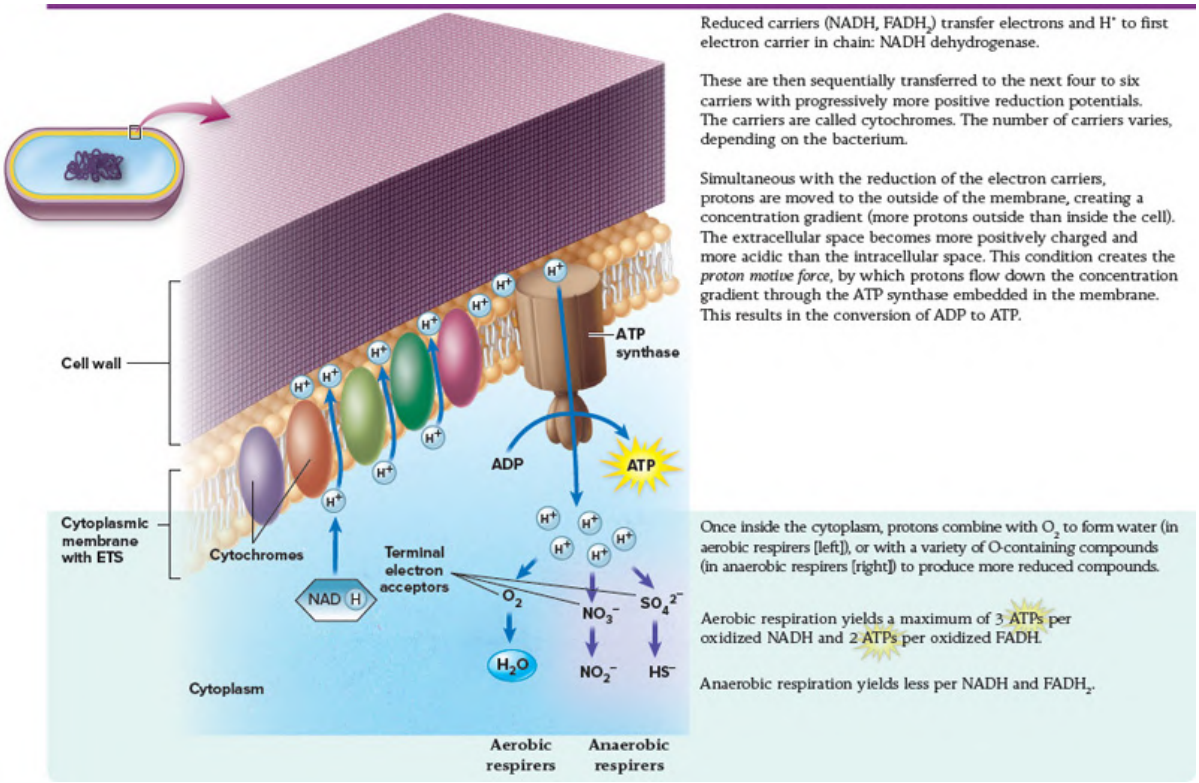
Table 7.3 The Krebs Cycle

Energy Lost or Gained	Overview	Details
Total yield per 2 pyruvate: CO_2: 2 Energy: 2 NADHs		The 3C pyruvate is converted to 2C acetyl CoA in one reaction.
Each acetyl CoA yields 1 ATP, 3 NADHs, 1 FADH, and 2 CO_2 molecules. Total Yield per 2 acetyl CoAs: CO_2: 4 Energy: 2 ATPs, 6 NADHs, 2 FADHs		In the first reaction, acetyl CoA donates 2Cs to the 4C molecule oxaloacetate to form 6C citrate. In the course of seven more reactions, citrate is manipulated to yield energy and CO_2 and oxaloacetate is regenerated. Intermediate molecules on the wheel can be shunted into other metabolic pathways as well.

a sequential and orderly fashion from one redox molecule to the next. The flow of electrons down this chain is highly energetic and allows the active transport of hydrogen ions to the outside of the membrane where the respiratory chain is located. In aerobic respiration, the step that finalizes the transport process is the acceptance of electrons and hydrogen by

oxygen, producing water. This process consumes oxygen. Some variability exists from one organism to another, but the principal compounds that carry out these complex reactions are NADH dehydrogenase, flavoproteins, coenzyme Q (ubiquinone), and **cytochromes** (sy′-toh-krohmz). The cytochromes contain a tightly bound metal atom at their center that is actively involved in accepting electrons and donating them to the next carrier in the series. The highly compartmentalized structure of the respiratory chain is an important factor in its function. Note in **table 7.4** that the electron transport carriers and enzymes are embedded in the cytoplasmic membrane in bacteria. The equivalent structure for housing them in eukaryotes is the inner mitochondrial membranes pictured in **figure 7.12**.

Table 7.4 The Respiratory (Electron Transport) Chain



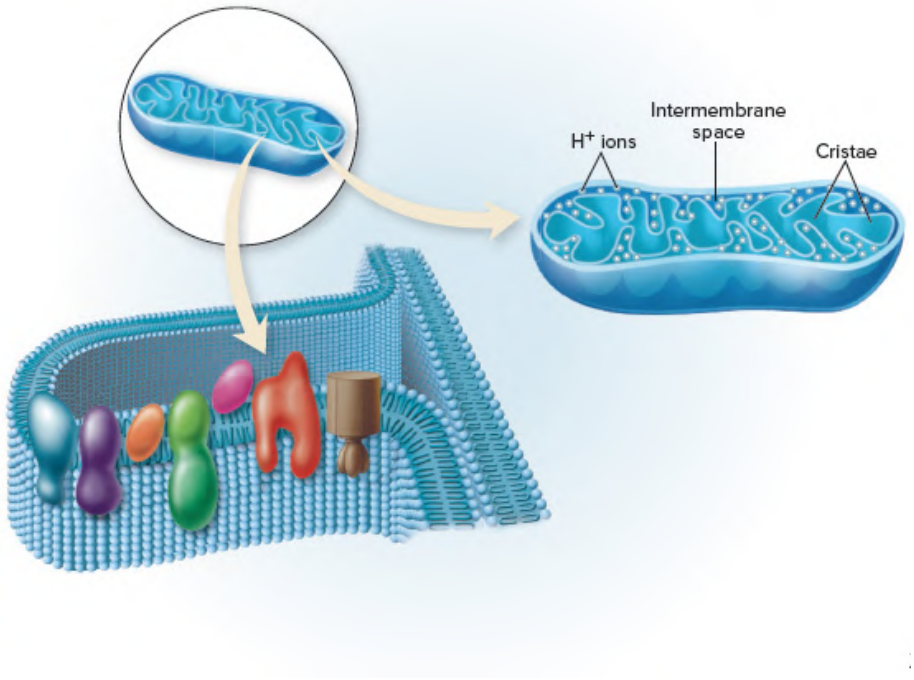


Figure 7.12 **The electron transport system on the inner membrane of the mitochondrial cristae in eukaryotes**.

Conveyance of the NADHs from glycolysis and the Krebs cycle to the first carrier sets in motion the remaining steps. The hydrogen ions sequestered outside the membrane travel back into the cell using the **ATP synthase** complex as a channel. When they do so, they provide the energy to add a phosphate to ADP, creating ATP in the process. Each NADH that enters electron transport can give rise to a maximum of 3 ATPs (though actual numbers are probably lower due to inefficiencies in the pathways). This coupling of ATP synthesis to electron transport is termed **oxidative phosphorylation**. Because the electrons from $FADH_2$ from the Krebs cycle enter the cycle at a later point than the NAD reactions, there is less energy to release, and only 2 ATPs are the result.

Medical Moment

Facultative Anaerobes

Some organisms are capable of utilizing either aerobic or anaerobic respiration, depending on what is available in their environment. As discussed in chapter 6, these are called facultative anaerobes. If oxygen is available, aerobic respiration is utilized as it is most efficient and yields more ATP than other metabolic pathways. In the absence of oxygen, these organisms use either anaerobic respiration or fermentation.

One example is *Listeria*. Foods ranging from dairy products and uncooked meats to fruits and vegetables have been identified to be contaminated with *Listeria monocytogenes*. Exposure to this bacterium causes foodborne illness and in those at high risk, may cause death. Being a facultative anaerobe contributes to the resiliency of *Listeria*, allowing it to survive and multiply in diverse environments. Most people are regularly exposed to this bacteria in their daily settings. Following safe food preparation and handling techniques can prevent foodborne illness.

Q. From this information, can you identify another way in which *Listeria* adapts to environmental conditions, in addition to oxygen utilization?

Answer in Appendix B.

The Final Step

Aerobic Respiration The final step in aerobic respiration, during which oxygen accepts the electrons, is catalyzed by cytochrome aa_3, also called cytochrome oxidase. This large enzyme complex is specifically adapted to receive electrons from cytochrome *c*, pick up hydrogens from the solution, and react with oxygen to form a molecule of water. This reaction, though in actuality more complex, can be summarized as follows:

$$2H^+ + 2e^- + \tfrac{1}{2}O_2 \rightarrow H_2O$$

Most eukaryotic aerobes have a fully functioning cytochrome system, but bacteria exhibit wide-ranging variations in this part of the system. Some species lack one or more of the redox steps; others have several alternative electron transport schemes. Because many bacteria lack cytochrome oxidase, this variation can be used to differentiate among certain genera of bacteria. An oxidase detection test can be used to help identify members of the genera *Neisseria* and *Pseudomonas* and some species of *Bacillus*.

A potential side reaction of the respiratory chain in aerobic organisms is the incomplete reduction of oxygen to superoxide ion (O_2^-) and hydrogen peroxide (H_2O_2). As mentioned in chapter 6, these toxic oxygen products can be very damaging to cells. Aerobes have neutralizing enzymes to deal with these products, including *superoxide dismutase* and *catalase*. One exception is the genus *Streptococcus*, which can grow well in oxygen yet lacks both cytochromes and catalase. The tolerance of these organisms to oxygen can be explained by the neutralizing effects of a special peroxidase. The lack of cytochromes, catalase, and peroxidases in anaerobes as a rule limits their ability to process free oxygen and contributes to its toxic effects on them.

Anaerobic Respiration The terminal step in anaerobic respiration utilizes inorganic compounds, rather than free oxygen, as the final electron acceptor in electron transport. Of these, the nitrate (NO_3^-) and nitrite (NO_2^-) reduction systems are best known. The reaction in species such as *Escherichia coli* is represented as:

$$\underset{\text{nitrate}}{NO_3^-} + NADH \xrightarrow[\text{Nitrate reductase}]{} \underset{\text{nitrate}}{NO_2^-} + H_2O + NAD^+$$

The enzyme nitrate reductase catalyzes the removal of oxygen from nitrate, leaving nitrite and water as products. A test for this reaction is one of the physiological tests used in identifying bacteria.

Some species of *Pseudomonas* and *Bacillus* possess enzymes that can further reduce nitrite to nitric oxide (NO), nitrous oxide (N_2O), and even nitrogen gas (N_2). This process,

called **denitrification**, is a very important step in recycling nitrogen in the biosphere. Other oxygen-containing nutrients reduced anaerobically by various

bacteria are carbonates and sulfates. None of the anaerobic pathways produce as much ATP as aerobic respiration. They produce a variable amount of ATP, from 2 to 36 ATPs.

After Pyruvic Acid The Fermentation Strategy

The definition of *fermentation* is the incomplete oxidation of glucose or other carbohydrates in the absence of oxygen. This process uses organic compounds as the terminal electron acceptors and yields a small amount of ATP (see figure 7.11). This pathway is used by organisms that do not have an electron transport chain and therefore cannot respire. Other organisms can repress the production of electron transport chain proteins when oxygen is lacking in their environment. They can then revert to fermentation.

Without an electron transport chain to churn out large quantities of ATP from reduced carriers, it may seem that fermentation would yield only meager amounts of energy (2 ATPs maximum per glucose), and that would slow down growth. What actually happens, however, is that many bacteria can grow as fast as they would in the presence of oxygen. This rapid growth is made possible by an increase in the rate of glycolysis. From another standpoint, fermentation permits independence from molecular oxygen and allows colonization of anaerobic environments. It also enables microorganisms with a versatile metabolism to adapt

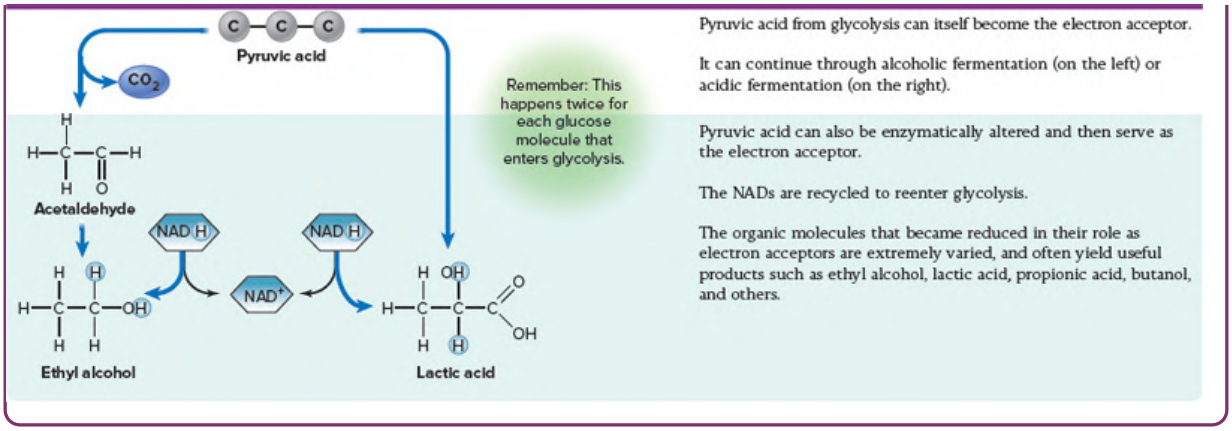
to variations in the availability of oxygen. For them, fermentation provides a means to grow even when oxygen levels are too low for aerobic respiration.

Bacteria that digest cellulose in the rumens of cattle are largely fermentative. After initially hydrolyzing cellulose to glucose, they ferment the glucose to organic acids, which are then absorbed as the bovine's principal energy source. Even human muscle cells can undergo a form of fermentation that permits short periods of activity after the oxygen supply in the muscle has been exhausted. Muscle cells convert pyruvic acid into lactic acid, which allows anaerobic production of ATP to proceed for a time. But this cannot go on indefinitely, and after a few minutes, the accumulated lactic acid causes muscle fatigue.

Table 7.5 gives an overview of fermentation.

Yeasts turn the sugar in grapes into alcohol through fermentation.
©Andrew Hagen/Shutterstock (Grapes); ©Ingram Publishing/SuperStock (wine barrels)

Table 7.5 Fermentation



Products of Fermentation in Microorganisms

Alcoholic beverages (wine, beer, whiskey) are perhaps the most well-known fermentation products. Note that the products of alcoholic fermentation are not only ethanol but also CO_2, a gas that accounts for the bubbles in champagne and beer and the rising of bread dough.

Other fermentation products are solvents (acetone, butanol), organic acids (lactic, acetic), dairy products, and many other foods. Derivatives of proteins, nucleic acids, and other organic compounds are fermented to produce vitamins, antibiotics, and even hormones such as hydrocortisone.

We have provided only a brief survey of fermentation products, but it is worth noting that microbes can be harnessed to synthesize a variety of other substances by varying the raw materials provided them. Large-scale industrial processes using microorganisms often utilize entirely different mechanisms from those described here, and they even occur aerobically, particularly in antibiotic, hormone, vitamin, and amino acid production. Even so, they are also referred to as *fermentation*.

Catabolism of Noncarbohydrate Compounds

We have given you one version of events for catabolism, using glucose, a carbohydrate, as our example. Other compounds serve as fuel, as well. The more complex polysaccharides are easily broken down into their component sugars, which can enter glycolysis at various points. Microbes also break down other molecules for their own use, of course. Two other major sources of energy and building blocks for microbes are lipids (fats) and proteins. Both of these must be broken down to their component parts to produce precursor metabolites and energy.

Recall from chapter 1 that fats are fatty acids joined to glycerol. Enzymes called **lipases** break these apart. The glycerol is then converted to dihydroxyacetone phosphate (DHAP), which can enter a step midway through glycolysis. The fatty acid component goes through a process called **beta oxidation**. Fatty acids have a variable number of carbons; in beta oxidation, 2-carbon units are successively transferred to coenzyme A, creating acetyl CoA,

which enters the Krebs cycle. This process can yield a large amount of energy. Oxidation of a 6-carbon fatty acid yields 50 ATPs, compared with 38 for a 6-carbon sugar.

Proteins are chains of amino acids. Enzymes called **proteases** break proteins down to their amino acid components, after which the amino groups are removed by a reaction called **deamination**. This leaves a carbon compound, which is easily converted to one of several Krebs cycle intermediates.

7.3 LEARNING OUTCOMES—Assess Your Progress

9. Name the three main catabolic pathways and the estimated ATP yield for each.
10. Construct a paragraph summarizing glycolysis.
11. Describe the Krebs cycle, with emphasis on what goes into it and what comes out of it.
12. Discuss the significance of the electron transport system, and compare the process between bacteria and eukaryotes.
13. State two ways in which anaerobic respiration differs from aerobic respiration.
14. Summarize the steps of microbial fermentation, and list three useful products it can create.
15. Describe how noncarbohydrate compounds are catabolized.

7.4 Anabolism and the Crossing Pathways of Metabolism

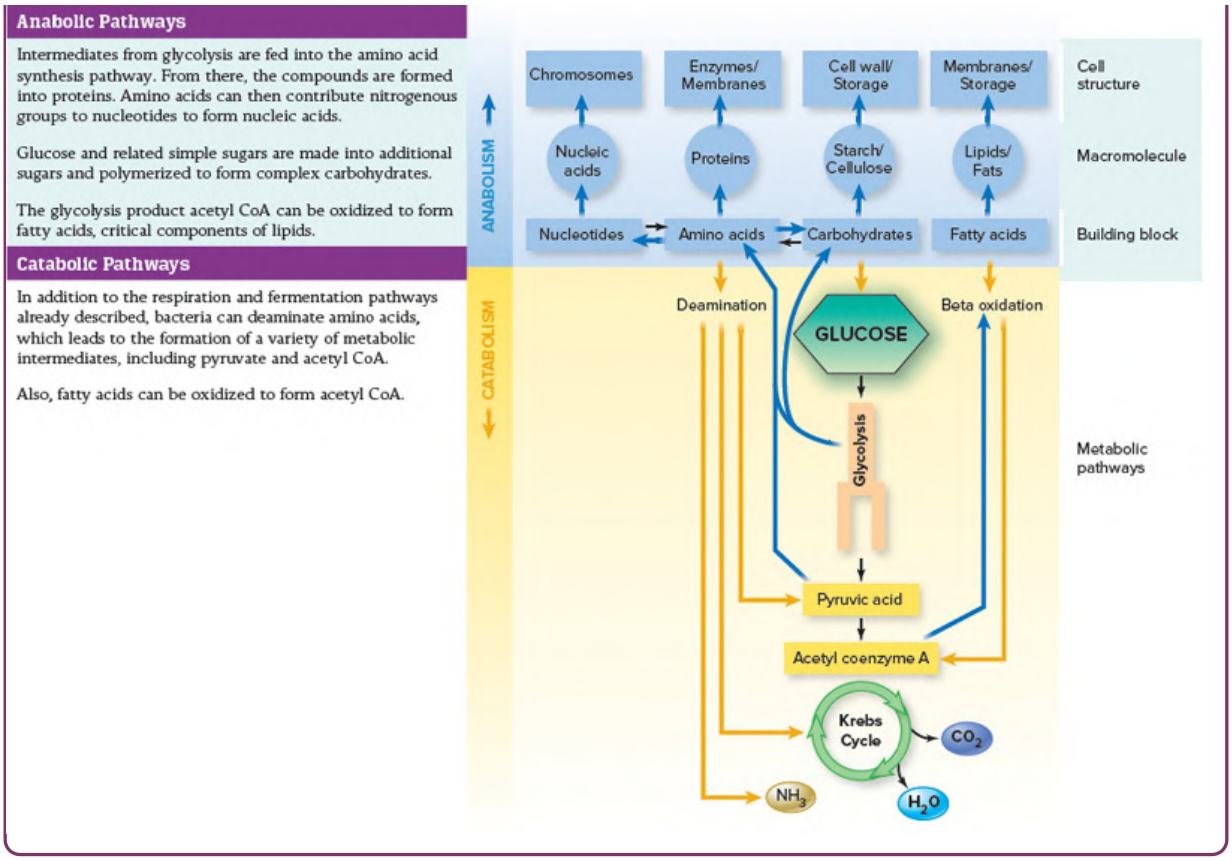
Our discussion now turns from catabolism and energy extraction to anabolic functions and biosynthesis. In this section, we present aspects of intermediary metabolism, including amphibolic pathways, the synthesis of simple molecules, and the synthesis of macromolecules.

The Efficiency of the Cell

It must be obvious by now that cells have mechanisms for careful management of carbon compounds. Rather than being dead ends, most catabolic pathways contain strategic molecular intermediates (metabolites) that can be diverted into anabolic pathways. In this way, a given molecule can serve multiple purposes, and the maximum benefit can be derived from all nutrients and metabolites of the cell pool. The ability of a system to integrate catabolic and anabolic pathways to improve cell efficiency is termed **amphibolism** (am-fee-bol′-izm).

At this point in the chapter, you can appreciate a more complex view of metabolism than that presented at the beginning in figure 7.1. **Table 7.6** demonstrates the amphibolic nature of intermediary metabolism.

Table 7.6 Amphibolic Pathways of Glucose Metabolism



Anabolism: Formation of Macromolecules

Monosaccharides, amino acids, fatty acids, nitrogen bases, and vitamins—the building blocks that make up the various macromolecules and organelles of the cell—come from two possible sources. They can enter the cell from the outside "ready to use," or they can be synthesized through various cellular pathways. The degree to which an organism can synthesize its own building blocks (simple molecules) is determined by its genetic makeup, a factor that varies tremendously from group to group. In chapter 6, you learned that autotrophs require only CO_2 as a carbon source, a few minerals to synthesize all cell substances, and no organic nutrients. Some heterotrophic organisms (*E. coli*, yeasts) are also very efficient in that they can synthesize all cellular substances from minerals and a single organic carbon source such as glucose. Compare this with a strict parasite that has few synthetic abilities of its own and derives most precursor molecules from the host.

Whatever their source, once these building blocks are added to the metabolic pool, they are available for the synthesis of polymers by the cell.

Carbohydrate Biosynthesis

Glucose has a central role in metabolism and energy utilization. For that reason, there are multiple pathways for manufacturing it in cells. Certain structures in the cell depend on an

adequate supply of glucose as well. It is the major component of the cellulose cell walls of some eukaryotes and of certain storage granules (starch, glycogen). One of the intermediaries in glycolysis, glucose-6-P, is used to form glycogen. Monosaccharides other than glucose are important in the synthesis of bacterial cell walls. Peptidoglycan contains a linked polymer of muramic acid and glucosamine. Fructose-6-P from glycolysis is used to form these two sugars. Carbohydrates (deoxyribose, ribose) are also essential building blocks in nucleic acids. Polysaccharides are the predominant components of cell surface structures such as capsules and the glycocalyx, and they are commonly found in slime layers.

Medical Moment

Amino Acids: Essential, Nonessential, and Conditionally Essential Amino Acids

Essential amino acids are those amino acids that must be obtained from our diet, as our bodies lack the ability to synthesize them, while nonessential amino acids are those that can be synthesized by our bodies.

However, as is often the case, there are exceptions to every rule just to keep things interesting! *Conditionally essential* amino acids are not normally required in the diet, except in specific populations that are unable to synthesize them in adequate amounts. For example, individuals with PKU (phenylketonuria), a genetic condition, must strictly restrict the levels of phenylalanine in their bodies to prevent serious complications such as developmental disabilities; however, because they cannot synthesize enough tyrosine from the trace amounts of phenylalanine in their bodies, tyrosine becomes an essential amino acid for these individuals.

Q. From the above, infer how tyrosine—nonessential in healthy people—is normally produced in the body.

Answer in Appendix B

Blood being drawn from the heel of an infant for PKU testing
©Marmaduke St. John/Alamy Stock Photo

Amino Acids, Protein Synthesis, and Nucleic Acid Synthesis

Proteins account for a large proportion of a cell's constituents. They are essential components of enzymes, the cytoplasmic membrane, the cell wall, and cell appendages. As a general rule, 20 amino acids are needed to make these proteins. Although some organisms (*E. coli*, for example) have pathways that will synthesize all 20 amino acids, others, including animals, lack some or all of the pathways for amino acid synthesis and must acquire the essential ones from their diets. Protein synthesis itself is a complex process that requires a genetic blueprint and the operation of intricate cellular machinery, as you will see in chapter 8.

DNA and RNA are responsible for the hereditary continuity of cells and the overall direction of protein synthesis. Because nucleic acid synthesis is a major topic of genetics and is closely allied to protein synthesis, it will likewise be covered in chapter 8.

Assembly of the Cell

The component parts of a bacteria cell are synthesized on a continuous basis, and catabolism is also taking place as long as nutrients are present and the cell is in a nondormant state. When anabolism produces enough macromolecules to serve two cells, and when DNA replication produces duplicate copies of the cell's genetic

material, the cell undergoes binary fission, which results in two cells from one parent cell. The two cells will need twice as many ribosomes, twice as many enzymes, and so on. The cell has created these during the anabolic phases we have described. Before cell division, the membrane(s) and the cell wall will have increased in size to create a cell that is almost twice as big as a "newborn" cell. Once synthesized, the phospholipid bilayer components of the membranes assemble themselves spontaneously with no energy input. Other assembly reactions require the input of energy. Proteins and other components must be added to the membranes. Growth of the cell wall, accomplished by the addition and coupling of sugars and peptides, requires energy input. The energy acquired during catabolic processes provides all the energy for these complex building reactions.

7.4 LEARNING OUTCOMES—Assess Your Progress

16. Provide an overview of the anabolic stages of metabolism.
17. Define *amphibolism*.

CASE FILE WRAP UP

©McGraw-Hill Education (health care worker); Source: CDC/Janice Haney Carr (*Staphylococcus aureus* bacteria)

In low oxygen states, the body uses other sources of energy. When organs are unable to extract the oxygen they need for energy production, the body converts to anaerobic metabolism to produce ATP. The by-product of this process is lactic acid. High levels of lactic acid can alter the patient's blood pH—a state called lactic acidosis. Generally a high serum lactate level indicates poor organ function.

Prompt identification and treatment of sepsis is associated with better outcomes and decreased mortality. The priority for sepsis management is early fluid resuscitation and antibiotic administration. It is essential to support hemodynamics (blood pressure and perfusion) to ensure adequate oxygen delivery to body tissues and prevent further organ damage.

In this case, the nurse correctly prioritized collection of body fluid samples for culture while waiting for the arrival of antibiotics. Collecting these samples after antibiotics have been given may alter the growth of bacteria, clouding the picture for treatment of a specific organism. Patients with septicemia are treated with broad-spectrum antibiotics until the specific causative organism is identified.

Electricity Eaters

This chapter describes metabolism, and it states right up front that metabolism has three goals:

- break down large molecules (usually sugars) to get building blocks,
- build molecules the cell needs, and
- harvest energy to do the building work.

Put another way, electron flow is necessary for energy to be gained. The flow goes like this:

an energy source → electron carriers → the electron transport chain

Well, nature continues to surprise us with its many different variations on central themes. In 2013 scientists discovered microbes that accomplish their metabolic goals using nothing but electrons. Recall from table 6.2 that many autotrophs run on CO_2 and sunlight or minerals. But these newly discovered bacteria are capable of metabolizing and growing using only electrons from electricity. We know that some bacteria can run the TCA cycle in reverse, creating acetyl CoA from carbon dioxide. However, the very newest bacteria discovered can live with no carbon input at all—not even CO_2.

Dr. Ken Nealson, at the University of Southern California, whose lab conducted these studies, states: "This is huge. What it means is there's a whole part of the microbial world that we don't know about." In addition there are a slew of practical applications, including waste recycling and creating biological fuel cells. Dr. Nealson is also guessing that this might be the predominant mode of life on other planets.

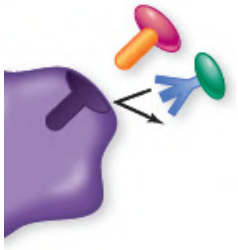
Electricity-eating bacteria have been found in many places with little oxygen but lots of minerals. One group was isolated from rocks on the sea bed near Catalina Island in California.
©incamerastock/Alamy Stock Photo

Chapter Summary

7.1 Metabolism and the Role of Enzymes

- Metabolism is the sum of cellular chemical and physical activities. It consists of anabolism, synthetic reactions that convert small molecules into large molecules, and catabolism, in which large molecules are degraded and energy is produced.

- Metabolism is made possible by organic catalysts called enzymes, that speed up reactions to rates compatible with biological processes.
- Enzymes are not consumed and can be reused. Each enzyme acts specifically upon its matching molecule or substrate.
- Substrate attachment occurs in the special pocket called the active, or catalytic, site.
- Enzymes are labile (unstable) and function only within narrow operating ranges of temperature, osmotic pressure, and pH, and they are especially vulnerable to denaturation.
- Enzymes are frequently the targets for physical and chemical agents used in control of microbes.
- Regulatory controls can act on enzymes directly or on the genetic process that gives rise to the enzymes.

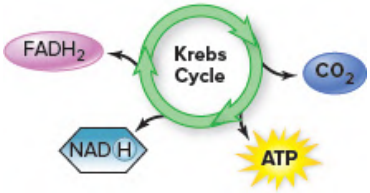


7.2 The Pursuit and Utilization of Energy

- Energy is the capacity of a system to perform work. It is consumed in endergonic reactions and is released in exergonic reactions.
- Extracting energy requires a series of electron carriers arrayed in a redox chain between electron donors and electron acceptors.

7.3 Catabolism

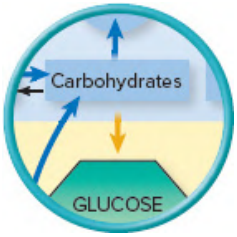
- Carbohydrates, such as glucose, are energy-rich because when catabolized they can yield a large number of electrons per molecule.
- Glycolysis is a pathway that degrades glucose to pyruvic acid without requiring oxygen.
- Pyruvic acid is processed in aerobic respiration via the Krebs cycle and its associated electron transport chain.



- Pyruvic acid undergoes further oxidation and decarboxylation in the Krebs cycle, which generates ATP, CO_2, and large amounts of reduced carriers (NADH and $FADH_2$).
- The respiratory chain completes energy extraction.
- The final electron acceptor in aerobic respiration is oxygen. In anaerobic respiration, compounds such as sulfate, nitrate, or nitrite serve this function.
- Fermentation is anaerobic respiration in which both the electron donor and final electron acceptors are organic compounds.
- Production of alcohol, vinegar, and certain industrial solvents relies upon fermentation.
- Glycolysis and the Krebs cycle are central pathways that link catabolic and anabolic pathways, allowing cells to break down different classes of molecules in order to synthesize compounds required by the cell.
- Intermediates, such as pyruvic acid, are convertible into amino acids, and amino acids can in turn be used as precursors to glucose and other carbohydrates.
- Two-carbon acetyl molecules from pyruvate can be used in fatty acid synthesis.

7.4 Anabolism and the Crossing Pathways of Metabolism

- The ability of a cell or system to integrate catabolic and anabolic pathways to improve efficiency is called amphibolism.
- Macromolecules, such as proteins, carbohydrates, and nucleic acids, are made of building blocks from two possible sources: from outside the cell (preformed) or via synthesis in one of the anabolic pathways.



SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2–Remember and Understand (Choose one.)	B. Bloom's Level 3, 4–Apply and Analyze	C. Bloom's Level 5, 6–Evaluate and Create

ASM Concept/Competency	A. Bloom's Level 1, 2–Remember and Understand (Choose one.)	B. Bloom's Level 3, 4–Apply and Analyze	C. Bloom's Level 5, 6–Evaluate and Create
Evolution	**1**. The electron transport system in bacteria is located on the _____ and in eukaryotic cells on the _____. **a**. plastid, chloroplast **b**. cytoplasmic membrane, mitochondrion **c**. cell wall, mitochondrion **d**. mitochondrion, cytoplasmic membrane	**2**. Speculate on why glycolysis, which is the beginning point of many catabolic pathways, does not require oxygen.	**3**. Provide evidence in support of or refuting the following statement: The evolution of aerobic respiration was driven by the success of photosynthetic microbes.
Cell Structure and Function	**4**. Many coenzymes are formed from **a**. metals. **b**. vitamins. **c**. proteins. **d**. substrates.	**5**. Describe the roles played by ATP and NAD in metabolism.	**6**. Explain why electron transport systems are always found in a membrane.
Metabolic Pathways	**7**. Energy is carried from catabolic to anabolic reactions in the form of **a**. ADP. **b**. high-energy ATP bonds. **c**. coenzymes. **d**. inorganic phosphate.	**8**. What is meant by the concept of the "final electron acceptor"?	**9**. Investigate the creation of lactic acid in human cells via fermentation, and write a paragraph contrasting it with microbial fermentation.

ASM Concept/Competency	A. Bloom's Level 1, 2–Remember and Understand (Choose one.)	B. Bloom's Level 3, 4–Apply and Analyze	C. Bloom's Level 5, 6–Evaluate and Create
Information Flow and Genetics	**10**. Enzyme action can be blocked by competitive molecules binding in the active site, by repressors binding in a distant site, and by **a**. product binding to the DNA used to make enzymes. **b**. substrates being in high concentration. **c**. incorrect temperature conditions. **d**. two of the above.	**11**. Compare and contrast the molecular structure of ATP with the molecular structure of an RNA nucleotide.	**12**. Suggest some reasons that pyruvate is central to so many metabolic strategies.
Microbial Systems	**13**. Enzymes that can be shut down or activated based on the presence of chemicals in their environment are called **a**. constitutive. **b**. repressed. **c**. holoenzymes. **d**. regulated.	**14**. Which of the three major catabolic strategies do you think the Last Common Ancestor possessed? Defend your answer.	**15**. Write a paragraph explaining why metabolic pathways that are amphibolic are advantageous for cells.

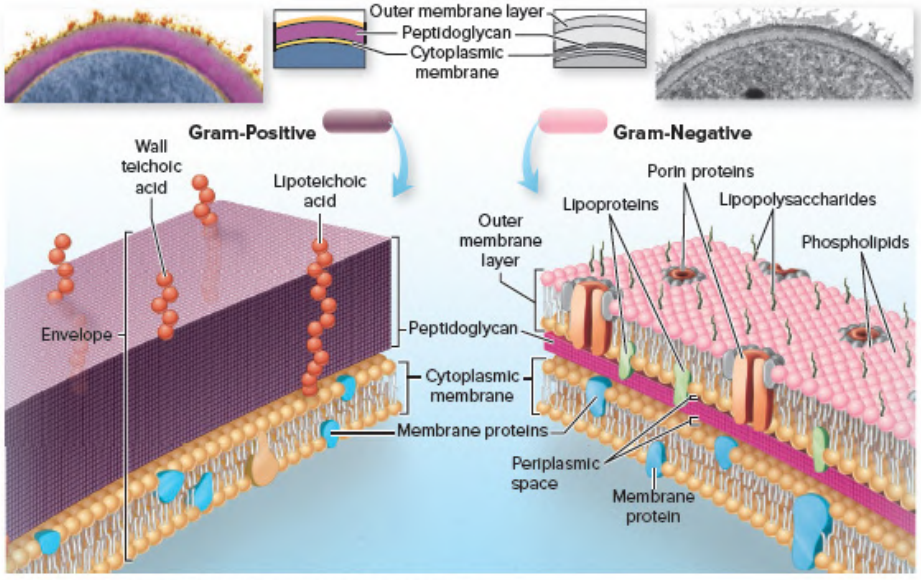
Impact of Microorganisms	**16**. The type of microbial metabolic pathway that is most often exploited to make acids and alcohols industrially is **a**. aerobic respiration. **b**. anaerobic respiration. **c**. fermentation. **d**. none of the above.	**17**. Defend this statement: Microbes (and their metabolic strategies) are absolutely essential to plant life on earth.	**18**. Construct an argument for the possibility that scientists will continue to discover novel metabolic strategies that are not yet known.
Scientific Thinking	**19**. Which of the following is true? **a**. The suffix "-ase" indicates an enzyme. **b**. Often enzymes are named for the substrates they act upon. **c**. Enzymes are larger than their substrates. **d**. All of the above are true.	**20**. Find three enzymatic capabilities that are commonly used to identify microbial species in a clinical lab.	**21**. Polymerase chain reaction (PCR) is a lab technology that requires high temperatures to make copies of DNA. The copies are made using an enzyme called a polymerase. PCR would not have been possible before thermophilic archaea were discovered. Explain why.

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From chapter 3, figure 3.15**. On these depictions of the gram-positive and gram-negative envelopes, draw protons in the proper compartment in such a way that creates a proton motive force.



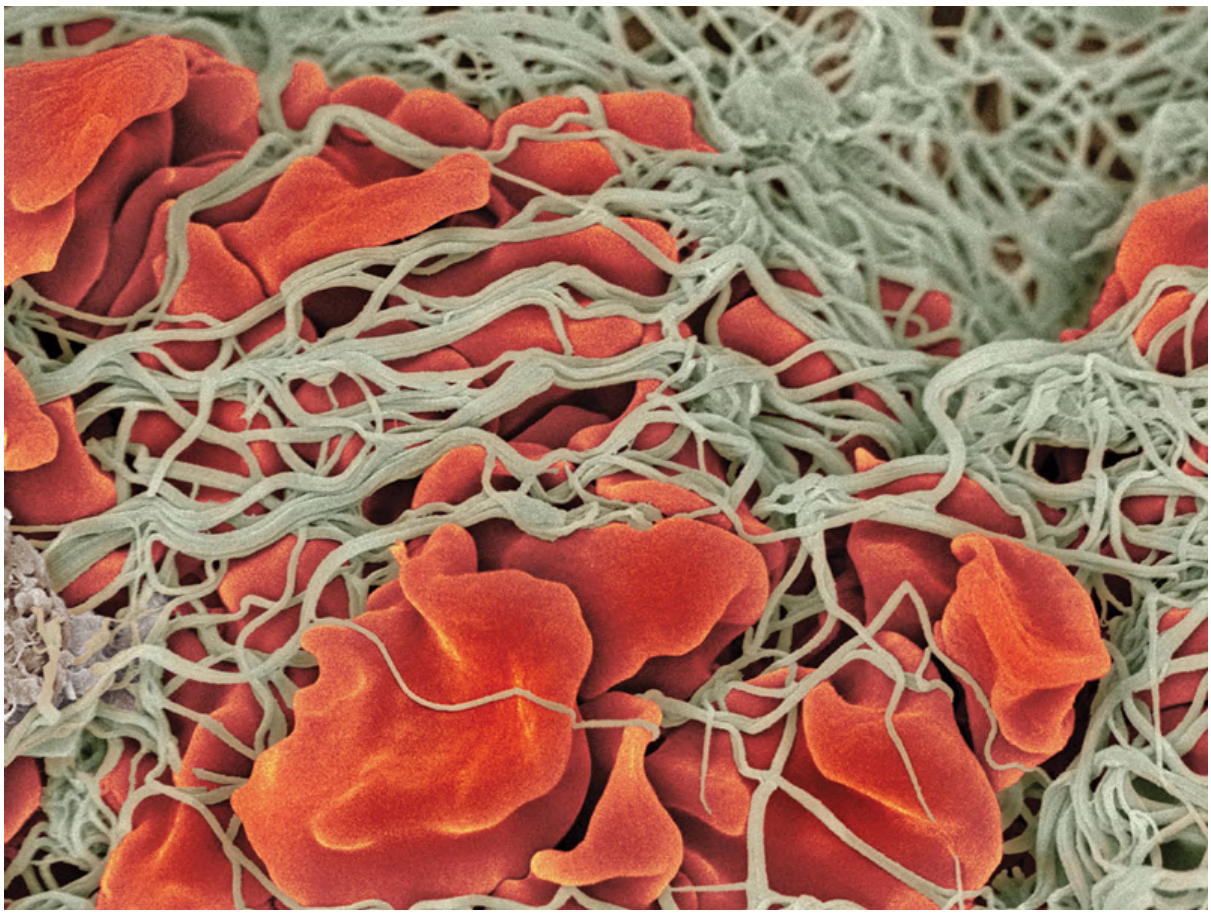
©Dr. Kari Lounatmaa/Science Source (gram-positive cell);
©Dennis Kunkel Microscopy, Inc./Medical Images (gram-negative cell)

connect Resources are available through Connect, including interactive learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

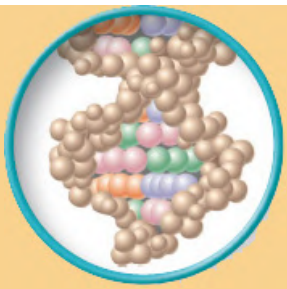
8

Microbial Genetics and Genetic Engineering



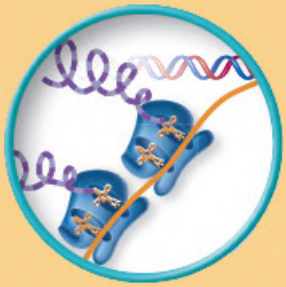
©Steve Gschmeissner/Science Source (blood cells and fibrin); ©Zmeel Photography/Getty Images (DNA analysis); ©Cathy Yeulet/123RF (health care worker)

IN THIS CHAPTER…



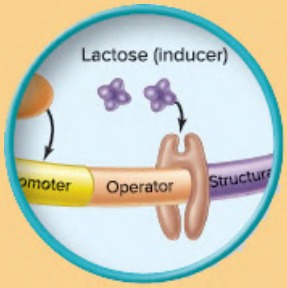
8.1 Introduction to Genetics and Genes

1. Define the terms *genome* and *gene*.
2. Differentiate between genotype and phenotype.
3. Draw a segment of DNA, labeling all important chemical groups within the molecule.
4. Summarize the steps of bacterial DNA replication, and identify the enzymes used in this process.
5. Compare and contrast the synthesis of leading and lagging strands during DNA replication.



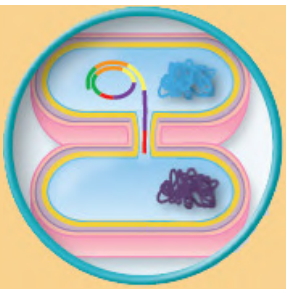
8.2 Transcription and Translation

6. Provide an overview of the relationship among DNA, RNA, and proteins.
7. Identify important structural and functional differences between RNA and DNA.
8. Draw a picture of the process of transcription.
9. List the three types of RNA directly involved in translation.
10. Define the terms *codon* and *anticodon*, and list three start and stop codons.
11. Identify the locations of the promoter, the start codon, and the A and P sites during translation.
12. Indicate how eukaryotic transcription and translation differ from these processes in bacteria.



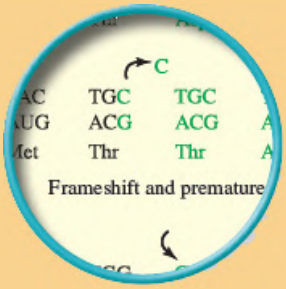
8.3 Genetic Regulation of Protein Synthesis

13. Define the term *operon*, and explain one advantage it provides to a bacterial cell.
14. Highlight the main points of *lac* operon operation.



8.4 DNA Recombination Events

15. Explain the defining characteristics of a recombinant organism.
16. Describe three forms of horizontal gene transfer used in bacteria.



8.5 Mutations: Changes in the Genetic Code

17. Define the term *mutation*, and discuss one positive and one negative example of it in microorganisms.
18. Differentiate among frameshift, nonsense, silent, and missense mutations.
19. Explain the influence of single-nucleotide polymorphisms on an organism.

8.6 Studying DNA in the Laboratory and Genetic Engineering

20. Explain the importance of restriction endonucleases to genetic engineering.
21. List the steps in the polymerase chain reaction.
22. Describe how you can clone a gene into a bacterium.
23. Differentiate between DNA profiling and DNA sequencing.
24. Provide a definition of *synthetic biology*.
25. Name two genetic techniques that are designed to treat human diseases.

CASE FILE

Factors to Consider

As a nurse for an elementary school, my responsibilities range from wellness education to caring for acute health needs of students and staff. I also help administer medications and manage medical needs for students with chronic conditions. One student whom I care for at the elementary school has hemophilia. I work closely with the patient, his parents, and school staff to ensure his medical needs are met while still allowing him to be at school.

The 7-year-old male student, Rayshawn, was diagnosed with Hemophilia A as a toddler. The condition is often inherited, but neither of his parents have hemophilia. After further genetic testing, it was determined that the student's hemophilia was caused by a spontaneous genetic mutation.

Having Hemophilia A means that Rayshawn is predisposed to frequent bleeding. He is not able to produce the blood clotting proteins necessary for normal clot formation. Small traumatic events could cause bleeding that is difficult to stop. Of greater concern is internal bleeding that could go unnoticed for some time and ultimately be life-threatening.

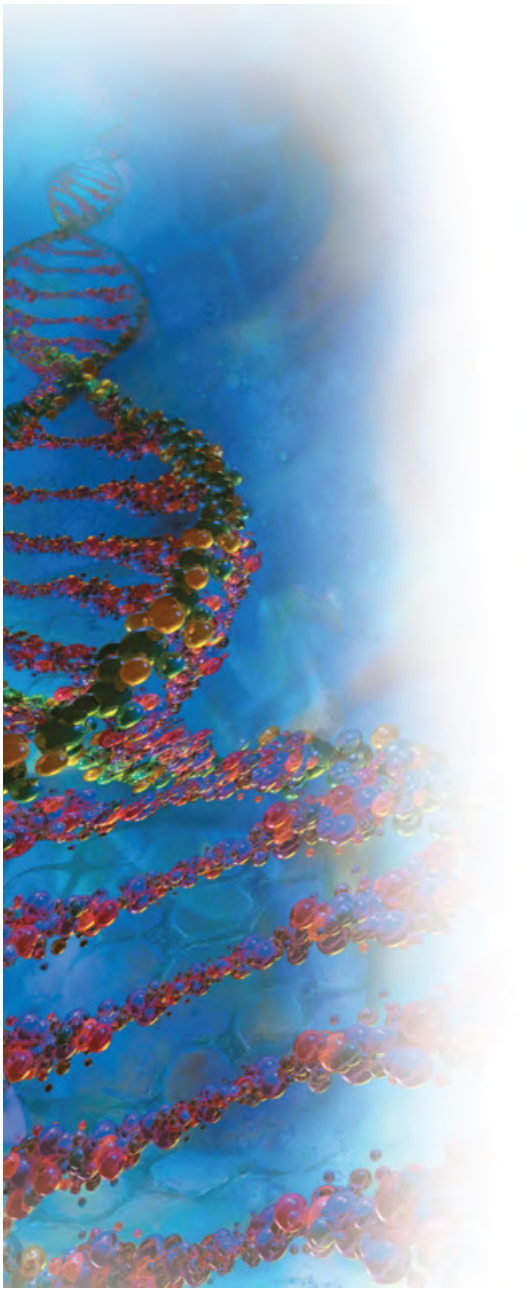
To manage his hemophilia, Rayshawn receives injections of clotting factor VIII three times per week. The infusions are administered at home, but I have vials of factor VIII at school in case it is needed during the school day. If the student has active bleeding, I bring him to the clinic to slowly infuse the intravenous (IV) factor replacement. He is restricted from high-contact activities but is allowed to participate in physical activities that have minimal risk of injury.

Another important part of my assessment of the patient is looking for joint swelling and tenderness. I have educated the school staff to be aware that spontaneous joint bleeds often occur with hemophilia and require immediate attention.

- Why does hemophilia cause uncontrollable bleeding?

- How is factor VIII produced?

Case File Wrap-Up appears at the end of the chapter.



©Adrian Neal/Getty Images

8.1 Introduction to Genetics and Genes

Genetics is the study of the inheritance, or **heredity**, of living things. It is a wide-ranging science that explores

- the transmission of biological properties (traits) from parent to offspring,
- how those traits are expressed in an organism,
- the structure and function of the genetic material, and
- how this material changes.

This chapter will explore DNA, which is the genetic material, and the proteins and other products that it gives rise to in a cell. Coming out of chapter 7, we should point out that the production of new DNA, RNA, and proteins is an example of an anabolic process.

The Nature of the Genetic Material

The genome is the sum total of genetic material of an organism. Although most of the genome exists in the form of chromosomes, genetic material can appear as nonchromosomal material as well **(figure 8.1)**. For example, bacteria and some fungi contain tiny extra pieces of DNA (plasmids), and certain organelles of eukaryotes (the mitochondria and chloroplasts) are equipped with their own DNA. Genomes of cells are composed exclusively of DNA, but viruses contain either DNA or RNA as the principal genetic material. Although the specific genome of an individual organism is unique, the general pattern of nucleic acid structure and function is similar among all organisms.

A **chromosome** is a distinct cellular structure composed of a neatly packaged DNA molecule. The chromosomes of eukaryotes and bacterial cells differ in several respects. The structure of eukaryotic chromosomes consists of a DNA molecule tightly wound around histone proteins, whereas a bacterial chromosome is condensed into a packet by proteins that are histone-like, but not actually histones. Eukaryotic chromosomes are located in the nucleus. They vary in number from a few to hundreds. They can occur in pairs (diploid) or singles (haploid), and they are double-stranded DNA in a more-or-less linear arrangement. In contrast, most bacterial chromosomes are circular double strands of DNA. Bacteria generally have one, two, or three chromosomes.

The chromosomes of all cells are subdivided into basic informational packets called genes. A **gene** can be defined from more than one perspective. In classical genetics, the term refers to the fundamental unit of heredity responsible for a given trait in an organism. In the molecular and biochemical sense, it is a site on the chromosome that provides information for a certain cell function. More specifically still, it has traditionally been characterized as a certain segment of DNA that contains the necessary code to make a protein or RNA molecule. Genes fall into three basic categories: (1) **structural genes** that code for proteins, (2) genes that code for the RNA machinery used in protein production, and (3) regulatory genes that control gene expression. The sum of all of these types of genes constitutes an organism's distinctive genetic makeup, or **genotype** (jee′-noh-typ). The expression of the genotype creates traits (certain structures or functions) referred to as the **phenotype** (fee′-noh-typ). Just as a person inherits a combination of genes (genotype) that gives a certain eye color or height (phenotypes), a bacterium inherits genes that direct the formation of a flagellum, and a virus contains genes for its capsid structure. All organisms contain more genes in their genotypes than are manifested as

a phenotype at any given time. In other words, the phenotype can change depending on which genes are “turned on” (expressed).

The Size and Packaging of Genomes

Genomes vary greatly in size. The smallest viruses have four or five genes; the bacterium *Escherichia coli* has a single chromosome containing 4,288 genes, and a human cell has about 19,000 to 20,000 genes on 23 chromosome pairs (46 chromosomes).

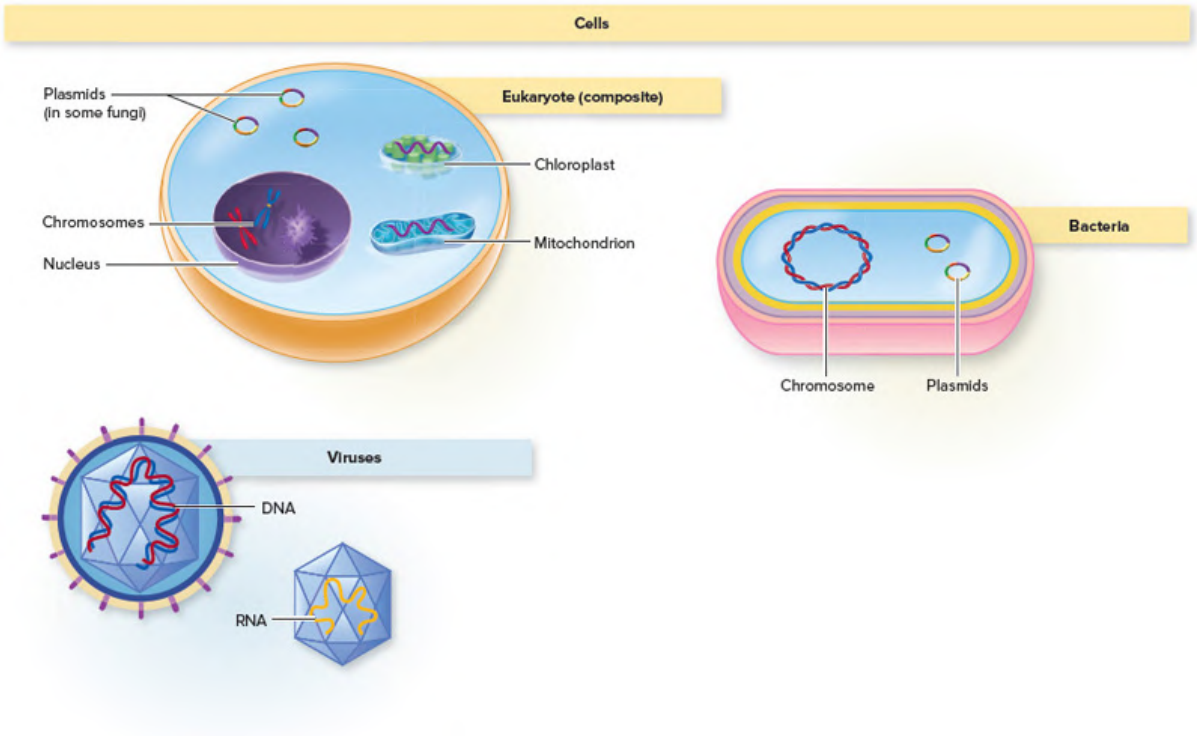


Figure 8.1 The locations and forms of the genome in cells and viruses (not to scale). Keep in mind that the cytosol is actually filled with ribosomes, other organelles, and a wide array of molecules. The drawing is simplified here to show the DNA.

The chromosome of *E. coli* would measure about 1 mm if unwound and stretched out linearly, and yet this fits within a cell that measures just over 1 μm across, making the stretched-out DNA 1,000 times longer than the cell **(figure 8.2)**. This is possible because the DNA is very tightly wound around protein in the cytoplasm. Still, the bacterial chromosome takes up only about one-third to one-half of the cell’s volume. Likewise, if the sum of all DNA making up the 46 human chromosomes in a cell were unraveled and laid end to end, it would measure about 6 feet.

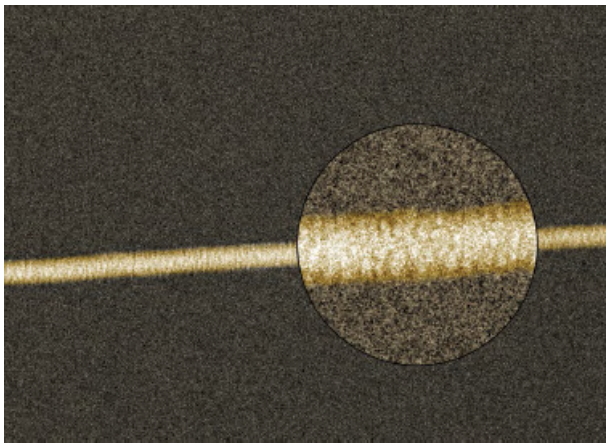


Figure 8.2 **Transmission electron micrograph of DNA**. The helical structure is clearly visible in the magnified area. ©Professor Enzo Di Fabrizio, IIT/Science Source

The DNA Code

The general structure of DNA is universal, except in some viruses that contain single-stranded DNA. The basic unit of DNA structure is a nucleotide, and a chromosome in a typical bacterium consists of several million nucleotides linked end to end. Each nucleotide is composed of a phosphate, a **deoxyribose**, and a **nitrogenous base**. The nucleotides covalently bond to each other in a sugar-phosphate linkage that becomes the backbone of each strand. Each sugar attaches in a repetitive pattern to two phosphates. One of the bonds is to the number 5′ (read "five prime") carbon on deoxyribose, and the other is to the 3′ carbon, which creates a certain order and direction on each strand **(figure 8.3)**. (In cyclical carbon molecules, like sugars, the carbons are numbered so we can keep track of them. Deoxyribose has 5 carbons numbered 1—5.)

The nitrogenous bases, **purines** and **pyrimidines**, attach along a strand by covalent bonds at the 1′ position of the sugar (figure 8.3a). They join with complementary

bases from the other strand using hydrogen bonds. Such weak bonds are easily broken, allowing the molecule to be "unzipped" into its complementary strands. This feature is of great importance in gaining access to the information encoded in the nitrogenous base sequence. Pairing of purines and pyrimidines is not random; it is dictated by the formation of hydrogen bonds between certain bases. Thus, in DNA, the purine adenine (A) always pairs with the pyrimidine thymine (T), and the purine guanine (G) always pairs with the pyrimidine cytosine (C). The bases are attracted to each other in this pattern because each has a complementary three-dimensional shape that matches its pair. Although the base-pairing partners generally do not vary, the sequence of base pairs along the DNA molecule can assume any order, resulting in a nearly infinite number of possible nucleotide sequences.

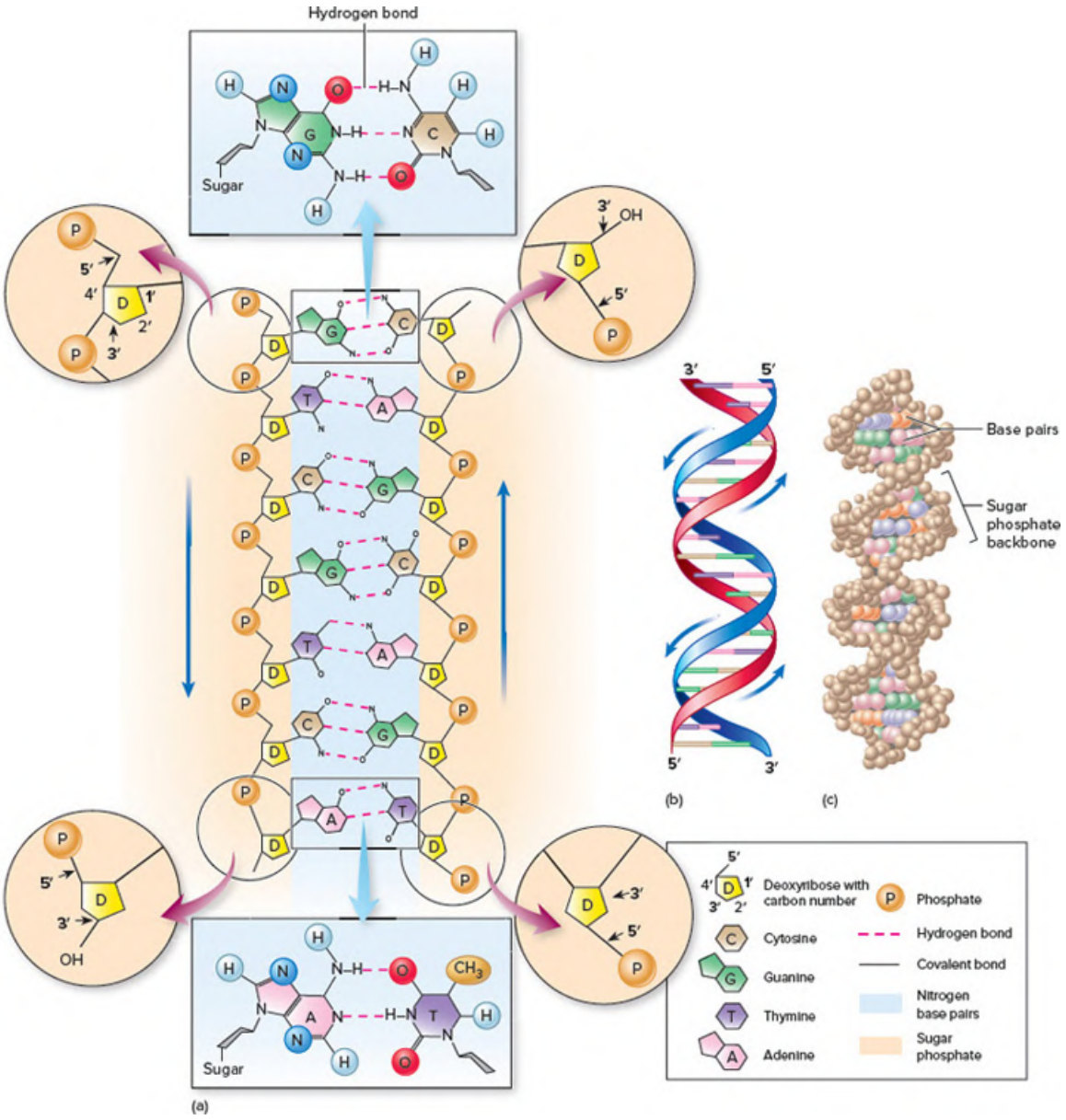


Figure 8.3 **Three views of DNA structure**. **(a)** A schematic nonhelical model, to show the arrangement of the molecules it is made of. Note that the order of phosphate and sugar bonds differs between the two strands, going from the 5′ carbon to the 3′ carbon on one strand, and from the 3′ carbon to the 5′ carbon on the other strand. Insets show details of the nitrogen *bases*. **(b)** Simplified model that highlights the antiparallel arrangement. **(c)** Space-filling model that more accurately depicts the three-dimensional structure of DNA.

Other important considerations of DNA structure concern the nature of the double helix itself. The halves are not oriented in the same direction. One side of the helix runs in the opposite direction of the other, in what is called an **antiparallel** arrangement (figure 8.3*b*). The order of the bond between the carbon on deoxyribose and the phosphates is used to keep track of the direction of the two sides of the helix. Thus, one helix runs from the 5′ to 3′ direction, and the other runs from the 3′ to 5′ direction. This characteristic is a significant factor in DNA synthesis and protein production.

NCLEX® PREP

1. Which of the following statements about genetic material are true? Select all that apply.
   a. *A chromosome is made up of a series of genes.*
   b. *The genotype describes the genetic composition of an organism, whereas the phenotype describes the expression of traits.*
   c. *RNA contains the genetic code for protein synthesis.*
   d. *In eukaryotic organisms, DNA is located in the nucleus of a cell.*

The Significance of DNA Structure

The English language, based on 26 letters, can create an infinite variety of words, but how can an apparently complex genetic language such as DNA be based on just four nitrogen base "letters"? A mathematical example can explain the possibilities. For a segment of DNA that is 1,000 nucleotides long, there are $4^{1,000}$ different sequences possible. Carried out, this number would approximate 1.5×10^{602}, a number so huge that it provides nearly endless degrees of variation.

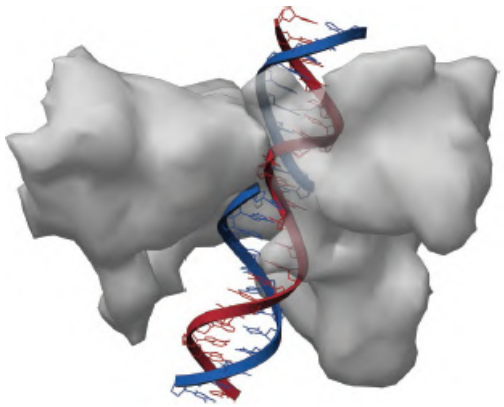
In this chapter, we will address two separate reactions DNA enters into: its own replication (facilitating cell division) and its role in producing proteins.

DNA Replication

The process of duplicating DNA is called *DNA replication*. In the following example, we will show replication in bacteria; but with some exceptions, it also applies to the process as it works in eukaryotes and some viruses.

The Overall Replication Process

DNA replication requires a careful orchestration of the actions of 30 different enzymes (partial list in **table 8.1**), which separate the strands of the existing DNA molecule, copy one strand, and produce two complete daughter molecules. A critical feature of DNA replication is that each daughter molecule will be identical to the parent in composition but neither one is completely new; one of the strands in each double-stranded daughter molecule comes from the parent DNA. The preservation of the parent molecule in this way, termed **semiconservative replication**—*semi-* meaning "half" as in *semicircle*—helps explain how the replication process maintains accuracy and fidelity during successive cycles.



DNA nestled in the active site of a topoisomerase
©McGraw-Hill Education/Jeramia Ory, Ph.D., photographer

Refinements and Details of Replication

The specific step of synthesizing a new daughter strand of DNA using the parental strand as a template is carried out by the enzyme DNA polymerase III. The process of replication can be most easily understood by keeping in mind a couple of points concerning both the structure of the DNA molecule and the limitations of DNA polymerase III:

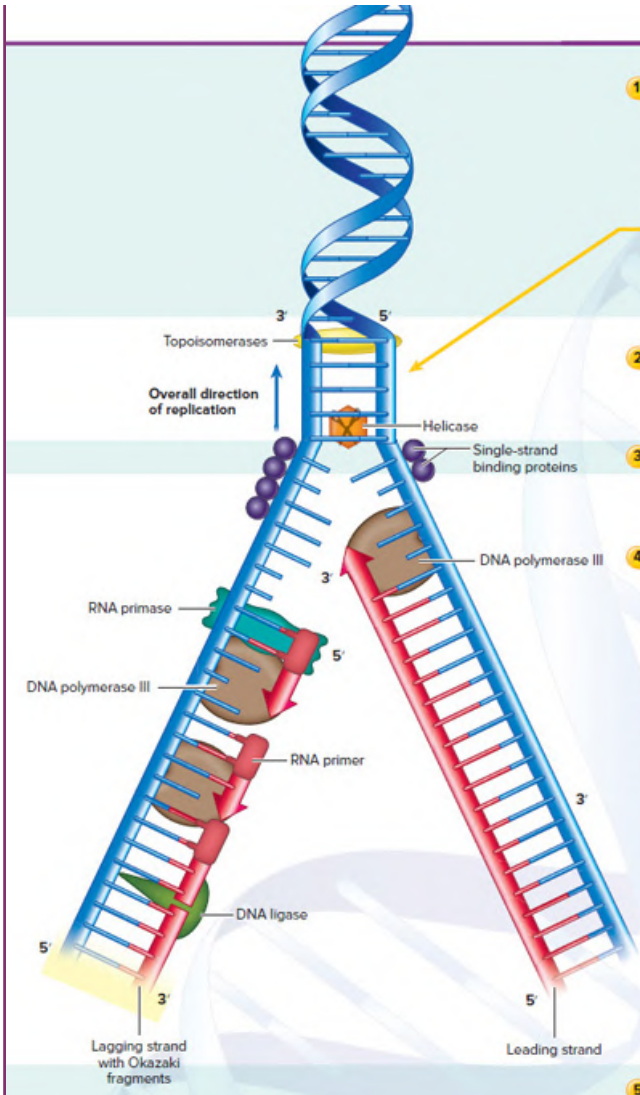
1. DNA polymerase III is unable to *begin* synthesizing a chain of nucleotides but can only continue to add nucleotides to an already existing chain.
2. DNA polymerase III can add nucleotides in only one direction, so new strands are always synthesized 5′ to 3′.

With these constraints in mind, we outline the process in **table 8.2**.

Table 8.1 Some Enzymes Involved in DNA Replication and Their Functions

Enzyme	Function
Helicase	Unzipping the DNA helix
Primase	Synthesizing an RNA primer
DNA polymerase III	Adding bases to the new DNA chain; proofreading the chain for mistakes
DNA polymerase I	Removing primer, closing gaps, repairing mismatches
Ligase	Final binding of nicks in DNA during synthesis and repair
Topoisomerases I and II	Supercoiling and untangling

Table 8.2 DNA Replication



1 The origin of replication is a short sequence rich in adenine and thymine bases. These base pairs are held together by only two hydrogen bonds rather than three. Because the origin of replication is AT-rich, less energy is required to separate the two strands than would be required if the origin were rich in guanine and cytosine.

During replication **topoisomerases** unwind the DNA helix, giving helicases (unzipping enzymes) access to bind to the dsDNA at the origin.

2 Helicases break the hydrogen bonds holding the two strands together, resulting in two separate strands.

3 *Single-stranded binding proteins* keep the strands apart.

4 DNA polymerase III adds nucleotides in accordance with the template pattern. Note that RNA primase has already added a short length of RNA.

Because DNA polymerase is correctly oriented for synthesis only in the 5′ to 3′ direction of the new molecule (blue) strand, only one strand, called the **leading strand,** can be synthesized as a continuous, complete strand. The strand with the opposite orientation (3′ to 5′) is termed the **lagging strand.** On this strand the polymerase adds nucleotides a few at a time in the direction away from the fork (5′ to 3′). As the fork opens up a bit, the next segment is synthesized backward to the point of the previous segment, a process repeated until synthesis is complete. In this way, the DNA polymerase is able to synthesize the two new strands simultaneously. This manner of synthesis produces one strand containing short fragments of DNA (100 to 1,000 bases long) called **Okazaki fragments.** These fragments are attached to the growing end of the lagging strand by another enzyme called DNA ligase.

5 In all cases, initiation of DNA synthesis requires "jump-starting" with a length of RNA manufactured by RNA primase. DNA polymerase can then add DNA nucleotides to that primer sequence. The primer sequence is later removed through enzymatic action.

The RNA primer is not seen on the leading strand as it appeared closer to the origin, which is not pictured. RNA primers are required at the beginning of each fragment of DNA synthesized on the lagging strand.

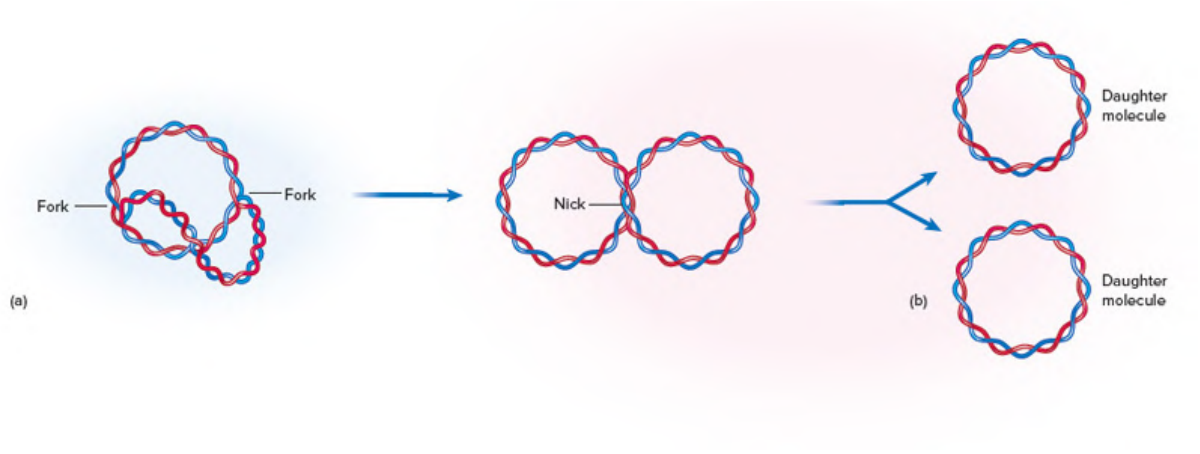


Figure 8.4 **Completion of chromosome replication in bacteria**. **(a)** As replication proceeds, one double strand loops away from the other. **(b)** Final separation is achieved through repair and the release of two completed molecules. The daughter cells receive these during binary fission.

Elongation and Termination of the Daughter Molecules The addition of nucleotides proceeds at an astonishing pace, estimated in some bacteria to be 750 bases per second at each fork! As replication proceeds, the newly produced double strand loops away from the original molecule **(figure 8.4*a*)**. DNA polymerase I removes the RNA primers used to initiate DNA synthesis and replaces them with DNA. When the forks come full circle and meet, ligases move along the lagging strand to begin the initial linking of the fragments and to complete synthesis and separation of the two circular daughter molecules **(figure 8.4*b*)**.

As with any language, DNA is occasionally "misspelled" when an incorrect base is added to the growing chain. In bacteria such mistakes are made once in approximately 10^9 to 10^{10} bases. Think of it this way: An overnight test tube culture of *E. coli* can be expected to have around 10^9 to 10^{10} bacteria per mL in it. That means a random mutation in every single base in the *E. coli* genome will be represented in at least one cell in the culture. Because continued cellular integrity is very dependent on accurate replication, cells have evolved their own proofreading function for DNA. DNA polymerase III, the enzyme that elongates the molecule, can detect incorrect, unmatching bases; excise them; and replace them with the correct base. DNA polymerase I can also proofread the molecule and repair damaged DNA.

Reminder: Be Sure to Read the Tables

If you skip the tables, you will miss much of the most important information about genetic processes. Follow the drawings and the accompanying text to save yourself time later catching up with important information.

8.1 LEARNING OUTCOMES—Assess Your Progress

1. Define the terms *genome* and *gene*.
2. Differentiate between genotype and phenotype.
3. Draw a segment of DNA, labeling all important chemical groups within the molecule.

4. Summarize the steps of bacterial DNA replication, and identify the enzymes used in this process.
5. Compare and contrast the synthesis of leading and lagging strands during DNA replication.

8.2 Transcription and Translation

Although the genome is full of critical information, the DNA itself does not perform cell processes directly. Its stored information is conveyed to RNA molecules, which carry out the instructions. The concept that genetic information flows from DNA to RNA to protein is a central theme of biology **(figure 8.5*a*)**. More precisely, it states that the master code of DNA is first used to synthesize an RNA molecule via

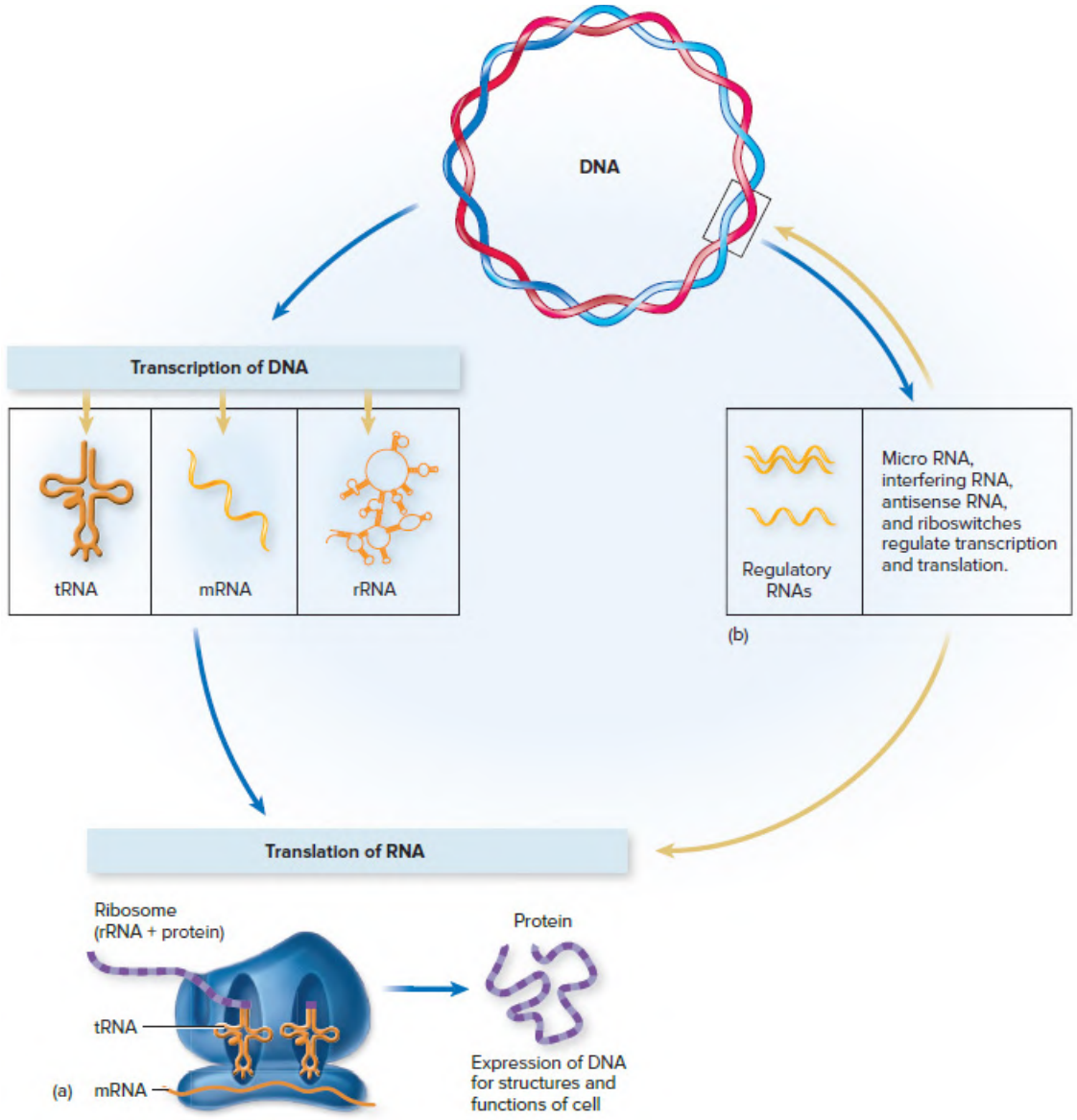


Figure 8.5 Summary of the flow of genetic information in cells. DNA is the ultimate storehouse and distributor of genetic information. **(a)** DNA must be deciphered into a usable cell language. It does this by transcribing its code into RNA helper molecules that translate that code into protein. **(b)** Other sections of the DNA produce very important RNA molecules that regulate genes and their products.

a process called **transcription**, and the information contained in the RNA is then used to produce proteins in a process known as **translation**. The principal exceptions to this pattern are found in RNA viruses, which convert RNA to other RNA, and in retroviruses, which convert RNA to DNA.

In addition to the RNA that is used to produce proteins, a wide variety of RNAs are used to regulate gene function. This means that vast amounts of DNA sequences code for RNAs that never get made into proteins **(figure 8.5*b*)**. The DNA that codes for these very crucial RNA molecules was called "junk" DNA until very recently, because we didn't understand its function, and only knew that it did not produce proteins. While there is also a varying amount

of junk DNA in cells (whose function is not yet understood), some of the junk DNA has been found to code for these very important regulatory RNAs.

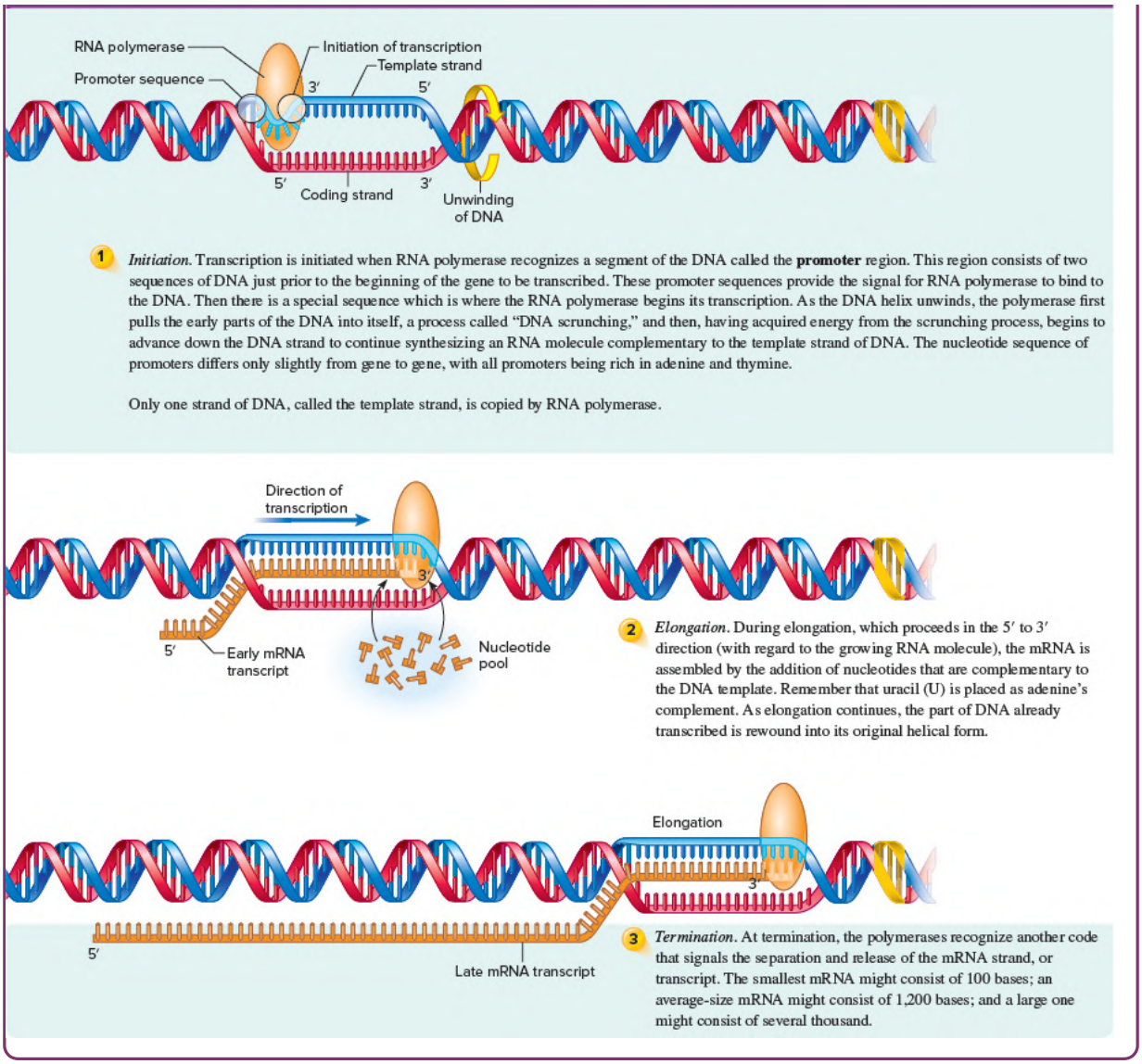
©Laguna Design/Getty Images

Transcription: The First Stage of Gene Expression

During transcription, the DNA code is converted to RNA in a stepwise fashion, directed by a large and very complex enzyme system, **RNA polymerase**. Only one strand of the DNA—the **template strand**—is used as the instructions for synthesis of a functioning polypeptide.

Table 8.3 describes transcription.

Table 8.3 Transcription



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2. The following are all true of RNA, except
- **a.** *it is a single-stranded molecule existing in helical form.*
- **b.** *it contains uracil rather than thymine as the complementary base-pairing mate for adenine.*
- **c.** *uracil does not follow the pairing rules; therefore, the inherent DNA code is changed.*
- **d.** *the sugar in RNA is ribose rather than deoxyribose.*

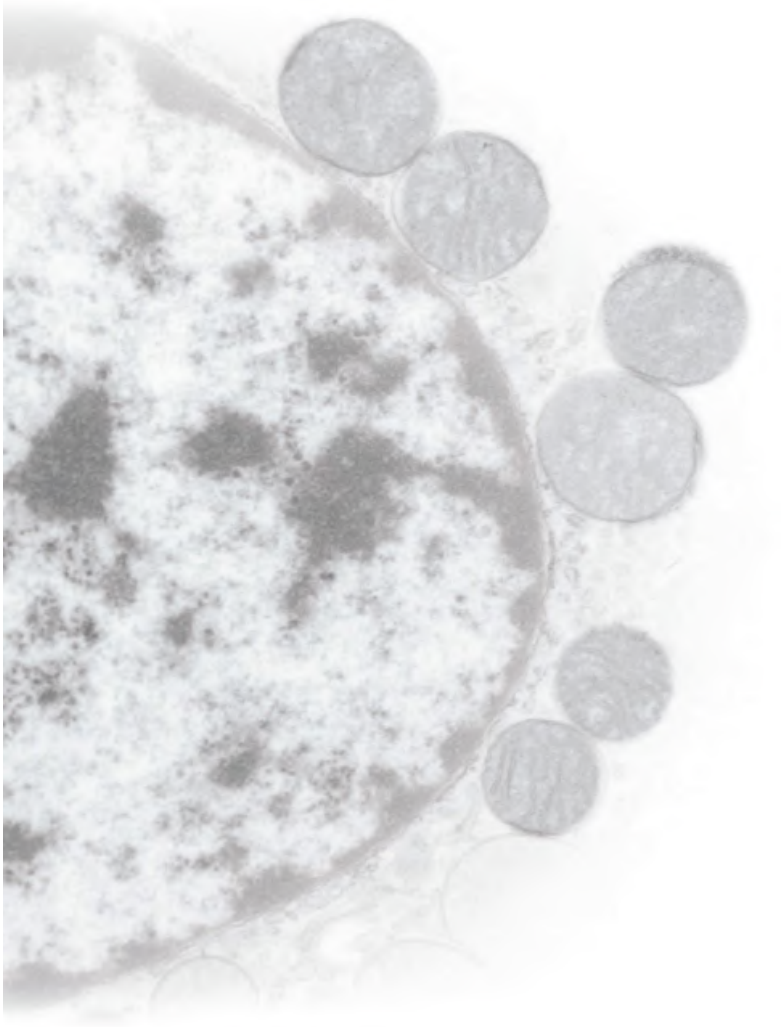
The RNAs

In terms of its general properties, ribonucleic acid is similar to DNA, but its general structure is different in several ways:

1. It is a single-stranded molecule that exists in helical form. This single strand can form secondary and tertiary levels of complexity due to bonds within the molecule, leading to specialized forms of RNA (tRNA and rRNA).
2. RNA contains *uracil (U)*, instead of thymine, as the complementary base-pairing mate for adenine. This does not change the inherent DNA code in any way because the uracil still follows the pairing rules.
3. Although RNA, like DNA, contains a backbone that consists of alternating sugar and phosphate molecules, the sugar in RNA is **ribose** rather than deoxyribose.

The products of transcription belong in two major categories: those that are necessary for translation, and those that have other functions in the cell. The translation machinery includes messenger RNA, transfer RNA, and ribosomal RNA. We will spend a lot of time on these molecules in the coming section, but first we will list the other RNA varieties:

- Regulatory RNAs: These are small RNA molecules, known as micro RNAs, antisense RNAs, riboswitches, and small interfering RNAs. They are important regulators of gene expression in bacteria and eukaryotes and also act in the coiling of chromatin in eukaryotic cells.
- Primer RNA: This RNA is laid down in DNA replication, as a template of sorts for the DNA sequence. Primer RNAs are operative in both bacterial and eukaryotic cells.



In eukaryotes, transcription occurs in the nucleus (pictured here) and translation occurs in the cytoplasm. In bacteria and archaea, both processes occur in the cytoplasm.

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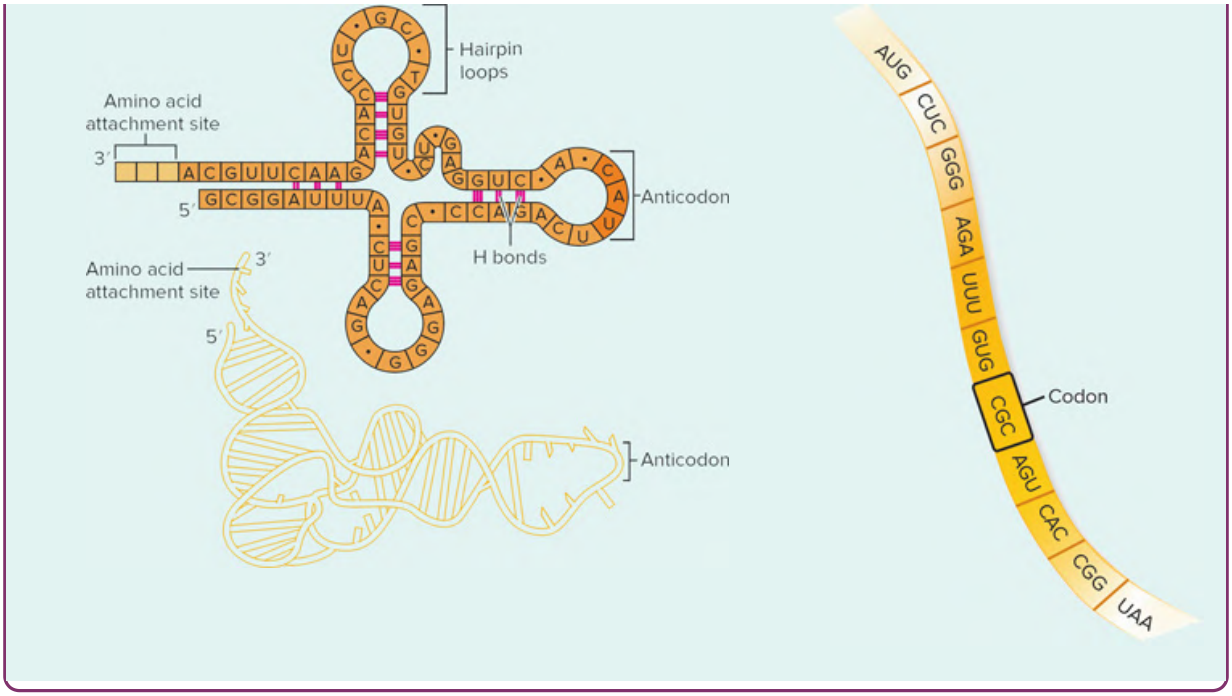
Table 8.4 The Three RNAs Involved in Translation

The bacterial (70S) ribosome is a macromolecule composed of tightly packaged **ribosomal RNA (rRNA)** (large gray and blue areas) and protein (smaller lavender and purple areas). A metabolically active bacterial cell can contain up to 20,000 of these minuscule factories—all actively engaged in reading the genetic program, taking in raw materials, and producing proteins at an impressive rate. Each ribosome has a large subunit and small subunit, each of which is composed of rRNA and protein.

©Center for Molecular Biology of RNA, UC-Santa Cruz

Transfer RNA (tRNA) is also a copy of a specific region of DNA; however, it differs from mRNA. Each one is only 75 to 95 nucleotides long, and contains sequences of bases that form hydrogen bonds with complementary sections within the same tRNA strand. At these points, the molecule bends back upon itself into several hairpin loops, giving the molecule a secondary cloverleaf structure that folds even further into a complex, three-dimensional helix. This compact molecule is an adaptor that converts RNA language into protein language. The bottom loop of the cloverleaf exposes a triplet, the **anticodon**, that both designates the specificity of the tRNA and complements mRNA's codons. At the opposite end of the molecule is a binding site for the amino acid that is specific for that tRNA's anticodon. For each of the 20 amino acids, there is at least one specialized type of tRNA to carry it.

Messenger RNA (mRNA) is a transcript (copy) of a structural gene or genes in the DNA. The complementary base-pairing rules ensure that the code will be faithfully copied in the mRNA transcript. The message of this transcribed strand is later read as a series of triplets called codons. The length of the mRNA molecule varies from about 100 nucleotides to several thousand. It carries the sequence that will dictate the eventual amino acid sequence of the protein.



- Ribozymes: These enzymes are made of RNA and, in eukaryotes, remove unneeded sequences from other RNAs.

All of these—and tRNA, mRNA, and rRNA—are products of the transcription of distinct genes in the chromosome.

Now, on to translation.

After Transcription: Translation

Three different RNA products of transcription are needed for the final step of protein expression. They are described in **table 8.4**.

The ribosomes of bacteria and eukaryotes are different sizes. Ribosomes in bacteria, as well as the ribosomes in chloroplasts and mitochondria of eukaryotes, are of a 70S size, made up of a 50S (large) subunit and a 30S (small) subunit. The "S" is a measurement of sedimentation rates, which is how ribosomes are characterized. It is a nonlinear measure; therefore, 30S and 50S add up to 70S. Eukaryotic ribosomes are 80S (a large subunit of 60S and a 40S small subunit). The small subunit binds to the 5′ end of the mRNA, and the large subunit supplies enzymes for making peptide bonds on the protein.

Translation: The Second Stage of Gene Expression

In translation, all of the elements needed to synthesize a protein, from the mRNA to the amino acids, are brought together on the ribosomes **(figure 8.6)**.

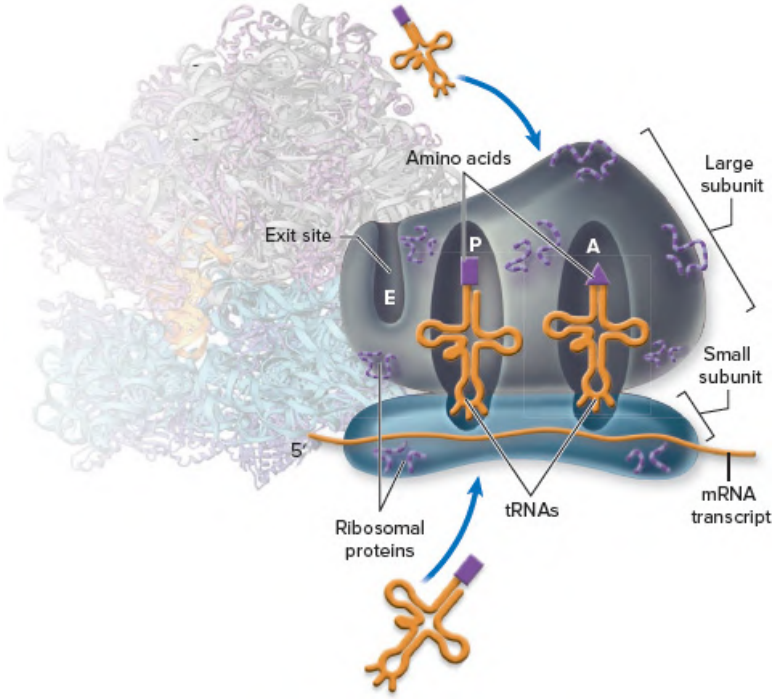


Figure 8.6 **The "players" in translation**. A ribosome serves as the stage for protein synthesis. Assembly of the small and large subunits results in specific sites for holding the mRNA and two tRNAs with their amino acids. This depiction of the ribosome matches the depiction on the left-hand page so you can see the connection between the molecular image and the image we will use in the book.

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3. The function of a codon in protein synthesis is best described as a three-nucleotide sequence
- **a.** *that specifies the initiation of translation*.
- **b.** *that specifies the termination of translation*.
- **c.** *on tRNA that complements the mRNA code*.
- **d.** *that dictates a specific amino acid*.

The Master Genetic Code: The Message in Messenger RNA

Translation relies on a central principle: The mRNA nucleotides are read in groups of three. Three nucleotides are called a **codon**, and it is the codon that dictates which amino acid is added to the growing peptide chain. In **figure 8.7**, the mRNA codons and their corresponding amino acid specificities are given. Because there are 64 different triplet codes and only 20 different amino acids, it is not surprising that some amino acids are represented by several codons. For example, leucine and serine can each be represented by any of six different triplets, and only tryptophan and methionine are represented by a single codon. This property is called **redundancy** and allows for the insertion of correct amino acids (sometimes) even when mistakes occur in the DNA sequence, as they do with regularity. Also, in codons such as those for leucine, only the first two nucleotides are required to encode the correct amino acid. Any of the four nucleotides can appear in the third position of the codon without changing the fact that

leucine is called for. This property, called **wobble**, is thought to permit some variation or mutation without altering the message. **Figure 8.8** shows the relationship between DNA sequence, RNA codons, tRNA, and amino acids.

Before newly made proteins can carry out their structural or enzymatic roles, they often require finishing touches. Even before the peptide chain is released from the ribosome, it begins folding upon itself to achieve its biologically active tertiary conformation. Other alterations, called **posttranslational** modifications, may be necessary. Some proteins must have the starting amino acid (formyl methionine) clipped off. Proteins destined to become complex enzymes have cofactors added; and some join with other completed proteins to form quaternary levels of structure.

In bacteria, the translation of mRNA starts while transcription is still occurring **(figure 8.9)**. A single mRNA is long enough to be fed through more than one ribosome simultaneously. This permits the synthesis of hundreds of protein molecules from the same mRNA transcript arrayed along a chain of ribosomes. This **polyribosomal complex** is like an assembly line for mass production of proteins. It occurs in bacteria, but not in eukaryotic cells, because there is no nucleus; and transcription and translation both occur in the cytoplasm. (In eukaryotes, transcription occurs in the nucleus.) Remember that all of the processes involved in gene expression are anabolic processes. Protein

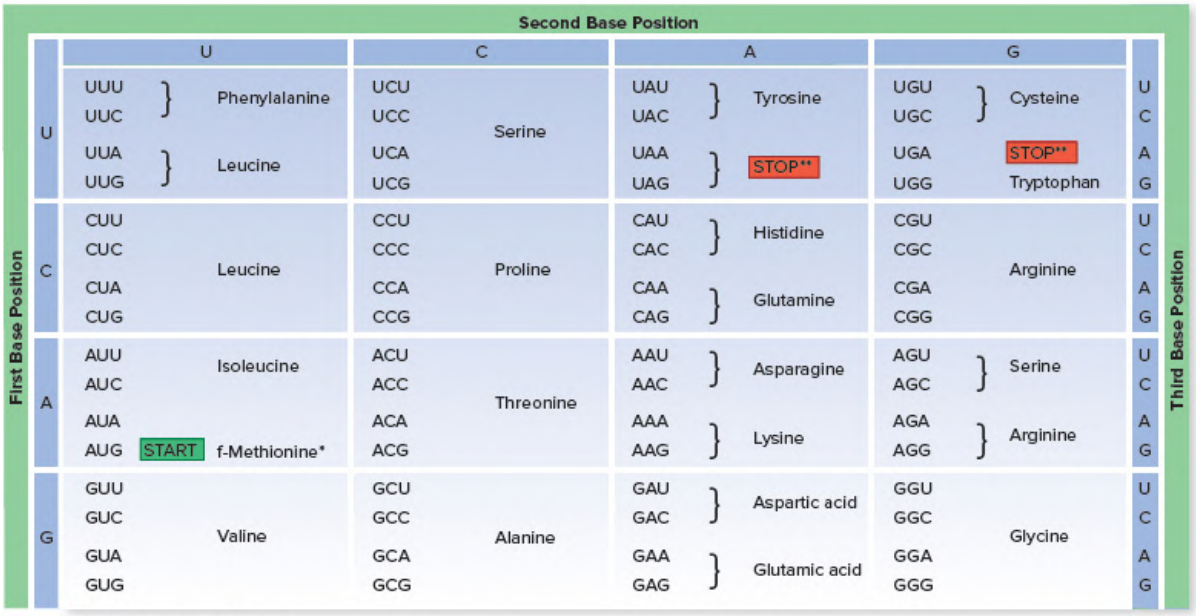


Figure 8.7 The genetic code: codons of mRNA that specify a given amino acid. The master code for translation is found in the mRNA codons.

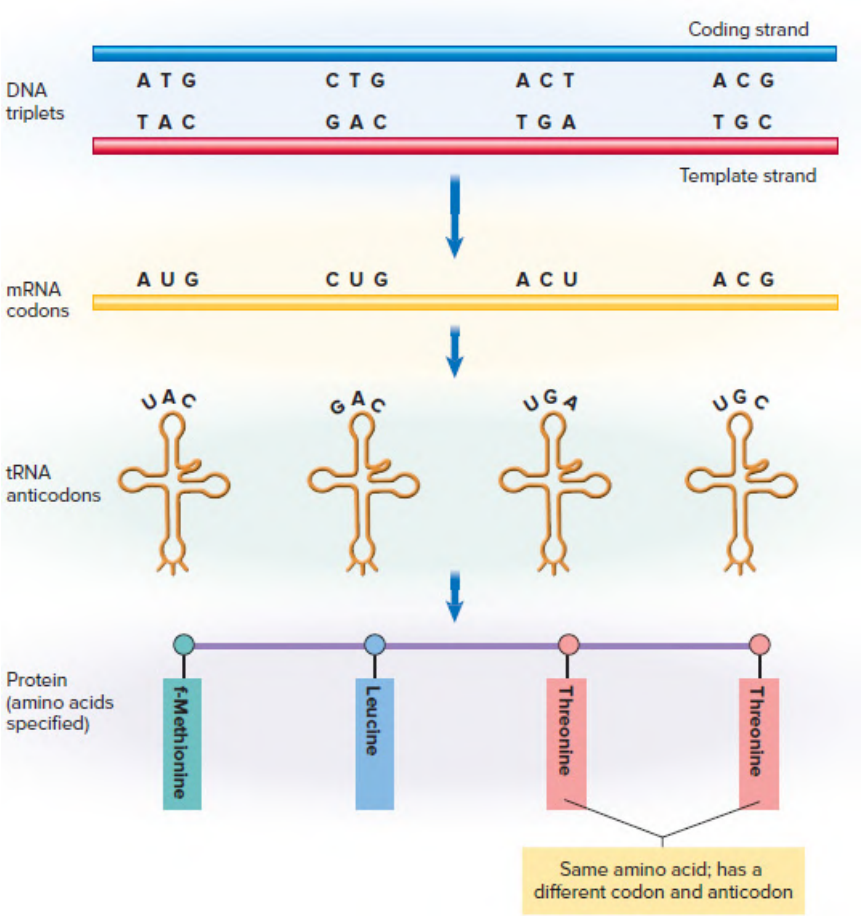


Figure 8.8 **Interpreting the DNA code**. If the DNA sequence is known, the mRNA codon can be surmised. If a codon is known, the anticodon and, finally, the amino acid sequence can be determined. The reverse is not as straightforward (determining the exact codon or anticodon from amino acid sequence) due to the redundancy of the code.

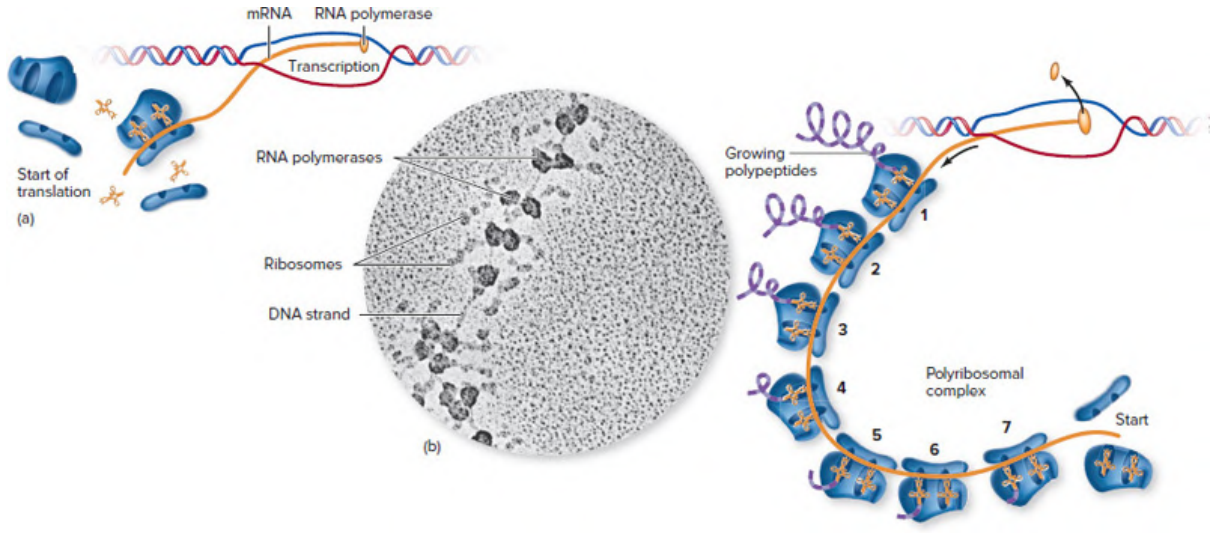
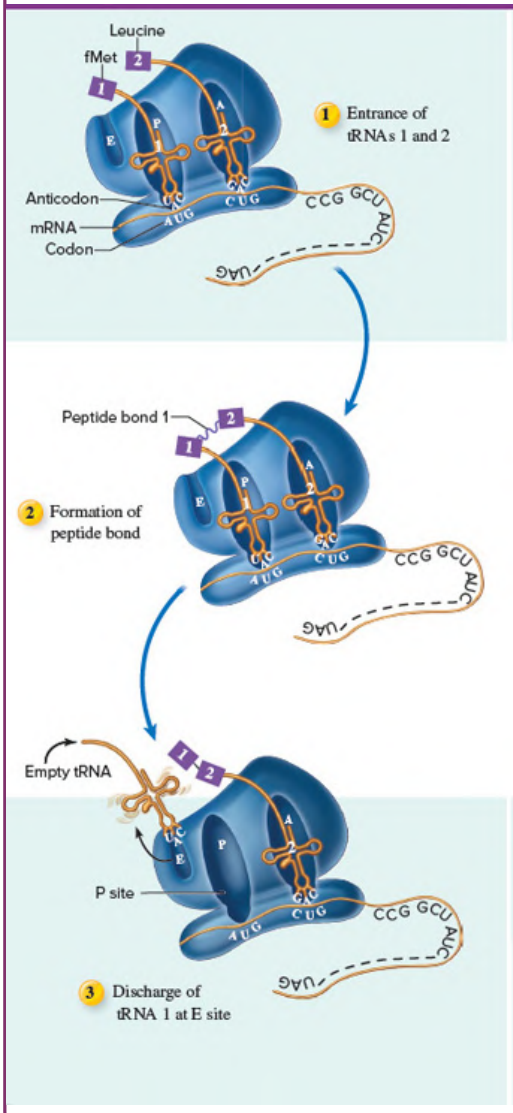


Figure 8.9 **Speeding up the protein assembly line in bacteria**. **(a)** The mRNA transcript encounters ribosomal parts immediately as it leaves the DNA. **(b)** A photomicrograph and a drawing illustrate the coupled processes of

transcription and translation. The ribosomal factories assemble along the mRNA in a chain, each ribosome reading the message and translating it into protein. Many products will thus be well along the synthetic pathway before transcription has even terminated. Note that the protein “tails” vary in length depending on the stage of translation. (b) Courtesy Steven McKnight, PhD

Table 8.5 Properties of Viruses



1 The mRNA molecule leaves the DNA transcription site and is transported to ribosomes in the cytoplasm. Ribosomal subunits come together and form sites to hold the mRNA and tRNAs. The ribosome begins to scan the mRNA by moving in the 5′ to 3′ direction along the mRNA. The first codon it encounters is called the START codon, which is almost always AUG. With the mRNA message in place on the assembled ribosome, the next step in translation involves entrance of tRNAs with their amino acids. The pool of cytoplasm contains a complete array of tRNAs, already charged by having the correct amino acid attached. The step in which the complementary tRNA meets with the mRNA code is guided by the two sites on the large subunit of the ribosome called the P site (left) and the A site (right). The ribosome also has an exit or E site where used tRNAs are released. (P stands for peptide site; A stands for aminoacyl (amino acid) site; E stands for exit site.)

2 Rules of pairing dictate that the anticodon of this tRNA must be complementary to the mRNA codon AUG; thus, the tRNA with anticodon UAC will be the first tRNA to occupy site P. It happens that the amino acid carried by the initiator tRNA in bacteria is formyl methionine. The ribosome shifts its “reading frame” to the right along the mRNA from one codon to the next. This brings the next codon into place on the ribosome and makes a space for the next tRNA to enter the A position. A peptide bond is formed between the amino acids on the adjacent tRNAs, and the polypeptide grows in length.

Elongation then begins with the filling of the A site by a second tRNA. The identity of this tRNA and its amino acid is dictated by the second mRNA codon.

3 The entry of tRNA 2 into the A site brings the two adjacent tRNAs in favorable proximity for a peptide bond to form between the amino acids (aa) they carry. The fMet is transferred from the first tRNA to aa 2, resulting in two coupled amino acids called a dipeptide.

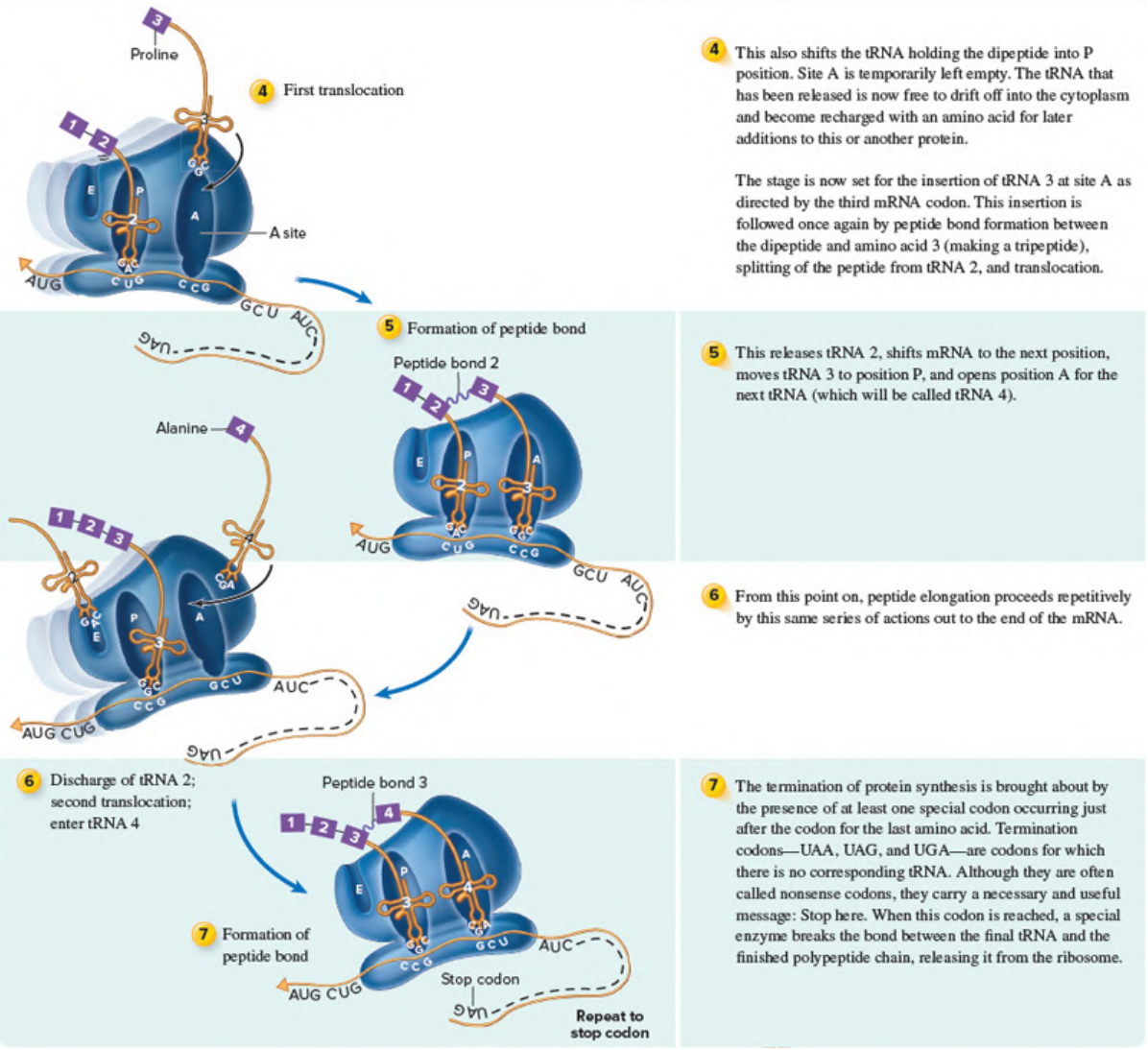
For the next step to proceed, some room must be made on the ribosome, and the next codon in sequence must be brought into position for reading. This process is accomplished by translocation, the enzyme-directed shifting of the ribosome to the right along the mRNA strand, which causes the blank tRNA 1 to be discharged from the ribosome at the E site.

synthesis consumes an enormous amount of energy. Nearly 1,200 ATPs are required just for the synthesis of an average-size protein. **Table 8.5** contains the details of translation.

Differences Between Eukaryotic and Bacterial Transcription and Translation

Eukaryotes and bacteria share many similarities in protein synthesis. The start codon in eukaryotes is also AUG, but eukaryotes use a different form of methionine.

Table 8.5 Translation (continued)



Another difference is that each eukaryotic mRNA codes for just one protein, unlike bacterial mRNAs, which often contain information from several genes in series.

As just mentioned, the presence of the DNA in a separate compartment (the nucleus) means that eukaryotic transcription and translation cannot be simultaneous. The mRNA transcript must pass through pores in the nuclear membrane and be carried to the ribosomes in the cytoplasm for translation.

We have given the simplified definition of a gene that works well for bacteria, but most eukaryotic genes (and, surprisingly, archaeal genes) do *not* exist as an uninterrupted

series of triplets coding for a protein. A eukaryotic gene contains the code for a protein, but located along the gene are one to several intervening sequences of bases, called **introns**, that do not code for protein. Introns are interspersed between coding regions, called **exons**, that will be translated into protein **(figure 8.10)**. We can use words as examples. A short section of colinear bacterial gene might read TOM SAW OUR DOG DIG OUT; a eukaryotic gene that codes for the same portion would read TOM SAW XZKP FPL OUR DOG QZWVP DIG OUT. The recognizable words are the exons, and the nonsense letters represent the introns.

This unusual genetic architecture, sometimes called a split gene, requires further processing for eukaryotes before translation. Transcription of the entire gene with both exons and introns occurs first, producing a pre-mRNA. A series of adenosines is added to the mRNA molecule. This protects the molecule and eventually directs it out of the nucleus for translation. Next, a type of RNA and protein called a **spliceosome** recognizes the exon-intron junctions and enzymatically cuts through them. The action of this splicer enzyme loops the introns into lariat-shaped pieces, excises them, and joins the exons end to end. By this means, a strand of mRNA with no intron material is produced. This completed mRNA strand can then proceed to the cytoplasm to be translated.

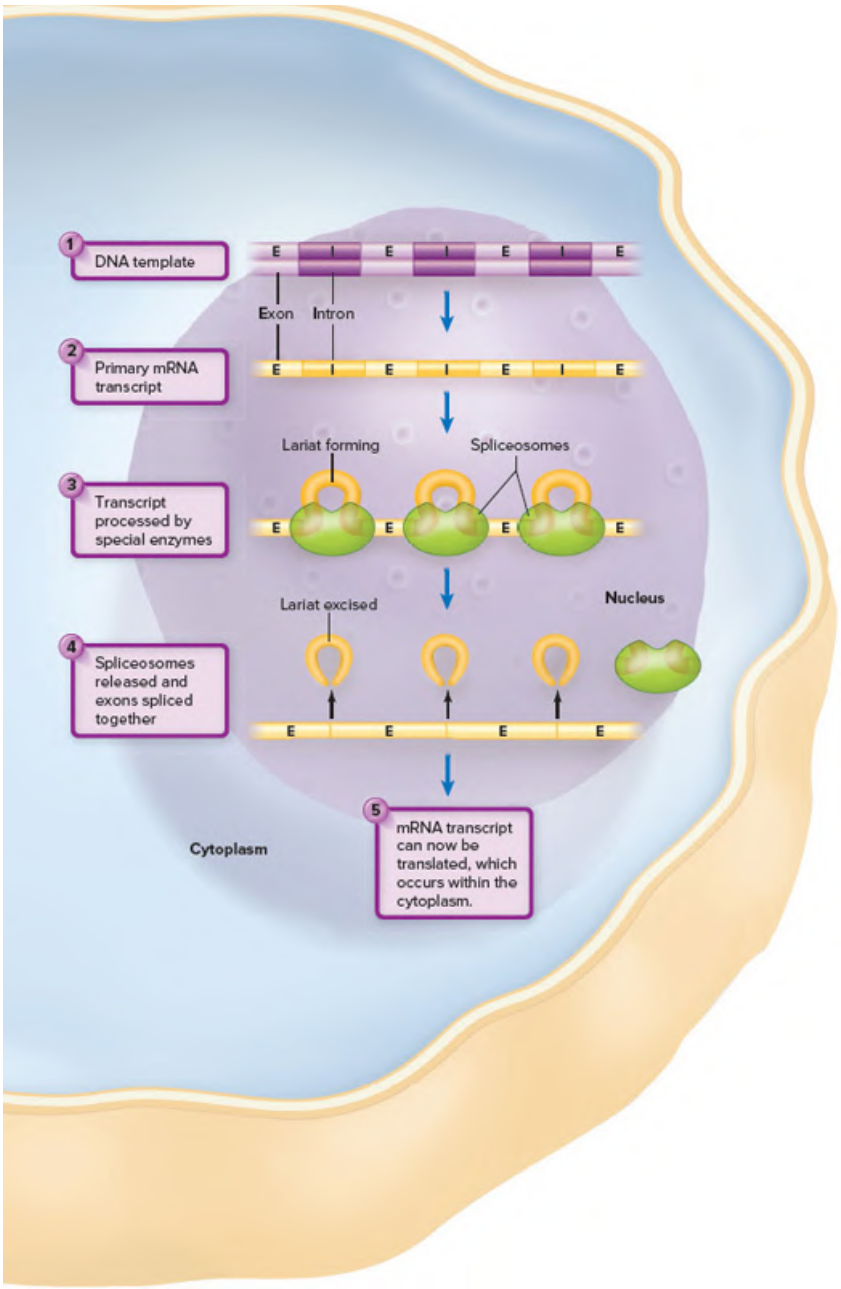


Figure 8.10 **The split gene of eukaryotes**. Eukaryotic genes have an additional complicating factor in their translation. Their coding sequences, or exons (E), are interrupted at intervals by segments called introns (I) that are not part of that protein's code. Introns are transcribed but not translated, which necessitates their removal by RNA splicing enzymes before translation.

8.2 LEARNING OUTCOMES—Assess Your Progress

6. Provide an overview of the relationship among DNA, RNA, and proteins.
7. Identify important structural and functional differences between RNA and DNA.
8. Draw a picture of the process of transcription.
9. List the three types of RNA directly involved in translation.

10. Define the terms *codon* and *anticodon*, and list three start and stop codons.
11. Identify the locations of the promoter, the start codon, and the A and P sites during translation.
12. Indicate how eukaryotic transcription and translation differ from these processes in bacteria.

8.3 Genetic Regulation of Protein Synthesis

In chapter 7, we surveyed the metabolic reactions in cells and the enzymes involved in those reactions. At that time, we mentioned that some enzymes are regulated and that one form of regulation occurs at the genetic level. Control mechanisms ensure that genes are active only when their products are required. In this way, enzymes will be produced as they are needed and prevent the waste of energy and materials in dead-end synthesis. Antisense RNAs, micro RNAs, and riboswitches provide regulation in bacteria, archaea, and eukaryotes. Bacteria and archaea have an additional strategy: They organize collections of genes into **operons**. Operons consist of a coordinated set of genes, all of which are regulated as a single unit. Operons are described

as either inducible or repressible. Many catabolic operons, or operons encoding enzymes that act in catabolism, are inducible, meaning that the operon is turned on (induced) by the substrate of the enzyme(s) for which the structural genes code. In this way, the enzymes needed to metabolize a nutrient (lactose, for example) are produced only when that nutrient is present in the environment. Repressible operons are normally in the "on" position, but are shut down in certain circumstances. Repressible operons often contain genes coding for anabolic enzymes, such as those used to synthesize amino acids. In the case of these operons, several genes in series are turned off (repressed) by the product synthesized by the enzyme.

The Lactose Operon: A Model for Inducible Gene Regulation in Bacteria

The best understood system for control through genetic induction is the **lactose (*lac*) operon**. This system, first described in 1961 by François Jacob and Jacques Monod, accounts for the regulation of lactose metabolism in *Escherichia coli*. Many other operons with similar modes of action have since been identified, and together they show us that the environment of a cell can have great impact on gene expression.

The lactose operon has three important features **(table 8.6):**

1. the **regulator**, composed of the gene that codes for a protein capable of repressing the operon (a **repressor**);
2. the control locus, composed of two areas, the **promoter** (recognized by RNA polymerase) and the **operator**, a sequence that acts as an on/off switch for transcription; and
3. the structural locus, made up of three genes, each coding for a different enzyme needed to catabolize lactose.

One of the enzymes, β-galactosidase, hydrolyzes the lactose into its monosaccharides; another, permease, brings lactose across the cytoplasmic membrane.

The operon provides an efficient strategy that permits genes for a particular metabolic pathway to be induced or repressed all together by a single regulatory element. The promoter, operator, and structural components usually lie adjacent to one another, but the regulator can be at a distant site.

Table 8.6 supplies the details of how the *lac* operon works.

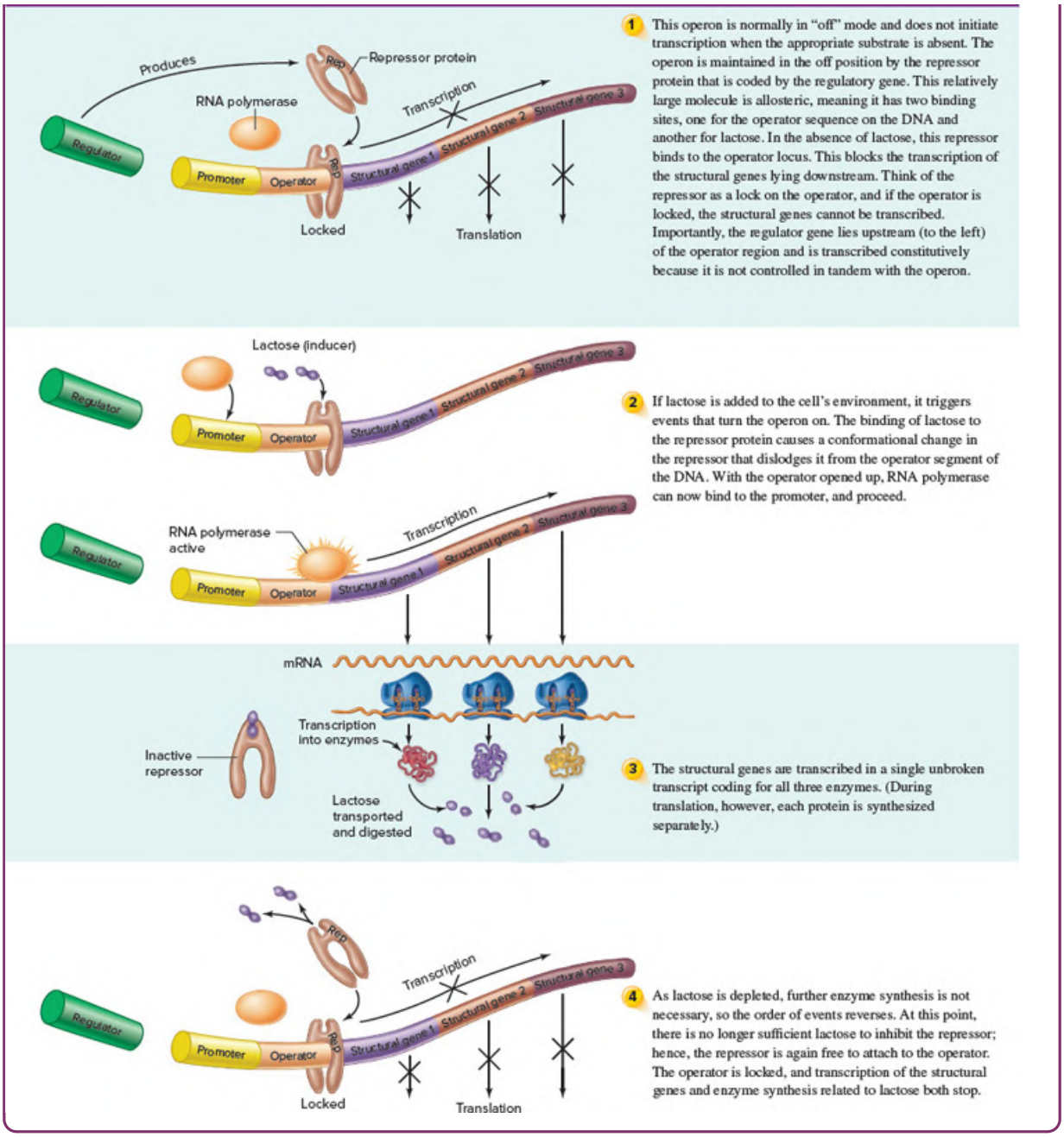
A fine but important point about the *lac* operon is that it functions only in the absence of glucose or if the cell's energy needs are not being met by the available glucose. Glucose is the preferred carbon source because it can be used immediately in growth and does not require induction of an operon. When glucose is present, a second regulatory system ensures that the *lac* operon is inactive, regardless of lactose levels in the environment.

Phase Variation

When bacteria turn on or off a complement of genes that leads to obvious phenotypic changes, it is sometimes called **phase variation**. Phase variation is a type of phenotypic variation, but it has its own name because it has some special characteristics, the most important of which is that the phenotype is heritable, meaning it is passed down to subsequent generations. The process of turning on genes is often mediated by regulatory proteins, as described with operons. The term *phase variation* is most often applied to traits affecting the bacterial cell surface and was originally coined to describe the ability of bacteria to change components of their surface that marked them for targeting by the host's immune system. Since these surface molecules also influenced the bacterium's ability to attach to surfaces, the ability to undergo phase variation allowed the microbes to adapt to—and stick in—different environments. Examples of phase variation include the ability of *Neisseria gonorrhoeae* strains to produce attachment fimbriae, and the ability of *Streptococcus pneumoniae* to produce a capsule.

Lactose used by bacteria is the same disaccharide found in the milk we drink.
©Ingram Publishing

Table 8.6 The *lac* Operon



8.3 LEARNING OUTCOMES—Assess Your Progress

13. Define the term *operon*, and explain one advantage it provides to a bacterial cell.
14. Highlight the main points of *lac* operon operation.

8.4 DNA Recombination Events

Genetic recombination through sexual reproduction is an important means of genetic variation in eukaryotes. Although bacteria have no exact equivalent to sexual reproduction, they exhibit a primitive means for sharing or recombining parts of their genome. An event in which one bacterium donates DNA to another bacterium is a type of genetic transfer termed **recombination**, the end result of which is a new strain different from both the donor and the original recipient strain. Recombination in bacteria depends in part on the fact that bacteria contain extrachromosomal DNA—that is, plasmids—and are adept at interchanging genes. Genetic exchanges have tremendous effects on the genetic diversity of bacteria. They provide additional genes for resistance to drugs and metabolic poisons, new nutritional and metabolic capabilities, and increased virulence and adaptation to the environment.

In general, any organism that contains genes that originated in another organism is called a **recombinant**.

Horizontal Gene Transfer in Bacteria

Any transfer of DNA that results in organisms acquiring new genes that did not come directly from parent organisms is called **horizontal gene transfer**. (Acquiring genes from parent organisms during reproduction would be vertical gene transfer.) Bacteria have been known to engage in horizontal gene transfer for decades. It is now becoming clear that eukaryotic organisms—including humans—also engage in horizontal gene transfer, often aided and abetted by microbes such as viruses. Earlier in this book we discussed the concept of "pangenomes," made necessary by the discovery that different representatives of the same species had dozens of genes that the reference organisms did not. This comes about mainly from horizontal gene transfer.

DNA transfer between bacterial cells typically involves small pieces of DNA in the form of plasmids or chromosomal fragments. Plasmids are small, circular pieces of DNA that contain their own origin of replication and therefore can replicate independently of the bacterial chromosome. Plasmids are found in many bacteria (as well as some fungi) and typically contain, at most, only a few dozen genes. Although plasmids are not necessary for bacterial survival, they often carry useful traits, such as antibiotic resistance. Chromosomal fragments that have escaped from a lysed bacterial cell are also commonly involved in the transfer of genetic information between cells. An important difference between plasmids and fragments is that while a plasmid has its own origin of replication and is stably replicated and inherited, chromosomal fragments must integrate themselves into the bacterial chromosome in order to be replicated and eventually passed to progeny cells.

Depending on the mode of transmission, the means of genetic recombination in bacteria is called conjugation, transformation, or transduction. *Conjugation* requires the attachment of two related species and the formation of a bridge that can transport DNA. *Transformation* entails the transfer of naked DNA and requires no special vehicle. *Transduction* is the transfer of DNA from one bacterium to another via a bacterial virus **(table 8.7)**.

Researchers have found DNA that originated in snakes in many different animals, including cows. They suspect it was transferred there by ticks or other parasites.

©Imagestate Media (John Foxx)/Imagestate (cow); ©Ingram Publishing/Fotosearch (snake)

Conjugation: Exchanging Genes

Conjugation is a mode of genetic exchange in which a plasmid or other genetic material is transferred by a donor to a recipient cell via a direct connection. Both gram-negative and gram-positive cells can conjugate. In gram-negative cells, the donor's plasmid (**called a fertility**,

Table 8.7 Types of Horizontal Gene Transfer in Bacteria

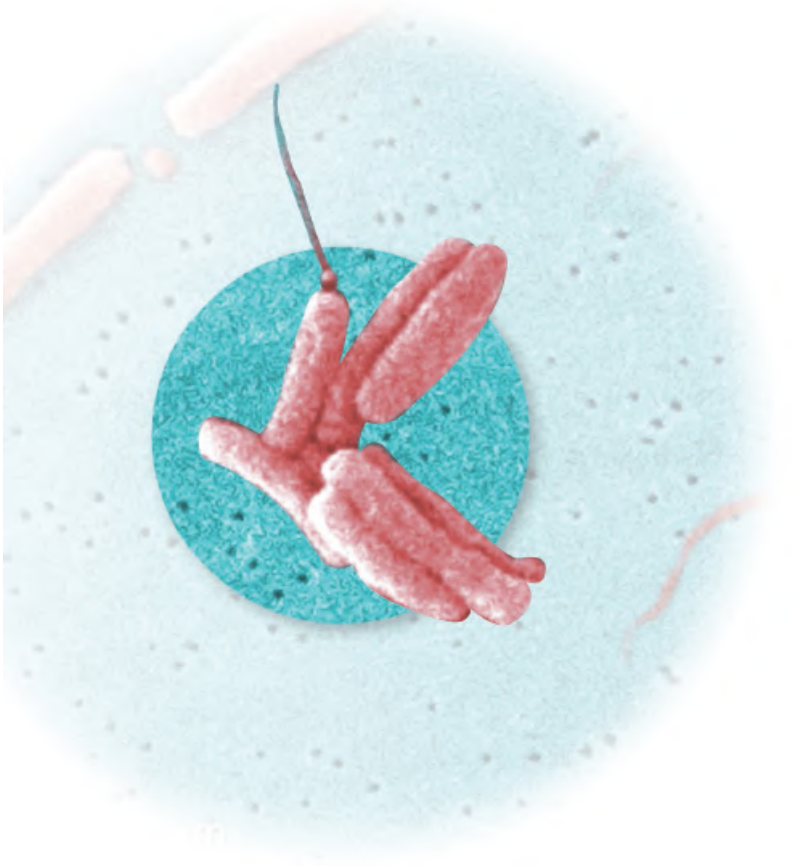
Examples of Mode	Factors Involved	Direct or Indirect*	Genes Commonly Transferred in Nature**
Conjugation	Donor cell with pilus Fertility plasmid in donor Both donor and recipient alive Bridge forms between cells to transfer DNA.	Direct	Drug resistance; resistance to metals; toxin production; enzymes; adherence molecules
Transformation	Free donor DNA (fragment) Live, competent recipient cell	Indirect	Polysaccharide capsule
Transduction	Donor is lysed bacterial cell. Defective bacteriophage is carrier of donor DNA. Live recipient cell of same species as donor	Indirect	Toxins; enzymes for sugar fermentation; drug resistance

*Direct means the donor and recipient are in contact during exchange; indirect means they are not.
**In the lab almost any gene can be transferred.

or **F factor**) has genes that direct the synthesis of a conjugative pilus. The recipient cell has a recognition site on its surface. A cell's role in conjugation is denoted by F^+ for the cell that has the F plasmid and by F^- for the cell that lacks it. Contact is made when a pilus grows out from the F^+ cell, attaches to the surface of the F^- cell, contracts, and draws the two cells together. In gram-positive cells, an opening is created between two adjacent cells, and the replicated DNA passes across from one cell to the other. Conjugation is a conservative process, in that the donor bacterium generally retains ("conserves") a copy of the genetic material being transferred.

There are hundreds of conjugative plasmids with some variations in their properties. One of the best understood plasmids is the F factor in *E. coli*, which can do either of two things **(figure 8.11):**

1. The donor (F^+) cell makes a copy of its F factor and transmits this to a recipient (F^-) cell. The F^- cell is thereby changed into an F^+ cell capable of producing a pilus and conjugating with other cells. No additional donor genes are transferred.
2. In high-frequency recombination (Hfr) donors, the plasmid becomes integrated into the F^+ donor chromosome, which, when replicated, begins to transfer to the recipient cell. This means that some chromosomal genes get transferred to the recipient. Plasmid genes may or may not be transferred.



F^+ and F^- bacteria share the same space in many settings, including the human gut.
Source: CDC/Janice Haney Carr

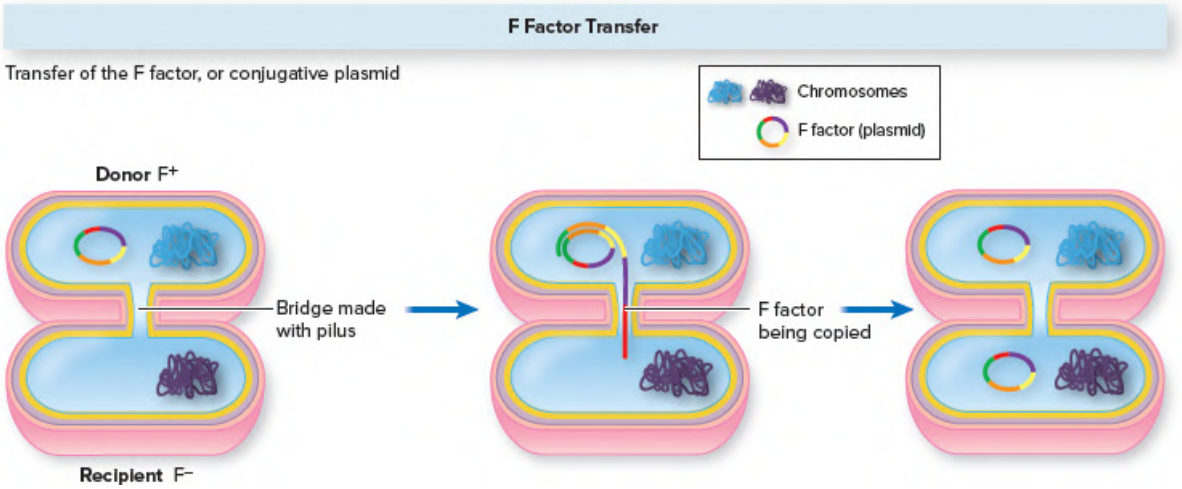


Figure 8.11 **Conjugation: genetic transmission through direct contact between two cells**.

Conjugation has great biomedical importance. Special **resistance (R) plasmids**, or **factors**, that bear genes for resisting antibiotics and other drugs are commonly shared among bacteria through conjugation. Transfer of R factors can confer multiple resistance to antibiotics such as

tetracycline, chloramphenicol, streptomycin, sulfonamides, and penicillin. Other types of R factors carry genetic codes for resistance to heavy metals (nickel and mercury) or for synthesizing virulence factors (toxins, enzymes, and adhesion molecules) that increase the pathogenicity of the bacterial strain.

Transformation: Capturing DNA from Solution

We now know that a chromosome released by a lysed cell breaks into fragments small enough to be accepted by a recipient cell. This nonspecific acceptance by a bacterial cell of small fragments of soluble DNA from the surrounding environment is termed *transformation*. Transformation is facilitated by special DNA-binding proteins on the cell wall that capture DNA from the surrounding medium. Cells that are capable of accepting genetic material through this means are termed **competent**. The new DNA is transported into the cytoplasm, where some of it is inserted into the bacterial chromosome. Transformation is a natural event found in several groups of gram-positive and gram-negative bacterial species.

Because transformation requires no special appendages, and the donor and recipient cells do not have to be in direct contact, the process is useful for certain types of recombinant DNA technology. With this technique, foreign genes from a completely unrelated organism are inserted into a plasmid, which is then introduced into a competent bacterial cell through transformation in the same way that small pieces are taken up naturally. These recombinations can be carried out in a test tube, and human genes can be experimented upon and even expressed outside the human body by placing them in a microbial cell. This same phenomenon in eukaryotic cells, termed **transfection**, is an essential aspect of genetically engineered yeasts, plants, and mice.

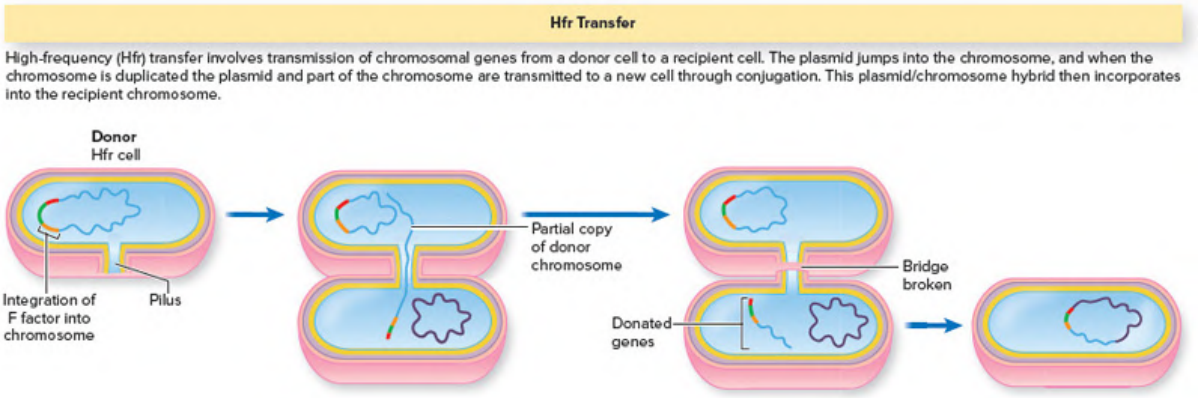
Source: CDC/Janice Haney Carr

Transduction: The Case of the Piggyback DNA

Earlier we described bacteriophages as bacterial parasites. Viruses can in fact serve as genetic vectors (an entity that can bring foreign DNA into a cell). The process by which a

bacteriophage serves as the carrier of DNA from a donor cell to a recipient cell is *transduction*. It occurs naturally in a broad spectrum of bacteria. The participating bacteria in a single transduction event must generally be the same species because of the specificity of viruses for host cells.

There are two versions of transduction. In *generalized transduction* **(figure 8.12)**, random fragments of disintegrating host DNA are taken up by the phage during assembly. Virtually any gene from the bacterium can be transmitted through this means. In *specialized*



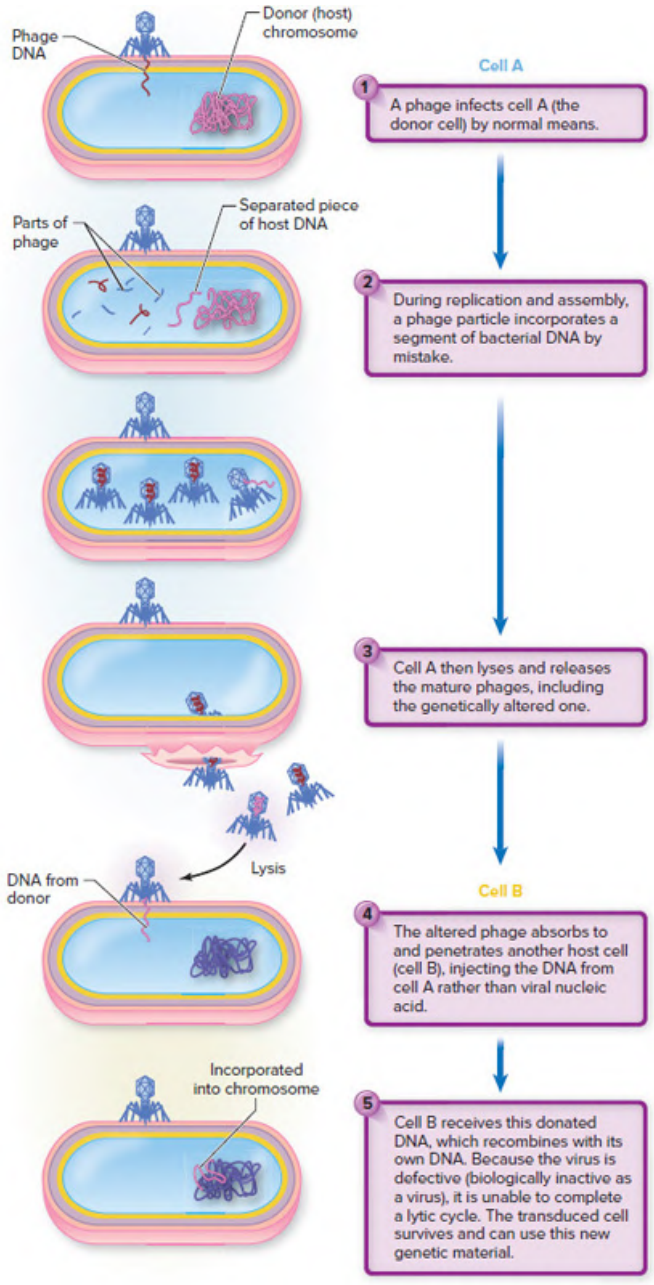


Figure 8.12 **Generalized transduction: genetic transfer by means of a virus carrier**.

transduction **(figure 8.13)**, a highly specific part of the host genome is regularly incorporated into the virus. This specificity is explained by the prior existence of a temperate prophage inserted in a fixed site on the bacterial chromosome. When activated, the prophage DNA separates from the bacterial chromosome, carrying a small segment of host

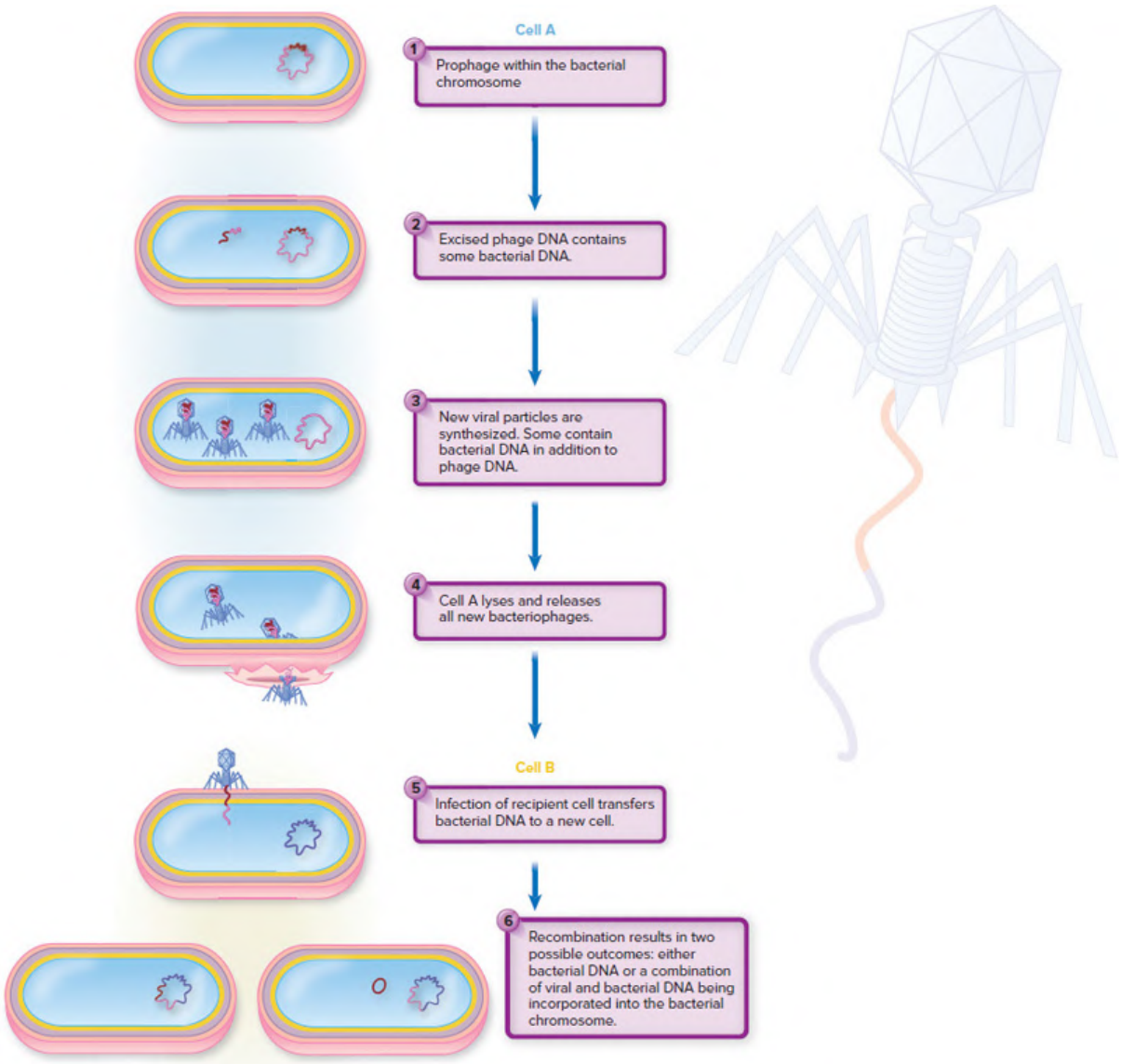


Figure 8.13 Specialized transduction: transfer of specific genetic material by means of a virus carrier. Specialized transduction begins with a cell that contains a prophage (a viral genome integrated into the host cell chromosome). Rarely, the virus enters a lytic cycle and, as it excises itself from its host cell, inadvertently includes some bacterial DNA. Replication and assembly result in production of a hybrid virus, containing some bacterial DNA. Release of the recombinant virus and subsequent infection of a new host result in transfer of bacterial DNA between cells.

genes with it. During a lytic cycle, these specific viral-host gene combinations are incorporated into the viral particles and carried to another bacterial cell.

Several cases of specialized transduction have biomedical importance. The virulent strains of bacteria such as *Corynebacterium diphtheriae, Clostridium* spp., and *Streptococcus pyogenes* all produce toxins with profound physiological effects. The nonvirulent strains do not produce toxins. It turns out that toxicity arises from the

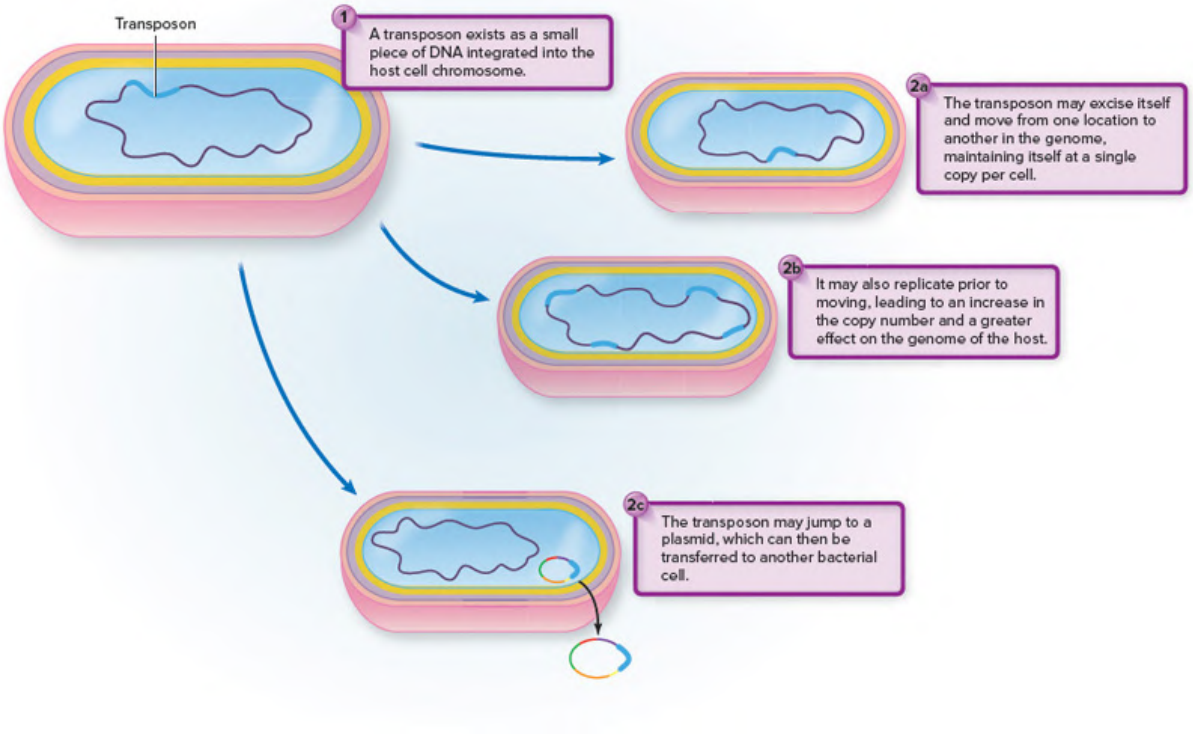


Figure 8.14 **Transposons: shifting segments of the genome**.

presence of bacteriophage genes that have been introduced by transduction. Only those bacteria infected with a temperate phage are toxin formers. (Details of toxin action are discussed in the organ system–specific disease chapters.)

Transposons

Another type of genetic transfer involves transposable elements, or **transposons**. Transposons have the distinction of shifting from one part of the genome to another and so are termed "jumping genes."

All transposons share the general characteristic of traveling from one location to another on the genome—from one chromosomal site to another, from a chromosome to a plasmid, or from a plasmid to a chromosome **(figure 8.14)**. Because transposons can occur in plasmids, they can also be transmitted from one cell to another in bacteria and a few eukaryotes. Some transposons replicate themselves before jumping to the next location, and others simply move without replicating first.

The overall effect of transposons—to scramble the genetic language—can be beneficial or adverse, depending upon such variables as where insertion occurs in a chromosome, what kinds of genes are relocated, and the type of cell involved. In bacteria, transposons are known to be involved in

- changes in traits such as colony morphology, pigmentation, and antigenic characteristics;
- replacement of damaged DNA; and

- the intermicrobial transfer of drug resistance (in bacteria).

8.4 LEARNING OUTCOMES—Assess Your Progress

15. Explain the defining characteristics of a recombinant organism.
16. Describe three forms of horizontal gene transfer used in bacteria.

8.5 Mutations: Changes in the Genetic Code

As precise and predictable as the rules of genetic expression seem, permanent changes do occur in the genetic code. Indeed, genetic change is the driving force of evolution. Any change to the nucleotide sequence in the genome is called a mutation. Mutations are most noticeable when the genotypic change leads to a change in phenotype. Mutations can involve the loss of base pairs, the addition of base pairs, or a rearrangement in the order of base pairs. Do not confuse this with genetic recombination, in which microbes transfer whole segments of genetic information among themselves.

A microorganism that exhibits a natural, nonmutated characteristic is known as a **wild type**, or wild strain with respect to that trait. If a microorganism bears a mutation, it is called a **mutant strain**. Mutant strains can show variance in morphology, nutritional characteristics, genetic control mechanisms, resistance to chemicals, temperature preference, and nearly any type of enzymatic function.

Causes of Mutations

Mutations can be spontaneous or induced, depending upon their origin. A **spontaneous mutation** is a random change in the DNA arising from errors in replication that occur randomly. The frequency of spontaneous mutations has been measured for a number of organisms. Mutation rates vary tremendously, from one mutation in 10^3 replications (a high rate) to one mutation in 10^{10} replications (a low rate). The rapid rate of bacterial reproduction allows these mutations to be observed more readily in bacteria than in most eukaryotes.

Induced mutations result from exposure to known **mutagens**, which are physical or chemical agents that interact with DNA in a disruptive manner. Examples of mutagens are some types radiation (UV light, X rays) and certain chemicals such as nitrous acid.

Barbara McClintock won the Nobel Prize for Physiology or Medicine in 1983 for the discovery of mobile genetic elements, or transposons, in the corn plant.

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Categories of Mutations

Mutations range from large mutations, in which large genetic sequences are gained or lost, to small ones that affect only a single base on a gene. These latter mutations, which involve addition, deletion, or substitution of single bases, are called **point mutations**.

To understand how a change in DNA influences the cell, remember that the DNA code appears in a particular order of triplets (three bases) that is transcribed into mRNA codons, each of which specifies an amino acid. A permanent alteration in the DNA that is copied faithfully into mRNA and translated can change the structure of the protein. A change in a protein can likewise change the morphology and physiology of a cell. Some mutations have a harmful effect on the cell, leading to cell dysfunction or death; these are called lethal mutations. Neutral mutations produce neither adverse nor helpful changes. A small number of mutations are beneficial in that they provide the cell with a useful change in structure or physiology.

Any change in the code that leads to placement of a different amino acid is called a **missense mutation**. A missense mutation can do one of the following:

- create a faulty, nonfunctional (or less functional) protein;
- produce a protein that functions in a different manner; or
- cause no significant alteration in protein function (see **table 8.8** to see what missense mutations look like).

A **nonsense mutation**, on the other hand, changes a normal codon into a stop codon that does not code for an amino acid and stops the production of the protein wherever it occurs. A nonsense mutation almost always results in a nonfunctional protein.(Table 8.8, row d, shows a

nonsense mutation resulting from a frameshift [described in next paragraph].) A **silent mutation**(table 8.8, row c) alters a base but does not change the amino acid and thus has no effect. For example, because of the redundancy of the code, ACU, ACC, ACG, and ACA all code for threonine, so a mutation that

Table 8.8 Categories of Point Mutations and Their Effects

Green letters indicate changes in the code due to mutation

(a)	DNA	TAC	TGG	CTG	CTC	TAC	TTT...	Normal gene
	RNA	AUG	ACC	GAC	GAG	AUG	AAA...	
	Protein	Met	Thr	Asp	Glu	Met	Lys...	
(b)	DNA	TAC	TGG	CTT	CTC	TAC	TTT...	Missense mutation: base substitution leading to amino acid switch (may or may not function well)
	RNA	AUG	ACC	GAA	GAG	AUG	AAA...	
	Protein	Met	Thr	Glu	Glu	Met	Lys...	
(c)	DNA	TAC	TGG	CTA	CTC	TAC	TTT...	Silent mutation: base substitution resulting in no change in amino acid (no change in function)
	RNA	AUG	ACC	GAU	GAG	AUG	AAA...	
	Protein	Met	Thr	Asp	Glu	Met	Lys...	
(d)	DNA	TAC	TGC (G)	TGC	TCT	ACT	TT	**Frameshift mutation**
	RNA	AUG	ACG	ACG	AGA	UGA	AAA...	Deletion mutation (d) ↓ Both lead to frameshifts and can lead to premature stop codons and/or poorly functioning protein ↑ Insertion mutation (e)
	Protein	Met	Thr	Thr	Arg	STOP		
				Frameshift and premature stop				
(e)	DNA	TAC	TGG	GCT	GCT	CTA	CTT...	
	RNA	AUG	ACC	CGA	CGA	GAU	GAA...	
	Protein	Met	Thr	Arg	Arg	Asp	Glu...	
				Frameshift				

changes only the last base will not alter the sense of the message in any way. A **back-mutation** occurs when a gene that has undergone mutation reverses (mutates back) to its original base composition.

Mutations also occur when one or more bases are inserted into or deleted from a newly synthesized DNA strand. This type of mutation, known as a **frameshift** (table 8.8, rows d and e), is so named because the reading frame of the mRNA has been changed. Frameshift mutations nearly always result in a nonfunctional protein because every amino acid after the mutation is different from what was coded for in the original DNA. Also note that insertion or deletion of bases in multiples of three (3, 6, 9, etc.) results in the addition or deletion of amino acids but does not disturb the reading frame.

UV light causes mutation of DNA in human skin cells also. This can lead to skin cancer.
©McGraw-Hill Education/Barry Barker, photographer

Single Nucleotide Polymorphism

One type of mutation recently found to be important in individual traits is called **single nucleotide polymorphism (SNP)** because only a single nucleotide is altered. This is a result of a point mutation at some point in the organism's ancestry, and it is passed on genetically. Tens of thousands of these differences at a single locus (when two different individuals are compared) are known to exist throughout the genome. The human genome contains 10 million SNPs. These variations are currently a hot area of research and commerce.

The ability to identify SNPs has proven critical to the new field of personalized medicine, which is customized to a person's genetic makeup. One example is when patients' genomes are examined for SNPs that have been found to be associated with a particular disease, to determine their risk. For example, in a condition called thrombophilia (a blood-clotting disorder), a point mutation in the gene for a clotting factor (factor V) causes an arginine to become a glutamine

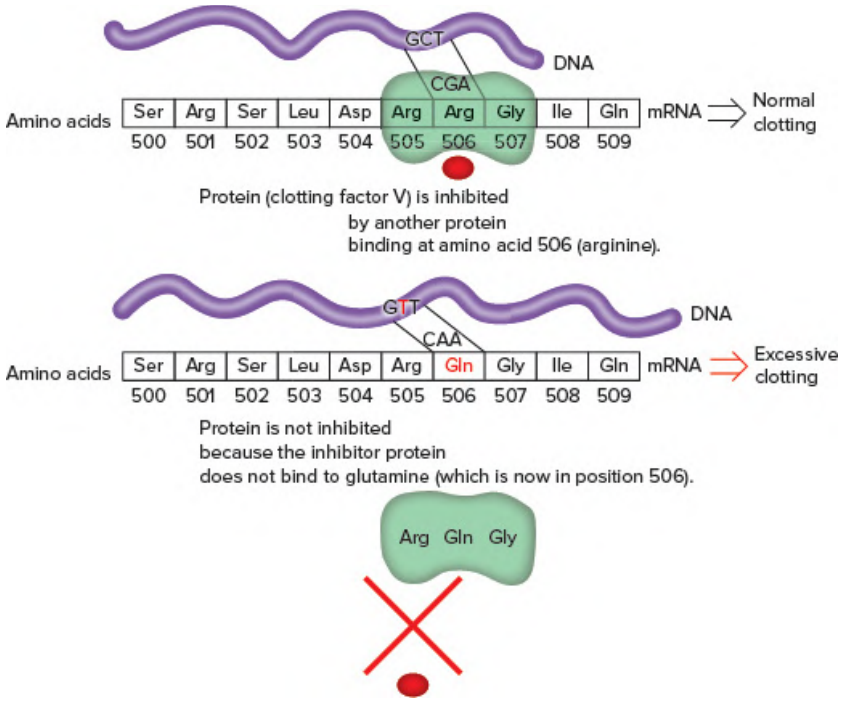

Figure 8.15 **Example of single-nucleotide polymorphism (SNP) associated with disease**. When the arginine at position 506 is instead a glutamine, the clotting factor is not inhibited. This leads to excessive clotting.

(figure 8.15). This leads to increased clotting in the patient. Also, SNPs can determine whether a patient will respond favorably to a particular treatment. The new field of pharmacogenomics tailors drug treatments using this knowledge of SNPs.

Repair of Mutations

Earlier we indicated that DNA has a proofreading mechanism to repair mistakes in replication that might otherwise become permanent. Because mutations are potentially life-threatening, the cell has systems for finding and repairing DNA that has been damaged by various mutagenic agents and processes. Most ordinary DNA damage is resolved by enzymatic systems specialized for finding and fixing such defects.

DNA that has been damaged by ultraviolet radiation can be restored by photoactivation—or light repair. This repair mechanism requires visible light and a light-sensitive enzyme, DNA photolyase, an enzyme which can detect and attach to the damaged areas (sites of abnormal pyrimidine binding). Ultraviolet repair mechanisms are successful only for a relatively small number of UV mutations. Cells cannot repair severe, widespread damage and will die.

Mutations can be excised, or cut out, by a series of enzymes that remove the incorrect bases and add the correct ones. This process is known as excision repair. First, enzymes break the bonds between the bases and the sugar-phosphate strand at the site of the error. A different enzyme subsequently removes the defective bases one at a time, leaving a gap that will be filled in by DNA polymerase I and ligase. A repair system can also locate mismatched bases that were missed during proofreading—for example, C mistakenly paired with A, or G with T.

Positive and Negative Effects of Mutations

Many mutations are not repaired. How the cell copes with them depends on the nature of the mutation and the strategies available to that organism. Mutations are permanent and heritable, meaning these changes will be passed on to the offspring of organisms and become a long-term part of the gene pool. Many mutations are harmful to organisms; others provide adaptive advantages.

Medical Moment

Gene Mutations

In human cellular function, even small changes in the genetic code can have devastating consequences. Consider the following examples of mutations, which are caused by an alteration in just one or two amino acids:

- *Alpers-Huttenlocher syndrome*: A point mutation causes the insertion of one incorrect nucleotide, resulting in the placement of amino acid threonine in place of alanine. This mutation inhibits DNA synthesis and causes decreased cellular energy. Patients with this syndrome present between ages 2 and 4 with developmental regression, liver problems, and recurrent seizures. The condition is progressive and eventually fatal.
- *Cystic fibrosis*: This disease affects cellular chloride channels, causing thick and sticky mucus production by secretory glands. Thick mucus causes problems for the airways, pancreas, reproductive organs, and digestive system. The mutation in the CFTR gene is most commonly caused by the deletion of a single amino acid. Though symptoms may be managed, patients with cystic fibrosis are vulnerable to infections and shorter life expectancy.
- *Epidermolysis bullosa*: A missense mutation is responsible for this inherited condition which causes skin fragility. Those affected develop blisters and lesions in response to even minor skin contact. The condition affects two nucleotides that code for amino acids that build keratin, a protein that gives skin stability and strength.

Q. Without the redundancy of the genetic code, would there be more or fewer cases of these diseases in the population?

Answer in Appendix B.

Although most spontaneous mutations are not beneficial, a small number contribute to the success of the individual and the population by creating variant strains with alternate ways of expressing a trait. Microbes are not “aware” of this advantage and do not direct these changes; they simply respond to the environment they encounter. A mutation is beneficial if it allows the organism to more readily survive or reproduce. In the long-range view, mutations and the variations they produce are the raw materials for change in the population and, thus, for adaptation and evolution.

Mutations that create variants occur frequently enough that any population contains mutant strains for a number of characteristics, but as long as the environment is stable, these mutants (for that particular trait) will never comprise more than a tiny percentage of the population. When the environment changes, however, it can become hostile for the survival of certain individuals, and only those microbes bearing protective mutations will be equipped to survive in the new environment. In this way, the environment naturally “selects” certain mutant strains that will reproduce, give rise to subsequent generations, and, in time, be the dominant strain in the population. Through these means, any change that confers an advantage due to this

selection pressure will be retained by the population. One of the clearest models for this sort of selection and adaptation is acquired drug resistance in bacteria (see chapter 10).

8.5 LEARNING OUTCOMES—Assess Your Progress

17. Define the *term mutation*, and discuss one positive and one negative example of it in microorganisms.
18. Differentiate among frameshift, nonsense, silent, and missense mutations.
19. Explain the influence of single-nucleotide polymorphisms on an organism.

8.6 Studying DNA in the Laboratory and Genetic Engineering

The knowledge of how DNA is manipulated within the cell to carry out the goals of a microbe allows scientists to utilize these processes to accomplish goals more to the liking of human beings. Since the 1970s, discoveries and advances have led to an explosion of new capabilities and, as a result, an explosion of new knowledge about microbes and about biology in general. In this section, we will highlight a few techniques that have relevance for microbiology and in particular for infectious diseases.

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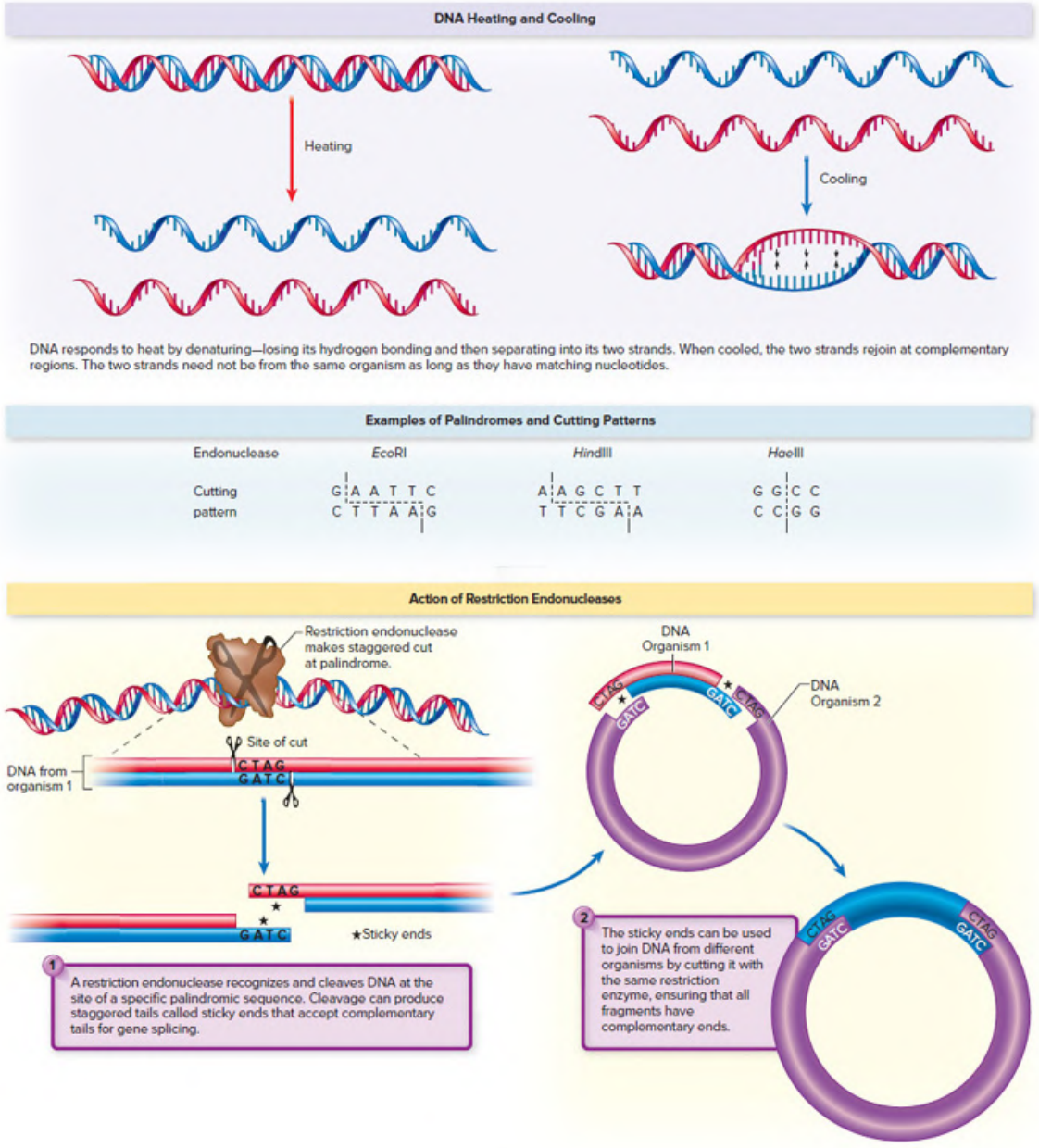
Working with DNA in the Laboratory

Two very important techniques were developed in the 1970s and 1990s that made massive advances in DNA study and manipulation possible. These are, first, the discovery of **restriction endonucleases**, and, second, the invention of the polymerase chain reaction.

The groundbreaking discovery in 1971 of restriction endonucleases made almost everything we discuss in this section possible. These enzymes come from bacterial cells. They recognize foreign DNA and are capable of breaking the phosphodiester bonds between adjacent nucleotides on both strands of DNA, leading to a break in the DNA strand. In the bacterial cell, this ability protects against the incompatible DNA of bacteriophages or plasmids. In the biotechnologist's lab, the enzymes can be used to cleave DNA at desired sites and are necessary for the techniques of recombinant DNA technology.

Hundreds of restriction endonucleases have been discovered in bacteria. Each type has a known sequence of 4 to 10 base pairs as its target, so sites of cutting can be finely controlled. These enzymes have the unique property of recognizing and clipping at base sequences called **palindromes** **(figure 8.16)**. Palindromes

Figure 8.16 **Some useful properties of DNA**.



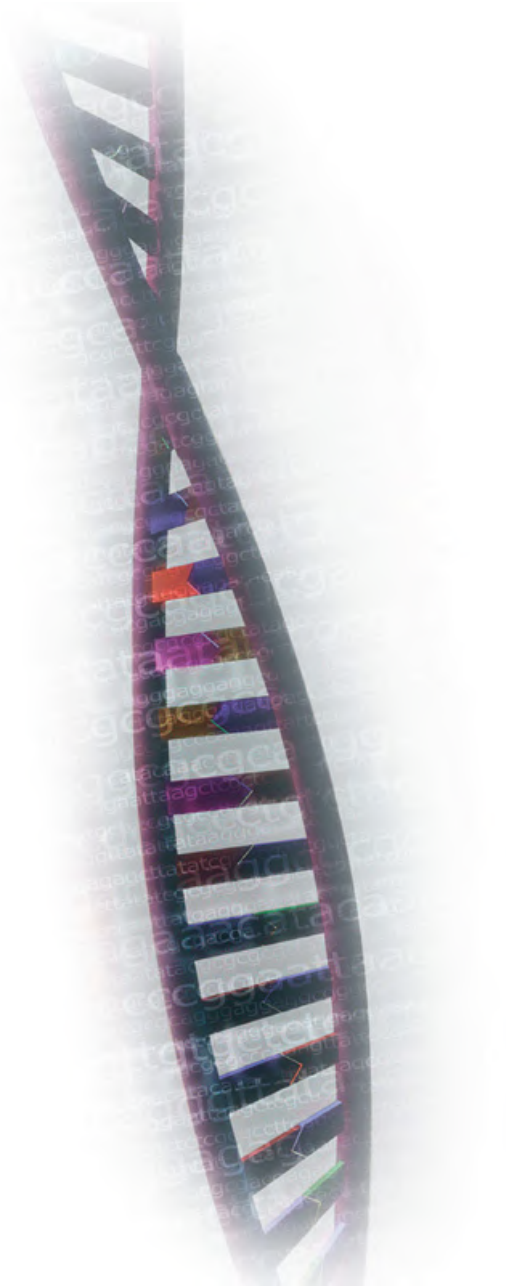
are sequences of DNA that are identical when read from the 5′ to 3′ direction on one strand and the 5′ to 3′ direction on the other strand.

Endonucleases are usually named by combining the first letter of the bacterial genus, the first two letters of the species, and the endonuclease number. Thus, *Eco*RI is the first endonuclease found in *Escherichia coli*, and *Hind*III is the third endonuclease discovered in *Haemophilus influenzae* type d (figure 8.16).

The pieces of DNA produced by restriction endonucleases are termed **restriction fragments**. Because DNA sequences vary, even among members of the same species, differences in the cutting pattern of specific restriction endonucleases give rise to restriction fragments of differing lengths, known as **restriction fragment length polymorphisms (RFLPs)**. RFLPs allow the direct comparison of the DNA of two different organisms at a specific site.

Another enzyme, called a **ligase**, is necessary to seal the sticky ends together by rejoining the phosphate-sugar bonds cut by endonucleases. Its main application is in final splicing of genes into plasmids and chromosomes.

An enzyme called **reverse transcriptase (RT)** is best known for its role in the replication of the AIDS virus and other retroviruses. It also provides geneticists with a valuable tool for converting RNA into DNA. Copies called **complementary DNA**, or **cDNA**, can be made from messenger, transfer, ribosomal, and other forms of RNA. The technique provides a valuable means of synthesizing eukaryotic genes from mRNA transcripts. The advantage is that the synthesized gene will be free of the intervening sequences (introns) that can complicate the management of eukaryotic genes in genetic engineering.



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NCLEX® PREP

4. A client is being treated with recombinant insulin, *Lispro*, and asks the nurse to explain what is meant by the term *recombinant* during their teaching session. Which statement should the nurse make to the client?

a. *This type of insulin is similar in structure to the insulin that is produced in your body, so there is less likelihood of developing a reaction than if a different type of insulin was used*.

b. *DNA recombinant medication utilizes genes that were transferred from one organism to another to cause a desired effect*.

c. *DNA recombinant medication, Lispro, is the same as other insulin preparations but has a longer onset of action.*

d. *Using Lispro will reduce the need for frequent Accu-chek testing during a 24-hour period.*

Polymerase Chain Reaction: A Molecular Xerox Machine for DNA

Some of the techniques used to analyze DNA and RNA are limited by the small amounts of test nucleic acid available. This problem was largely solved by the invention of a simple, versatile way to amplify DNA called the polymerase chain reaction (PCR). This technique rapidly increases the amount of DNA in a sample without the need for making cultures or carrying out complex purification techniques. It is so sensitive that it holds the potential to detect cancer from a single cell or to diagnose an infection from a single gene copy. It is comparable to being able to pluck a single DNA "needle" out of a "haystack" of other molecules and make unlimited copies of the DNA. The rapid rate of PCR makes it possible to replicate a target DNA from a few copies to billions of copies in a few hours.

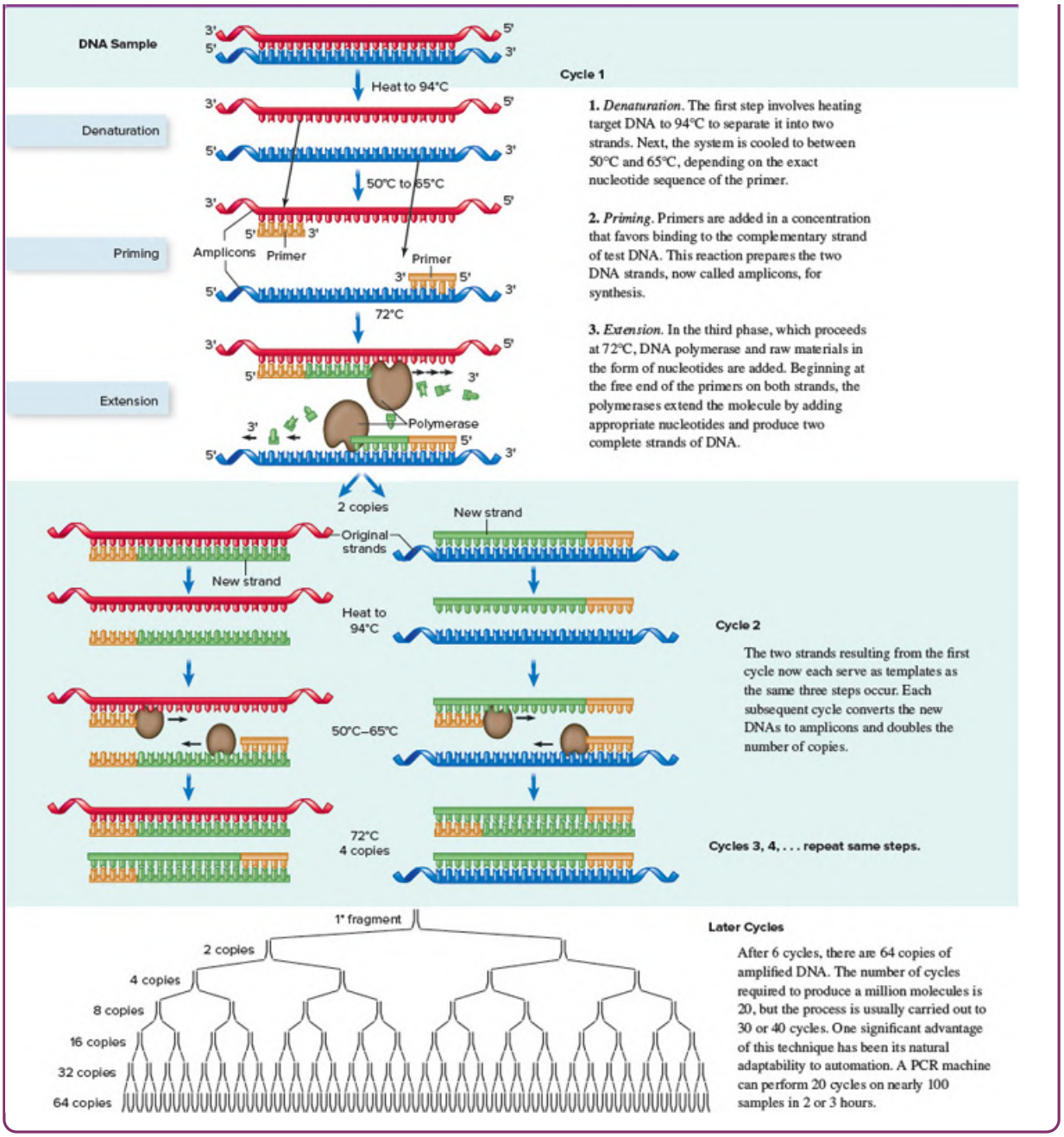
To understand the idea behind PCR, it will be instructive to review table 8.2, which describes synthesis of DNA as it occurs naturally in cells. The PCR method uses essentially the same events, with the opening up of the double strand, using the exposed strands as templates, the addition of primers, and the action of a DNA polymerase.

Initiating the reaction requires a few specialized ingredients. As we saw earlier, the **primers** are synthetic oligonucleotides (short DNA strands) of a known sequence of 15 to 30 bases that serve as landmarks to indicate where DNA amplification will begin. To keep the DNA strands separated, processing must be carried out at a relatively high temperature. This necessitates the use of special **DNA polymerases** isolated from thermophilic bacteria. The most commonly used is Taq polymerase obtained from *Thermus aquaticus*. (Taq is an abbreviation of the genus and species name of this microbe, from which the enzyme was isolated.) Enzymes isolated from this thermophilic organism remain active at the elevated temperatures used in PCR. Another vital component of PCR is a machine called a thermal cycler that automatically performs the cyclic temperature changes.

The PCR technique operates by repetitive cycling of three basic steps: denaturation, priming, and extension, as outlined in **table 8.9**.

In conventional PCR, the nucleic acid products have to be visualized in some way, usually via gel electrophoresis. Improvements to PCR have made that step unnecessary. Real-time PCR techniques incorporate fluorescence into the amplification

Table 8.9 Polymerase Chain Reaction



* For simplicity's sake, we have omitted the elongation of the complete original parent strand during the first cycles. Ultimately, templates that correspond only to the smaller fragments dominate and become the primary population of replicated DNA.

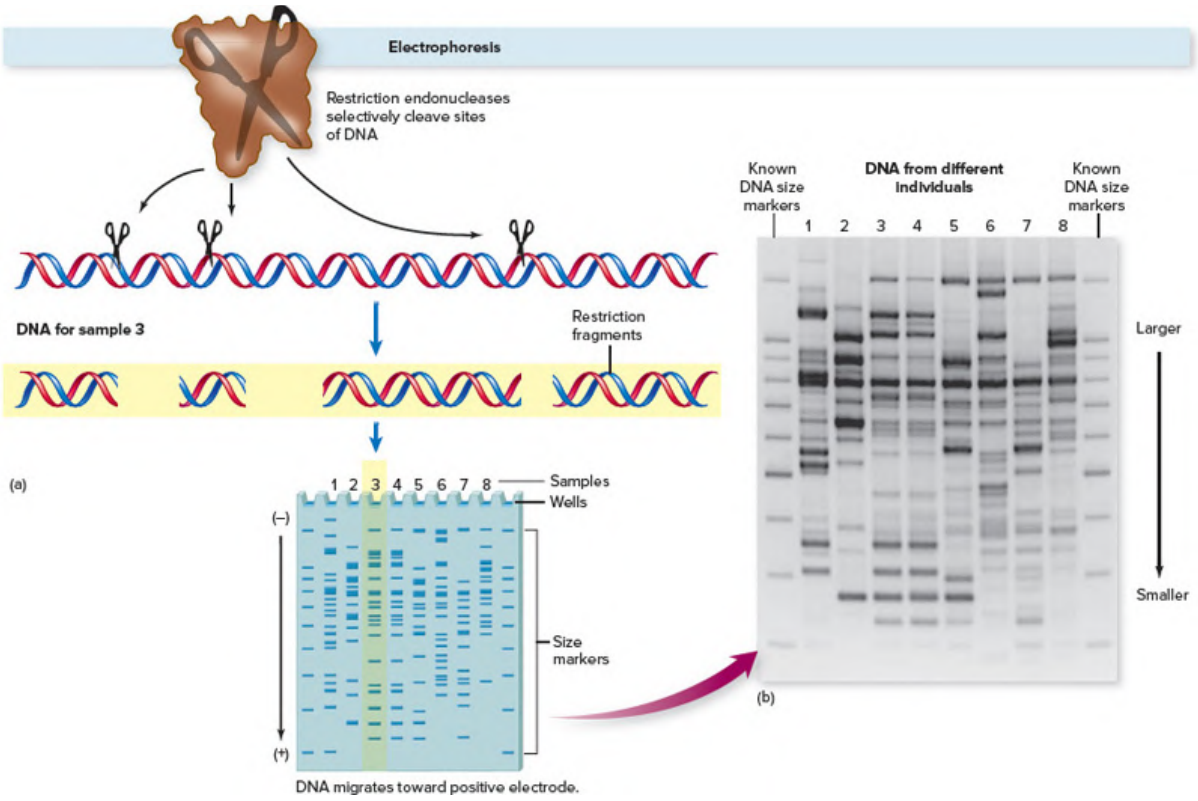


Figure 8.17 **Revealing the patterns of DNA with electrophoresis**. **(a)** After cleavage into fragments, DNA is loaded into wells on one end of an agarose gel. When an electrical current is passed through the gel (from the negative pole to the positive pole), the DNA, being negatively charged, migrates toward the positive pole. The larger fragments, measured in numbers of base pairs, migrate more slowly and remain nearer the wells than the smaller (shorter) fragments. **(b)** An actual stained gel reveals a separation pattern of the fragments of DNA. The size of a given DNA band can be determined by comparing the distance it traveled to the distance traveled by a set of DNA fragments of known size (both outer lanes).

(b) ©Zmeel Photography/Getty Images

mix itself, and an automated reader records the creation of the amplicons in real time. There is more detailed information about the newer PCR techniques in chapter 15.

The polymerase chain reaction quickly became prominent as a powerful workhorse of molecular biology, medicine, and biotechnology. It is taking on an important role in diagnosis of infectious diseases, as well.

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Analysis of DNA

There are many reasons scientists would want to know the characteristics of an organism's DNA. Knowing the entire genome sequence of an organism (such as a human) can reveal genetic abnormalities, ancestry, and so forth. Sometimes the point is to determine if one sample of DNA is the same as another sample. If you know the DNA patterns of a pathogen causing an outbreak, for example, you can look at the

DNA patterns of a microbe isolated from a patient to see if they were made ill by that same pathogen. Also, you can look at the pattern of DNA left at a crime scene, and match it against a suspect's DNA. There are multiple ways to analyze DNA, and we will highlight two of them: **DNA profiling** and **DNA sequencing**.

DNA Profiling

You've no doubt seen images like the one in **figure 8.17*b***, with its ladderlike images, on TV crime shows. These are examples of DNA profiles. They rely on the fact that the DNA in different individuals—even of the same species—contain small differences. This is true for humans and it is true for bacteria of the same species. When the individual's DNA is digested with a set of restriction enzymes, the small sequence differences will cause the DNA to be cut up at different places, resulting in fragments of different lengths in the digested samples of different individuals. To actually see the array of different fragments, and to compare the fragments of different individuals, scientists use a technique called **gel electrophoresis**.

In this technique, samples are placed in compartments (wells) in a soft agar gel and subjected to an electrical current. The phosphate groups in DNA give the entire molecule an overall negative charge, which causes the DNA to move toward the positive pole in the gel. The rate of movement is based primarily on the size of the fragments. The larger fragments move more slowly and remain nearer the top of the gel, whereas the smaller fragments migrate faster and end up farther from the wells. The positions of DNA fragments are determined by staining the DNA fragments in the gel **(figure 8.17*a*)**.

DNA Sequencing

There are many instances in which a detailed picture of the entire sequence of a genome is needed.

By far the most detailed maps of a genome are **sequence maps**, which give an exact order of bases in a plasmid, a chromosome, or an entire genome. Genome sequencing projects have been highly successful. Genomes of thousands of organisms have been sequenced, including viruses, bacteria, and eukaryotic organisms (including humans). One of the remarkable discoveries in this huge enterprise has been how similar the genomes of relatively unrelated organisms are. Humans share approximately 80% of their DNA codes with mice, about 60% with rice, and even 30% with the worm *C. elegans*.

So how is this sequencing performed? We can illustrate the process by describing an older method, called shotgun sequencing **(figure 8.18)**. Shotgun sequencing can be broken down into seven steps:

1. First, the genome of an organism is broken down into smaller, manageable fragments.
2. The fragments are separated through gel electrophoresis.
3. Each fragment is inserted into a plasmid and is cloned into an *E. coli* cell. This produces a complete library of fragments. The library exists in the bacterial cultures, which can be preserved indefinitely and sampled repeatedly.
4. The plasmids are purified and the DNA fragments are sequenced by automated sequencers, machines that add labeled primers to each fragment. The primers usually recognize the plasmid sequences that flank the genome insert, so that the machine can tell where the fragment begins and ends. Each section of the genome ends up being sequenced multiple times in an overlapping fashion.
5. A computer program takes all the sequence data and is able to find where the sequence overlaps. This automated process results in a larger, contiguous set (contigs) of nucleotide sequences.

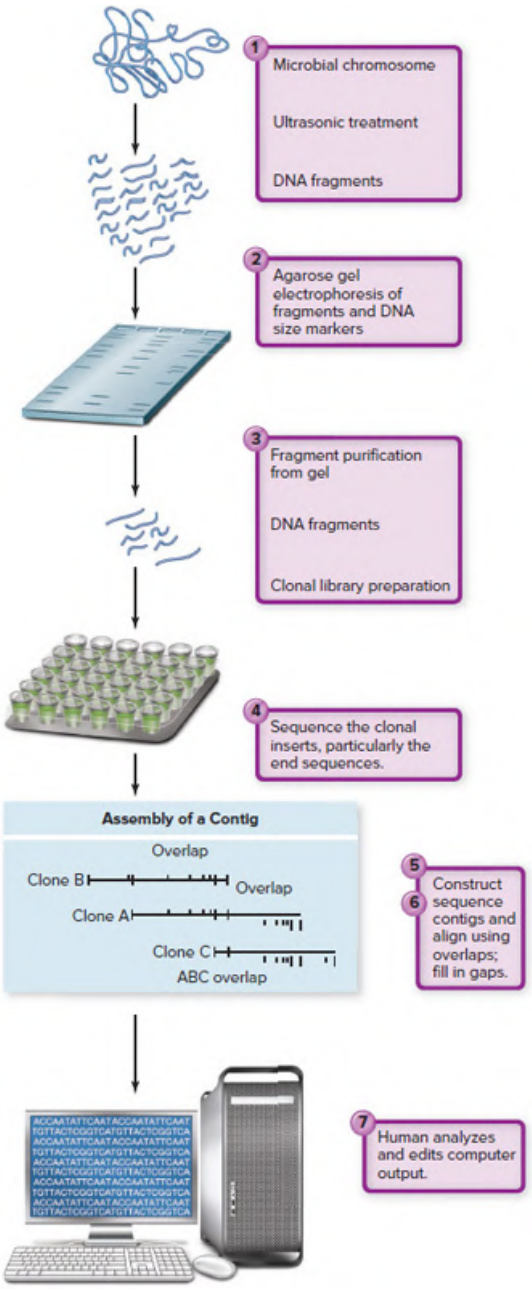


Figure 8.18 **Whole-genome shotgun sequencing**. See text for explanation of numbers.

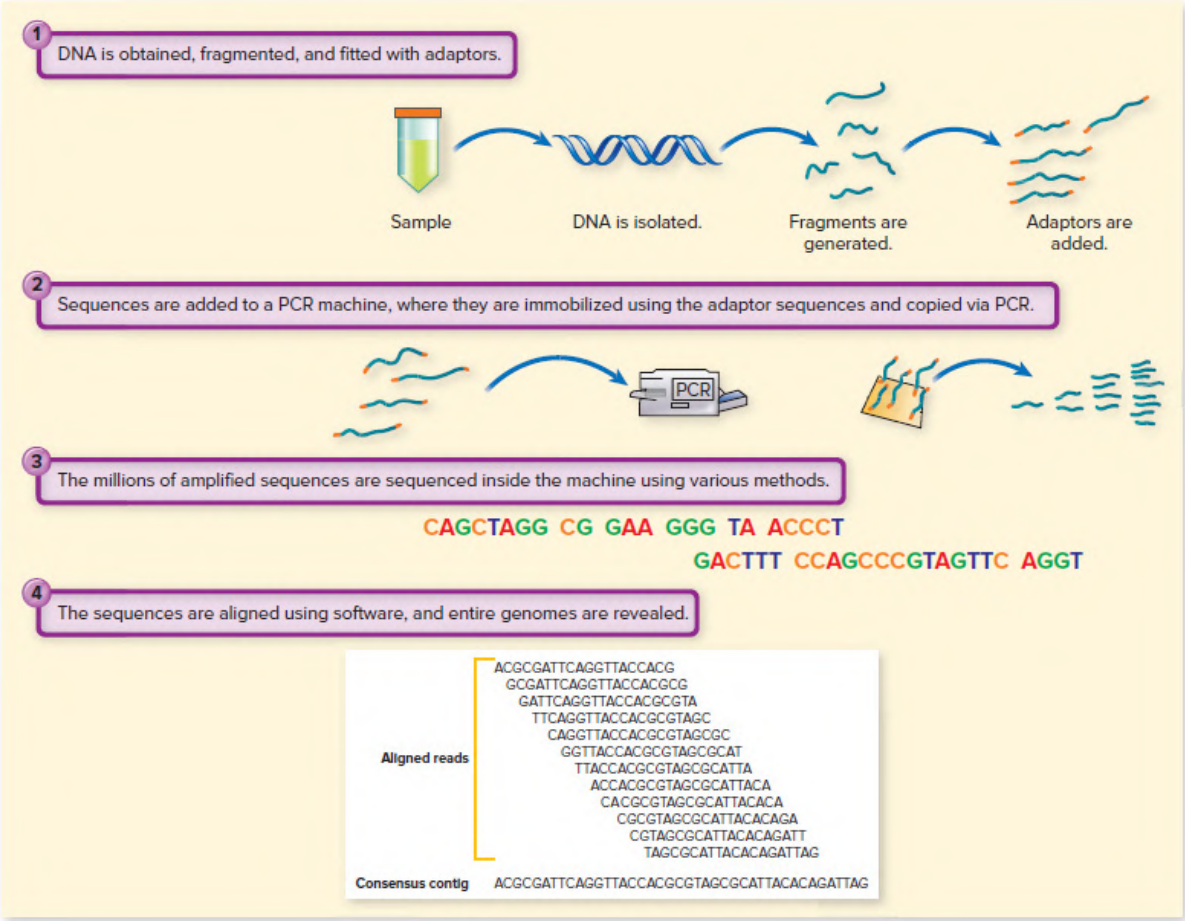


Figure 8.19 **High-throughput sequencing**.

6. The contigs are put in the proper order to determine the entire sequence. This step is tricky; there are often gaps between the contigs, but there are a variety of methods to resolve this issue.

7. An important last step is editing. A human examines the sequence, looking for irregularities, frameshifts, and ambiguities.

In modern sequencing, similar principles are used, but the whole process is scaled up in what is called "high throughput" genome sequencing. High-throughput sequencing (also called "deep sequencing" or "next generation sequencing") requires four steps **(figure 8.19)**: (1) A library of DNA is prepared by fragmenting the DNA and fitting each fragment with some common adaptors, or short DNA sequences that are designed to match the probes in the next step. (2) The collection of sequences is placed in a flow cell, whose surface is coated with the probes that will bind with adaptor molecules on the DNA. Inside the flow cell, the conditions and reagents are provided to use one or another form of PCR to amplify each fragment many times. (3) Then all of the amplified fragments are sequenced so that each nucleotide is "read" thousands of times, each time it is present on a sequence of any length. The details of how this happens differ for each of the automated systems, but all rely on techniques that originated in the Sanger method. (4) Finally,

the millions of sequence lengths—also called "reads"—are aligned using bioinformatics software. Putting all the reads together, the entire sequence can be deduced.

This whole process is mostly automated, using machines developed over the course of the last decade.

Recombinant DNA Technology

The primary intent of recombinant DNA technology is to deliberately remove genetic material from one organism and combine it with that of a different organism. Its origins can be traced to 1970, when microbiologists first began to duplicate the clever tricks bacteria do naturally with bits of extra DNA such as plasmids, transposons, and proviruses. As mentioned earlier, humans have been trying to artificially influence genetic transmission of traits for centuries. The discovery that bacteria can readily accept, replicate, and express foreign DNA made them powerful agents for studying the genes of other organisms in isolation. The practical applications of this work were soon realized by biotechnologists. Bacteria could be genetically engineered to mass produce substances such as hormones, enzymes, and vaccines that were difficult to synthesize by the usual industrial methods.

This process is called molecular cloning, or gene cloning. (Don't confuse it with another use of the word "clone," namely, the cloning of whole organisms.) Gene cloning involves the removal of a selected gene from an animal, plant, or microorganism (the genetic donor) followed by its propagation in a different host organism. Cloning requires that the desired donor gene first be selected, excised by restriction endonucleases, and isolated. The gene is next inserted into a **vector** (usually a plasmid or a virus) that will insert the DNA into a **cloning host**. The cloning host is usually a bacterium or a yeast that can replicate the gene and translate it into the protein product for which it codes. In the next section, we examine the elements of gene isolation, vectors, and cloning hosts and show how they participate in a complete recombinant DNA procedure.

Medical Moment

Growing Hormones

One medical use of recombinant DNA technology is for the production of human growth hormone. Growth hormone (GH), or somatotropin, is secreted by the pituitary gland and allows for cell regeneration at the ends of growing bone. Low levels of GH in children causes short stature, such as in dwarfism. It may also cause other developmental delays and hinder sexual maturation. Replacement of the hormone when a child is still in the stages of bone growth offers potential for more normal height for age.

Recombinant DNA technology has allowed for mass production of synthetic somatotropin. It is synthesized in *E. coli* and compounded into an injectable form. The synthetic form is nearly identical to the growth hormone produced by the pituitary. The patient injects the medication on a regular schedule until reaching a normal height or until skeletal growth plates have closed.

Somatotropin has many side effects, some potentially severe, and a physician must monitor the patient closely. The drug has been used for performance enhancement by some athletes, despite it being designated as a prohibited substance by many athletic governing agencies. Advanced laboratory testing now is able to differentiate natural growth hormone levels from synthetic somatotropin.

Q. Before recombinant human growth hormone was made available in 1985, growth hormone extracted from the pituitary glands of human cadavers was used to treat children with growth disorders. Can you name two

possible disadvantages to that practice?

Answer in Appendix B.

Construction of a Recombinant, Insertion into a Cloning Host, and Genetic Expression

Table 8.10 is a step-by-step guide to cloning a gene.

The New "-omics"

The ability to obtain the entire sequences of organisms has spawned new vocabulary that refers to the "total picture" of some aspect of a cell or organism.

genomics The systematic study of an organism's genes and their functions.

proteomics The study of an organism's complement of proteins (its "proteome") and functions mediated by the proteins.

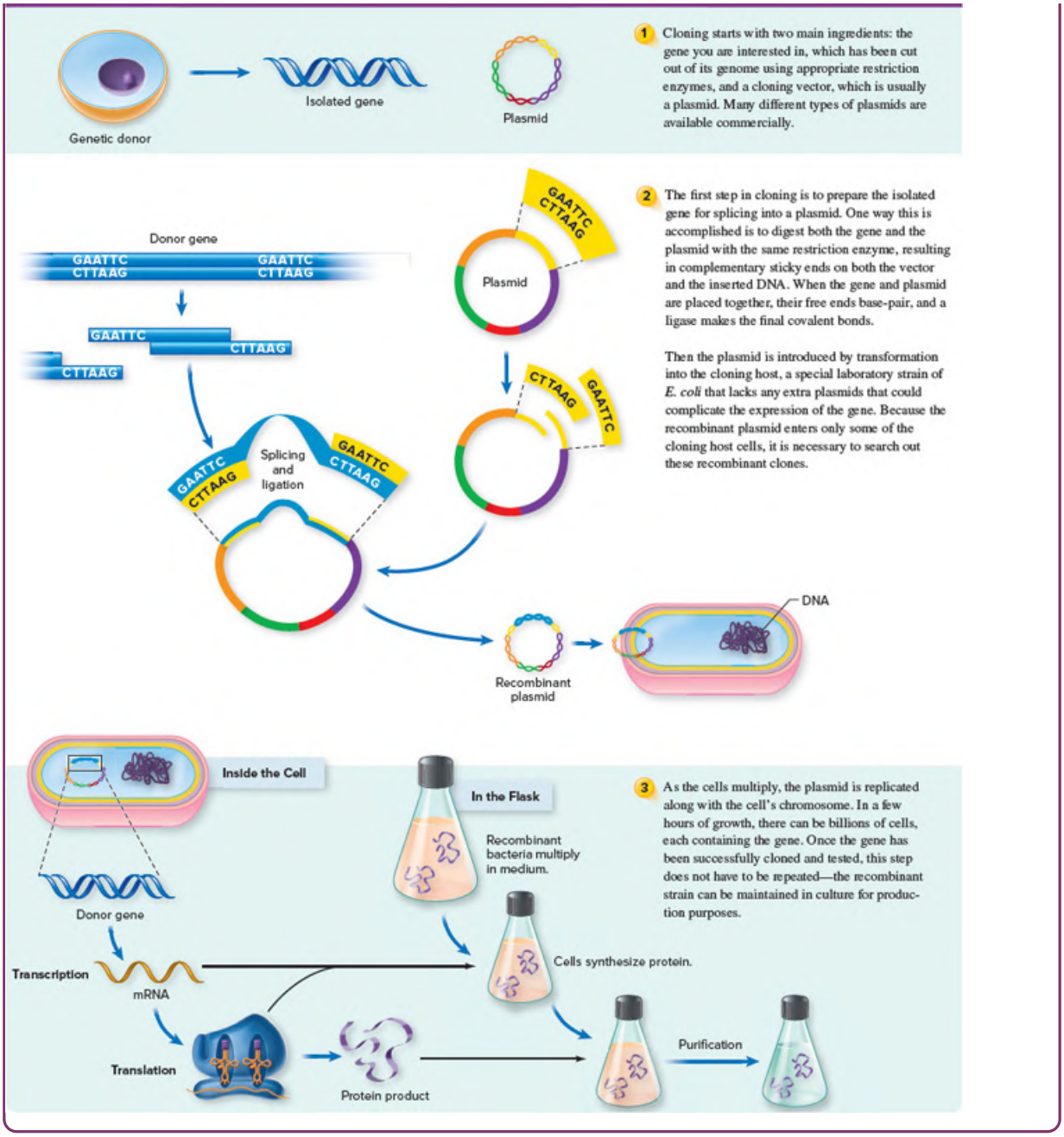
metagenomics (also called "community genomics") The study of all the genomes in a particular ecological niche, as opposed to individual genomes from single species.

metabolomics The study of the complete complement of small chemicals present in a cell at any given time. Provides a snapshot of the physiological state of the cell and the end products of its metabolism.

Synthetic Biology

In recent years, researchers have staked out entirely new territory in genetic manipulation: They are creating new biological molecules and organisms from scratch. This field is called *synthetic biology*. A pioneer in the field is one of the same men who sequenced the human genome, Craig Venter. In 2010, he successfully created a self-replicating bacterial cell from four bottles of chemicals: the four nucleotides of DNA. This was a breakthrough of major proportions, as it was the first time a living, replicating cell had been synthesized from chemicals. Synthetic biology uses engineering-type methods to assemble molecules and cells. Medical science is poised to be revolutionized when

Table 8.10 Gene Cloning



scientists can create precise chemicals to replace those missing in disease, assemble customized immune components, or construct biological molecules that can precisely target cancerous cells or pathogenic microbes. Synthetic biology also holds promise for alternative energy production and for offering new and different manufacturing processes. Of course, the ability of scientists to "create" life, in a sense, makes many people nervous. The scientific community, and those who monitor it, are engaged in intense conversations about the ethics—as well as security issues—of synthetic biology.

Using Genetic Techniques to Treat Disease

Advances in genetic manipulation have allowed the development of several new strategies to treating diseases such as cancer and other debilitating or life-threatening conditions. Two of these are **gene therapy** and **CRISPR**.

Gene therapy has been attempted since 1990, and has only recently found significant success. It involves replacing a faulty gene that is responsible for disease with a gene from a healthy organism. For example, a blood condition called beta-thalassemia comes about when a gene for beta-globulin is defective. Patients require monthly blood transfusions for survival. But in 2010 an adult was cured of the condition when gene therapy supplied a healthy copy of the gene. Other successes have followed.

There are various strategies for this therapy. In general, the normal gene is cloned in vectors such as retroviruses (mouse leukemia virus) or adenoviruses that are infectious but relatively harmless. In one technique, tissues can be removed from the patient and incubated with these genetically modified viruses to transfect them with the normal gene. The transfected cells are then reintroduced into the patient's body by transfusion **(figure 8.20)**. Alternatively, naked DNA or a virus vector is directly introduced into the patient's tissues. This is the basis of a successful immunotherapy treatment for melanoma today.

Experimentation with various types of gene therapy, or clinical testing, is performed on human volunteers with the particular genetic condition. Thousands of these trials have been and are being carried out in the United States and other countries. Most trials target cancer, single-gene defects, and infections; and most gene deliveries are carried out by virus vectors. Early therapeutic trials were hampered by

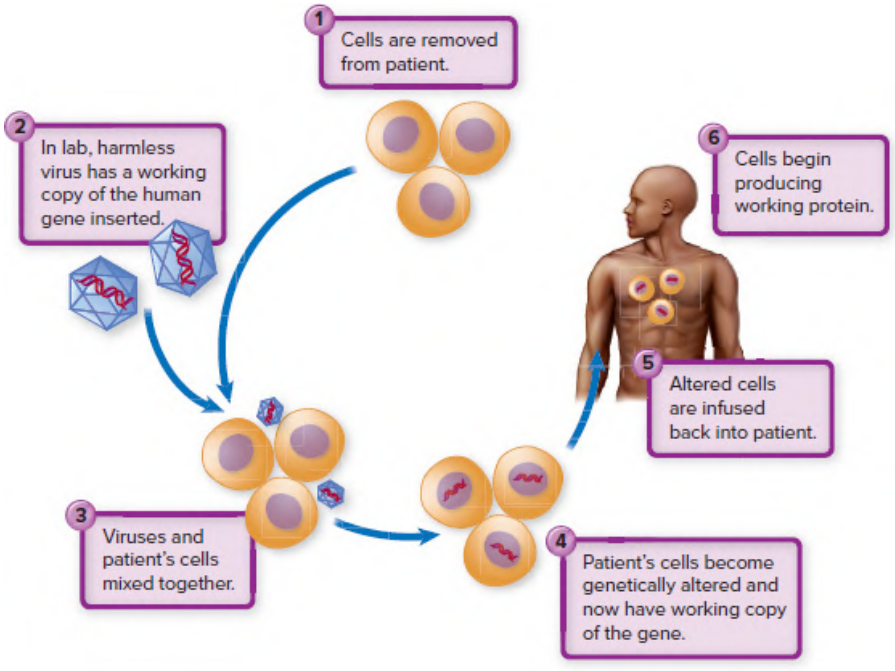


Figure 8.20 **Gene therapy example**.

several difficulties relating to effectiveness and safety. Some of the safety issues were related to the use of (seemingly safe) viruses as delivery vehicles, which then ended up causing malignancies.

The strategies described so far are called *somatic cell* gene therapy. This means that the changes are permanent in the individual who is treated, but they are not passed on to offspring. The ultimate sort of gene therapy is germline therapy, in which genes are inserted into an egg, sperm, or early embryo. In this type of therapy, the new gene will be present in all cells of the individual. The therapeutic gene is also heritable (that is, can be passed on to subsequent generations).

There is another system found in bacteria and archaea that can be exploited by scientists to alter genomes. The system is called CRISPR, which stands for *clustered regularly interspaced short palindromic repeats*. In the bacteria and archaea, these are short lengths of DNA with repeating nucleotides. After the repeats, short segments of spacer DNA are found. These turn out to be the vestiges of DNA left behind by "invading" bacteriophages or plasmids. The CRISPR areas of the genome are capable of recognizing and cutting this foreign DNA, keeping the bacterium or archaea from being invaded. It is thought to be an adaptive immune system used by bacteria. In other words, the bacteria "learn" the identity of an attacking phage by placing bits of its DNA in its own genome, and in the future can cut it up before it causes trouble.

The system turns out to be highly adaptable for laboratory use, and scientists have started using CRISPR in many genetic engineering applications. It is cheap, relatively easy to perform, and very powerful. Researchers need only design a correct **guide RNA** that targets specific gene sequences and mix it with nucleases associated with the CRISPR system, and they can cut DNA in just about any organism exactly where they want to. There are many voices within and outside of the scientific community that have ethical concerns about the CRISPR system. One reason is that off-target changes—affecting other areas of the genome—have been documented. Another concern is that the CRISPR system can be used in a process called **gene drive**. In this scenario, CRISPR is used to artificially cause an organism's offspring to accrue a particular mutation at a much accelerated rate. This might be a good thing, for instance, if CRISPR can cause mosquitoes to no longer be susceptible to the malaria protozoan. The fear is that this speeded-up evolution could have many unintended consequences.

Researcher loading a robotic liquid handling device to perform PCR (the polymerase chain reaction).
Source: CDC/Hsi Liu, Ph.D., MBA, James Gathany

8.6 LEARNING OUTCOMES—Assess Your Progress

20. Explain the importance of restriction endonucleases to genetic engineering.
21. List the steps in the polymerase chain reaction.
22. Describe how you can clone a gene into a bacterium.
23. Differentiate between DNA profiling and DNA sequencing.
24. Provide a definition of *synthetic biology*.
25. Name two genetic techniques that are designed to treat human diseases.

CASE FILE WRAP-UP

©Cathy Yeulet/123RF (health care worker); ©Steve Gschmeissner/Science Source (blood cells and fibrin)

The clotting cascade is the complex process that leads to formation of a blood clot and prevents uncontrolled bleeding. It involves numerous steps and the activation of multiple clotting factors and other cofactors. Different types of hemophilia inhibit different proteins that form clotting factors. Hemophilia A is characterized by low factor VIII clotting activity. Diagnosis is made by laboratory tests measuring factor levels.

To replace the missing clotting factor, IV infusions are administered. Depending on the severity of hemophilia, patients may require regular scheduled infusions to prevent internal bleeding. Some patients with mild hemophilia only require factor infusions when there are signs of injury or bleeding.

The administered factor VIII is produced using recombinant DNA technology. The synthetic product is nearly identical to that encoded by the human factor VIII gene. The gene coding for the factor is cloned into a plasmid vector and then animal cells in a laboratory are injected with the vector. They then produce mass quantities of the clotting protein. This recombinant factor VIII has advantages over factor products derived from human blood sources, as it cannot transmit potentially dangerous microorganisms.

The Microbiome

Host Genetics and the Microbiome

The composition of the human microbiota shows a lot of variability from person to person. Of course, we know that humans themselves show a lot of variation, which comes from their different genetic makeup. This led scientists to wonder whether the composition of the microbiota is influenced by the host's genetics.

One good way to test this is to look at two different types of pairs of people: monozygotic (identical) twins and dizygotic (fraternal) twins. Fraternal twins do not share the same genes, but identical twins do. If the microbiomes of identical twins were

significantly more similar than the microbiomes of fraternal twins, it would suggest that the human genome influences what microbiome the person acquires.

To ask this question the way scientists do, you would construct a hypothesis: The degree of difference between the microbiota of fraternal twins will be no greater than the degree of difference between the microbiota of identical twins. (This is written as a null hypothesis, meaning it is a statement that there will be no difference between two groups.) Then you would set up an experiment to test the hypothesis, using a large number of pairs of both types of twins. In this study, 416 pairs of twins were examined.

In this study, the identical twins turned out to have more similar microbiomes than the fraternal twins. They had what they called "a hub of heritable taxa," chief among them a newly discovered bacterial group named *Christensenellaceae*.

So the hypothesis was disproven; there was a significant difference between the two groups. The paper's authors suggest that a person's microbiome is heritable, like, having blue eyes. Only here it is a bit more indirect—a person's genotype is heritable, which determines his or her phenotype, which may determine his or her microbiome.

There is a saying in science, "Chance favors the prepared mind." In the case of this study, the scientists found something extra they were not counting on: The presence of *Christensenellaceae* was associated with low body mass index (BMI). Because this was just an association and the study could not prove causation, they did another experiment in which they deliberately exposed mice to *Christensenellaceae*. Those mice had reduced weight gain compared to mice not fed *Christensenellaceae*. So the studies continue. This is what many scientists love about their jobs: discovering surprises, and finding answers to questions that practically ask themselves!

Source: *Cell Host and Microbe*, vol. 19, pgs. 731–743, 2016.

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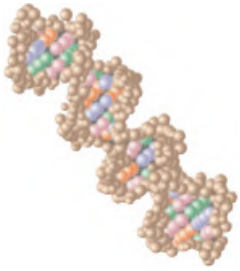
Chapter Summary

8.1 Introduction to Genetics and Genes

- Nucleic acids contain the blueprints of life in the form of genes. DNA is the blueprint molecule for all cellular organisms. The blueprints of viruses can be either DNA or RNA.
- The total amount of DNA in an organism is termed its genome (also genotype). Not all genes are expressed all the time; the ones that are expressed determine an organism's phenotype.
- Bacterial DNA consists of a few thousand genes in one circular chromosome. Eukaryotic genomes range from thousands to tens of thousands of genes.
- DNA copies itself just before cellular division by the process of semiconservative replication. Semiconservative replication means that each "old" DNA strand is the template upon which each "new" strand is synthesized.
- The circular bacterial chromosome is replicated at two forks as directed by DNA polymerase III. At each fork, two new strands are synthesized—one continuously and one in short fragments called Okazaki fragments.



8.2 Transcription and Translation

- Information in DNA is converted to proteins by the processes of transcription and translation.
- DNA also contains a great number of non-protein-coding sequences. These sequences are often transcribed into RNA that serves to regulate cell function.
- Eukaryotes transcribe DNA in the nucleus, remove its introns, and then translate it in the cytoplasm. Bacteria transcribe and translate simultaneously because the DNA is not sequestered in a nucleus and the bacterial DNA is free of introns.

8.3 Genetic Regulation of Protein Synthesis

- Operons are collections of genes in bacteria that code for products with a coordinated function.
- Nutrients can combine with regulator gene products to turn a set of structural genes on (inducible genes) or off (repressible genes). The *lac* (lactose) operon is an example of an inducible operon.

8.4 DNA Recombination Events

- Genetic recombination occurs in eukaryotes through sexual reproduction and through horizontal gene transfer.
- In bacteria, recombination occurs only through horizontal gene transfer.
- The three main types of horizontal gene transfer in bacteria are transformation, conjugation, and transduction.
- Transposons are genes that can relocate from one part of the genome to another, causing rearrangement of genetic material.

8.5 Mutations: Changes in the Genetic Code

- Changes in the genetic code can occur by two means: mutation and recombination.
- Mutations are changes in the nucleotide sequence of the organism's genome.
- Mutations can be either spontaneous or induced by exposure to some external mutagenic agent.
- Single-nucleotide polymorphisms are single base mutations that can have big effects on an organism.

- All cells have enzymes that repair damaged DNA. When the degree of damage exceeds the ability of the enzymes to make repairs, mutations occur.

8.6 Studying DNA in the Laboratory and Genetic Engineering

- Scientists use a set of tools and techniques that allows them to study and also manipulate the genetic content of organisms.
- Two basic tools that make modern genetic science possible are restriction enzymes and PCR, the polymerase chain reaction.
- The PCR technique amplifies small amounts of DNA into much larger quantities for further analysis.
- DNA is analyzed in many ways; two important ones are DNA profiling and DNA sequencing.
- Cloning is the process by which genes are removed from the original host and duplicated for transfer into a cloning host by means of cloning vectors.
- Synthetic biology uses an engineering approach to biology to create genomes from scratch.
- Genetic advances have led to two new approaches to treating human disease: gene therapy and a strategy known as CRISPR.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. Single nucleotide polymorphisms are found in **a**. DNA. **b**. RNA. **c**. plasmids. **d**. siRNA.	**2**. Using your knowledge of DNA from this chapter, imagine two different ways antibiotic resistance may develop in a bacterium.	**3**. Conduct research on CRISPR and explain in layperson's terms why there are ethical concerns around its use in genetic engineering.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Cell Structure and Function	**4**. Which of the following is a characteristic of RNA? **a**. RNA is double-stranded. **b**. RNA contains thymine, which pairs with adenine. **c**. RNA contains deoxyribose. **d**. RNA molecules are necessary for translation and gene regulation.	**5**. List some advantages and disadvantages to a cell having DNA in the cytoplasm as opposed to in a nucleus.	**6**. Construct an argument for why tRNA contains a lot of secondary structure but mRNA does not.
Metabolic Pathways	**7**. The lac operon is usually in the _____ position and is activated by a/an _____ molecule. **a**. on; repressor **b**. off; inducer **c**. on; inducer **d**. off; repressor	**8**. Discuss the intersection between the "metabolome" and phenotype.	**9**. Defend this statement: *All of biology is dependent on binding reactions*.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Information Flow and Genetics	**10**. DNA is semiconservative because the _____ strand will become half of the _____ molecule. **a**. RNA; DNA **b**. template; finished **c**. sense; mRNA **d**. codon; anticodon	**11**. Examine the DNA triplets here and determine the amino acid sequence they code for. Then provide a different DNA sequence that will produce the same protein. TAC CAG ATA CAC TCC CCT	**12**. Using the same piece of DNA in question 11 (TAC CAG ATA CAC TCC CCT) create the following: (i) a frameshift mutation; (ii) a silent mutation; and (iii) a nonsense mutation.
Microbial Systems	**13**. Which of the following is not a mechanism of horizontal gene transfer? **a**. spontaneous mutations **b**. transformation **c**. transduction **d**. conjugation	**14**. Why is it advisable to use reverse transcriptase to create DNA from an mRNA transcript when preparing eukaryotic genes for use in bacterial cells? Why not use the eukaryotic DNA directly?	**15**. Metagenomics is providing insight into the possible causes of some difficult to understand diseases, such as irritable bowel syndrome. Develop a possible explanation for why metagenomic approaches might be better than traditional diagnostic approaches.

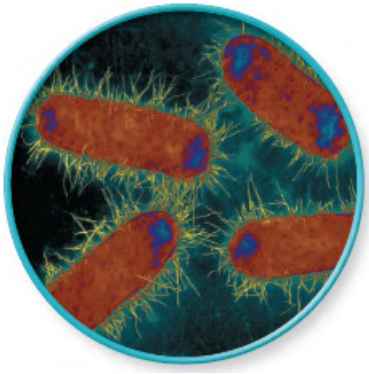
Impact of Microorganisms	**16.** The creation of biological molecules and cells entirely from chemicals is called **a.** recombination. **b.** sequencing. **c.** artificial biology. **d.** synthetic biology.	**17.** You are a public health official trying to determine if 20 different people in your city were sickened by the same microbe, or if they were unrelated. Which technique from this chapter would you use, and why?	**18.** Genetically modified organisms (GMOs)—especially in foods—are considered by popular culture to be “bad.” Construct a counterargument.
Scientific Thinking	**19.** How many cycles of PCR would it take to create 16 copies of a DNA molecule? **a.** two **b.** three **c.** four **d.** eight	**20.** Construct an analogy using your clothes closet to describe the difference between genotype and phenotype.	**21.** What experiment could you devise to determine if a particular phage engaged in general or specialized transduction?

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From chapter 3, figure 3.10*a***. Speculate on why these cells contain two chromosomes (shown in blue).



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connect® Resources are available through Connect, including interactive learning tools, animations, and more.

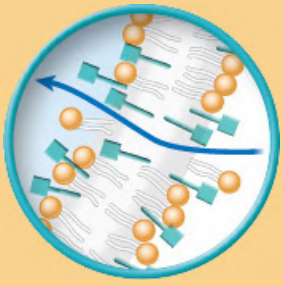
Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

9

Physical and Chemical Control of Microbes

©ERproductions Ltd/Blend Images LLC (forceps); ©shapecharge/E+/Getty Images (health care worker); ©McGraw-Hill Education/Charles D. Winters, photographer (boiling water); ©Kathleen Talaro (heavy metals)

IN THIS CHAPTER…



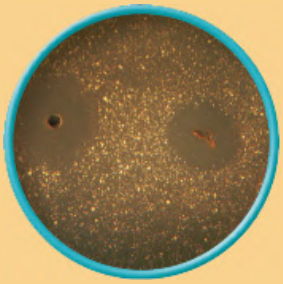
9.1 Controlling Microorganisms

1. Clearly define the terms *sterilization, disinfection, decontamination, sanitization, antisepsis*, and *degermation*.

2. Identify the microorganisms that are most resistant and least resistant to control measures.
3. Compare the action of microbicidal and microbistatic agents.
4. Name four categories of cellular targets for physical and chemical agents.

9.2 Methods of Physical Control

5. Name six methods of physical control of microorganisms.
6. Discuss both moist and dry heat methods, and identify multiple examples of each.
7. Define *thermal death time* and *thermal death point*.
8. Explain methods of moist heat control.
9. Explain two methods of dry heat control.
10. Identify advantages and disadvantages of cold and desiccation.
11. Differentiate between the two types of radiation control methods.
12. Explain how filtration and osmotic pressure function as control methods.



9.3 Chemical Agents in Microbial Control

13. Name the desirable characteristics of chemical control agents.
14. Discuss chlorine and iodine and their uses.
15. List advantages and disadvantages to phenolic compounds.
16. Explain the mode of action of chlorhexidine.
17. Explain the applications of oxidizing agents.
18. Identify some heavy metal control agents.
19. Discuss the disadvantages of aldehyde agents.
20. Identify applications for ethylene oxide sterilization.

CASE FILE

Control of Microorganisms in the Operating Room

As a nurse in the operating room, I assist with all phases of surgical procedures. Before the surgery begins, I prepare the operation suite. During the procedure, I assist the surgeons by ensuring they have the supplies needed. Following the procedure, I prepare surgical supplies to be appropriately cleaned or disposed.

One day, I was the surgical nurse for a kidney transplant. The patient was a 55-year-old male with chronic kidney disease who was receiving a donor organ from a relative. Prior to the surgery, I met with the family and explained my role in the procedure. I ensured that their questions were answered and appropriate consent was obtained.

Prior to the procedure, I prepared the operating room with the supplies necessary for surgery. First, I cleaned large metal carts with a bleach solution. I spread large plastic sterile drapes over the carts, ensuring that I did not touch or contaminate them. I opened the packages containing trays of sterilized equipment and, using proper technique, transferred them to the tables. For this procedure, even ice made from sterile water was prepared.

When the patient was in the operating room and we were ready to begin the procedure, I donned surgical scrubs, a mask, hair covering, and shoe covers. I then scrubbed my hands, arms, and elbows with antimicrobial soap and water, followed by alcohol-based hand cleaner, and finally a scrub brush with antiseptic. Keeping my hands raised in the air, I was assisted by another nurse in putting on a sterile gown and gloves. I then used an iodine-impregnated sponge to scrub the patient's surgical site.

During the procedure, my role was to be the surgical assisting nurse. As my personal protective equipment was sterile, I only touched supplies that were on the sterile field and table. Throughout the case, I handed the necessary supplies to the surgeons and kept the equipment organized and counted. If supplies were needed that were not on the operating table, a circulating nurse obtained them and opened the package onto the sterile field in a

manner that prevented contamination of the contents. I was then able to handle the supplies and give them to the surgeons as they were needed.

- Why is intraoperative sterile technique important?
- How is equipment sterilized to be reused between cases?

Case File Wrap-Up appears at the end of the chapter.

NCLEX® PREP

1. An RN on a medical surgical unit would expect to perform all of the following antimicrobial control methods during her care of patients, except:
   a. *sterilization*.
   b. *antisepsis*.
   c. *disinfection*.
   d. *sanitization*.

9.1 Controlling Microorganisms

Much of the time in the developed world, we take for granted tap water that is drinkable, food that is not spoiled, shelves that are full of products to eradicate "germs," and drugs that are always available to treat infections. Controlling our degree of exposure to potentially harmful microbes is a monumental concern in our lives. A prime example of this occurred when letters containing endospores of *Bacillus anthracis* (anthrax) were delivered and opened in the Hart Office Building of the U.S. Senate in 2001. The EPA (Environmental Protection Agency) had to determine a method of removing all traces of a lethal, highly infectious, endospore-forming bacterium from an enormous space, which presented a huge challenge. Although this example is extreme, controlling our exposure to harmful microbes is always an ongoing concern. The ancient Greeks learned to burn corpses and clothing during epidemics; the Egyptians embalmed the bodies of their dead, using strong salts and pungent oils. These methods may seem rather archaic by modern measures, but these examples illustrate that controlling microbes has been a concern for several centuries.

The methods of microbial control used outside of the body are designed to result in four possible outcomes:

1. sterilization,
2. disinfection,
3. decontamination (also called sanitization), or
4. antisepsis (also called degermation).

These terms are differentiated in **table 9.1**. While it may seem clumsy to have more than one word for some of these processes, it is important that you recognize them when you hear them used so we include them here. To complicate matters, the everyday use of some of these terms

can at times be vague and inexact. For example, occasionally one may be directed to "sterilize" or "disinfect" a patient's skin, even though this usage does not fit the technical definition of either term. We also provide a flowchart **(figure 9.1)** to summarize the major applications and aims in microbial control.

©Photographee.eu/Shutterstock

Table 9.1 Concepts in Antimicrobial Control

Techniques and chemicals that are capable of sterilizing are highlighted with a pink background.

Term	Definition	Key Points	Examples of Agents
Sterilization	Process that destroys or removes all viable microorganisms (including viruses)	The term *sterile* should be used only in the strictest sense to refer to materials that have been subjected to the process of sterilization (there is no such thing as slightly sterile). Generally reserved for inanimate objects as it would be impractical or dangerous to sterilize parts of the human body Common uses: surgical instruments, syringes, commercially packaged food	Heat (autoclave) Sterilants (chemical agents capable of destroying endospores)
Disinfection	Physical process or a chemical agent to destroy vegetative pathogens but not bacterial endospores Removes harmful products of microorganisms (toxins) from material	Normally used on inanimate objects because the concentration of disinfectants required to be effective is harmful to human tissue Common uses: boiling food utensils, applying 5% bleach solution to an examining table, immersing thermometers in an iodine solution between uses	Bleach Iodine Heat (boiling)
Decontamination/Sanitization	Cleansing technique that mechanically removes microorganisms as well as other debris to reduce contamination to safe levels	Important to restaurants, dairies, breweries, and other commercial entities that handle large numbers of soiled utensils/containers Common uses: Cooking utensils, dishes, bottles, and cans must be sanitized for reuse.	Soaps Detergents Commercial dishwashers

Term	Definition	Key Points	Examples of Agents
Antisepsis/Degermation	Reduces the number of microbes on the human skin A form of decontamination but on living tissues	Involves scrubbing the skin (mechanical friction) or immersing it in chemicals (or both)	Alcohol Surgical hand scrubs

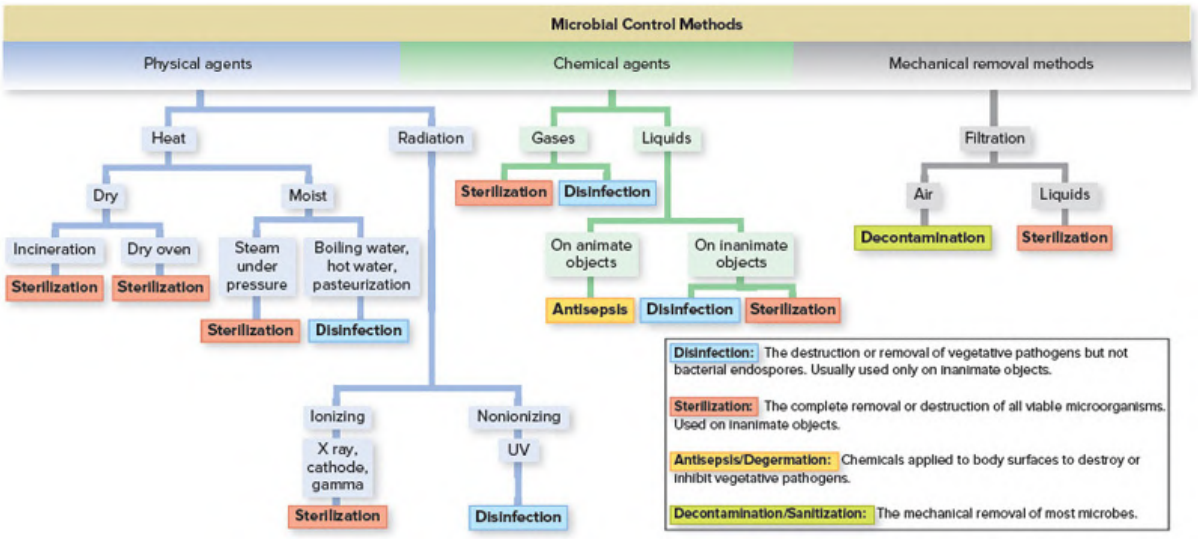


Figure 9.1 **Microbial control methods**.

Note: In all of the tables in this chapter, agents that are capable of *sterilizing* will feature a red/pink background.

Relative Resistance of Microbial Forms

The primary targets of microbial control are microorganisms capable of causing infection or spoilage that are present in the external environment and on the human body. This targeted population is rarely simple or uniform; in fact, it often contains mixtures of microbes with extreme differences in resistance and harmfulness. **Figure 9.2** compares the general resistance these forms have to physical and chemical methods of control.

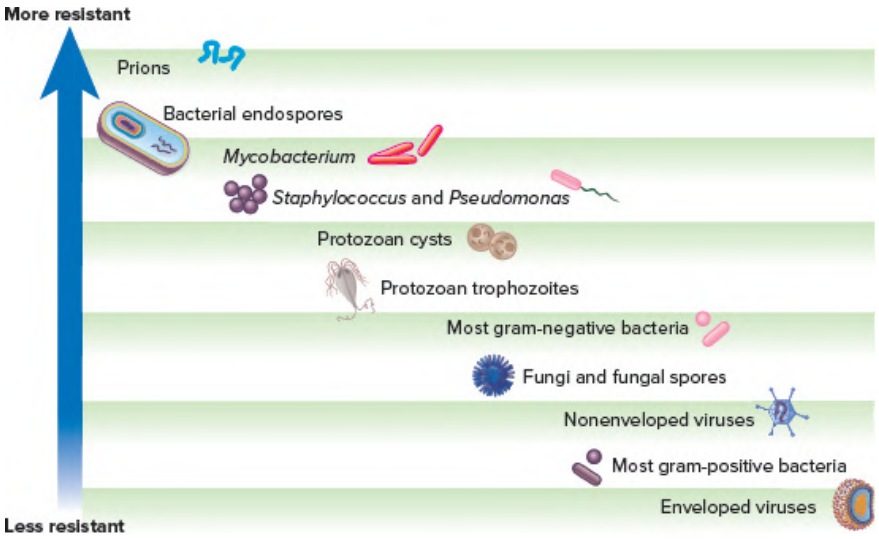


Figure 9.2 **Relative resistance of different microbial types to microbial control agents**. This is a very general hierarchy; different control agents are more or less effective against the various microbes.

A Note About Prions

Prions are in a class of their own when it comes to "sterilization" procedures. This chapter defines "sterile" as the absence of all viable microbial life—but none of the procedures described in this chapter are necessarily sufficient to destroy prions. Prions are extraordinarily resistant to heat and chemicals. If instruments or other objects become contaminated with these unique agents, they must either be discarded as biohazards or, if this is not possible, a combination of chemicals and heat must be applied in accordance with the Centers for Disease Control and Prevention (CDC) guidelines. The guidelines themselves are constantly evolving as new information becomes available. In the meantime, this chapter discusses sterilization using bacterial endospores as the toughest form of microbial life. When tissues, fluids, or instruments are suspected of containing prions, consultation with infection control experts and/or the CDC is recommended when determining effective sterilization conditions. Chapter 17 describes prions in detail.

Table 9.2 Comparative Resistance of Bacterial Endospores and Vegetative Cells to Control Agents

Method	Required to Destroy	Endospores Required to Destroy Vegetative Forms	Endospores Are ____× More Resistant
Heat (moist)	120°C	80°C	1.5
Radiation (X-ray) dosage	4,000 Grays	1,000 Grays	4
Sterilizing gas (ethylene oxide)	1,200 mg/L	700 mg/L	1.7
Sporicidal liquid (2% glutaraldehyde)	3 h	10 min	18

Actual comparative figures on the requirements for destroying various groups of microorganisms are shown in **table 9.2**. Bacterial endospores have traditionally been considered the most resistant microbial entities, being as much as 18 times harder to destroy than their counterpart vegetative cells. Because of their resistance to microbial control, their destruction is

the goal of *sterilization* because any process that kills endospores will invariably kill all less resistant microbial forms. (Prions are a special case; see note in the margin.) Other methods of control (disinfection, antisepsis) act primarily upon microbes that are less hardy than endospores.

Agents Versus Processes

The terms *sterilization, disinfection*, and so on refer to processes. You will encounter other terms that describe the agents used in the process. Two examples of these are the terms *bactericidal* and *bacteristatic*. The root *-cide*, meaning "having the capacity to kill," can be combined with other terms to define an antimicrobial agent aimed at destroying a certain group of microorganisms. For example, a **bactericide** is a chemical that destroys bacteria except for those in the endospore stage. It may or may not be effective on other microbial groups. A *fungicide* is a chemical that can kill fungal spores, hyphae, and yeasts. A *virucide* is any chemical known to inactivate viruses, especially on living tissue. A *sporicide* is an agent capable of destroying bacterial endospores. **Germicide** and **microbicide** are additional terms for chemical agents that kill microorganisms.

Sepsis is defined as the growth of microorganisms in the blood and other tissues. The term **asepsis** refers to any practice that prevents the entry of infectious agents into sterile tissues and thus prevents infection. Aseptic techniques commonly practiced in health care range from sterile methods that exclude all microbes to *antisepsis*. In antisepsis, chemical agents called **antiseptics** are applied directly to exposed body surfaces (skin and mucous membranes), wounds, and surgical incisions to destroy or inhibit pathogens. Examples of antisepsis include preparing the skin before surgical incisions with iodine compounds, swabbing an open root canal with hydrogen peroxide, and ordinary hand washing with a germicidal soap.

The Greek words *stasis* and *static* mean "to stand still." They can be used in combination with various prefixes to indicate a state in which microbes are

temporarily prevented from multiplying but are not killed outright. Although killing or permanently inactivating microorganisms is the usual goal of microbial control, microbistasis does have meaningful applications. **Bacteristatic** agents prevent the growth of bacteria on tissues or on objects in the environment, and *fungistatic* chemicals inhibit fungal growth. Materials used to control microorganisms in the body (antiseptics and drugs) often have **microbistatic** effects because many microbicidal compounds can be highly toxic to human cells. Note that even a *-cidal* agent doesn't necessarily result in sterilization, depending on how it is used.

Crops, like these soybeans, are grown using fungicidal chemicals.
©Doug Sherman/Geofile

Practical Matters in Microbial Control

Numerous factors should be taken into consideration when selecting a workable method of microbial control. One useful framework for determining how devices that come in contact with patients should be handled is whether they are considered *critical, semicritical*, or *noncritical*. *Critical* medical devices are those that are expected to come into contact with sterile tissues. A good example of this would be a syringe needle or an artificial hip. These must be sterilized before use. *Semicritical* devices are those that come into contact with mucosal membranes. An endoscopy tube is an example. These must receive at least high-level disinfection and preferably

should be sterilized. *Noncritical* items are those that do not touch the patient or are only expected to touch intact skin, such as blood pressure cuffs or crutches. They require only low-level disinfection unless they become contaminated with blood or body fluids.

A remarkable variety of substances can require sterilization. They range from durable solids such as rubber to sensitive liquids such as serum. Hundreds of situations requiring sterilization confront the network of persons involved in health care, whether technician, nurse, doctor, or manufacturer, and no universal method works well in every case.

Considerations such as cost, effectiveness, and method of disposal are all important. For example, disposable plastic items such as catheters and syringes that are used in invasive medical procedures have the potential for infecting the tissues. These must be sterilized during manufacture by a nonheating method (gas or radiation), because heat can damage plastics. After these items have been used, it is often necessary to destroy or decontaminate them before they are discarded because of the potential risk to the handler. Steam sterilization, which is quick and sure, is a sensible choice at this point, because it does not matter if the plastic is destroyed.

What Is Microbial Death?

Death is a phenomenon that involves the permanent termination of an organism's vital processes. Signs of life in complex organisms such as animals are self-evident, and death is made clear by loss of nervous function, respiration, or heartbeat. In contrast, death in microscopic organisms that are composed of just one or a few cells is often hard to detect, because they reveal no conspicuous vital signs to begin with.

Usually the most practical way to detect this damage is to determine if a microbial cell can still reproduce when exposed to a suitable environment. If the microbe has sustained metabolic or structural damage to such an extent that it can no longer reproduce, even under ideal environmental conditions, then it is no longer viable. The permanent loss of reproductive capability, even under optimum growth conditions, has become the accepted microbiological definition of death.

Factors Affecting Death Rate

The individual cells of a culture show marked variation in susceptibility to a given microbicidal agent. Death of the whole population is not instantaneous but begins when a certain threshold of microbicidal agent (some combination of time and concentration) is met. Death continues in a logarithmic manner as the time or concentration of the agent is increased.

Because many microbicidal agents target the cell's metabolic processes, active cells (younger, rapidly dividing) tend to die more quickly than those that are less metabolically active (older, inactive). Eventually, a point is reached at which survival of any cells is highly unlikely; this point is equivalent to sterilization.

The effectiveness of a particular agent is governed by several factors besides time. These additional factors influence the action of antimicrobial agents:

1. The number of microorganisms. A higher load of contaminants requires more time to destroy.

2. The nature of the microorganisms in the population. In most actual circumstances of disinfection and sterilization, the target population is not a single species of microbe but a mixture of bacteria, fungi, endospores, and viruses, presenting a broad spectrum of microbial resistance.
3. The temperature and pH of the environment.
4. The concentration (dosage, intensity) of the agent. For example, ultraviolet (UV) radiation is most effective at 260 nanometers (nm), and most disinfectants are more active at higher concentrations.
5. The mode of action of the agent. How does it kill or inhibit the microorganism?
6. The presence of solvents, interfering organic matter, and inhibitors. Saliva, blood, and feces can inhibit the actions of disinfectants and even of heat.

The influence of these factors is discussed in greater detail in subsequent sections.

Modes of Action of Antimicrobial Agents

An antimicrobial agent's adverse effect on cells is known as its *mode* (or *mechanism*) *of action*. Agents affect one or more cellular targets, inflicting damage progressively until the cell is no longer able to survive. Antimicrobials have a range of cellular targets, with the agents that are least selective in their targeting tending to be effective against the widest range of microbes (examples include heat and radiation). More selective agents (drugs, for example) tend to target only a single cellular component and are much more restricted as to the microbes they are effective against.

The cellular targets of physical and chemical agents fall into four general categories:

1. the cell wall,
2. the cytoplasmic membrane,
3. cellular synthetic processes (DNA, RNA), and
4. proteins.

Table 9.3 depicts the effects of various agents on cellular structures and processes. For example, **figure 9.3** illustrates what happens to a membrane when it is exposed to surfactants.

Table 9.3 Actions of Various Physical and Chemical Agents upon the Cell

Cellular Target	Effects of Agents	Examples of Agents Used
Cell wall	Chemical agents can damage the cell wall by • blocking its synthesis, or • digesting it	Chemicals Detergents Alcohol
Cytoplasmic membrane	Agents physically bind to lipid layer of the cytoplasmic membrane. This opens up the cytoplasmic membrane and allows damaging chemicals to enter the cell and important ions to exit the cell.	Detergents
Cellular synthesis	Agents can interrupt the synthesis of proteins via the ribosomes, inhibiting proteins needed for growth and metabolism and preventing multiplication. Agents can change genetic codes (mutation).	Formaldehyde Radiation Ethylene oxide

Cellular Target	Effects of Agents	Examples of Agents Used
Proteins	Some agents are capable of denaturing proteins (breaking of protein bonds, which results in breakdown of the protein structure). Agents may attach to the active site of a protein, preventing it from interacting with its chemical substrate.	Moist heat Alcohol Phenolics

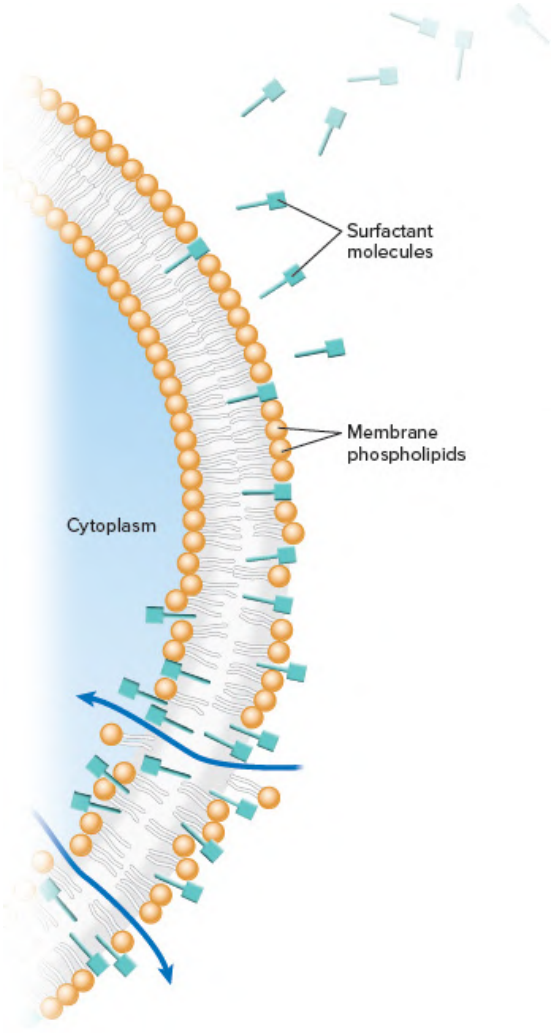


Figure 9.3 **Mode of action of surfactants on the cytoplasmic membrane**. Surfactants inserting in the lipid bilayer disrupt it and create abnormal channels that alter permeability and cause leakage both into and out of the cell.

9.1 LEARNING OUTCOMES—Assess Your Progress

1. Clearly define the terms *sterilization, disinfection, decontamination, sanitization, antisepsis*, and *degermation*.
2. Identify the microorganisms that are most resistant and least resistant to control measures.
3. Compare the action of microbicidal and microbistatic agents.
4. Name four categories of cellular targets for physical and chemical agents.

©Bruce Chambers, The Orange County Register/Newscom

9.2 Methods of Physical Control

We can divide our methods of controlling microorganisms into two broad categories: physical and chemical. We'll start with physical methods. Microorganisms have adapted to the tremendous diversity of habitats the earth provides, even severe conditions of temperature, moisture, pressure, and light. For microbes that normally withstand such extreme physical conditions, our attempts at control would probably have little effect. Fortunately for us, we are most interested in controlling microbes that flourish in the same environment in which humans live. The vast majority of these microbes are readily controlled by abrupt changes in their environment. Most prominent among antimicrobial physical agents is heat. Other less widely used agents include radiation, filtration, ultrasonic waves, and even cold. The following sections examine some of these methods and explore their practical applications in medicine, commerce, and the home.

Heat

As a rule, elevated temperatures (exceeding the maximum growth temperature) are microbicidal, whereas lower temperatures (below the minimum growth temperature) are microbistatic. We'll start with heat. Heat can be applied in either moist or dry forms. *Moist heat* occurs in the form of hot water, boiling water, or steam (vaporized water). In practice, the temperature of moist heat usually ranges from 60°C to 135°C. As we shall see, the temperature of steam can be regulated by adjusting its pressure in a closed container. *Dry heat* refers to hot air (such as in an oven) or an open flame. In practice, the temperature of dry heat ranges from 160°C to several thousand degrees Celsius.

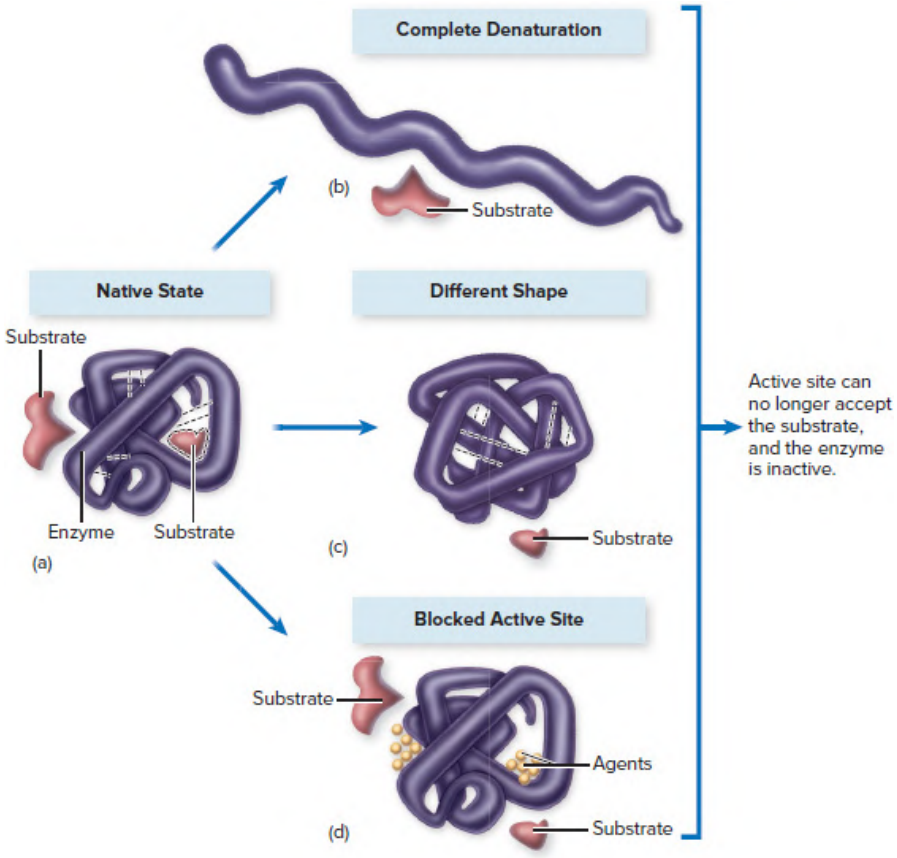


Figure 9.4 **The action of heat and chemicals on proteins.** **(a)** The native (functional) state is maintained by bonds that create active sites to fit the substrate. Heat and agents denature the protein by breaking all or some secondary and tertiary bonds. Results are **(b)** complete unfolding or **(c)** random bonding and incorrect folding. **(d)** Some agents react with functional groups on the active site and interfere with bonding.

Mode of Action and Relative Effectiveness of Heat

Moist heat and dry heat differ in their modes of action as well as in their efficiency. Moist heat can achieve the same effectiveness as dry heat but with lower temperatures and shorter exposure times **(table 9.4)**. Although many cellular structures are damaged by moist heat, its most lethal effect is the coagulation and denaturation of proteins, which quickly and permanently halts cellular metabolism.

Dry heat dehydrates the cell, removing the water necessary for metabolic reactions, and it also denatures proteins **(figure 9.4)**. However, the lack of water actually increases the stability of some protein conformations, necessitating the use of higher temperatures when dry heat is employed as a method of microbial control. At very high temperatures, dry heat oxidizes cells, burning them to ashes. This method is the one used in the laboratory when a loop is flamed or in industry when medical waste is incinerated.

Table 9.4 Comparison of Times and Temperatures to Achieve Sterilization with Moist and Dry Heat

	Temperature (°C)	Time to Sterilize (Min)
Moist heat	121	15
	125	10
	134	3
Dry heat	121	600
	140	180
	160	120
	170	60

Pasteurization increases the shelf life of dairy products.
©Fuse/Getty Images

Heat Resistance and Thermal Death: Endospores and Vegetative Cells

Bacterial endospores exhibit the greatest resistance and the vegetative forms of bacteria and fungi are the least resistant to both moist and dry heat. Destruction of endospores usually requires temperatures above boiling.

Vegetative cells also vary in their sensitivity to heat. Among bacteria, the death times with moist heat range from 50°C for 3 minutes (*Neisseria gonorrhoeae*) to 60°C for 60 minutes (*Staphylococcus aureus*). It is worth noting that vegetative cells of endospore formers are just as susceptible as vegetative cells of non-endospore-formers, and that pathogens are neither more nor less susceptible than nonpathogens. Other microbes, including fungi, protozoa, and worms, are rather similar in their sensitivity to heat. Viruses are surprisingly resistant to heat, with a tolerance range extending from 55°C for 2 to 5 minutes (adenoviruses) to 60°C for 600 minutes (hepatitis A virus).

Susceptibility of Microbes to Heat: Thermal Death Measurements

A combination of the two variables, heat and time, constitutes the **thermal death time**, or **TDT**, defined as the shortest length of time required to kill all test microbes at a specified temperature. The TDT has been experimentally determined for the microbial species that are common or important contaminants in various heat-treated materials. Another way to compare the susceptibility of microbes to heat is the **thermal death point (TDP)**, defined as the lowest temperature required to kill all microbes in a sample in 10 minutes.

Many perishable substances are processed with moist heat. Some of these products are intended to remain on the shelf at room temperature for several months or even years. The chosen heat treatment must render the product free of agents of spoilage or disease. At the same time, the quality of the product and the speed and cost of processing must be considered. For example, in the commercial preparation of canned green beans, one of the manufacturer's greatest concerns is to prevent growth of the agent of botulism. From several possible TDTs (i.e., combinations of time and temperature) for *Clostridium botulinum* endospores, the factory must choose one that kills all endospores but does not turn the beans to mush. Out of these many considerations emerges an optimal TDT for a given processing method. Commercial canneries heat low-acid foods at 121°C for 30 minutes, a treatment that sterilizes these foods. Because of such strict controls in canneries, cases of botulism due to commercially canned foods are rare.

Moist Heat Control Methods

The three ways that moist heat is employed to control microbes are described in **table 9.5**.

©Kevin McNeal/Getty Images

Table 9.5 Moist Heat Methods

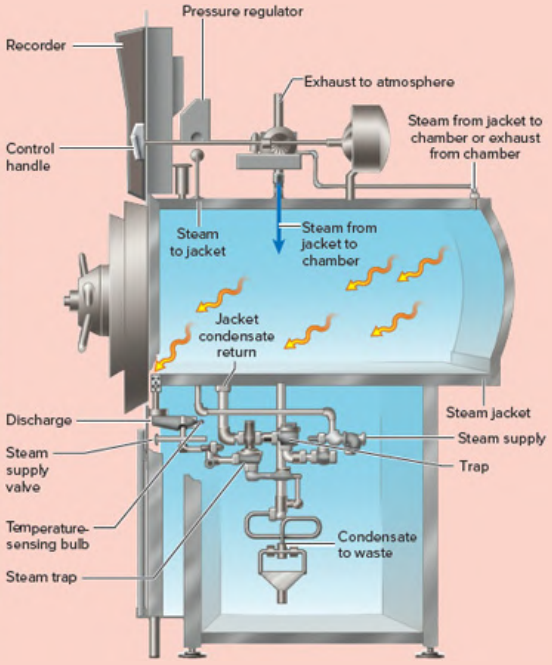
Techniques and chemicals that are capable of sterilizing are highlighted with a pink background.

Method	Applications
Boiling Water: Disinfection A simple boiling water bath can quickly decontaminate items in the clinic and home. Because a single processing at 100°C will not kill all resistant cells, this method can be relied on only for disinfection and not for sterilization. Exposing materials to boiling water for 30 minutes will kill most non-endospore-forming pathogens, including resistant species such as the tubercle bacillus and staphylo cocci. Probably the greatest disadvantage with this method is that the items can be easily recontaminated when removed from the water. ©McGraw-Hill Education/Charles D. Winters, photographer	Useful in the home for disinfection of water, materials for babies, food and utensils, bedding, and clothing from the sickroom

Method	Applications
Pasteurization: Disinfection of Beverages Fresh beverages such as milk, fruit juices, beer, and wine are easily contaminated during collection and processing. Because microbes have the potential for spoiling these foods or causing illness, heat is frequently used to reduce the microbial load and destroy pathogens. **Pasteurization** is a technique in which heat is applied to liquids to kill potential agents of infection and spoilage, while at the same time retaining the liquid's flavor and nutritional value. Ordinary pasteurization techniques require special heat exchangers that expose the liquid to 71.6°C for 15 seconds (flash method) or to 63°C to 66°C for 30 minutes (batch method). The first method is preferable because it is less likely to change flavor and nutrient content, and it is more effective against certain resistant pathogens such as *Coxiella* and *Mycobacterium*. Although these treatments inactivate most viruses and destroy the vegetative stages of 97% to 99% of bacteria and fungi, they do not kill endospores or particularly heat-resistant microbes. Milk is not sterile after regular pasteurization. In fact, it can contain 20,000 microbes per milliliter or more, which explains why even an unopened carton of milk will eventually spoil. (Newer techniques can also produce *sterile milk* that has a storage life of 3 months. This milk is processed with ultrahigh temperature [UHT]—134°C—for 1 to 2 seconds.) This is not generally considered pasteurization, so we don't consider pasteurization a sterilization method. ©James King-Holmes/Science Source	Milk, wine, beer, other beverages ©John A. Rizzo/Getty Images

Method	Applications
Steam Under Pressure: Autoclaving At sea level, normal atmospheric pressure is 15 pounds per square inch (psi), or 1 atmosphere. At this pressure, water will boil (change from a liquid to a gas) at 100°C, and the resultant steam will remain at exactly that temperature, which is unfortunately too low to reliably kill all microbes. In order to raise the temperature of steam, the pressure at which it is generated must be increased. As the pressure is increased, the temperature at which water boils and the temperature of the steam produced both rise. For example, at a pressure of 20 psi (5 psi above normal), the temperature of steam is 109°C. As the pressure is increased to 10 psi above normal, the steam's temperature rises to 115°C, and at 15 psi above normal (a total of 2 atmospheres), it will be 121°C. It is not the pressure by itself that is killing microbes but the increased temperature it produces.	Heat-resistant materials such as glassware, cloth (surgical dressings), metallic instruments, liquids, paper, some media, and some heat-resistant plastics. If items are heat-sensitive (plastic Petri dishes) but will be discarded, the autoclave is still a good choice. However, it is ineffective for sterilizing substances that repel moisture (oils, waxes), or for those that are harmed by it (powders).

Method	Applications
Such pressure-temperature combinations can be achieved only with a special device that can subject pure steam to pressures greater than 1 atmosphere. Health and commercial industries use an **autoclave** for this purpose, and a comparable home appliance is the pressure cooker. The most efficient pressure-temperature combination for achieving sterilization is 15 psi, which yields 121°C. It is important to avoid overpacking materials or haphazardly loading the chamber, which prevents steam from circulating freely around the contents and prevents the full contact that is necessary. The duration of the process is adjusted according to the bulkiness of the items in the load (thick bundles of material or large flasks of liquid) and how full the chamber is. The range of autoclaving times varies from 10 minutes for light loads to 40 minutes for heavy or bulky ones; the average time is 20 minutes.	



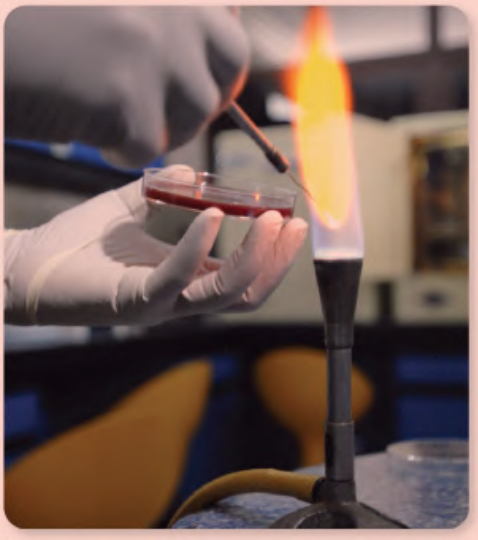
Dry Heat: Hot Air and Incineration

Dry heat is not as versatile or as widely used as moist heat, but it has several important sterilization applications. The temperatures and times employed in dry heat vary according to the particular method, but in general, they are greater than with moist heat. **Table 9.6** describes the two methods.

Table 9.6 Dry Heat Methods

Techniques and chemicals that are capable of sterilizing are highlighted with a pink background.

Method	Applications

Method	Applications
Incineration in a flame is perhaps the most rigorous of all heat treatments. The flame of a Bunsen burner reaches 1,870°C at its hottest point, and furnaces/incinerators operate at temperatures of 800°C to 6,500°C. Direct exposure to such intense heat ignites and reduces microbes and other substances to ashes and gas. Incineration of microbial samples on inoculating loops and needles using a Bunsen burner is a very common practice in the microbiology laboratory. This method is fast and effective, but it is also limited to metals and heat-resistant glass materials. This method also presents hazards to the operator (an open flame) and to the environment (contaminants on needle or loop often spatter when placed in flame). Tabletop infrared incinerators have replaced Bunsen burners in many labs for these reasons. Large incinerators are regularly employed in hospitals and research labs for complete destruction of infectious materials.  ©UIG via Getty Images	Bunsen burners/small incinerators: laboratory instruments such as inoculating loops. Large incinerators: syringes, needles, culture materials, dressings, bandages, bedding, animal carcasses, and pathology samples.
The hot-air oven provides another means of dry-heat sterilization. The so-called *dry oven* is usually electric (occasionally gas) and has coils that radiate heat within an enclosed compartment. Heated, circulated air transfers its heat to the materials in the oven. Sterilization requires exposure to 150°C to 180°C for 2 to 4 hours, which ensures thorough heating of the objects and destruction of endospores. ©RayArt Graphics/Alamy Stock Photo	Glassware, metallic instruments, powders, and oils that steam does not penetrate well. Not suitable for plastics, cotton, and paper, which may burn at the high temperatures, or for liquids, which will evaporate.

©PhotoAlto/PunchStock

The Effects of Cold and Desiccation

The principal benefit of cold treatment is to slow down the growth of cultures and microbes in food during processing and storage. *It must be emphasized that cold can only be counted on to be microbistatic, not microbicidal*. Although it is true that some microbes are killed by cold temperatures, most are not adversely affected by gradual cooling, long-term refrigeration, or deep-freezing. In fact, freezing temperatures, ranging from −70°C to −135°C, are often used in research labs to preserve cultures of bacteria, viruses, and fungi for long periods. Some psychrophiles grow very slowly even at freezing temperatures and

A wide variety of foods are irradiated to control microbial growth.
Source: USDA - Agricultural Research Service

can continue to secrete toxic products. Ignorance of these facts is probably responsible for numerous cases of food poisoning from frozen foods that have been defrosted at room temperature and then inadequately cooked. Pathogens able to survive several months in the refrigerator are *Staphylococcus aureus, Clostridium* species (endospore formers), *Streptococcus* species, and several types of yeasts, molds, and viruses. Outbreaks of *Salmonella* food infection traced backed to refrigerated foods such as ice cream, eggs, and tiramisu are testimony to the inability of cold temperatures to reliably kill pathogens.

Vegetative cells directly exposed to normal room air gradually become dehydrated, or **desiccated**. Delicate pathogens such as *Streptococcus pneumoniae*, the spirochete of syphilis, and *Neisseria gonorrhoeae* can die after a few hours of air drying, but many others are not killed and some are even preserved. Endospores of *Bacillus* and *Clostridium* are viable for thousands of years under extremely dry conditions. Staphylococci and streptococci in dried secretions and the tubercle bacillus surrounded by sputum can remain viable in air and dust for lengthy periods. Many viruses (especially nonenveloped) and fungal spores can also withstand long periods of desiccation. Desiccation can be a valuable way to preserve foods because it greatly reduces the amount of water available to support microbial growth.

It is interesting to note that a combination of freezing and drying—**lyophilization** (ly-off″-il-ih-za′-shun)—is a common method of *preserving* microorganisms and other cells in a viable state for many years. Pure cultures are frozen instantaneously and exposed to a vacuum that rapidly removes the water (it goes right from the frozen state into the vapor state). This method avoids the formation of ice crystals that would damage the cells. Although not all cells survive this process, enough of them do to permit future reconstitution of that culture.

As a general rule, chilling, freezing, and desiccation should not be construed as methods of disinfection or sterilization because their antimicrobial effects are erratic and uncertain, and one cannot be sure that pathogens subjected to them have been killed.

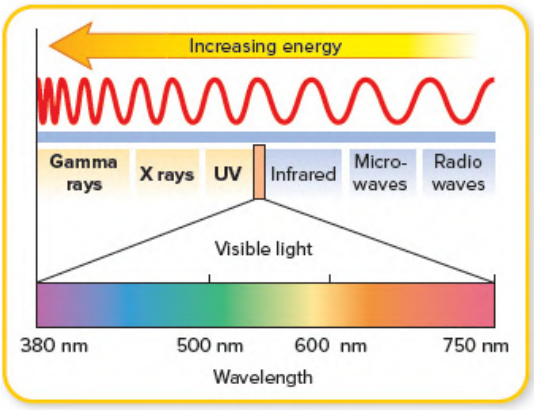


Figure 9.5 **The electromagnetic spectrum, showing different types of radiation**.

Figure 9.6 **A UV light disinfection unit for hospital rooms**.
©Essdras M Suarez/The Boston Globe via Getty Images

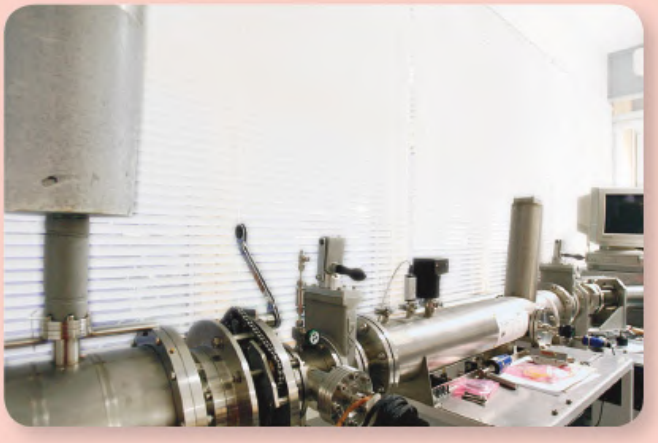
Radiation

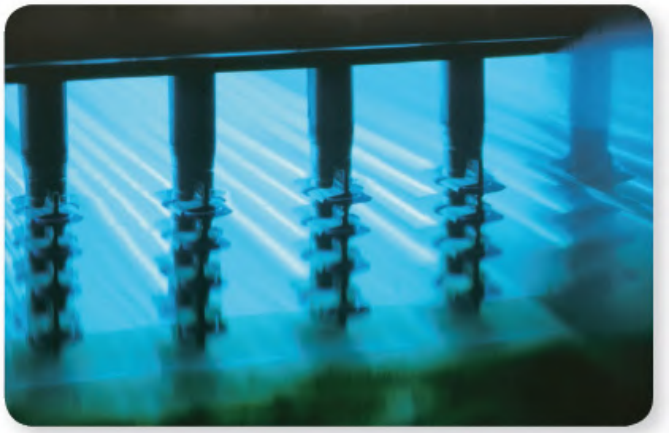
Energy, in the form of radiation, is a useful source of antimicrobial activity. **Radiation** is defined as energy emitted from atomic activities and dispersed at high velocity through matter or space. **Figure 9.5** illustrates the different wavelengths of radiation. In our discussion, we consider only those types suitable for microbial control: gamma rays, X rays, and ultraviolet radiation. These are the forms with shorter wavelengths than those of visible light. There are several especially useful practices that take advantage of these forms of radiation **(table 9.7)**. Hospitals have begun using specialized UV-light machines to clean hospital rooms after patients have been released and the rooms have been cleaned with traditional methods **(figure 9.6)**. The UV apparatus must be moved to more than one position so that the radiation reaches each area of the room, but it can disinfect areas that are not usually reached by hand cleaning methods.

Table 9.7 **Radiation Methods**

Techniques and chemicals that are capable of sterilizing are highlighted with a pink background.

Method	Applications

Method	Applications
Ionizing Radiation: Gamma Rays and X Rays Ionizing radiation is a highly effective alternative for sterilizing materials that are sensitive to heat or chemicals. Devices that emit ionizing rays include gamma-ray machines containing radioactive cobalt, X-ray machines similar to those used in medical diagnosis, and cathode-ray machines. Items are placed in these machines and irradiated for a short time with a carefully chosen dosage. The dosage of radiation is measured in *Grays* (which has replaced the older term, *rads*). Depending on the application, exposure ranges from 5 to 50 kiloGrays (kGray; a kiloGray is equal to 1,000 Grays). Although all ionizing radiation can penetrate liquids and most solid materials, gamma rays are most penetrating, X rays are intermediate, and cathode rays are least penetrating. Foods have been subject to **irradiation** in limited circumstances for more than 50 years. From flour to pork and ground beef, to fruits and vegetables, radiation is used to kill not only bacterial pathogens but also insects and worms and even to inhibit the sprouting of white potatoes. Irradiation may lead to a small decrease in the amount of thiamine (vitamin B1) in food, but this change is small enough to be inconsequential. The irradiation process does produce short-lived free radical oxidants, which disappear almost immediately (this same type of chemical intermediate is produced through cooking as well). Certain foods do not irradiate well and are not good candidates for this type of antimicrobial control. The white of eggs becomes milky and liquid, grapefruit gets mushy, and alfalfa seeds do not germinate properly. Lastly, it is important to remember that food is not made radioactive by the irradiation process, and many studies, in both animals and humans, have concluded that there are no ill effects from eating irradiated food. In fact, NASA relies on irradiated meat for its astronauts.  ©Adam Hart-Davis/Science Source	Drugs, vaccines, medical instruments (especially plastics), syringes, surgical gloves, tissues such as bone and skin, and heart valves for grafting. After the anthrax attacks of 2001, mail delivered to certain Washington, D.C., ZIP Codes was irradiated with ionizing radiation. Its main advantages include speed, high penetrating power (it can sterilize materials through outer packages and wrappings), and the absence of heat. Its main disadvantages are potential dangers to radiation machine operators from exposure to radiation and possible damage to some materials.

Method	Applications
Nonionizing Radiation: Ultraviolet Rays **Ultraviolet (UV) radiation** ranges in wavelength from approximately 100 to 400 nm. It is most lethal from 240 to 280 nm (with a peak at 260 nm). Owing to its lower energy state, UV radiation is not as penetrating as ionizing radiation. Because UV radiation passes readily through air, slightly through liquids, and only poorly through solids, the object to be disinfected must be directly exposed to it for full effect. Ultraviolet rays are a powerful tool for destroying fungal cells and spores, bacterial vegetative cells, protozoa, and viruses. Bacterial spores are about 10 times more resistant to radiation than are vegetative cells, but they can be killed by increasing the time of exposure. Even though it is possible to sterilize with UV, it is so technically challenging that we don't regularly call it a sterilizing technology.  ©Tom Pantages	Usually directed at disinfection rather than sterilization. Germicidal lamps can cut down on the concentration of airborne microbes as much as 99%. They are used in hospital rooms, operating rooms, schools, food preparation areas, and dental offices. Ultraviolet disinfection of air has proved effective in reducing postoperative infections, preventing the transmission of infections by respiratory droplets, and curtailing the growth of microbes in food-processing plants and slaughterhouses. Ultraviolet irradiation of liquids requires special equipment to spread the liquid into a thin, flowing film that is exposed directly to a lamp. This method can be used to treat drinking water and to purify other liquids (milk and fruit juices) as an alternative to heat. The photo shows a UV treatment system for the disinfection of water.

UV radiation damages cells by causing the inappropriate formation of bonds between two adjacent bases on a DNA strand **(figure 9.7)**. It affects pyrimidines (Ts and Cs). If the new "dimers" are not repaired, they can prevent that segment of DNA from being correctly replicated or transcribed. Massive dimerization is lethal to cells.

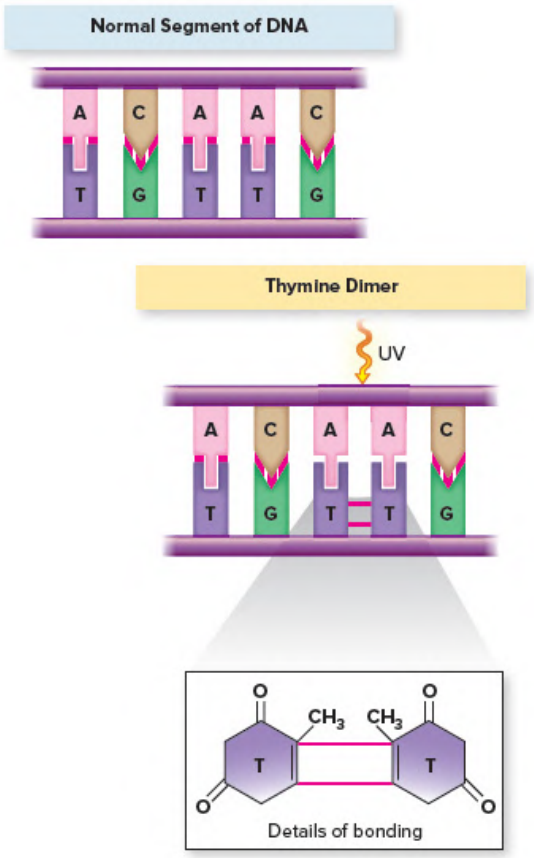


Figure 9.7 **Formation of pyrimidine dimers by the action of ultraviolet (UV) radiation**. This shows what occurs when two adjacent thymine bases on one strand of DNA are induced by UV rays to bond laterally with each other. The result is a thymine dimer (shown in greater detail). Dimers can also occur between adjacent cytosines, and thymine and cytosine bases.

Other Physical Methods: Filtration

Filtration is an effective method to remove microbes from air and liquids. In practice, a fluid is strained through a filter with openings large enough for the fluid to pass through but too small for microorganisms to pass through **(figure 9.8*a*)**.

Most modern microbiological filters are thin membranes of cellulose acetate, polycarbonate, and a variety of plastic materials (Teflon, nylon) whose pore size can be carefully controlled and standardized. Ordinary substances such as charcoal, diatomaceous earth, or unglazed porcelain are also used in some applications. Viewed microscopically, most filters are perforated by very precise, uniform pores **(figure 9.8*b*)**. The pore diameters vary from coarse (8 μm) to ultrafine (0.02 μm), permitting selection of the minimum particle size to be trapped. Those with even smaller pore diameters permit true sterilization by removing viruses, and some will even remove large proteins. A sterile liquid filtrate is typically produced by suctioning the liquid through a sterile filter into a presterilized container. These filters are also used to separate mixtures of microorganisms and to enumerate bacteria in water analysis.

Filtration is used to prepare liquids that cannot withstand heat, including serum and other blood products, vaccines, drugs, IV fluids, enzymes, and media. Filtration has been employed as an alternative method for decontaminating milk and beer without altering their flavor. It is also

an important step in water purification. Its use extends to filtering out particulate impurities (crystals, fibers, and so on) that can cause severe reactions in the body. It has the disadvantage of not removing small soluble molecules (such as toxins) that can cause disease.

Filtration is also an efficient means of removing airborne contaminants that are a common source of infection and spoilage. High-efficiency particulate air (HEPA) filters are widely used to provide a flow of decontaminated air to hospital rooms and

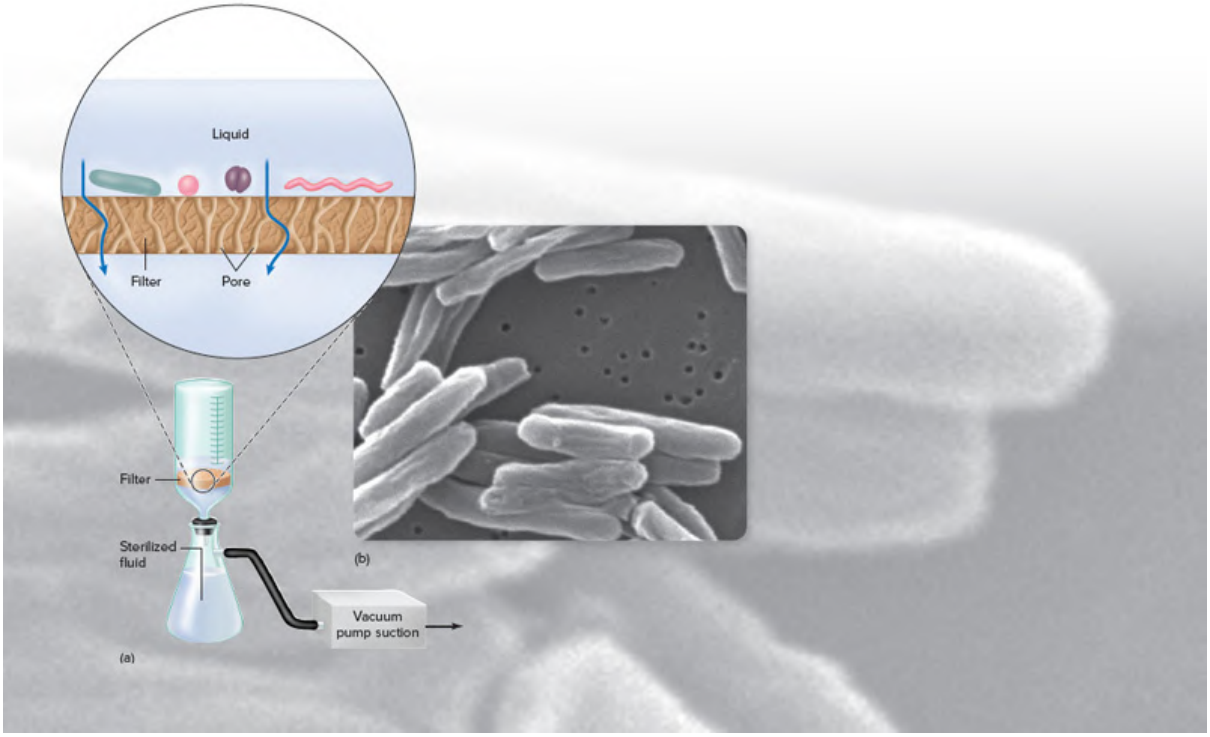


Figure 9.8 **Membrane filtration**. **(a)** Vacuum assembly for achieving filtration of liquids through suction. The surface of the filter is shown magnified in the blown-up section, with tiny passageways (pores) too small for the microbial cells to enter but large enough for liquid to pass through. **(b)** Scanning electron micrograph (21,000) of filter, showing relative size of pores and bacteria trapped on its surface.
Source: CDC/Dr. Ray Butler; Janice Carr (b)

sterile rooms. A vacuum with a HEPA filter was even used to remove anthrax endospores from the Senate offices most heavily contaminated after the terrorist attack in late 2001.

Canned sardines remain edible for 2–3 years, preserved by the osmotic pressure of high salt.
©Burke/Triolo Productions/Getty Images

Osmotic Pressure

In chapter 6, you learned about the effects of osmotic pressure on cells. This fact has long been exploited as a means of preserving food. Adding large amounts of salt or sugar to foods creates a hypertonic environment for bacteria in the foods, causing plasmolysis and making it impossible for the bacteria to multiply. People knew that these techniques worked long before the discovery of bacteria. Even in ancient times, people used pickling, smoking, and drying of foods to control growth of microorganisms. This is why meats are "cured," or treated with high salt concentrations so they can be kept for long periods without refrigeration. High sugar concentrations in foods like jellies have the same effect. Osmotic pressure, however, is never a sterilizing technique.

9.2 LEARNING OUTCOMES—Assess Your Progress

5. Name six methods of physical control of microorganisms.
6. Discuss both moist and dry heat methods, and identify multiple examples of each.
7. Define *thermal death time* and *thermal death point*.
8. Explain methods of moist heat control.
9. Explain two methods of dry heat control.
10. Identify advantages and disadvantages of cold and desiccation.
11. Differentiate between the two types of radiation control methods.

12. Explain how filtration and osmotic pressure function as control methods.

9.3 Chemical Agents in Microbial Control

Antimicrobial chemicals occur in the liquid, gaseous, or even solid state, and they range from disinfectants and antiseptics to sterilants and preservatives (chemicals that inhibit the deterioration of substances). For the sake of convenience (and sometimes safety), many solid or gaseous antimicrobial chemicals are dissolved in water, alcohol, or a mixture of the two to produce a liquid solution. Solutions containing pure water as the solvent are termed **aqueous**, whereas those dissolved in pure alcohol or water-alcohol mixtures are termed **tinctures**.

Selecting a Microbicidal Chemical

The choice and appropriate use of antimicrobial chemical agents are of constant concern in medicine and dentistry. Although actual clinical practices of chemical decontamination vary widely, some desirable qualities in a germicide have been identified, including the following:

- rapid action even in low concentrations
- solubility in water or alcohol and long-term stability
- broad-spectrum microbicidal action without being toxic to human and animal tissues
- penetration of inanimate surfaces to sustain a cumulative or persistent action
- resistance to becoming inactivated by organic matter
- not corrosive and nonstaining
- sanitizing and deodorizing properties
- affordability and availability

Germicides are evaluated in terms of their effectiveness in destroying microbes in medical and dental settings. The three levels of chemical decontamination procedures are *high, intermediate*, and *low*. High-level germicides kill endospores and, if properly used, are sterilants. Materials that necessitate high-level control are medical devices—for

©Paul Velgos/Getty Images

example, catheters, heart-lung equipment, and implants—that are not heat-sterilizable and are intended to enter body tissues during medical procedures. Intermediate-level germicides kill fungal (but not bacterial) spores, resistant pathogens such as the tubercle bacillus, and viruses. They are used to disinfect items (respiratory equipment, thermometers) that come into intimate contact with the mucous membranes but are noninvasive. Low levels of disinfection eliminate only vegetative bacteria, vegetative fungal cells, and some viruses. They are used to clean materials such as electrodes, straps, and pieces of furniture that touch the skin surfaces but not the mucous membranes.

Factors Affecting the Germicidal Activity of Chemicals

Factors that control the effect of a germicide include the nature of the microorganisms being treated, the nature of the material being treated, the degree of contamination, the time of

exposure, and the strength and chemical action of the germicide. The variations in concentration and time needed can be quite wide **(table 9.8)**.

Medical Moment

The Use of Alcohol-Based Hand Cleansers

Hand washing is recognized as one of the most important methods of preventing infection in hospitals, yet studies have shown that many health care workers (HCWs) do not wash their hands as often as they should, or fail to wash their hands in accordance with guidelines. For this reason, the use of alcohol-based hand antiseptics between hand washes has become common practice. Most alcohol-based hand antiseptics contain isopropanol, n-propanol, or ethanol. Some contain combinations of two of these substances. A concentration of 60% to 80% alcohol is deemed most effective due to the fact that proteins require water in order for denaturation to occur. Higher concentrations of alcohol contain less water, so they are less effective.

Q. Would you expect an enveloped virus or a nonenveloped virus to be more likely to be inactivated by an alcohol hand antiseptic? Explain your answer.

Answer in Appendix B.

The Chemical Categories

The modes of action of most germicides are to attack the cellular targets discussed earlier: proteins, nucleic acids, the cell wall, and the cytoplasmic membrane. **Table 9.9** on the following pages provides details about the most commonly used chemicals and their modes of action.

In 2016 the Food and Drug Administration (FDA) placed a ban (effective fall 2017) on 19 active ingredients which may no longer be used in consumer products such as soaps or cleaning solutions. They did this because many of the active ingredients can select for resistance in microbes, and some of them can even increase the likelihood that microbes will become resistant to antibiotics themselves. There has

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2. An RN is preparing to access a patient's IV catheter for infusion of a medication. What is the most appropriate agent for cleaning the plastic cap of the IV catheter prior to connecting the medication tubing?

a. *alcohol*
b. *hydrogen peroxide*
c. *chlorhexidine*
d. *iodine*

Table 9.8 Required Concentrations and Times for Chemical Destruction of Selected Microbes

Organism	Concentration	Time
Agent: Chlorine		
Mycobacterium tuberculosis	50 ppm	50 sec
Entamoeba cysts (protozoa)	0.1 ppm	150 min
Hepatitis A virus	3 ppm	30 min
Agent: Ethyl Alcohol		

Organism	Concentration	Time
Staphylococcus aureus	70%	10 min
Escherichia coli	70%	2 min
Poliovirus	70%	10 min
Agent: Hydrogen Peroxide		
Staphylococcus aureus	3%	12.5 sec
Neisseria gonorrhoeae	3%	0.3 sec
Herpes simplex virus	3%	12.8 sec
Agent: Quaternary Ammonium Compound		
Staphylococcus aureus	450 ppm	10 min
Salmonella typhi	300 ppm	10 min
Agent: Ethylene Oxide Gas		
Streptococcus faecalis	500 mg/L	2–4 min
Influenza virus	10,000 mg/L	25 h

The main antimicrobial ingredient in many mouthwashes is alcohol.
©Stockbyte/PunchStock

Table 9.9 Germicidal Categories According to Chemical Group

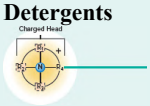
Techniques and chemicals that are capable of sterilizing are highlighted with a pink background.

Agent	Target Microbes	Form(s)	Mode of Action	Indications for Use	Limitations

Agent	Target Microbes	Form(s)	Mode of Action	Indications for Use	Limitations
Halogens: chlorine	Can kill endospores (slowly); all other microbes	Liquid/gaseous chlorine (Cl_2), hypochlorites (OCl), chloramines (NH_2Cl)	In solution, these compounds combine with water and release hypochlorous acid (HOCl); denature enzymes permanently and suspend metabolic reactions	Chlorine kills bacteria, endospores, fungi, and viruses; gaseous/liquid chlorine: used to disinfect drinking water, sewage and waste water; hypochlorites: used in health care to treat wounds, disinfect bedding and instruments, sanitize food equipment and in restaurants, pools, and spas; chloramines: alternative to pure chlorine in treating drinking water; also used to treat wounds and skin surfaces	Less effective if exposed to light, alkaline pH, and excess organic matter
Halogens: iodine	Can kill endospores (slowly); all other microbes	Free iodine in solution (I_2) Iodophors (complexes of iodine and alcohol)	Penetrates cells of microorganisms where it interferes with a variety of metabolic functions; interferes with the hydrogen and disulfide bonding of proteins	2% iodine, 2.4% sodium iodide (aqueous iodine) used as a topical antiseptic 5% iodine, 10% potassium iodide used as a disinfectant for plastic and rubber instruments, cutting blades, etc. Iodophor products contain 2% to 10% of available iodine, which is released slowly; used to prepare skin for surgery, in surgical scrubs, to treat burns, and as a disinfectant	Can be extremely irritating to the skin and is toxic when absorbed **Many iodophors banned in consumer products in 2017**.
Oxidizing agents	Kill endospores and all other microbes	Hydrogen peroxide, peracetic acid	Oxygen forms free radicals (—OH), which are highly toxic and reactive to cells	As an antiseptic, 3% hydrogen peroxide used for skin and wound cleansing, mouth washing, bedsore care Used to treat infections caused by anaerobic bacteria 35% hydrogen peroxide used in low temperature sterilizing cabinets for delicate instruments	Sporicidal only in high concentrations

Agent	Target Microbes	Form(s)	Mode of Action	Indications for Use	Limitations
Aldehydes	Kill endospores and all other microbes	Organic substances bearing a—CHO functional group on the terminal carbon	Glutaraldehyde can irreversibly disrupt the activity of enzymes and other proteins within the cell Ortho-phthalaldehyde	Glutaraldehyde kills rapidly and is broad-spectrum; used to sterilize respiratory equipment, scopes, kidney dialysis machines, dental instruments Ortho-phthalaldehyde is safer than glutaraldehyde and just as effective	Glutaraldehyde is somewhat unstable, especially with increased pH and temperature Ortho-phthalaldehyde is much more expensive than glutaraldehyde
Gaseous sterilants/disinfectants	Ethylene oxide kills endospores; other gases less effective	Ethylene oxide is a colorless substance that exists as a gas at room temperature	Ethylene oxide reacts vigorously with functional groups of DNA and proteins, blocking both DNA replication and enzymatic actions Chlorine dioxide is a strong alkylating agent	Ethylene oxide is used to disinfect plastic materials and delicate instruments; can also be used to sterilize syringes, surgical supplies, and medical devices that are prepackaged	Ethylene oxide is explosive—it must be combined with a high percentage of carbon dioxide or fluorocarbon It can damage lungs, eyes, and mucous membranes if contacted directly Ethylene oxide is rated as a carcinogen by the government

Agent	Target Microbes	Form(s)	Mode of Action	Indications for Use	Limitations
Phenol (carbolic acid)	Some bacteria, viruses, fungi	Derived from the distillation of coal tar Phenols consist of one or more aromatic carbon rings with added functional groups	In high concentrations, they are cellular poisons, disrupting cell walls and membranes, proteins In lower concentrations, they inactivate certain critical enzyme systems	Phenol remains one standard against which other (less toxic) phenolic disinfectants are rated; the phenol coefficient quantitatively compares a chemical's antimicrobial properties to those of phenol Phenol is now used only in certain limited cases, such as in drains, cesspools, and animal quarters	Toxicity of many phenolics makes them dangerous to use as antiseptics Many phenols banned in consumer products in 2017, including triclosan and triclocarban.

Agent	Target Microbes	Form(s)	Mode of Action	Indications for Use	Limitations
Chlorhexidine	Most bacteria, viruses, fungi	Complex organic base containing chlorine and two phenolic rings	Targets bacterial membranes, where selective permeability is lost, bacterial cell walls, and proteins, resulting in denaturation	Mildness, low toxicity and rapid action make chlorhexidine a popular choice of agents Used in hand scrubs, prepping skin for surgery, as an obstetric antiseptic, as a mucous membrane irrigant, etc.	Effects on viruses and fungi are variable
Alcohol 	Most bacteria, viruses, fungi	Colorless hydrocarbons with one or more—OH functional groups Ethyl and isopropyl alcohol are suitable for antimicrobial control	Concentrations of 50% and higher dissolve membrane lipids, disrupt cell surface tension, and compromise membrane integrity	Germicidal, nonirritating, and inexpensive Routinely used as skin degerming agents (70% to 95% solutions)	Rate of evaporation decreases effectiveness Inhalation of vapors can affect the nervous system
Detergents 	Some bacteria, viruses, fungi	Polar molecules that act as surfactants Anionic detergents have limited microbial power Cationic detergents, such as quaternary ammonium compounds ("quats"), are much more effective antimicrobials	Positively charged end of the molecule binds well with the predominantly negatively charged bacterial surface proteins Long, uncharged hydrocarbon chain allows the detergent to disrupt the cytoplasmic membrane Cytoplasmic membrane loses selective permeability, causing cell death	Effective against viruses, algae, fungi, and gram-positive bacteria Rated only for low-level disinfection in the clinical setting Used to clean restaurant utensils, dairy equipment, equipment surfaces, restrooms	Ineffective against tuberculosis bacterium, hepatitis virus, *Pseudomonas*, and endospores Activity is greatly reduced in presence of organic matter Detergents function best in alkaline solutions **Some quats banned in consumer products in 2017**.

Agent	Target Microbes	Form(s)	Mode of Action	Indications for Use	Limitations
Heavy metal compounds	Some bacteria, viruses, fungi	Heavy metal germicides contain either an inorganic or an organic metallic salt; may come in tinctures, soaps, ointment, or aqueous solution	Mercury, silver, and other metals exert microbial effects by binding onto functional groups of proteins and inactivating them	Organic mercury tinctures are fairly effective antiseptics Organic mercurials serve as preservatives in cosmetics, ophthalmic solutions, and other substances Silver nitrate solutions are used for topical germicides and ointments	Microbes can develop resistance to metals Not effective against endospores Can be toxic if inhaled, ingested, or absorbed May cause allergic reactions in susceptible individuals

©Richard Hutchings (bleach); ©McGraw-Hill Education/Jill Braaten, photographer (hydrogen peroxide); ©Richard Hutchings (alcohol); ©McGraw-Hill Education/Stephen Frisch, photographer (heavy metals)

been a proliferation of antimicrobial chemicals in everyday products, such as hand lotions and detergents. Most scientists agree that soaps and detergents are sufficient on their own for the cleaning they are expected to do, and that "spiking" them with antimicrobial agents causes more harm than good in the home environment. Of course, the hospital environment is a different situation. But in order for the antimicrobial agents used to clean surfaces and skin in a hospital to remain effective, we need to decrease their overall presence in the (outpatient) environment. The agents banned in the FDA ruling are pointed out in tables 9.9 and 9.10.

A chemical's strength or concentration is expressed in various ways, depending on accepted practice and the method of preparation. In dilutions, a small volume of the liquid chemical (solute) is diluted in a larger volume of solvent to achieve a certain ratio. For example, a common laboratory phenolic disinfectant such as Lysol is usually diluted 1:200; that is, one part of chemical has been added to 200 parts of water by volume. Solutions such as chlorine that are effective in very diluted concentrations are expressed in parts per million (ppm). In percentage solutions, the solute is added to water by weight or volume to achieve a certain percentage in the solution. Alcohol, for instance, is used in percentages ranging from 50% to 95%.

Another factor that contributes to germicidal effectiveness is the length of exposure. Most compounds require adequate contact time to allow the chemical to penetrate and to act on the microbes present. The composition of the material being treated must also be considered. Smooth, solid objects are more reliably disinfected than are those with pores or pockets that can trap soil. An item contaminated with common biological matter such as serum, blood, saliva, pus, fecal material, or urine presents a problem in disinfection. Large amounts of organic material can hinder the penetration of a disinfectant and, in some cases, can form bonds that reduce its activity. Adequate cleaning of instruments and other reusable materials must occur before use of the germicide or sterilant. Otherwise, there is no way to predict whether your procedure will be effective.

For a look at the antimicrobial chemicals found in some common household products, see **table 9.10**.

Medical Moment

Zap VAP: The Role of Chlorhexidine

Patients in the intensive care unit often require support for their breathing. A plastic tube is inserted in the nose or mouth so a ventilator can deliver oxygen and breaths. However, the breathing tube establishes a path by which microbes may easily invade the lower respiratory tract and lungs, as well as restricts the patient's natural ability to clear secretions. Therefore, patients with an artificial airway are at risk for hospital-acquired lung infection, or ventilator-associated pneumonia (VAP).

There are many recommendations for care that are implemented by nurses and the care team to prevent VAP. One of the interventions is routine oral care with an antiseptic, such as chlorhexidine. The surfaces of the oral cavity are rinsed with the antiseptic wash to kill organisms in the mouth that could migrate to the lungs.

Chlorhexidine has a low toxicity and is safe for use on mucous membranes. It is applied directly to the teeth and surfaces of oral cavity by a soft sponge or irrigation. The use of chlorhexidine in patients on ventilator support has been shown to decrease the incidence of VAP.

Q. What is the mode of action of chlorhexidine?

Answer in Appendix B.

Table 9.10 Active Ingredients of Various Commercial Antimicrobial Products

Product	Specific Chemical Agent	Antimicrobial Category
Lysol® Sanitizing Wipes	Dimethyl benzyl ammonium chloride	Detergent (quat)
Clorox® Disinfecting Wipes	Dimethyl benzyl ammonium chloride	Detergent (quat)
Tilex® Mildew Remover	Sodium hypochlorites	Halogen
Lysol® Mildew Remover	Sodium hypochlorites	Halogen
Ajax® Antibacterial Hand Soap	Triclosan; banned beginning in fall 2017	Phenolic
Dawn® Antibacterial Hand Soap	Triclosan; banned beginning in fall 2017	Phenolic
Dial® Antibacterial Hand Soap	Triclosan; banned beginning in fall 2017	Phenolic
Lysol® Disinfecting Spray	Alkyl dimethyl benzyl ammonium saccharinate/ethanol	Detergent (quats)/alcohol
ReNu® Contact Lens Solution	Polyaminopropyl biguanide	Chlorhexidine
Wet Ones® Antibacterial Moist Towelettes	Benzethonium chloride	Detergents (quat)
Noxzema® Triple Clean	Triclosan; banned beginning in fall 2017	Phenolic
Scope® Mouthwash	Ethanol	Alcohol
Purell® Instant Hand Sanitizer	Ethanol	Alcohol
Pine-Sol®	Phenolics and surfactant	Mixed
Allergan® Eye Drops	Sodium chlorite	Halogen

9.3 LEARNING OUTCOMES—Assess Your Progress

13. Name the desirable characteristics of chemical control agents.
14. Discuss chlorine and iodine and their uses.
15. List advantages and disadvantages to phenolic compounds.
16. Explain the mode of action of chlorhexidine.
17. Explain the applications of oxidizing agents.
18. Identify some heavy metal control agents.

19. Discuss the disadvantages of aldehyde agents.
20. Identify applications for ethylene oxide sterilization.

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3. In the health care environment, use of alcohol-based hand sanitizer is appropriate in all of the following situations, except:
- **a.** *An RN is entering a patient's room to administer an oral medication.*
- **b.** *An RN has removed his gloves and is exiting a patient's room.*
- **c.** *An RN is preparing to don sterile gloves for a procedure.*
- **d.** *An RN has performed a dressing change and his hands are visibly soiled.*

CASE FILE WRAP-UP

©McGraw-Hill Education (wound); ©shapecharge/E+/Getty Images (health care worker); ©ERproductions Ltd/Blend Images LLC (forceps); ©Ingram Publishing/AGE Fotostock (tray of surgical tools)

At the end of the case, the sterile equipment and surgical supplies were counted to ensure that none had been left in the patient. Human tissue waste and blood-saturated supplies were disposed of in biohazard waste containers. Waste that was potentially pathogenic was sent to be sterilized by an autoclave prior to disposal. Plastic supplies, syringes, gauze sponges, and other single-use equipment were discarded. The surgical tools were rinsed of visible debris and placed in large trays for sterilization.

Some surgical equipment is reused after appropriate sterilization. In the hospital sterile processing department, an autoclave is used to eliminate all viable microorganisms, including bacterial endospores and viruses. (Prions may not be destroyed by this process.) The surgical tools are packed loosely onto a tray covered with cloth and loaded into the autoclave. There it is exposed to high heat under pressure. To ensure the equipment within the packaging has been brought to the appropriate temperature, indicator tape is applied to the outside of the package. This autoclave tape changes color when a high enough temperature has been maintained for the necessary time for sterilization to be complete.

The prevention of surgical site infections (SSIs) is one of the major benchmarks of quality in a hospital. SSIs create complications for the postoperative patient with increases in morbidity and hospital length of stay, and can even be fatal.

Hand Hygiene

How many times have you heard that one of the best ways to prevent infections in hospitals is for health care workers to wash their hands? OK, but now that we know about how important our resident microbiota is, we should ask what the frequent hand washing does to that. A recent study followed 34 health care workers in a surgical intensive care unit. Twenty-four of them were registered nurses, six were respiratory therapists, and four were nurse technologists.

During a typical 12-hour shift,

53% of them reported washing their hands with soap and water between 6 and 20 times,
41% used alcohol rubs more than 20 times, and
62% donned more than 40 pairs of gloves.

The researchers then tested for common healthcare-associated pathogens and found that approximately 45% of the health care workers' dominant hands were positive for *Staphylococcus aureus*. They detected methicillin-resistant *Staphylococcus aureus* (MRSA) on 3.9% of the dominant hands. About 4% of hands were positive for the fungus *Candida albicans*.

These rates look very similar to the rates found in the general population—those of us who are not washing our hands 20 times a day. This might be an illustration of the difference between *colonization*—when microbes become part of your "normal" microbiota—and *contamination*—when you pick up microbes from your environment and they stay only transiently. Contaminants are usually easily washed off, while colonizers are embedded deeper in your skin oils and the top layers of epithelial cells.

One question raised, but not answered, by this study is whether altering the normal microbiome of the hand by frequent hand hygiene practices might make it (a) easier or (b) more difficult for pathogens to contaminate and/or colonize caregivers' hands.

Source: Rosenthal, et al. "Healthcare Workers' Hand Microbiome May Mediate Carriage of Hospital Pathogens," *Pathogens*, vol. 3, no. 4, pgs. 1–13. March, 2014.

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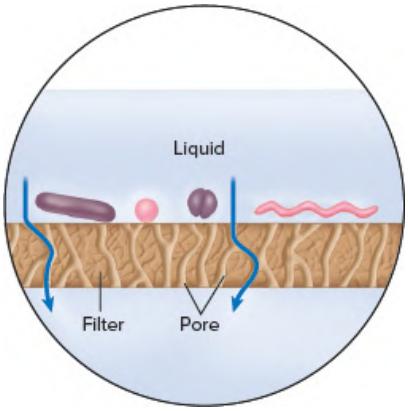
Chapter Summary

9.1 Controlling Microorganisms

- Microbial control methods involve the use of physical and chemical agents to eliminate or reduce the numbers of microorganisms from a specific environment to prevent the spread of infectious agents, retard spoilage, and keep commercial products safe.
- The population of microbes that cause spoilage or infection varies widely, so microbial control methods must be adjusted to fit individual situations.
- The type of microbial control is indicated by the terminology used. Sterilization agents destroy all viable organisms, including viruses. Antisepsis, disinfection, decontamination/sanitization, and antisepsis/degermation reduce the numbers of viable microbes to a specified level.
- Antimicrobial agents are described according to their ability to destroy or inhibit microbial growth. Microbicidal agents cause microbial death. They are described by what they are *–cidal* for: sporicides, bactericides, fungicides, and viricides.
- An antiseptic agent is applied to living tissue to destroy or inhibit microbial growth.
- A disinfectant agent is used on inanimate objects to destroy vegetative pathogens but not bacterial endospores.
- Decontamination/sanitization reduces microbial numbers on inanimate objects to safe levels by physical or chemical means.
- Microbial death is defined as the permanent loss of reproductive capability in microorganisms.
- Antimicrobial agents attack specific cell sites to cause microbial death or damage. The four major cell targets are the cell wall, the cytoplasmic membrane, biosynthesis pathways for DNA or RNA, or protein (enzyme) function.

9.2 Methods of Physical Control

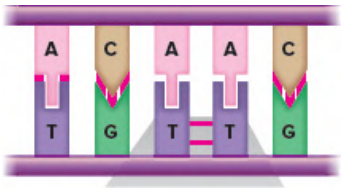
- Physical methods of microbial control include heat, cold, radiation, drying, filtration, and osmotic pressure.
- Heat is the most widely used method of microbial control. It is used in combination with water (moist heat) or as dry heat (oven, flames).
- The thermal death time (TDT) is the shortest length of time required to kill all microbes at a specific temperature.
- The thermal death point (TDP) is the lowest temperature at which all microbes are killed in a specified length of time (10 minutes).
- Autoclaving, or steam sterilization, is the process by which steam is heated under pressure to sterilize a wide range of materials in a comparatively short time (minutes to hours).
- Boiling water and pasteurization of beverages disinfect but do not sterilize materials.
- Dry heat is microbicidal under specified times and temperatures. Flame heat, or incineration, is microbicidal.
- Chilling, freezing, and desiccation are microbistatic but not microbicidal. They are not considered true methods of disinfection because they are not consistent in their effectiveness.
- Ionizing radiation (cold sterilization) by gamma rays and X rays is used to sterilize medical products, meats, and spices. It damages DNA and cell organelles by producing disruptive ions.



- Ultraviolet light, or nonionizing radiation, has limited penetrating ability. But it is proving to be very useful for terminal disinfection of hospital rooms.
- Decontamination by filtration removes microbes from heat-sensitive liquids and circulating air. The pore size of the filter determines what kinds of microbes are removed.
- The addition of high amounts of salt or sugar to food results in preservation through osmotic pressure.

9.3 Chemical Agents in Microbial Control

- Chemical agents of microbial control are classified by their physical state and chemical nature.
- Chemical agents can be either microbicidal or microbistatic. They are also classified as high-, medium-, or low-level germicides.
- Factors that determine the effectiveness of a chemical agent include the type and numbers of microbes involved, the material involved, the strength of the agent, and the exposure time.
- Halogens are effective chemical agents at both microbicidal and microbistatic levels. Chlorine, iodine, and iodophors are examples.
- Phenols are strong microbicidal agents used in general disinfection.
- Alcohols dissolve membrane lipids and destroy cell proteins. Their action depends upon their concentration, but they are generally only microbistatic.
- Oxidizing agents are versatile microbicides that can be used as antiseptics for wounds and disinfectants for utensils. A high concentration is an effective sporicide.
- Detergents reduce cytoplasmic membrane surface tension, causing membrane rupture. Cationic detergents, or quats, are low-level germicides limited by the amount of organic matter present and the microbial load.
- Aldehydes are potent sterilizing agents and high-level disinfectants that irreversibly disrupt microbial enzymes.
- Ethylene oxide and chlorine dioxide are gaseous sterilants that work by alkylating protein and DNA.



SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three

questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. DNA repair mechanisms can help alleviate the effects of **a**. UV radiation. **b**. alcohol disinfection. **c**. autoclaving. **d**. dry heat.	**2**. Explain why all cells in a population do not die instantaneously when exposed to an antimicrobial agent, even when they are of a single species.	**3**. Triclosan and other antimicrobial compounds have been banned from consumer products. Explain why we should not be using every chemical available to us to combat microbes.
Cell Structure and Function	**4**. Microbial control methods that kill _____ are able to sterilize. **a**. viruses **b**. the tuberculosis bacteria **c**. endospores **d**. cysts	**5**. Why are enveloped viruses generally more susceptible to disinfection, when they have an extra "layer"?	**6**. Construct an argument about why some antimicrobial agents are toxic for skin and human tissue.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Metabolic Pathways	**7**. Cytoplasmic enzymes are most likely to be disrupted by **a**. UV light. low temperatures. high temperatures. detergents. **d**.	**8**. Most antimicrobials that target protein function are nonselective as to the microbes they affect. Explain why that might be so.	**9**. There is a bacterium that has a metabolic pathway that enables it to survive in the presence of acid. What kind of pathway/or product would you expect in this case?
Information Flow and Genetics	**10**. Transcription is targeted most directly by **a**. quats. **b**. detergents. **c**. UV radiation. **d**. alcohol.	**11**. Do you think UV radiation is a good way to disinfect living tissue? Why or why not?	**12**. Suggest a possible mechanism for how a microbe that becomes resistant to an antimicrobial chemical can transfer that to another microorganism, and also transfer resistance to antibiotics in the process.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Microbial Systems	**13**. Disinfection procedures must take into account **a**. the mixture of microbes being targeted. **b**. whether the microbes in biofilms are dispersed. **c**. the amount of organic matter associated with the microbes. **d**. all of the above.	**14**. Provide a rationale for how a particular microbe could be considered a serious contaminant in one situation and completely harmless in another.	**15**. What additional considerations would you have to address if the device you were to trying to disinfect—such as a water line in a dental office—was known to contain a microbial biofilm?

Impact of Microorganisms	**16**. Sanitization is a process by which **a**. the microbial load on an object is reduced. **b**. objects are made sterile with chemicals. **c**. utensils are scrubbed. **d**. skin is debrided.	**17**. Tissue transplantation carries a risk of transferring microbes in the tissue to the recipient. What are the most appropriate ways of ensuring that does not happen?	**18**. Defend this statement: It is possible to make our environment too clean.
Scientific	**19**. The most	**20**. Precisely what is	**21**. Devise an

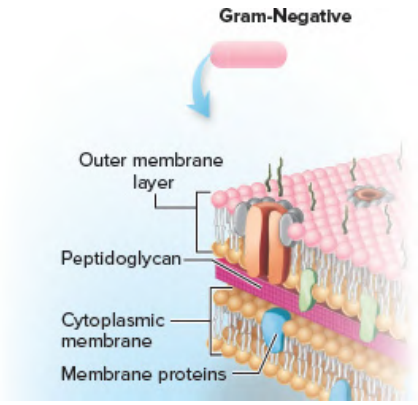
Thinking	versatile method for sterilizing heat-sensitive liquids is **a**. UV radiation. **b**. exposure to ozone. **c**. peracetic acid. **d**. filtration.	microbial death?	experiment that will differentiate between bactericidal versus bacteriostatic effects.

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From chapter 3, figure 3.15**. Study this illustration of a gram-negative cell envelope. In what ways could alcohol damage these two membranes? How would that harm the cell?



connect Resources are available through Connect, including interactive learning tools, animations, and more.

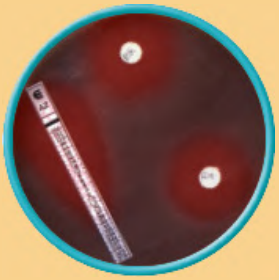
Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

10

Antimicrobial Treatment

©Ryan McVay/Getty Images (health care worker); ©Glow Images (pills); Source: CDC/Dr. Richard Facklam (Etest)

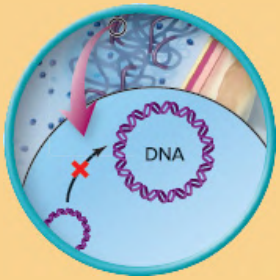
IN THIS CHAPTER…



10.1 Principles of Antimicrobial Therapy

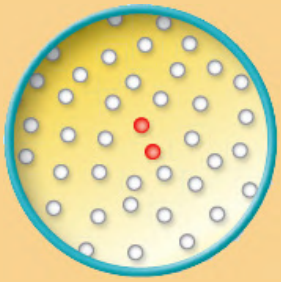
1. State the main goal of antimicrobial treatment.
2. Identify the sources for the most commonly used antimicrobials.
3. Describe two methods for testing antimicrobial susceptibility.

4. Define *therapeutic index*, and identify whether a high or a low index is preferable.



10.2 Interactions Between Drug and Microbe

5. Explain the concept of selective toxicity.
6. List the five major targets of antimicrobial agents.
7. Identify which categories of drugs are most selectively toxic and why.
8. Distinguish between broad-spectrum and narrow-spectrum antimicrobials, and explain the significance of the distinction.
9. Identify the microbes against which the various penicillins are effective.
10. Explain the mode of action of penicillinases and their role in treatment decisions.
11. Identify two antimicrobials that act by inhibiting protein synthesis.
12. Explain how drugs targeting folic acid synthesis work.
13. Identify one example of a fluoroquinolone.
14. Describe the mode of action of drugs that target the cytoplasmic or cell membrane.
15. Discuss how treatments of biofilm and nonbiofilm infections differ.
16. Name the four main categories of antifungal agents.
17. Explain why antiprotozoal and antihelminthic drugs are likely to be more toxic than antibacterial drugs.
18. List the three major targets of action of antiviral drugs.



10.3 Antimicrobial Resistance

19. Discuss two main ways that microbes acquire antimicrobial resistance.
20. List five cellular or structural mechanisms that microbes use to resist antimicrobials.
21. Discuss at least two novel antimicrobial strategies that are under investigation.

10.4 Interactions Between Drug and Host

22. Distinguish between drug toxicity and allergic reactions to drugs.
23. Explain what a superinfection is and how it occurs.

CASE FILE

Not What We Were Expecting

I was working in a pediatric hospital in the emergency room. A 2-year-old girl with an obvious rash was brought in by her mother, who wondered whether the rash might be chickenpox.

I took the child and her mother back to a cubicle and began to take the child's history. The child was healthy and had no major health problems. Her vaccinations were all current, including the chickenpox vaccine, which made a diagnosis of chickenpox unlikely. She had recently had otitis media, an ear infection, and was on her second-to-last day of antibiotic therapy with Ceclor, a cephalosporin antibiotic. The child had taken Ceclor on one other occasion for an ear infection.

The vital signs were normal. She did not have a fever. The rash was maculopapular, a flat red rash with tiny pimplelike eruptions in the center. The rash covered her face, chest, back, arms, and legs. In fact, it covered almost her entire body except the palms of her hands and soles of her feet. The child was clearly uncomfortable and was scratching exposed areas of skin.

The mother reported that she had taken the child for a haircut in the afternoon and noticed a few spots on the child's neck. She did not think much of the spots at the time. She dropped the little girl off at her mother-in-law's for child care while she went to work as a waitress. When she returned to her mother-in-law's house to pick the child up after her shift, the child was awake, irritable, and covered in the rash. Alarmed, the mother brought the child immediately to the emergency room.

After recording the history and the child's vital signs, I went to find the physician and reported my findings. He told me that he felt he knew what the problem was but would

quickly examine the child first before telling me his diagnosis. The doctor looked at the patient's rash and told the mother, "Just as I thought. The rash is a reaction to the Ceclor your daughter has been taking. Stop the Ceclor and the rash will go away." The mother was surprised because she had always thought that an allergic reaction to a drug would start with the first dose. The physician told her that an allergic reaction could begin at any time, even after taking the same drug numerous times.

The child's ears were checked and there was no sign of infection. The girl and her mother were discharged after receiving a prescription for an antihistamine to help control the itching.

- What category of antibiotic does Ceclor fall under?
- What is the mechanism underlying the allergic response to an antibiotic?

Case File Wrap-Up appears at the end of the chapter.

10.1 Principles of Antimicrobial Therapy

A hundred years ago in the United States, one out of three children was expected to die of an infectious disease before the age of 5. Early death or severe lifelong debilitation from scarlet fever, diphtheria, tuberculosis, meningitis, and many other bacterial diseases was a fearsome yet undeniable fact of life to most of the world's population. The introduction of modern drugs to control infections in the 1940s was a medical revolution that has added significantly to the life span and health of humans. It is no wonder that, for many years, antibiotics were regarded as miracle drugs. But even though antimicrobial drugs have greatly reduced the incidence of certain infections, they have definitely not eradicated infectious disease and probably never will. In fact, we are dangerously close to a postantibiotic era, where the drugs we have are no longer effective.

The goal of antimicrobial chemotherapy is deceptively simple: Administer a drug to an infected person, which destroys the infective agent without harming the host's cells. In actuality, this goal is rather difficult to achieve, because many (often contradictory) factors must be taken into account. The ideal drug should be easy to administer, yet be able to reach the infectious agent anywhere in the body; be toxic to the infectious agent, while being nontoxic to the host. It should remain active in the body as long as needed, yet be safely and easily broken down and excreted. Additionally, microbes in biofilms often require different drugs than when they are not in biofilms. In short, the perfect drug does not exist—but by balancing drug characteristics against one another, a satisfactory compromise can often be achieved **(table 10.1)**.

Antimicrobial agents are characterized with regard to their origin, range of effectiveness, and whether they are naturally produced or chemically synthesized. A few of the more important terms you will encounter are found in **table 10.2**.

In this chapter, we describe different types of antimicrobial drugs, their mechanism of action, and the types of microbes on which they are effective. The organ system chapters 16 through 21 list specific disease agents and the drugs used to treat them.

Scarlet fever is an uncommon sequel to strep throat. It is treatable with antibiotics. Strawberry tongue is one of its symptoms.

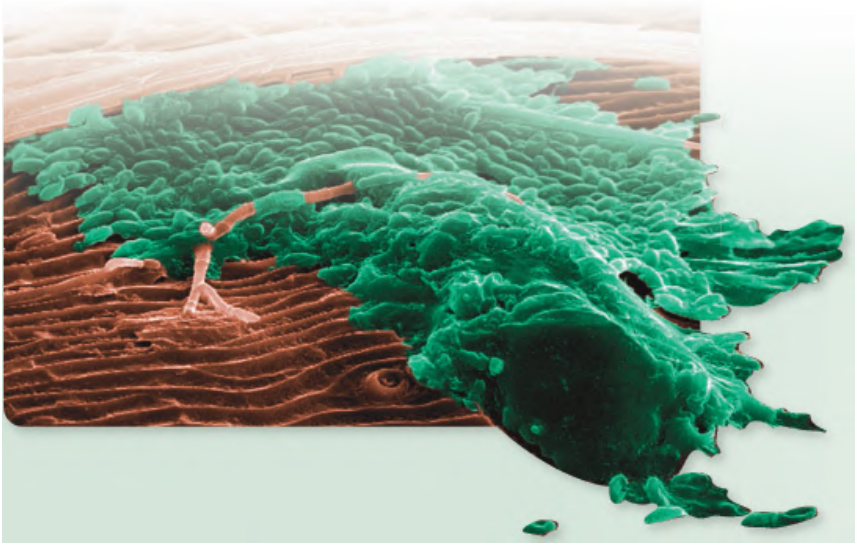
©BSIP/Universal Images Group/Getty Images

NCLEX® PREP

1. An RN is caring for a 26-year-old male who is being treated for meningococcal disease. His family members have been started on an antibiotic to prevent them contracting the disease. This is an example of
   a. *chemotherapy*.
   b. *prophylaxis*.
   c. *herd immunity*.
   d. *host defense*.

Table 10.1 Characteristics of the Ideal Antimicrobial Drug

• Toxic to the microbe but nontoxic to host cells
• Microbicidal rather than microbistatic
• Relatively soluble; functions even when highly diluted in body fluids
• Remains potent long enough to act and is not broken down or excreted prematurely
• Does not lead to the development of antimicrobial resistance
• Complements or assists the activities of the host's defenses
• Remains active in tissues and body fluids
• Readily delivered to the site of infection
• Reasonably priced
• Does not disrupt the host's health by causing allergies or predisposing the host to other infections



Bacterial biofilm formed on the surface of a spider.
Source: CDC/Janice Haney Carr

Table 10.2 Terminology of Antimicrobials

Prophylaxis	Use of a drug to prevent infection of a person at risk
Antimicrobial Chemotherapy	The use of drugs to control infection
Antimicrobials	All-inclusive term for any antimicrobial drug, regardless of its origin
Antibiotics	Substances produced by the natural metabolic processes of some microorganisms that can inhibit or destroy other microorganisms; generally, the term is used for drugs targeting bacteria and not other types of microbes
Semisynthetic Drugs	Drugs that are chemically modified in the laboratory after being isolated from natural sources
Synthetic Drugs	Drugs produced entirely by chemical reactions
Narrow-Spectrum (Limited Spectrum)	Antimicrobials effective against a limited array of microbial types—for example, a drug effective mainly against gram-positive bacteria
Broad-Spectrum (Extended Spectrum)	Antimicrobials effective against a wide variety of microbial types—for example, a drug effective against both gram-positive and gram-negative bacteria

The Origins of Antimicrobial Drugs

Nature is a prolific producer of antimicrobial drugs. Antibiotics, after all, are natural metabolic products of bacteria and fungi. By inhibiting the growth of other microorganisms in the same habitat (antagonism), antibiotic producers presumably enjoy less competition for nutrients and space. The greatest numbers of current antibiotics are derived from bacteria in the genera *Streptomyces* and *Bacillus* and from molds in the genera *Penicillium* and *Cephalosporium*. Also, chemists have created new drugs by altering the structure of naturally occurring antibiotics, and they are actively searching for metabolic compounds with antimicrobial effects in species other than bacteria and fungi.

Identifying the Microbe and Starting Treatment

Before actual antimicrobial therapy can begin, it is important that at least three factors be known:

1. the identity of the microorganism causing the infection,
2. the degree of the microorganism's susceptibility (also called sensitivity) to various drugs, and
3. the overall medical condition of the patient.

Identification of infectious agents from body specimens should be attempted as soon as possible. It is especially important that such specimens be taken before any antimicrobial drug is given, before the drug reduces the numbers of the infectious agent. Direct examination of body fluids, sputum, or stool is a rapid initial method for detecting and perhaps even identifying bacteria or fungi. A doctor often begins the therapy on the basis of such immediate findings, or even on the basis of an informed best guess. For instance, if a sore throat appears to be caused by *Streptococcus pyogenes*, the physician might prescribe penicillin, because this species seems to be almost universally sensitive to it so far. If the infectious agent is not or cannot be isolated, epidemiological statistics may be required to predict the most likely agent in a given infection. For example, *Streptococcus pneumoniae* accounts for the majority of cases of bacterial meningitis in children, followed by *Neisseria meningitidis* (discussed in detail in chapter 17).

First mass-produced in 1944, penicillin saved many lives in WWII.
Source: Library of Congress [LC-USZC4-1986]

Testing for the Drug Susceptibility of Microorganisms

Some bacteria require antimicrobial sensitivity testing and some do not. Testing is essential in those groups of bacteria commonly showing resistance, such as *Staphylococcus* species, *Neisseria gonorrhoeae, Streptococcus pneumoniae, Enterococcus faecalis*, and the aerobic gram-negative intestinal bacilli. On the other hand, drug testing in fungal or protozoal infections is difficult and is often unnecessary because the antimicrobial agents generally target all representatives of these groups.

Selecting the right antimicrobial treatment relies on testing the *in vitro* activity of various drugs against the isolated microbe in the laboratory. In general, these tests involve exposing a pure culture of the bacterium to several different drugs and observing the effects of the drugs on growth.

The *Kirby-Bauer* technique is an agar diffusion test that provides useful data on antimicrobial susceptibility. In this test, the surface of a plate of special medium is spread with the test bacterium, and small discs containing a premeasured amount of antimicrobial are dispensed onto the bacterial lawn. After incubation, the zone of inhibition surrounding the discs is measured and compared with a standard for each drug **(table 10.3** and **figure 10.1)**. This profile of antimicrobial sensitivity is called an antibiogram. The Kirby-Bauer procedure is less effective for bacteria that are anaerobic, highly fastidious, or slow-growing (*Mycobacterium*). An alternative diffusion system that provides additional information on drug effectiveness is the E-test **(figure 10.2)**.

More sensitive and quantitative results can be obtained with tube dilution tests. First the antimicrobial is diluted serially in tubes of broth (often miniaturized tubes called wells in 96-well plates). Then each tube is inoculated with a small uniform sample of pure culture, incubated, and examined for growth (turbidity). The smallest concentration (highest dilution) of drug that visibly inhibits growth is called the **minimum inhibitory concentration**, or **MIC**. The MIC is useful in determining the

Penicillins damage bacterial cell walls.
Source: CDC/Janice Haney Carr

Source: CDC/Janice Haney Carr

Table 10.3 Results of a Sample Kirby-Bauer Test

	Zone Sizes (mm) Required for			
Drug	**Susceptibility (S)**	**Resistance (R)**	**Example Results (mm) for *Staphylococcus aureus***	**Evaluation**
Bacitracin	>13	<8	15	S
Chloramphenicol	>18	<12	20	S
Erythromycin	>18	<13	15	I
Gentamicin	>13	<12	16	S
Kanamycin	>18	<13	20	S

Drug	Zone Sizes (mm) Required for Susceptibility (S)	Zone Sizes (mm) Required for Resistance (R)	Example Results (mm) for *Staphylococcus aureus*	Evaluation
Neomycin	>17	<12	12	R
Penicillin G	>29	<20	10	R
Polymyxin B	>12	<8	10	I
Streptomycin	>15	<11	11	R
Vancomycin	>12	<9	15	S
Tetracycline	>19	<14	25	S

R = resistant, I = intermediate, S = sensitive

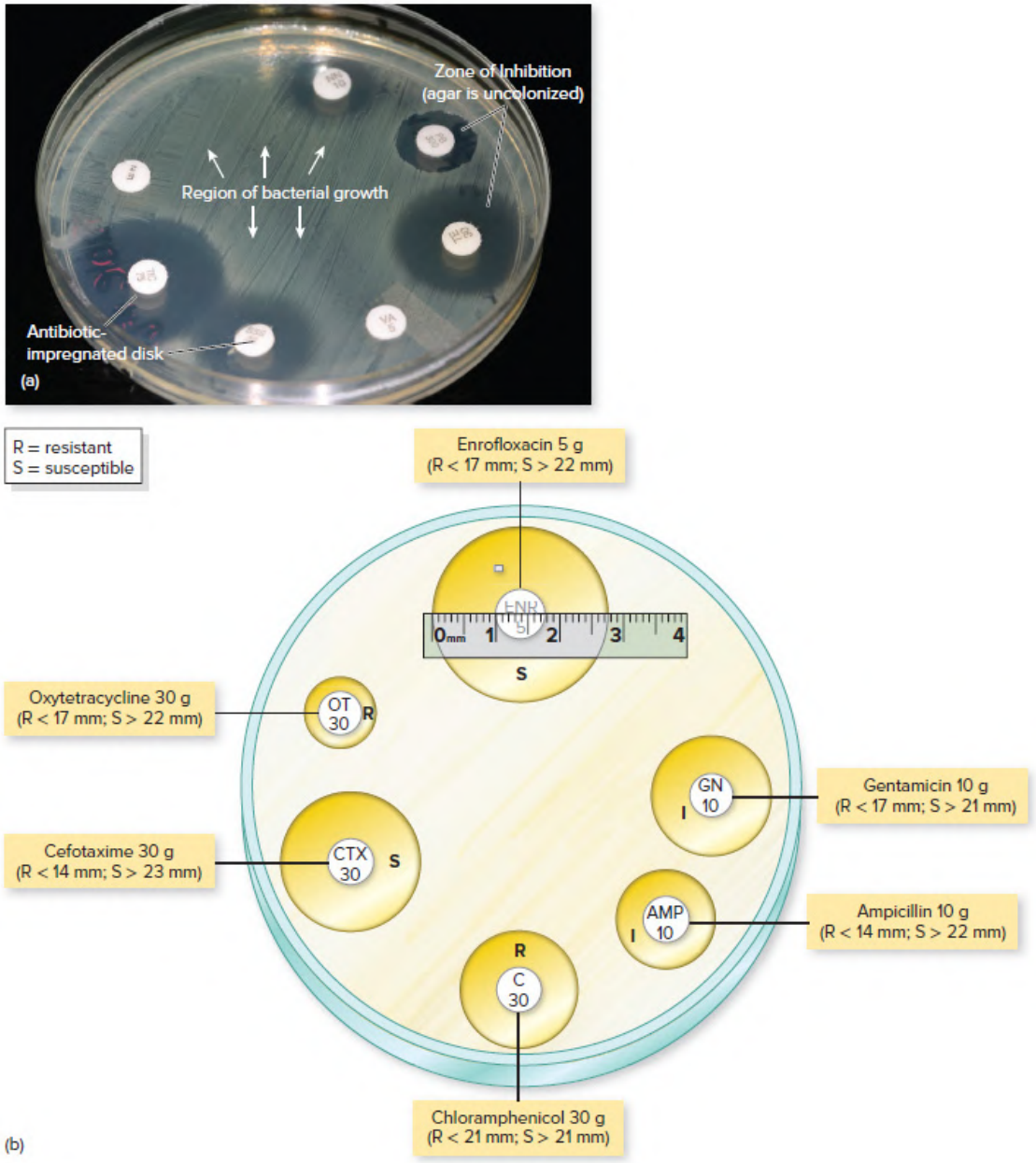


Figure 10.1 **Technique for preparation and interpretation of disc diffusion tests**. **(a)** Photograph of an agar dish with a bacterial isolate distributed evenly all over its surface. After inoculation, the antibiotic-containing disks are dropped onto the agar and it is incubated. The damp agar moistens the discs and antibiotic diffuses out of them in widening arcs. The concentration of the antibiotic decreases as it gets farther from the disc. If the test bacterium is sensitive to a drug, a zone of inhibition develops around its disc. Roughly speaking, the larger the size of this zone, the greater is the bacterium's sensitivity to the drug. The diameter of each zone is measured in millimeters and evaluated for susceptibility or resistance by means of a comparative standard (see table 10.3). **(b)** The principles illustrated in a drawing.

(a) ©McGraw-Hill Education/Don Rubbelke, photographer

smallest effective dosage of a drug and in providing a comparative index against other antimicrobials **(figure 10.3)**. In many clinical laboratories, these antimicrobial testing procedures are performed in automated machines that can test dozens of drugs simultaneously.

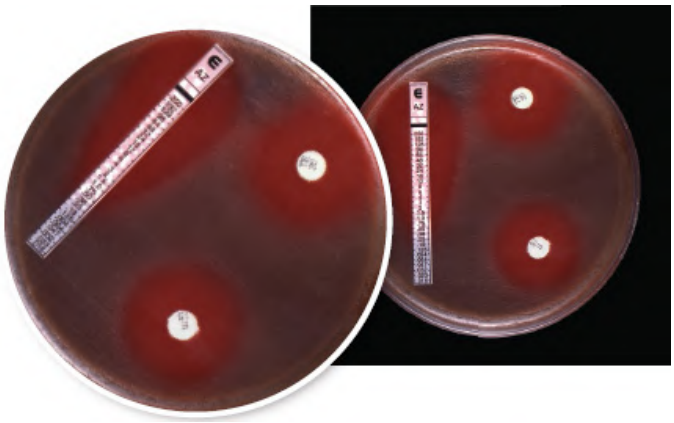
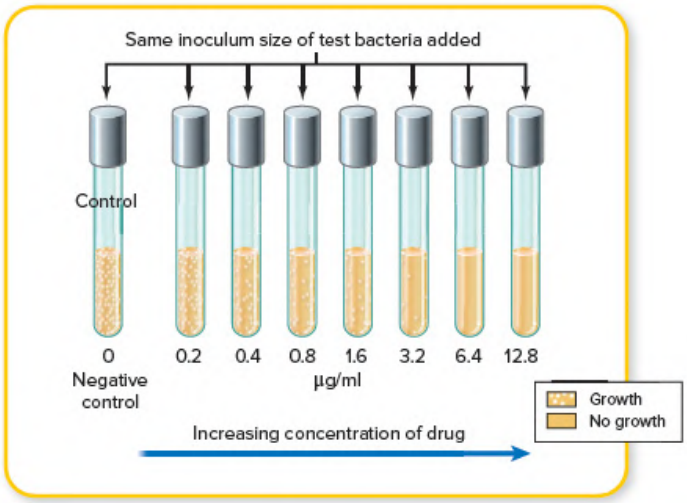
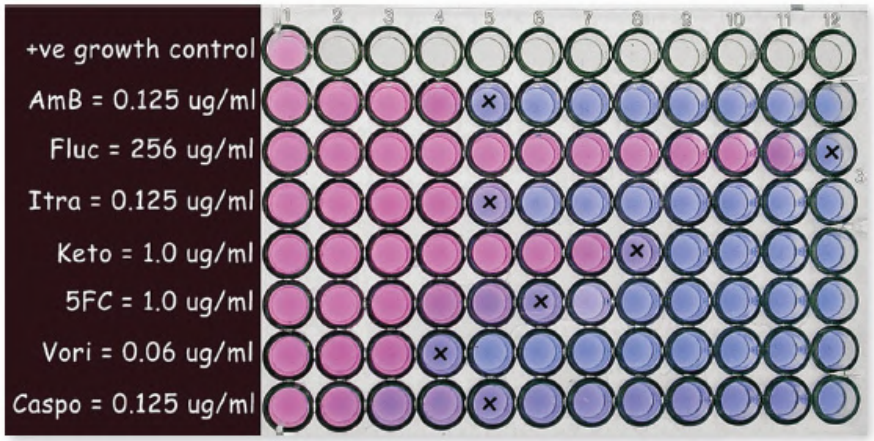


Figure 10.2 **Alternative to the Kirby-Bauer procedure**. Another diffusion test is the E-test, which uses a strip to produce the zone of inhibition. The advantage of the E-test is that the strip contains a gradient of drug calibrated in micrograms. This way, the MIC can be measured by observing the mark on the strip that corresponds to the edge of the zone of inhibition.

Source: CDC/Dr. Richard Facklam



(a)



(b)

Figure 10.3 **Tube dilution test for determining the minimum inhibitory concentration (MIC).** **(a)** The antibiotic is diluted serially through tubes of liquid nutrient from right to left. All tubes are then inoculated with an identical amount of a test bacterium and then incubated. The first tube on the left is a control that lacks the drug and shows maximum growth. The dilution of the first tube in the series that shows no growth (no turbidity) is the MIC. **(b)** Microbroth dilution in a multiwell plate adapted for eukaryotic pathogens. Here, amphotericin B, flucytosine, and several azole drugs are tested on a pathogenic yeast. Pink indicates growth and blue, no growth. Numbers indicate the dilution of the MIC, and Xs show the first well without growth.

(b) Courtesy David Ellis

©Terry Vine/Blend Images LLC

The MIC and Therapeutic Index

The results of antimicrobial sensitivity tests guide the physician's choice of a suitable drug. If treatment has already been started, it is important to determine if the tests bear out the use of that particular drug. Once therapy has begun, it is important to observe the patient's clinical response, because the *in vitro* activity of the drug is not always correlated with its *in vivo* effect. When antimicrobial treatment fails, the failure is due to one or more the following:

- the inability of the drug to diffuse into that body compartment (the brain, joints, skin);
- resistant microbes in the infection that didn't make it into the sample collected for testing;
- an infection caused by more than one pathogen (mixed), some of which are resistant to the drug; or
- in outpatient situations you have to also consider the possibility that the patient did not take the antimicrobials correctly.

If therapy does fail, a different drug, combined therapy, or a different method of administration must be considered.

Because drug toxicity to the host is a concern, it is best to choose the one with high selective toxicity for the infectious agent and low human toxicity. The **therapeutic index (TI)** is defined as the ratio of the dose of the drug that is toxic to humans as compared to its minimum effective (therapeutic) dose. The closer these two figures are (the smaller the ratio), the greater is the potential for toxic drug reactions. For example, a drug that has a therapeutic index of

$$\frac{10\ \mu g/mL\ (\text{toxic dose})}{9\ \mu g/mL\ (\text{MIC})}\ \text{TI} = 1.1$$

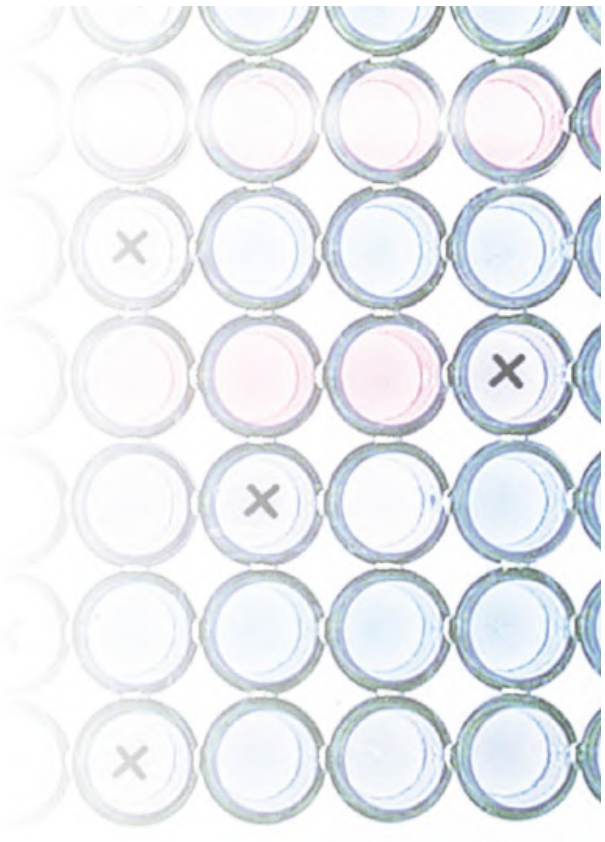
is a riskier choice than one with a therapeutic index of

$$\frac{10\ \mu g/mL}{1 \mu g/mL} \text{TI} = 10$$

When a series of drugs being considered for therapy have similar MICs, the drug with the highest therapeutic index usually has the widest margin of safety.

The physician must also take a careful history of the patient to discover any preexisting medical conditions that will influence the activity of the drug or the response of the patient. A history of allergy to a certain class of drugs precludes the use of that drug and any drugs related to it. Underlying liver or kidney disease will ordinarily require changing the drug therapy, because these organs play such an important part in metabolizing or excreting the drug. Infants, the elderly, and pregnant women require special precautions. For example, age can diminish gastrointestinal absorption and organ function, and most antimicrobial drugs cross the placenta and could affect fetal development.

Patients must be asked about other drugs they are taking, because incompatibilities can result in increased toxicity or failure of one or more of the drugs. For example, the combination of aminoglycosides and cephalosporins can be toxic to kidneys; antacids reduce the absorption of isoniazid; and the interaction of tetracycline or rifampin with oral contraceptives can abolish the contraceptive's effect. Some drug combinations (penicillin with certain aminoglycosides, or amphotericin B with flucytosine) act synergistically, so that reduced doses of each can be used in combined therapy. Patients are also often taking over-the-counter medicines and supplements, many of which can affect the way antimicrobials work.



Courtesy David Ellis

The Art and Science of Choosing an Antimicrobial Drug

Even when all the information is in, the final choice of a drug is not always easy or straightforward. Consider the hypothetical case of an elderly alcoholic patient with pneumonia caused by *Klebsiella* and complicated by diminished liver and kidney function. All drugs must be given by injection because of prior damage to the gastrointestinal lining and poor absorption. Drug tests show that the infectious agent is sensitive to third-generation cephalosporins, gentamicin, imipenem, and azlocillin. The patient's history shows previous allergy to the penicillins, so these would be ruled out. Drug interactions occur between alcohol and the cephalosporins, which are also associated with serious bleeding in elderly patients, so this may not be a good choice. Aminoglycosides such as gentamicin are toxic to the kidneys and poorly cleared by damaged kidneys. Imipenem causes intestinal discomfort, but it has less toxicity and would be a viable choice.

In the case of a cancer patient with severe systemic *Candida* infection, there will be fewer criteria to weigh. Intravenous amphotericin B or fluconazole are the only possible choices, despite drug toxicity and other possible adverse side effects. In a life-threatening situation in which a dangerous chemotherapy is perhaps the only chance for survival, the choices are reduced and the priorities are different.

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While choosing the right drug is an art and a science, requiring the consideration of many different things, the process has been made simpler—or at least more portable—with the advent of smartphones and applications ("apps"). Most doctors now have the information literally at their fingertips, when they pull their smartphones out of their pockets.

10.1 LEARNING OUTCOMES—Assess Your Progress

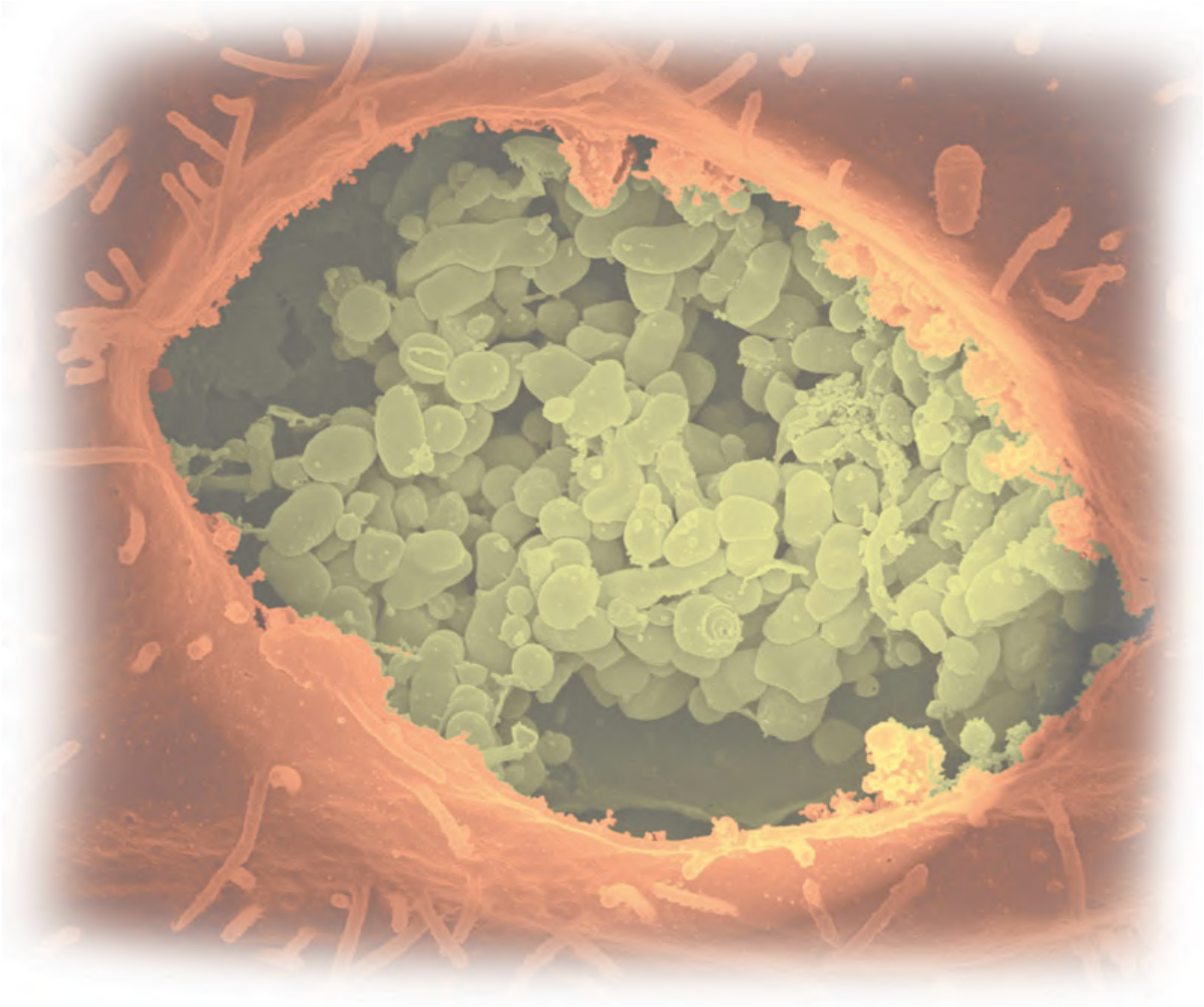
1. State the main goal of antimicrobial treatment.
2. Identify the sources for the most commonly used antimicrobials.
3. Describe two methods for testing antimicrobial susceptibility.
4. Define *therapeutic index*, and identify whether a high or a low index is preferable.

10.2 Interactions Between Drug and Microbe

The goal of antimicrobial drugs is either to disrupt the cell processes or structures of bacteria, fungi, and protozoa or to inhibit virus replication. Many antimicrobial drugs interfere with the function of enzymes required to synthesize or assemble macromolecules, or they destroy structures already formed in the cell. Above all, drugs should be **selectively toxic**, which means they should kill or inhibit microbial cells without simultaneously damaging host tissues. This concept of selective toxicity is central to antibiotic treatment, and the best drugs in current use are those that block the actions or synthesis of molecules in microorganisms but not in vertebrate cells. Examples of drugs with excellent selective toxicity are those that block the synthesis of the cell wall in bacteria (e.g., penicillins). They have low toxicity and few direct effects on human cells because human cells lack the chemical peptidoglycan and are thus unaffected by this action of the antibiotic. Among the most toxic to human cells are drugs that act upon a structure common to both the infective agent and the host cell, such as the cytoplasmic membrane (e.g., amphotericin B, used to treat fungal infections). As the characteristics of the infectious agent become more and more similar to those of the host cell, selective toxicity becomes more difficult to achieve, and undesirable side effects are more likely to occur.

Mechanisms of Drug Action

If the goal of chemotherapy is to disrupt the structure or function of an organism to the point where it can no longer survive, then the first step toward this goal is to identify the structural and metabolic needs of a living cell. Once the requirements



Coxiella burnetii multiplying inside a vacuole of a human cell.
Source: National Institute of Allergy and Infectious Diseases (NIAID)/NIH/USHHS

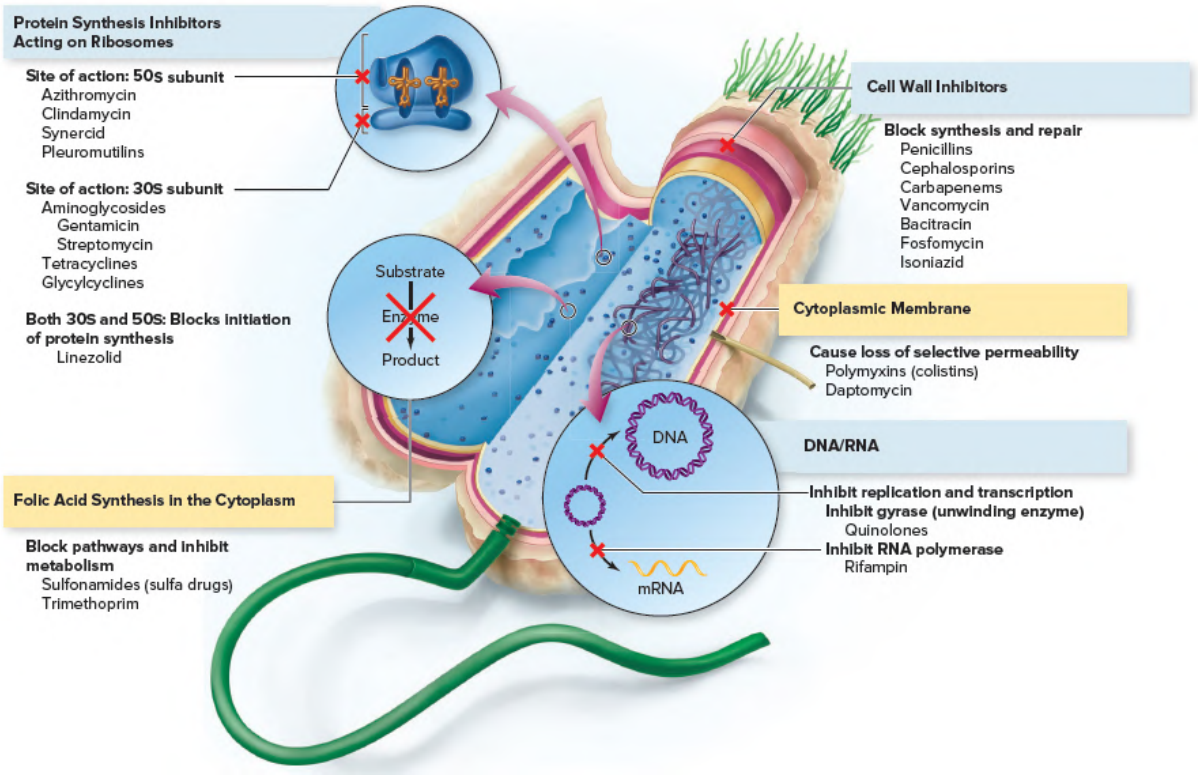


Figure 10.4 **Primary sites of action of antimicrobial drugs on bacterial cells**.

of a living cell have been determined, methods of removing, disrupting, or interfering with these requirements can be used as potential antimicrobial strategies. The metabolism of an actively dividing cell is marked by the production of new cell wall components (in most cells), DNA, RNA, proteins, and cytoplasmic membrane. Consequently, current widely used antimicrobial drugs are divided into categories based on which of these metabolic targets they affect. These categories are outlined in **figure 10.4** and include the following:

1. inhibition of cell wall synthesis,
2. inhibition of nucleic acid (RNA and DNA) structure and function,
3. inhibition of protein synthesis,
4. interference with cytoplasmic membrane structure or function, and
5. inhibition of folic acid synthesis.

As you will see, these categories are not completely discrete, and some effects can overlap. **Table 10.4** describes these categories, as well as common drugs comprising each of these categories.

NCLEX® PREP

2. A patient is admitted to the hospital with pneumonia. A chest x-ray is obtained and a specimen is sent to the laboratory for culture and sensitivity testing. The provider orders antibiotic therapy to begin. Which of the following statements by the RN is appropriate when providing patient education about antibiotic therapy?

a. *"We will be giving you antibiotics because respiratory viruses are common in the community right now and we suspect you have a viral pneumonia."*

b. *"We are starting broad-spectrum and narrow-spectrum antibiotics so we can be sure to treat all the organisms that could be causing your infection."*

c. *"Your chest x-ray results appear to show bacterial pneumonia. As results from the lab specimen return, your antibiotic therapy may be changed."*

d. *"It is important to notify me immediately if you develop nausea or diarrhea as it indicates the antibiotic is not working."*

Table 10.4 Specific Drugs and Their Metabolic Targets

Drug Class/Mechanism of Action	Subgroups	Uses/Characteristics
Drugs That Target the Cell Wall		
Penicillins	Penicillins G and V	Most important natural forms used to treat gram-positive cocci, some gram-negative bacteria (meningococci, syphilis, spirochetes)
	Ampicillin, carbenicillin, amoxicillin	Have a broader spectrum of action, are semisynthetic; used against gram-negative enteric rods
©j4m3z/Getty Images	Nafcillin, cloxacillin	Useful in treating infections caused by some penicillinase-producing bacteria (penicillinase is one type of beta-lactamase, a class of enzymes that destroy the beta-lactam ring in some antibiotics; some bacteria can produce these enzymes, making them resistant to these types of antibiotics)
	Clavulanic acid	Inhibits beta-lactamase enzymes; added to penicillins to increase their effectiveness in the presence of penicillinase-producing bacteria
Cephalosporins	Cefazolin	First generation*; most effective against gram-positive cocci, few gram-negative bacteria
	Cefaclor	Second generation; more effective than first generation against gram-negative bacteria such as *Enterobacter, Proteus*, and *Haemophilus*
	Cephalexin, cefotaxime	Third generation; broad-spectrum, particularly against enteric bacteria that produce beta-lactamases
	Ceftriaxone	Third generation; semisynthetic broad-spectrum drug that treats wide variety of urinary, skin, respiratory, and nervous system infections
	Cefepime	Fourth generation
	Cegtaroline	Fifth generation; used against methicillin-resistant *Staphylococcus aureus* (MRSA) and also against penicillin-resistant gram-positive and gram-negative bacteria
Carbapenems	Doripenem, imipenem	Powerful but potentially toxic; reserved for use when other drugs are not effective
	Aztreonam	Narrow-spectrum; used to treat gram-negative aerobic bacilli causing pneumonia, septicemia, and urinary tract infections; effective for those who are allergic to penicillin
Miscellaneous Drugs That Target the Cell Wall	Bacitracin	Narrow-spectrum; used to combat superficial skin infections caused by streptococci and staphylococci; main ingredient in Neosporin
	Isoniazid	Used to treat *Mycobacterium tuberculosis*, but only against growing cells; used in combination with other drugs in active tuberculosis
	Vancomycin	Narrow-spectrum of action; used to treat staphylococcal infections in cases of penicillin and methicillin resistance or in patients with an allergy to penicillin
	Fosfomycin tromethamine	Phosphoric acid agent; effective as an alternative treatment for urinary tract infection caused by enteric bacteria

Drug Class/Mechanism of Action	Subgroups	Uses/Characteristics
Drugs That Target Protein Synthesis		
Aminoglycosides Insert on sites on the 30S subunit and cause the misreading of the mRNA, leading to abnormal proteins	Streptomycin	Broad-spectrum; used to treat infections caused by gram-negative rods, certain gram-positive bacteria; used to treat bubonic plague, tularemia, and tuberculosis

*New improved versions of drugs are referred to as new "generations."

©Peter Dazeley/Photographer's Choice/Getty Images

Drug Class/Mechanism of Action	Subgroups	Uses/Characteristics
Drugs That Target Protein Synthesis (continued)		
Tetracyclines Block the attachment of tRNA on the A acceptor site and stop further protein synthesis	Tetracycline	Effective against gram-positive and gram-negative rods and cocci, aerobic and anaerobic bacteria, mycoplasmas, rickettsias, and spirochetes
Glycylcyclines	Tigecycline	Newer derivative of tetracycline; effective against bacteria that have become resistant to tetracyclines
Macrolides Inhibit translocation of the subunit during translation (erythromycin)	Erythromycin, clarithromycin, azithromycin	Relatively broad-spectrum, semisynthetic; used in treating ear, respiratory, and skin infections, as well as *Mycobacterium* infections in AIDS patients
Miscellaneous Drugs That Target Protein Synthesis	Clindamycin	Broad-spectrum antibiotic used to treat penicillin-resistant staphylococci, and serious anaerobic infections of the stomach and intestines unresponsive to other antibiotics
	Quinupristin + dalfopristin (Synercid)	A combined antibiotic from the streptogramin group of drugs; effective against *Staphylococcus* and *Enterococcus* species causing endocarditis and surgical infections, including resistant strains
	Linezolid	Synthetic drug from the oxazolidinones; a novel drug that inhibits the initiation of protein synthesis; used to treat antibiotic-resistant organisms such as MRSA and VRE
Drugs That Target Folic Acid Synthesis		
Sulfonamides Interfere with folate metabolism by blocking enzymes required for the synthesis of tetrahydrofolate, which is needed by the cells for folic acid synthesis and eventual production of DNA, RNA, and amino acids	Sulfamethoxazole	Used to treat shigellosis, acute urinary tract infections, certain protozoal infections
	Silver sulfadiazine	Used to treat burns, eye infections (in ointment and solution forms)

Drug Class/Mechanism of Action	Subgroups	Uses/Characteristics
©Ingram Publishing	Trimethoprim	Inhibits the enzymatic step in an important metabolic pathway that comes just before the step inhibited by sulfonamides; trimethoprim often given in conjunction with sulfamethoxazole because of this synergistic effect; used to treat *Pneumocystis jiroveci* in AIDS patients
Drugs That Target DNA or RNA		
Fluoroquinolones Inhibit DNA unwinding enzymes or helicases, thereby stopping DNA transcription	Ciprofloxacin, ofloxacin	Second generation
	Levofloxacin	Third generation; used against gram-positive organisms, including some that are resistant to other drugs
Miscellaneous Drugs That Target DNA or RNA	Rifampin	Limited in spectrum because it cannot pass through the cell envelope of many gram-negative bacilli; mainly used to treat infections caused by gram-positive rods and cocci and a few gram-negative bacteria; used to treat leprosy and tuberculosis
Drugs That Target Cytoplasmic or Cell Membranes		
Polymyxins (colistins) Interact with membrane phospholipids; distort the cell surface and cause leakage of protein and nitrogen bases, particularly in gram-negative bacteria	Polymyxin B	Used to treat drug-resistant *Pseudomonas aeruginosa* and severe urinary tract infections caused by gram-negative rods
	Daptomycin	Most active against gram-positive bacteria

Spectrum of Activity

One of the most useful ways of categorizing antimicrobials, which you have already encountered in the previous section, is to designate them as either **broad-spectrum** or **narrow-spectrum**. Broad-spectrum drugs are effective against more than one group of bacteria, whereas narrow-spectrum drugs generally target a specific group. **Table 10.5** demonstrates that tetracyclines are broad-spectrum, whereas polymyxin and even penicillins are narrow-spectrum agents.

Since penicillin is such a familiar antibiotic, and since the alterations in the molecule over the years illustrate how antibiotics are developed and improved upon, we provide an overview in **table 10.6**. Here you will see that original penicillin was narrow-spectrum and susceptible to microbial counterattacks. Later penicillins were developed to overcome those two limitations.

Unfortunately, we don't seem to be getting it right often enough. A 2016 study by the Centers for Disease Control and Prevention (CDC) and the Pew Charitable Trust looked at the three most common infections in the United States: sinus infections, sore throats, and ear infections. The study found that 48% of these patients were not prescribed the antibiotics recommended by national standards for these infections. While we just discussed reasons the first-line antibiotic might not be prescribed, experts say that only about 20% of patients are likely to have reasons the first-line drug should not be used. Many times the nonrecommended antibiotics are more broad-spectrum than the recommended ones, and are therefore more likely to lead to microbiome disruption and increased antibiotic resistance.

Referring back to table 10.4, you can view details about various antimicrobial drugs based on which of the five major mechanisms they target.

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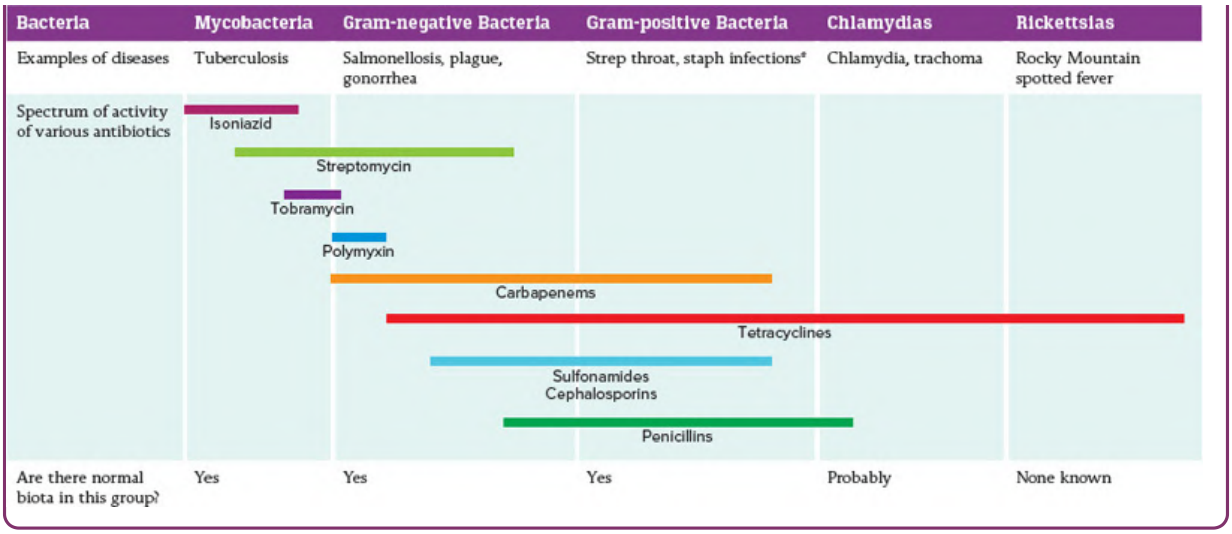
Antibiotics and Biofilms

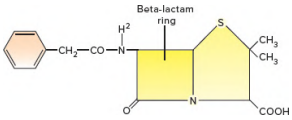
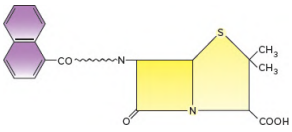
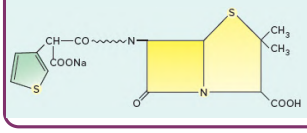
As you read in chapter 6, biofilm inhabitants behave differently than their free-living counterparts. One of the major ways they differ—at least from a medical perspective—is that they are often unaffected by the same antimicrobials that work against them

Table 10.5 Spectrum of Activity for Antibiotics



* Note that some members of a bacterial group may not be affected by the antibiotics indicated, due to acquired or natural resistance. In other words, exceptions do exist.

Table 10.6 Characteristics of Selected Penicillin Drugs

Name	Spectrum of Action	Uses, Advantages	Disadvantages
Penicillin G 	Narrow	Best drug of choice when bacteria are sensitive; low cost; low toxicity	Can be hydrolyzed by penicillinase; allergies occur; requires injection
Penicillin V	Narrow	Good absorption from intestine; otherwise, similar to Penicillin G	Hydrolysis by penicillinase; allergies
Methicillin, nafcillin 	Narrow	Not usually susceptible to penicillinase	Poor absorption; allergies; growing resistance
Ampicillin	Broad	Works on gram-negative bacilli	Can be hydrolyzed by penicillinase; allergies; only fair absorption
Amoxicillin	Broad	Gram-negative infections; good absorption	Hydrolysis by penicillinase; allergies
Azlocillin, mezlocillin, ticarcillin 	Very broad	Effective against *Pseudomonas* species; low toxicity compared with aminoglycosides	Allergies; susceptible to many betalactamases

when they are free-living. When this was first recognized, it was assumed that it was a problem of penetration, that the (often ionically charged) antimicrobial drugs could not penetrate the sticky extracellular material surrounding biofilm organisms. While that is a factor, there is something more important contributing to biofilm resistance: the different phenotype expressed

by biofilm bacteria. When secured to surfaces, they express different genes and therefore have different antibiotic susceptibility profiles.

Years of research have so far not yielded an obvious solution to this problem, though there are several partially successful strategies. One of these involves interrupting the quorum-sensing pathways that mediate communication between cells and may change phenotypic expression. Daptomycin (trade name: Cubicin), a lipopeptide that is effective in deep tissue infections with resistant bacteria, has also shown some success in biofilm infection treatment. Also, some researchers have found that adding DNase to their antibiotics can help with penetration of the antibiotic through the extracellular debris—apparently some of which is DNA from lysed cells.

Many biofilm infections can be found on biomaterials inserted in the body, such as cardiac or urinary catheters. These can be impregnated with antibiotics prior to insertion to prevent colonization. This, of course, cannot be done with biofilm infections of natural tissues, such as the prostate or middle ear.

Interestingly, it appears that chemotherapy with some antibiotics—notably aminoglycosides—can cause bacteria to form biofilms at a higher rate than they otherwise would. Obviously there is much more to come in understanding biofilms and their control.

Agents to Treat Fungal Infections

Because the cells of fungi are eukaryotic, they present special problems in chemotherapy. For one, the great majority of chemotherapeutic drugs are designed to act on bacteria and are generally ineffective in combating fungal infections. For another, the similarities between fungal and human cells often mean that drugs toxic to fungal cells are also capable of harming human tissues. A few agents with special antifungal properties have been developed for treating systemic and superficial fungal infections. Four main drug groups currently in use are the macrolide polyene antibiotics, the azoles, the echinocandins, and allylamines. **Table 10.7** describes in further detail the antifungal drug groups and their actions.

Agents to Treat Protozoal Infections

The enormous diversity among protozoal and helminthic parasites and their corresponding therapies reach far beyond the scope of this textbook; however, a few of the more common drugs are surveyed here and described again for particular diseases in the organ systems chapters.

Antimalarial Drugs: Quinine and Its Relatives

Quinine, extracted from the bark of the cinchona tree, was the principal treatment for malaria for hundreds of years, but it has been replaced by the synthesized quinolones, mainly chloroquine and primaquine, which have less toxicity to humans. Because there are several species of *Plasmodium* (the malaria parasite) and many stages in its life cycle, no single drug is universally effective for every species and stage. Furthermore, the geographical region where the malaria infection was acquired must be taken into account, as different parts of the world harbor strains of the parasite with differing drug resistances. A drug called artemisinin, which comes originally from a plant called sweet wormwood, used for centuries in Chinese traditional medicine, has become the staple for malaria treatment in most parts of the world. It was discovered to be effective against malaria by a woman named Tu Youyou, a Chinese scientist. She was awarded

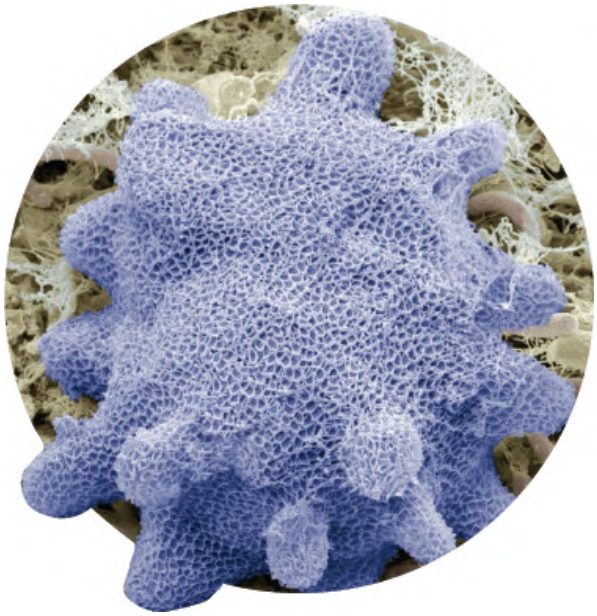
the Nobel Prize in 2015 for her breakthrough. Artemisinin combination therapy (ACT) is now recommended for the treatment of certain types of malaria today. It involves the use of artemisinin with quinine derivatives or other drugs.

Chemotherapy for Other Protozoal Infections

A widely used amoebicide, metronidazole (Flagyl), is effective in treating mild and severe intestinal infections and hepatic disease caused by *Entamoeba histolytica*. Given orally, it also has applications for infections by *Giardia lamblia* and *Trichomonas vaginalis* (described in chapters 20 and 21, respectively). Other drugs with antiprotozoal activities are quinacrine (a quinine-based drug), sulfonamides, and tetracyclines.

Agents to Treat Helminthic Infections

Treating helminthic infections has been one of the most difficult and challenging of all chemotherapeutic tasks. Flukes, tapeworms, and roundworms are much larger parasites than other microorganisms and, being animals, have greater similarities to human physiology. Also, the usual strategy of using drugs to block their reproduction



Korotnevella, an amoeba.
©Science Photo Library/Alamy Stock Photo

Table 10.7 Agents Used to Treat Fungal Infections

Drug Group	Drug Examples	Action
Macrolide polyenes	Amphotericin B (shown above in gray)	• Bind to fungal membranes, causing loss of selective permeability; extremely versatile • Can be used to treat skin, mucous membrane lesions caused by *Candida albicans* • Injectable form of the drug can be used to treat histoplasmosis and *Cryptococcus* meningitis

Drug Group	Drug Examples	Action
Azoles	Ketoconazole, fluconazole, miconazole, and clotrimazole	• Interfere with sterol synthesis in fungi • Ketoconazole—cutaneous mycoses, vaginal and oral candidiasis, systemic mycoses • Fluconazole—AIDS-related mycoses (aspergillosis, *Cryptococcus* meningitis) • Clotrimazole and miconazole—used to treat infections in the skin, mouth, and vagina
Echinocandins	Micafungin, caspofungin	• Inhibit fungal cell wall synthesis • Used against *Candida* strains and aspergillosis
Allylamines	Terbinafine, naftifine	• Inhibit enzyme critical for ergosterol synthesis • Used to treat ringworm and other cutaneous mycoses

is usually not successful in eradicating the adult worms. The most effective drugs immobilize, disintegrate, or inhibit the metabolism in all stages of the life cycle.

Mebendazole and albendazole are broad-spectrum antiparasitic drugs used for several types of roundworm intestinal infestations. These drugs work locally in the intestine to inhibit the function of the microtubules of worms, eggs, and larvae. This means the parasites can no longer utilize glucose, which leads to their demise. The compound pyrantel paralyzes the muscles of intestinal roundworms. Consequently, the worms are unable to maintain their grip on the intestinal wall and are expelled along with the feces by the normal peristaltic action of the bowel. Two newer antihelminthic drugs are praziquantel, a treatment for various tapeworm and fluke infections, and ivermectin, a veterinary drug now used for strongyloidiasis and oncocercosis in humans. Helminthic diseases are described in chapter 20 because these organisms spend a large part of their life cycles in the digestive tract.

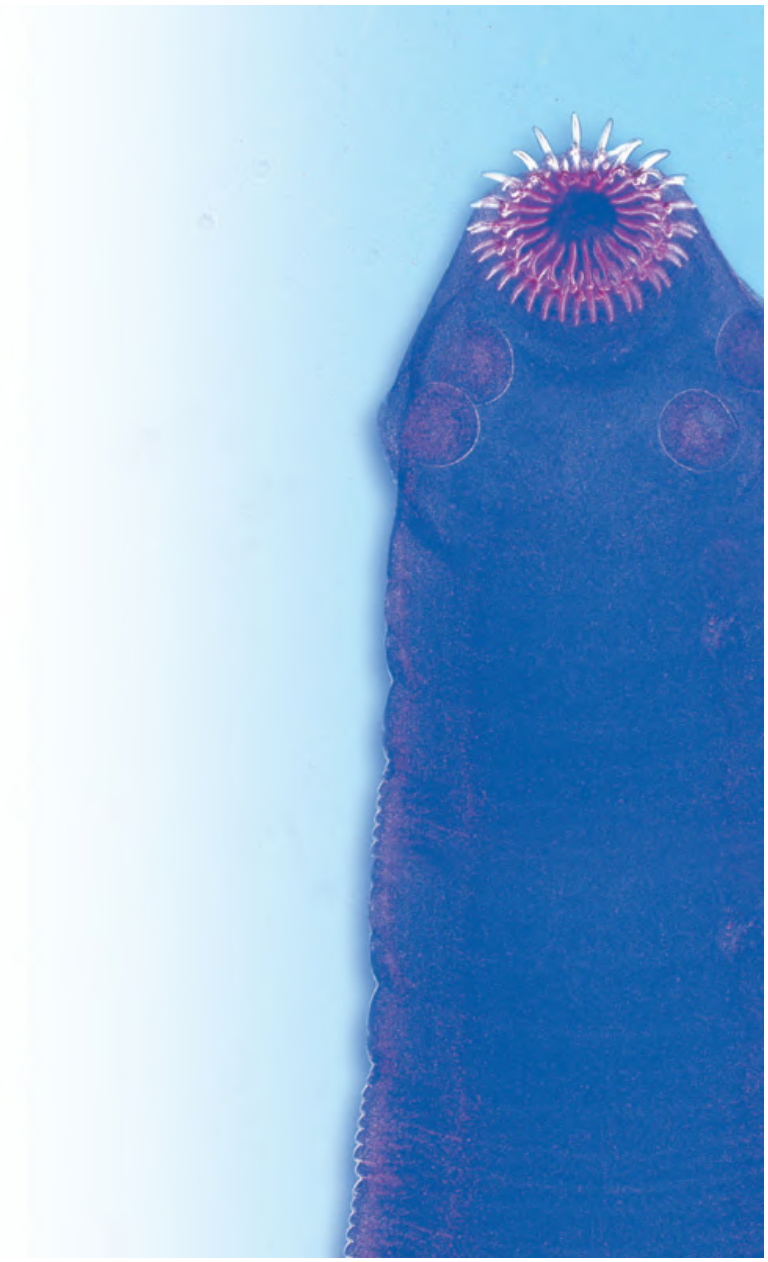
Agents to Treat Viral Infections

The treatment of viral infections presents unique problems. With a virus, we are dealing with an infectious agent that relies upon the host cell for the vast majority of its metabolic functions. With currently used drugs, disrupting viral metabolism requires that we disrupt the metabolism of the host cell to a much greater extent than is desirable. Put another way, selective toxicity with regard to viral infection is difficult to achieve because a single metabolic system is responsible for the well-being of both virus and host. Although viral diseases such as measles, mumps, and hepatitis are routinely prevented by the use of effective vaccinations, epidemics of AIDS, influenza, and even the "commonness" of the common cold attest to the need for more effective medications for the treatment of viral pathogens.

The currently used antiviral drugs were developed to target specific points in the infectious cycle of viruses. Three major modes of action are as follows:

1. barring penetration of the virus into the host cell,
2. blocking the transcription and translation of viral molecules, and
3. preventing the maturation of viral particles.

Table 10.8 presents an overview of antivirals from each of these categories. Very few viral infections have effective *treatments*. Currently only infections with HIV, hepatitis B and C, and herpesviruses are routinely treated. Influenza drugs are available but are not highly effective.



Tapeworm
Source: NHPA/M. I. Walker

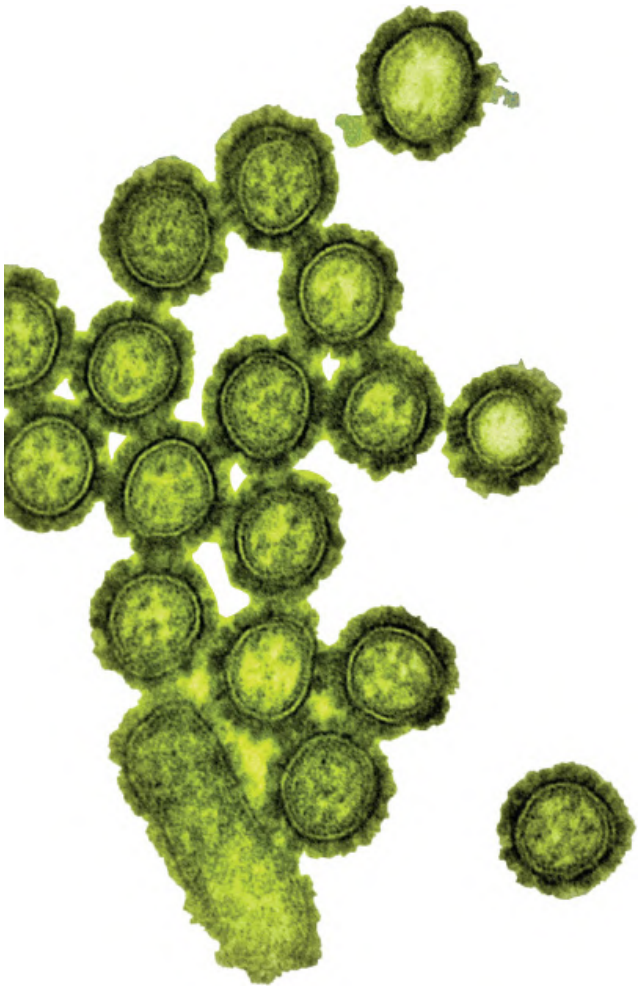
Table 10.8 Actions of Antiviral Drugs

Mode of Action	Examples	Effects of Drug
Inhibition of Virus Entry Receptor/fusion/uncoating inhibitors	Enfuvirtide (Fuzeon®)	Blocks HIV infection by preventing the binding of viral proteins to cell receptor, thereby preventing fusion of virus with cell
	Amantadine (Symmetrel®)	Blocks entry of influenza virus by interfering with fusion of virus with cell membrane

Mode of Action	Examples	Effects of Drug
Inhibition of Nucleic Acid Synthesis	Acyclovir (Zovirax®), other "cyclovirs," vidarabine	Nucleoside analogs; take the place of actual nucleosides and terminate DNA replication in **herpesviruses**
	Ribavirin	Purine analog, used for respiratory syncytial virus (RSV) and some hemorrhagic fever viruses
	Zidovudine (AZT), lamivudine (3TC), didanosine (ddI), zalcitabine (ddC), and stavudine (d4T)	Nucleotide analog reverse transcriptase (RT) inhibitors; stop the action of reverse transcriptase in HIV, blocking viral DNA production
	Nevirapine, efavirenz, delavirdine	Nonnucleotide analog reverse transcriptase inhibitors; attach to HIV RT binding site, stopping its action
Inhibition of Viral Assembly/Release	Indinavir, saquinavir	Protease inhibitors; insert into HIV protease, stopping its action and resulting in inactive noninfectious viruses
	Zanamivir (Relenza®) and oseltamivir (Tamiflu®)	Neuraminidase inhibitors; block exit of influenza viruses from host cells

10.2 LEARNING OUTCOMES—Assess Your Progress

5. Explain the concept of selective toxicity.
6. List the five major targets of antimicrobial agents.
7. Identify which categories of drugs are most selectively toxic and why.
8. Distinguish between broad-spectrum and narrow-spectrum antimicrobials, and explain the significance of the distinction.
9. Identify the microbes against which the various penicillins are effective.
10. Explain the mode of action of penicillinases and their role in treatment decisions.
11. Identify two antimicrobials that act by inhibiting protein synthesis.
12. Explain how drugs targeting folic acid synthesis work.
13. Identify one example of a fluoroquinolone.
14. Describe the mode of action of drugs that target the cytoplasmic or cell membrane.
15. Discuss how treatments of biofilm and nonbiofilm infections differ.
16. Name the four main categories of antifungal agents.
17. Explain why antiprotozoal and antihelminthic drugs are likely to be more toxic than antibacterial drugs.
18. List the three major targets of action of antiviral drugs.



The influenza virus
Source: National Institute of Allergy and Infectious Diseases (NIAID)/NIH/USHHS

10.3 Antimicrobial Resistance

One unfortunate outcome of the use of antimicrobials is the development of microbial **drug resistance**, an adaptive response in which microorganisms begin to tolerate an amount of drug that would ordinarily be inhibitory. The ability to circumvent or inactivate antimicrobial drugs is due largely to the genetic versatility and adaptability of microbial populations. The property of drug resistance can be intrinsic as well. Resistance is called intrinsic when it is a fixed trait of a microbe. For example, all microbes are intrinsically resistant to antibiotics they themselves produce. Of much greater importance is the

acquisition of resistance to a drug by a microbe that was previously sensitive to the drug. In our context, the term *drug resistance* will refer to this last type of acquired resistance.

How Does Drug Resistance Develop?

Contrary to popular belief, antibiotic resistance is an ancient phenomenon. In 2012, 93 bacterial species were discovered in a cave in New Mexico that had been cut off from the surface for

millions of years. Most of these species were found to have resistance to multiple antibiotics—antibiotics naturally produced by other microbes. Because most of our oldest therapeutically used antibiotics are natural products from fungi and bacteria, resistance to them has been a survival strategy for *other* microbes for as long as the microbes have been around. The scope of the problem in terms of using the antibiotics as treatments for humans became apparent in the 1980s and 1990s, when scientists and physicians observed treatment failures on a large scale. What the New Mexico data and other recent findings tell us is that the acquisition of drug resistance is not always a result of exposure to the drug. This adds another dimension to the efforts to prolong antibiotic effectiveness, which so far have focused on limiting the amount of antibiotic in the environment. We see now that this is important but not enough to prevent microorganisms from developing resistance altogether.

Whether antibiotics are present or not, microbes become newly resistant to a drug after one of the following two events occurs:

1. spontaneous mutations in critical chromosomal genes, or
2. acquisition of entire new genes or sets of genes via horizontal transfer from another species.

There may be a third mechanism of acquiring resistance to a drug, which is a phenotypic, not a genotypic, adaptation. Recent studies suggest that bacteria can "go to sleep" when exposed to antibiotics, meaning they will slow or stop their metabolism so that they cannot be harmed by the antibiotic. They can then rev back up after the antibiotic concentration decreases. Sometimes these bacteria are called "persisters." (This is one reason biofilm bacteria are less susceptible to antibiotics than free-living bacteria are.)

Also, some fungi have an additional option for becoming antibiotic-resistant, as discovered in 2014. In these species of fungi, a small regulatory RNA known as interfering RNA—or RNAi—has been found to bind to a genetic sequence temporarily. When it is bound, the gene is silenced and the target of the antibiotic is not manufactured by the fungus, thus rendering it temporarily resistant to that drug. This provides the fungus more flexibility, allowing it to express the gene later when the antibiotic is no longer present. This reversible mechanism is called an **epigenetic** event, and the gene silencing is called an **epimutation**. In the next sections, we will focus on the two genetic changes that can result in acquired resistance.

Drug resistance that is found on chromosomes usually results from spontaneous random mutations in bacterial populations. The chance that such a mutation will be advantageous is minimal, and the chance that it will confer resistance to a specific drug is lower still. Nevertheless, given the huge numbers of microorganisms in any population and the constant rate of mutation, such mutations do occur. The end result varies from slight changes in microbial sensitivity, which can be overcome by larger doses of the drug, to complete loss of sensitivity.

Resistance occurring through horizontal transfer originates from plasmids called resistance (R) factors that are transferred through conjugation, transformation, or transduction. Such traits are "lying in wait" for an opportunity to be expressed and to confer adaptability on the species. Many bacteria also maintain transposable drug resistance sequences (transposons) that are duplicated and inserted from one plasmid to another or from a plasmid to the chromosome. Chromosomal genes and plasmids containing codes for drug resistance are often faithfully replicated and inherited by all subsequent progeny. This sharing of resistance genes accounts for the rapid proliferation of drug-resistant species. As you have read in earlier chapters, gene

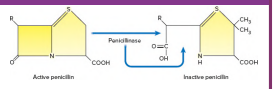
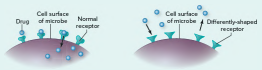
transfers are extremely frequent in nature, with genes coming from totally unrelated bacteria, viruses, and other organisms living in the body's normal biota and the environment.

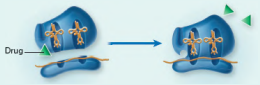
Conjugating bacteria
©L. Caro/SPL/Science Source

Specific Mechanisms of Drug Resistance

Mutations and horizontal transfer, just described, result in mutants that have acquired one of several mechanisms of drug resistance. The actual changes that take place inside the cell as a result of these gene changes mostly fall into five categories **(table 10.9)**. Think of the two pathways to acquiring resistance as the "how" and these five mechanisms as the "what."

Table 10.9 Mechanisms of Drug Resistance

Mechanism	Example	
New enzymes are synthesized, inactivating the drug (occurs when new genes are acquired).	Bacterial exoenzymes called beta-lactamases or penicillinases hydrolyze the betalactam ring structure of some penicillins and cephalosporins, rendering the drugs inactive.	
Permeability or uptake of the drug into the bacterium is decreased (occurs via mutation).		
Drug is immediately eliminated (occurs through the acquisition of new genes).	Many bacteria possess multidrug-resistant (MDR) pumps that actively transport drugs out of cells, conferring drug resistance on many gram-positive and gram-negative pathogens.	

Mechanism	Example	
Binding sites for drugs are decreased in number and/or affinity (occurs via mutation or through the acquisition of new genes).	Erythromycin and clindamycin resistance is associated with an alteration on the 50S ribosomal binding site.	
An affected metabolic pathway is shut down, or an alternative pathway is used (occurs via mutation of original enzymes).	Sulfonamide and trimethoprim resistance develop when microbes deviate from the usual patterns of folic acid synthesis.	

Natural Selection and Drug Resistance

So far, we have been considering drug resistance at the cellular and molecular levels, but its full impact is felt only if this resistance occurs throughout the cell population. Let us examine how this might happen and its long-term therapeutic consequences.

Any large population of microbes is likely to contain a few individual cells that are already drug resistant because of prior mutations or transfer of plasmids **(figure 10.5)**. While we now know that many things can cause these "odd balls" to start overtaking the population, one of the most reliable ways to make this happen is for the correct antibiotic to be present. Sensitive individuals are inhibited or destroyed, and resistant forms survive and proliferate. During subsequent population growth, offspring of these resistant microbes will inherit this drug resistance. In time, the replacement population will have a preponderance of the drug-resistant forms and can eventually become completely resistant (figure 10.5). In ecological terms, the environmental factor (in this case, the drug) has put selection pressure on the population, allowing the more "fit" microbe (the drug-resistant one) to survive, and the population has evolved to a condition of drug resistance.

An Urgent Problem

Textbooks generally avoid using superlatives and exclamation points. But the danger of antibiotic resistance can hardly be overstated. The CDC issued a "Threat Report" about this issue for the first time in 2013, and they continue to monitor the situation, which they label "potentially catastrophic." Now it is such a large problem that an economist recently predicted that worldwide deaths from antibiotic-resistant microbes will surpass deaths from cancer by 2050. This crisis has the world's attention. In September 2016 a meeting of the General Assembly of the United Nations was held on this topic, only the fourth time in history a health issue was the topic of such a meeting. (The other issues were HIV, Ebola, and noncommunicable diseases.)

Even though the antibiotic era began less than 80 years ago, we became so confident it would be permanent that we may have forgotten what it was like before antibiotics were available. Certain types of pneumonia had a 50% fatality rate. Strep throat could turn deadly overnight. Infected wounds often required amputations or led to death. Yet the effectiveness of our currently available antibiotics is declining, in some cases very rapidly. There is a real possibility that we will enter a postantibiotic era, in which some infections will be untreatable. In 2015 the World Health Organization adopted a yearlong campaign to combat antibiotic resistance, due to the urgency of the situation.

The United Nations building in New York City
©Fuse/Getty Images

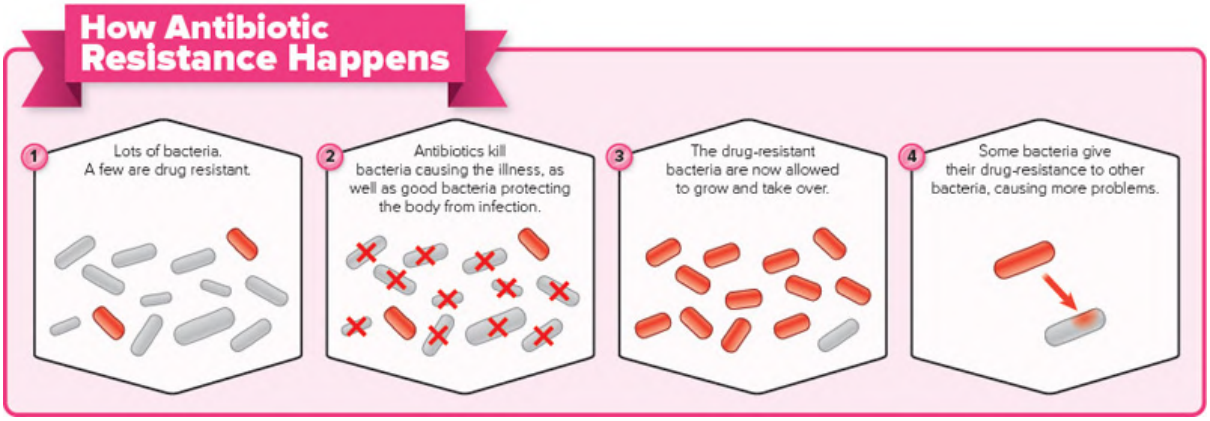


Figure 10.5 **How antibiotic resistance happens**.

New and effective antibiotics have been slow to come to market. There are a variety of reasons for this, including the economic reality that antibiotics (taken in short courses) are not as lucrative for drug manufacturers as drugs for chronic diseases, which must often be taken for life, even though they are just as time-consuming and expensive to develop. Policy-makers are starting to create incentives for the discovery and manufacture of new antibiotics.

The CDC has categorized resistant bacteria into three groups, termed "hazard levels." The three hazard levels are *concerning, serious*, and *urgent*. We will look at them individually in the disease chapters later in the book.

Urgent Threats

- *Clostridium difficile* (*C. diff*)
- Carbapenem-resistant Enterobacteriaceae (CRE)
- Drug-resistant *Neisseria gonorrhoeae*

Serious Threats

- Multidrug-resistant *Acinetobacter*
- Drug-resistant *Campylobacter*
- Fluconazole-resistant *Candida* (a fungus)

- Extended spectrum β-lactamase producing Enterobacteriaceae (ESBLs)
- Vancomycin-resistant *Enterococcus* (VRE)
- Multidrug-resistant *Pseudomonas aeruginosa*
- Drug-resistant non-typhoidal *Salmonella*
- Drug-resistant *Salmonella* typhi
- Drug-resistant *Shigella*
- Methicillin-resistant *Staphylococcus aureus* (MRSA)
- Drug-resistant *Streptococcus pneumoniae*
- Drug-resistant tuberculosis

Concerning Threats

- Vancomycin-resistant *Staphylococcus aureus* (VRSA)
- Erythromycin-resistant Group A *Streptococcus*
- Clindamycin-resistant Group B *Streptococcus*

In the United States alone, 2 million people a year become infected with resistant bacteria, and at least 23,000 deaths are attributed to them.

In the fall of 2015 bacteria containing a gene (*mcr-1*) that makes them resistant to colistin (also known as polymyxin), which is considered a last-resort antibiotic for bacteria that are multiply-drug resistant, were found in China. Subsequently they have been found in humans and animals in the United States. The gene can easily be spread to other bacteria. This raises the possibility of the spread of infections that can no longer be treated by any drug. The CDC is monitoring the spread of this gene closely for that reason.

New Approaches to Antimicrobial Therapy

The pace of antibiotic discovery and creation was rapid in the few decades after penicillin revolutionized the practice of medicine. But new antibiotics stopped entering the pipeline in the latter part of the 20th century. Now that we are in a crisis situation with antibiotic resistance, researchers are taking novel approaches to developing new drugs that they hope can replace, and improve upon, the ones that are losing their effectiveness.

RNA Interference

RNA interference, you recall from chapter 8, refers to small pieces of RNA that regulate the expression of genes. This is being exploited in attempts to shut down the metabolism of pathogenic microbes. There have been several human trials of RNA

interference, including trials to evaluate the effectiveness of synthetic RNAs in treating hepatitis C and respiratory syncytial virus.

Defense Peptides

Other researchers are looking into proteins called defense peptides. Host defense peptides are peptides of 20 to 50 amino acids that are secreted as part of the mammalian innate immune system. They have names such as defensin, magainins, and protegrins. Some bacteria produce

similar peptides. These are called bacteriocins and lantibiotics. Both host and bacterial defense peptides have multiple activities against bacteria—inserting in their membranes and also targeting other structures in the cells. Other peptides target viruses. Fuzeon, a drug used in HIV infections, is a peptide that inhibits fusion of the virus to human cells.

Researchers have also had success with artificially engineered peptides. A molecule called SNAPP—structurally nanoengineered antimicrobial peptide polymer—has shown promise in killing multiple types of bacteria that have become resistant to other drugs.

CRISPR

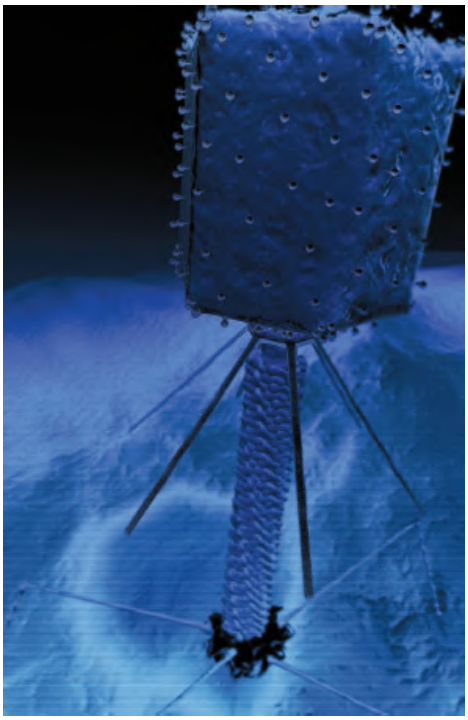
The CRISPR system, which you read about in chapter 8, provides the first true hope that science may be capable of overcoming antibiotic resistance. CRISPR is a system found in bacteria that can cause very specific cuts in genes. Researchers turned this into a powerful genetic engineering tool and one possible way to use it is as a method to treat antibiotic-resistant infections, together with an antibiotic. Scientists in Israel delivered CRISPR packaged in a phage vector that only infected the offending bacteria. CRISPR then destroyed the genes that were causing the resistance, allowing the antibiotics to be effective.

Drugs from Noncultivable Bacteria

Our current antibiotics were either derived from or inspired by natural chemicals secreted by microorganisms themselves. But, considering the fact that more than 99% of all microbes are noncultivable, some researchers have reasoned that there could be a treasure chest of new antimicrobial substances in soil and elsewhere in the environment that will never be identified with traditional methods. Researchers in Massachusetts designed a unique chamber in which to allow soil bacteria to grow in their natural environment and then harvested an antibiotic they call teixobactin. It inhibits cell wall synthesis using a unique mechanism. Most strikingly, bacteria seem unable to—or at least very slow to—develop resistance to the drug.

Bacteriophages

Sometimes the low-tech solution can be the best one. Before antibiotics were discovered and developed in the 1930s and 1940s, scientists in the former Soviet Union and other regions were treating patients with mixtures of bacteriophages, whose host bacterium was presumed to be causing the illness. The antibiotic era put a temporary end to serious consideration of this strategy, but it is receiving renewed attention as we search for alternatives to antibiotics. One recent human trial used a mixture of bacteriophages specific for *Pseudomonas aeruginosa* to treat ear infections caused by the bacterium. These infections are found in the form of biofilms and have been extremely difficult to treat. The phage preparation called Biophage-PA successfully treated patients who had experienced long-term antibiotic-resistant infections. Other researchers are incorporating phages into wound dressings. One clear advantage to bacteriophage treatments is the extreme specificity of the phages—only one species of bacterium is affected, leaving the normal inhabitants of the body, and the body itself, alone. Phage treatments are currently licensed to decontaminate food and food-processing facilities, as well as to treat poultry for *Salmonella*.



©MedicalRF.com

Helping Nature Along

Other novel approaches to controlling infections include the use of **probiotics** and **prebiotics**. Probiotics are preparations of live microorganisms that are fed to animals and humans to improve the intestinal biota. This can serve to replace microbes lost

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3. An RN in a family medicine office provides education for ill patients being prescribed antibiotic therapy. Consumption of yogurt is encouraged as it is a _____, containing live microorganisms that can improve intestinal biota.

a. *prebiotic*
b. *natural antimicrobial*
c. *superbiotic*
d. *probiotic*

Medical Moment

Why Do Antibiotics Cause Diarrhea?

You are prescribed an antibiotic for strep throat. You take it as prescribed. Then, suddenly, you have diarrhea to go along with your fever and sore throat—just what you didn't need!

Why do we often get diarrhea when we take antibiotics? We have resident microbial biota in our intestines. We can refer to these helpful bacteria as "good" bacteria and the potentially illness-causing bacteria as "bad" bacteria.

When we take antibiotics, we upset the delicate balance between numbers of good and bad bacteria so that the bad begin to outnumber the good. This may result in diarrhea, an unpleasant side effect of many antibiotics.

Q. Is postantibiotic diarrhea considered drug toxicity, an allergic reaction, or neither?

Answer in Appendix B.

Figure 10.6 Examples of probiotic grocery items.
©Kathleen Talaro

during antimicrobial therapy or simply to augment the biota that is already there. This is a slightly more sophisticated application of methods that have long been used in an empiric fashion, for instance, by people who consume yogurt because of the beneficial microbes it contains. Recent years have seen a huge increase in the numbers of probiotic products sold in ordinary grocery stores **(figure 10.6)**. Experts generally find these products safe, and in some cases they can be effective. Probiotics are thought to be useful for the management of food allergies. Their role in the stimulation of mucosal immunity is also being investigated.

Prebiotics are nutrients that encourage the growth of beneficial microbes in the intestine. For instance, certain sugars such as fructans are thought to encourage the growth of the beneficial *Bifidobacterium* in the large intestine and to discourage the growth of potential pathogens.

A technique that is gaining mainstream acceptance is the use of fecal transplants in the treatment of recurrent *Clostridium difficile* infection and ulcerative colitis. This procedure involves the transfer of feces from healthy patients via colonoscopy. This is, in fact, just an adaptation of probiotics. But instead of a few beneficial bacterial species being given orally with the hope that they will establish themselves in the intestines, a rich microbiota is administered directly to the site it must colonize—the large intestine. Work is also underway to develop a pill containing the appropriate species, with a coating that will enable it to remain intact as it traverses the stomach and small intestine and releases the bacteria in the lower intestine. Clearly, the use of these agents is a different type of antimicrobial strategy than we are used to, but it may have its place in a future in which traditional antibiotics are more problematic.

10.3 LEARNING OUTCOMES—Assess Your Progress

19. Discuss two main ways that microbes acquire antimicrobial resistance.
20. List five cellular or structural mechanisms that microbes use to resist antimicrobials.
21. Discuss at least two novel antimicrobial strategies that are under investigation.

10.4 Interactions Between Drug and Host

Until now, this chapter has focused on the interaction between antimicrobials and the microorganisms they target. During an infection, the microbe is living in or on a host; therefore, the drug is administered to the host even though its target is the microbe. Therefore, the effect of the drug on the host must always be considered.

It is estimated that at least 5% of all persons taking an antimicrobial drug experience some type of serious adverse reaction to it. The major side effects of

An allergic reaction to an antimicrobial medication.
Source: CDC

drugs fall into one of three categories: direct damage to tissues through toxicity, allergic reactions, and disruption in the balance of normal microbial biota. The damage incurred by antimicrobial drugs can be short term and reversible or permanent, and it ranges in severity from cosmetic to lethal.

Toxicity to Organs

Drugs most often adversely affect the following organs: the liver (hepatotoxic), kidneys (nephrotoxic), gastrointestinal tract, cardiovascular system and blood-forming tissue (hemotoxic), nervous system (neurotoxic), respiratory tract, skin, bones, and teeth. The potential toxic effects of drugs on the body, along with the responsible drugs, are detailed in **table 10.10**.

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4. Which medication could be used against gram-negative bacteria, gram-positive bacteria, chlamydias, and rickettsias?
   a. *tobramycin*
   b. *penicillin*
   c. *tetracyclines*
   d. *cephalosporins and sulfonamides*

Table 10.10 Major Adverse Toxic Reactions to Common Drug Groups

Antimicrobial Drug	Primary Damage or Abnormality Produced
Antibacterials	
Penicillin G	Rash, hives, watery eyes
Carbenicillin	Abnormal bleeding
Ampicillin	Diarrhea and enterocolitis
Cephalosporins	Inhibition of platelet function Decreased circulation of white blood cells; nephritis
Tetracyclines	Diarrhea and enterocolitis Discoloration of tooth enamel Reactions to sunlight (photosensitivity)
Sulfonamides	Formation of crystals in kidney; blockage of urine flow Hemolysis Reduction in number of red blood cells
Polymyxin (colistin)	Kidney damage Weakened muscular responses
Quinolones (ciprofloxacin, norfloxacin)	Headache, dizziness, tremors, GI distress
Rifampin	Damage to hepatic cells Dermatitis
Antifungals	
Amphotericin B	Disruption of kidney function
Flucytosine	Decreased number of white blood cells
Antiprotozoal Drugs	
Metronidazole	Nausea, vomiting
Antihelminthics	
Pyrantel	Intestinal irritation Headache, dizziness
Antivirals	
Acyclovir	Seizures, confusion Rash
Amantadine	Nervousness, light-headedness Nausea
AZT	Immunosuppression, anemia

Allergic Responses to Drugs

One of the most frequent drug reactions is **allergy**. This reaction occurs because the drug acts as an antigen (a foreign material capable of stimulating the immune system) and stimulates an

allergic response. This response can be provoked by the intact drug molecule or by substances that develop from the body's metabolic alteration of the drug. In the case of penicillin, for instance, it is not the penicillin molecule itself that causes the allergic response but a product, *benzylpenicilloyl*. Allergic reactions have been reported for every major type of antimicrobial drug, but the penicillins account for the greatest number of antimicrobial allergies, followed by the sulfonamides.

People who are allergic to a drug become sensitized to it during the first contact, usually without symptoms. Once the immune system is sensitized, a second (or later) exposure to the drug can lead to a reaction such as a skin rash (hives), respiratory inflammation, and, rarely, anaphylaxis, an acute, overwhelming allergic response that develops rapidly and can be fatal. (This topic is discussed in greater detail in chapter 14.)

Suppression and Alteration of the Microbiota by Antimicrobials

Most normal, healthy body surfaces, such as the skin, large intestine, outer openings of the urogenital tract, and oral cavity, provide numerous habitats for a virtual "garden" of microorganisms. These normal colonists, or residents, called the **biota**, or microbiota, consist mostly of harmless or beneficial bacteria, but a small number can potentially be pathogens. Although we defer a more detailed discussion of this topic to chapter 11 and later chapters, here we focus on the general effects of drugs on this population.

If a broad-spectrum antimicrobial is introduced into a host to treat infection, it will destroy microbes regardless of their roles as normal biota, affecting not only the targeted infectious agent but also many others in sites far removed from the original infection **(figure 10.7)**. When this therapy destroys beneficial resident species, other microbes that were once in small numbers begin to overgrow and cause disease. This complication is called a **superinfection**. (This topic is also addressed in the Microbiome feature at the end of the chapter.)

Some common examples demonstrate how a disturbance in microbial biota leads to replacement biota and superinfection. A broad-spectrum cephalosporin used to treat a urinary tract infection by *Escherichia coli* will cure the infection, but it will also destroy the lactobacilli in the vagina that normally maintain a protective acidic environment there. The drug has no effect, however, on *Candida albicans*, a yeast that also resides in normal vaginas. Released from the inhibitory environment provided by lactobacilli, the yeasts proliferate and cause symptoms. *Candida* can cause similar superinfections of the oropharynx (thrush) and the large intestine.

Oral therapy with tetracyclines, clindamycin, and broad-spectrum penicillins and cephalosporins is associated with a serious and potentially fatal condition known as *antibiotic-associated colitis* (pseudomembranous colitis). This condition has become known as *C. diff*, caused by the overgrowth of the endospore-forming bacterium *Clostridium difficile*. It invades the intestinal lining and releases toxins that induce diarrhea, fever, and abdominal pain. It has become an urgent public health problem, as you read earlier.

An Antimicrobial Drug Dilemma

The remarkable progress in treating many infectious diseases has spawned a view of antimicrobials as a "cure-all" for infections as diverse as the common cold and acne.

In the past, many physicians tended to use a "shotgun" antimicrobial therapy for minor infections, which involves administering a broad-spectrum drug instead

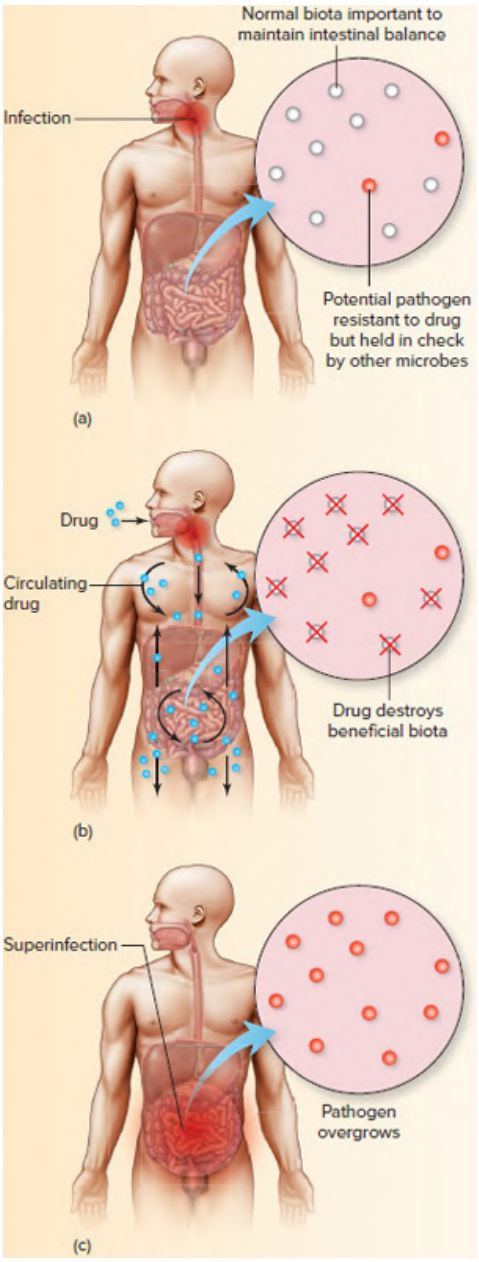

Figure 10.7 **The role of antimicrobials in disrupting microbial biota and causing superinfections**. **(a)** A primary infection in the throat is treated with an oral antibiotic. **(b)** The drug is carried to the intestine and is absorbed into the circulation. **(c)** The primary infection is cured, but drug-resistant pathogens have survived and create an intestinal superinfection.

of a more specific narrow-spectrum one. This practice led to superinfections and other adverse reactions. Importantly, it also caused the development of resistance in "bystander" microbes (normal biota) that were exposed to the drug as well. This helped to spread antibiotic resistance to pathogens. With growing awareness of the problems of antibiotic resistance, this practice is less frequent.

Tons of excess antimicrobial drugs produced in this country are exported to other countries, where controls are not as strict. Nearly 200 different antibiotics are sold over the counter in Latin

America and Asian countries. It is common for people in these countries to self-medicate without understanding the correct medical indication. Drugs used in this way are largely ineffectual, but, worse yet, they are known to be responsible for emergence of drug-resistant bacteria that subsequently cause epidemics.

In the final analysis, every allied health professional should be critically aware not only of the admirable and utilitarian nature of antimicrobials but also of their limitations.

10.4 LEARNING OUTCOMES—Assess Your Progress

22. Distinguish between drug toxicity and allergic reactions to drugs.

23. Explain what a superinfection is and how it occurs.

Infections caused by *Streptococcus pyogenes*, such as "strep throat," are treatable with penicillin, but many other streptococcal infections are not.

Source: CDC

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5. Mary has a urinary tract infection and is prescribed cephalexin for 10 days. Toward the end of her course of treatment, Mary develops a vaginal yeast infection. The yeast infection is an example of a/an

a. *superinfection*.
b. *expected complication*.
c. *allergic reaction*.
d. *toxic reaction*.

CASE FILE WRAP-UP

©Design Pics/Kristy-Anne Glubish (pills in boxes); ©Ryan McVay/Getty Images (health care worker); ©Glow Images (pills); ©Markos Dolopikos/Alamy Stock Photo (pills in package)

Cefaclor, which goes by a variety of brand names, including Ceclor, is a second-generation cephalosporin antibiotic used to treat gram-negative bacteria. When it first came out, it was popular among physicians for treating otitis media infections; however, cefaclor caused rash in a large number of patients. It has now fallen out of favor as newer cephalosporins have come along.

People with an allergy to penicillin may not be able to take cefaclor, as there is a possibility of a cross-reaction occurring. This is due to a similarity in the side chain structure of penicillins and some cephalosporins. The choice of whether to avoid the use of cephalosporins in individuals who are allergic to penicillin is often based on the allergic manifestations and the drug under consideration. Some people are able to take cephalosporins without suffering any adverse effects but should be aware of the possibility of reaction, however remote.

Allergic response to an antibiotic occurs because the drug acts as an antigen, a foreign agent that stimulates the immune response. People who are allergic to antibiotics usually become sensitized during the first contact, usually without suffering any noticeable symptoms. Once the body has become sensitized, subsequent exposure to the drug leads to an allergic response. Each subsequent exposure can result in more severe symptoms.

The Microbiome

How Antibiotics Impact the Microbiome

While scientists have long had an appreciation of the fact that our “normal biota” can be beneficial, it is only in the past several years that we have discovered the absolutely integral role the microbiome plays in human health. Now consider this: For the last 80 years we have been dousing ourselves with antibiotics. Antibiotics were and are a modern medical miracle, one of the most important public health advances in history. But know we have to start

considering what effects they have on the entire microbiome, not just the one causing the offending infection.

One study in 2016 tried to do just this. A group from Washington University (WU) in St. Louis examined the collateral effects of antibiotics on the microbiome. We have already discovered that a disrupted microbiome can lead to negative consequences such as altered nutrient metabolism, decreased vitamin production, protection from pathogenic microbes, among other things. So the WU group collected information from many sources to provide an overview of the effects of antibiotic usage on the microbiome over the life of a human. This table summarizes some of their key findings.

©Design Pics/LJM Photo/Getty Images

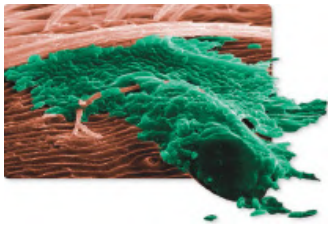
Birth	The guts of babies born by cesarean section are colonized by skin bacteria as opposed to the vaginal biota which colonizes vaginal-born babies. This has been associated with higher rates of immunological disorders, asthma, and type 1 diabetes. Antibiotics are also routinely administered to the mother during a cesarean section, further disrupting the formation of a healthy gut microbiota in the infant. Premature birth results in aggressive administration of broad-spectrum antibiotics. This is associated with increased risk of sepsis and other serious consequences.
Childhood	The risk of developing type 2 diabetes has been found to be increased with the repeated use of some types of antibiotics, reflecting the role of the microbiome in metabolic diseases such as diabetes.
Adulthood	Some antibiotic-induced changes observed: (i) increased inappropriate immune activity leading to asthma, allergies, and autoimmune disease; (ii) increased susceptibility to infection and diarrhea due to decreased diversity of gut microbiome; (iii) vitamin K deficiency leading to uncontrolled bleeding due to depletion of vitamin-producing bacteria.

Source: *Genome Medicine*, vol 8, p. 39, 2016.

Chapter Summary

10.1 Principles of Antimicrobial Therapy

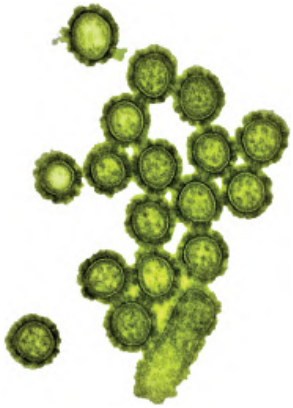
- Antimicrobial treatment involves the use of drugs to control infection on or in the body.
- Antimicrobial drugs are produced either synthetically or from natural sources.
- Broad-spectrum antimicrobials are effective against many types of microbes. Narrow-spectrum antimicrobials are effective against a limited group of microbes.
- Bacteria and fungi are the primary sources of most currently used antibiotics. The molecular structures of these compounds can be chemically altered or mimicked in the laboratory.
- The three major considerations necessary to choose an effective antimicrobial are the identity of the infecting microbe, the microbe's sensitivity to available drugs, and the overall medical status of the infected host.
- The Kirby-Bauer test identifies antimicrobials that are effective against a specific infectious bacterial isolate.
- The MIC (minimum inhibitory concentration) identifies the smallest effective dose of an antimicrobial toxic to the infecting microbe.
- The therapeutic index is a ratio of the amount of drug toxic to the infected host and the MIC. The smaller the ratio, the greater the potential for toxic host-drug reactions.



Source: CDC/Janice Haney Carr

10.2 Interactions Between Drug and Microbe

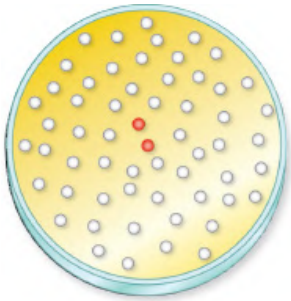
- Antimicrobials are classified into approximately 20 major drug families, based on chemical composition, source or origin, and their site of action.
- There are a great number of antibacterial drugs but a limited number that are effective against protozoa, helminths, fungi, and viruses.
- Currently there are five main cellular targets for antibiotics in microbes: cell wall synthesis, nucleic acid structure and function, protein synthesis, cytoplasmic membranes, and folic acid synthesis.
- Penicillins, cephalosporins, carbapenems, and vancomycin block cell wall synthesis.
- Aminoglycosides, tetracyclines, oxazolidinone, and pleuromutilins block protein synthesis in bacteria.
- Sulfonamides, trimethoprim, and the fluoroquinolones are synthetic antimicrobials effective against a broad range of microorganisms. They block steps in the synthesis of nucleic acids or folic acid.
- Polymyxins and daptomycin are the major drugs that disrupt cell membranes.
- Bacteria in biofilms respond differently to antibiotics than when they are free-floating. It is therefore difficult to eradicate biofilms in the human body.
- Fungal antimicrobials, such as macrolide polyenes, azoles, echinocandins, and allylamines, must be monitored carefully because of the potential toxicity to the infected host.
- There are fewer antiprotozoal drugs than antibacterial drugs because protozoa are eukaryotes like their human hosts, and they have several life stages, some of which can be resistant to the drug.
- Antihelminthic drugs immobilize or disintegrate infesting helminths or inhibit their metabolism in some manner.
- Antiviral drugs interfere with viral replication by blocking viral entry into cells, blocking the replication process, or preventing the assembly of viral subunits into complete virions.



Source: National Institute of Allergy and Infectious Diseases (NIAID)/NIH/USHHS

10.3 Antimicrobial Resistance

- Microorganisms are said to have acquired antibiotic resistance when they are no longer inhibited by an antimicrobial to which they were previously sensitive.
- Antibiotic resistance is an urgent problem that has resulted in widespread vigilance and policy actions.
- Microbes acquire genes that code for methods of inactivating or escaping the antimicrobial, or acquire mutations that affect the drug's impact.
- Mechanisms of microbial drug resistance include drug inactivation, decreased drug uptake, decreased drug receptor sites, and modification of metabolic pathways formerly attacked by the drug.
- Widespread indiscriminate use of antimicrobials is one factor that has resulted in an explosion of microorganisms resistant to all common drugs.
- Probiotics and prebiotics are methods of crowding out pathogenic bacteria and providing a favorable environment for the growth of beneficial bacteria.



10.4 Interactions Between Drug and Host

- The three major side effects of antimicrobials are toxicity to organs, allergic reactions, and problems resulting from alteration of normal biota.
- Antimicrobials that destroy most but not all normal biota can allow the unaffected normal biota to overgrow, causing a superinfection.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

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ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. Microbial resistance to drugs is acquired through **a**. conjugation. **b**. transformation. **c**. transduction. **d**. all of these.	**2**. A friend was recently diagnosed with strep throat. One week after his treatment, his symptoms returned. Your friend tells you, "I must have become immune to the drug the doctor gave me." Discuss the validity of your friend's statement, providing evidence in support of or refuting his claim.	**3**. You are invited to participate in debate about the concept of evolution. Construct an opening statement supporting evolution, using antibiotic resistance in microorganisms as an example.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Cell Structure and Function	**4**. Drugs that prevent the formation of the bacterial cell wall are **a**. quinolones. **b**. penicillins. **c**. tetracyclines. **d**. aminoglycosides.	**5**. Why does the penicillin group of antibiotics have milder toxicity than other antibiotics?	**6**. This chapter discussed five major cellular targets for antibiotic action. Can you imagine a different one? Which one and why?
Metabolic Pathways	**7**. A compound synthesized by bacteria or fungi that destroys or inhibits the growth of other microbes is a/an **a**. synthetic drug. **b**. antibiotic. **c**. interferon. **d**. competitive inhibitor.	**8**. Conduct research to find out why drugs blocking folic acid synthesis are highly selective (not harmful to humans).	**9**. What kind of organisms do you suppose first possessed beta-lactamases, and for what reason?

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Information Flow and Genetics	**10**. R factors are _____ that contain a code for _____. **a**. genes, replication **b**. plasmids, drug resistance transposons, interferon plasmids, conjugation **d**.	**11**. You take a sample from a growth-free portion of the zone of inhibition in the Kirby-Bauer test and inoculate it onto a plate of nonselective medium. What does it mean if growth occurs on the new plate?	**12**. Develop an argument about why the CRISPR technique could potentially provide a permanent solution to antibiotic resistance, where other "fixes" have been temporary.

Microbial Systems	**13**. Treating malarial infections is theoretically difficult because **a**. the protozoal parasite is eukaryotic and therefore similar to human cells. **b**. there are several species of *Plasmodium*. **c**. no single drug can target all the life stages of *Plasmodium*. **d**. all of the above are true.	**14**. Can you think of a situation in which it would be better for a drug to be microbistatic rather than microbicidal? Discuss thoroughly.	**15**. Scientists have found evidence that exposing some bacteria to antibiotics makes them much more likely to form biofilms. Construct an argument for why this might be.

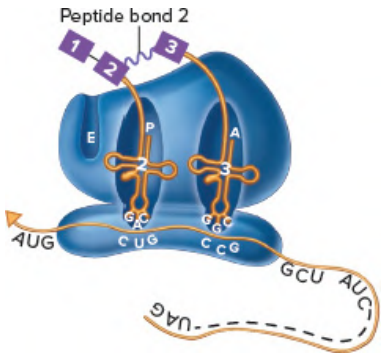
Impact of Microorganisms	**16**. Pick the true statement about antibiotics. **a**. Antibiotics were invented by chemists in the 1830s. **b**. Antibiotics are natural products of microorganisms. **c**. Antibiotics are inherently dangerous for the environment. **d**. None of the above are true.	**17**. Speculate on whether having large concentrations of antibiotics in water sources or farmland would be a positive or negative phenomenon. Defend your answer.	**18**. Imagine a postantibiotic era in which there are no drug treatments for microbial infections. What alternative strategies will make up our arsenal against morbidity and mortality due to microbes?
Scientific Thinking	**19**. An antimicrobial drug with a _____ therapeutic index is a better choice than one with a _____ therapeutic index. **a**. low; high **b**. high; low	**20**. A critically ill patient enters your emergency room, exhibiting signs and symptoms of severe septic shock. In this case, should you immediately begin treatment with a broad-spectrum drug or a narrow-spectrum drug? Explain your answer.	**21**. You are the director of the Centers for Disease Control and Prevention. What are your top three recommendations for halting the spread of *mcr-1*-containing bacteria?

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From chapter 8, table 8.5**. Place Xs over this figure in places where bacterial protein synthesis might be inhibited by drugs.

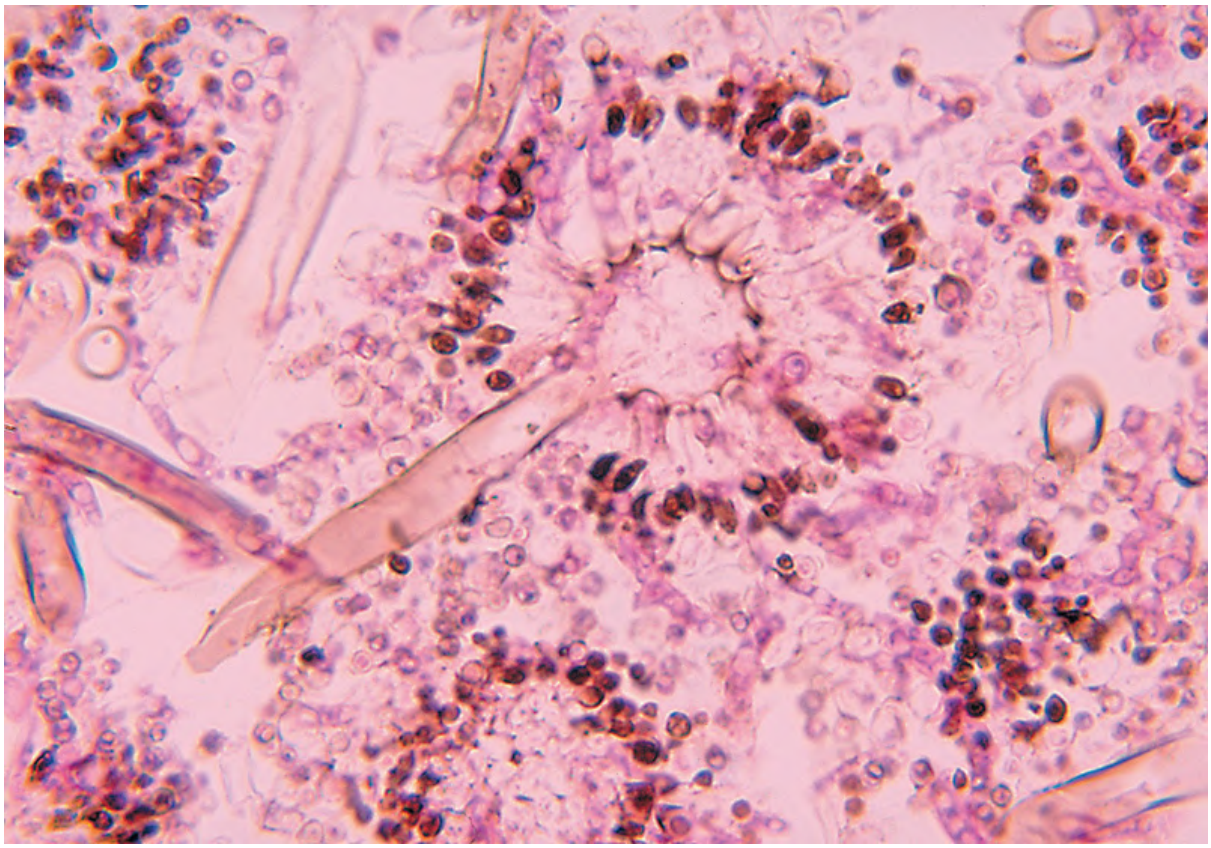


connect Resources are available through Connect, including interactive learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

11

Interactions Between Microbes and Humans



Source: CDC (histopathologic features); Source: NIH Roadmap for Medical Research website (Human Microbiome Project); ©McGraw-Hill Education/Christopher Kerrigan, photographer (person breathing); ©ERproductions Ltd/Blend Images LLC (health care worker)

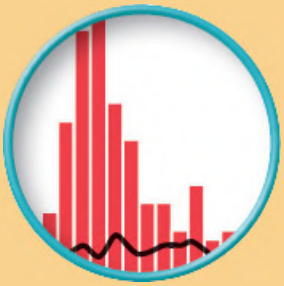
IN THIS CHAPTER…

11.1 The Human Microbiome

1. Differentiate among the terms *colonization, infection*, and *disease*.
2. Enumerate the sites where normal biota is found in humans.
3. Discuss how the Human Microbiome Project is changing our understanding of normal biota.

11.2 When Colonization Leads to Disease

4. Differentiate between a microbe's pathogenicity and its virulence.
5. List the steps a microbe has to take to get to the point where it can cause disease.
6. Explain the significance of polymicrobial infections.
7. List several portals of entry and exit.
8. Define *infectious dose*, and explain its role in establishing infection.
9. Describe three ways microbes cause tissue damage.
10. Compare and contrast major characteristics of endotoxin and exotoxins.
11. Provide a definition of *virulence factors*.
12. Draw a diagram of the stages of disease in a human.
13. Differentiate among the various types of reservoirs, providing examples of each.
14. List several different modes of transmission of infectious agents.
15. Define *healthcare-associated infection*, and list the three most common types.
16. List Koch's postulates, and discuss when they might not be appropriate in establishing causation.



11.3 Epidemiology: The Study of Disease in Populations

17. Summarize the goals of epidemiology, and differentiate it from traditional medical practice.
18. Explain what is meant by a disease being "notifiable" or "reportable," and provide examples.
19. Define *incidence and prevalence*, and explain the difference between them.
20. Discuss the three major types of epidemics, and identify the epidemic curve associated with each.

CASE FILE

Seizing the Opportunity

When I worked as a nurse on a bone marrow transplant unit, I cared for patients who had undergone treatment for cancer or other blood disorders. Bone marrow transplant (BMT) offered the cure to many of my patients' diseases, but the treatment was not without risk. During the BMT procedure, a patient's bone marrow, which produces blood cells, is intentionally destroyed. It is replaced with blood stem cells that will produce red blood cells, white blood cells, and platelets. Because white blood cells are virtually eliminated during the procedure, patients are unable to fight infection until they are replenished. We followed strict isolation precautions to protect patients from exposure to pathogens as their blood cells regenerated.

One day, I cared for Cindy, a 62-year-old female who had undergone bone marrow transplant to treat acute myeloid leukemia. She was 10 days posttransplant and had a new fever. We had a protocol for treating patients with a low white blood cell count and new-onset fever. I collected blood cultures, gave medication to treat her fever, and assessed her clinical presentation.

Cindy reported mild chest pain and seemed to be working harder to breathe. She had developed a cough 24 hours ago. Her respiratory rate was faster than normal. I found that her oxygen saturation was low and so I applied oxygen by a nasal cannula. When I listened to her lungs, I could hear wheezing and diminished breath sounds on one side. I called the physician to the bedside.

The doctor ordered blood work, a chest x-ray, and CT scan to assess Cindy's lung fields. This imaging revealed an abnormal area on one of her lungs. The doctor decided to transfer the patient to the intensive care unit for further management.

Once Cindy was admitted to the intensive care unit, a full infectious workup was done. Additional samples of body fluids were sent for culture, in addition to other blood tests for conditions affecting the immunocompromised population. Because of her fever and neutropenia (low white blood cell count), an infectious cause seemed likely for her respiratory distress. The medical team initiated antibiotics, knowing that her body's ability to fight infection was impaired.

The next week I found out that Cindy had been diagnosed with aspergillosis.

- What causes aspergillosis?
- How did Cindy get sick despite careful precautions to prevent exposure to infectious agents?

Case File Wrap-Up appears at the end of the chapter.

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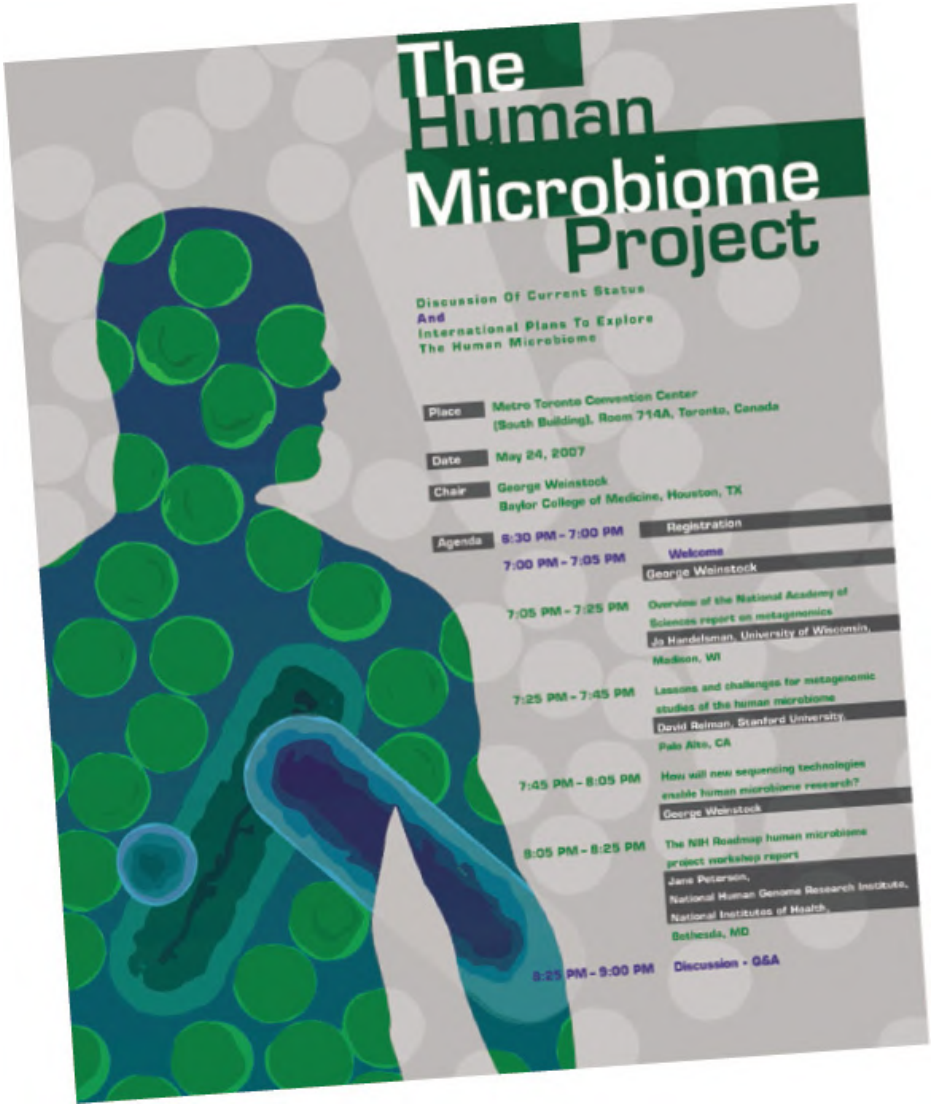
1. Which of the following factors would promote progression of an infection? Select all that apply.
   a. *low microbial virulence*
   b. *proper portal of entry*
   c. *genetic profile of host resistance to microbe*
   d. *no previous exposure to this infection*
   e. *host immunosuppression*

11.1 The Human Microbiome

It is easy to think of humans and other mammals as discrete, stand-alone organisms that are also colonized by some nice, nonharmful microorganisms. In fact, that's what scientists thought for the last 150 years or so. But the truer picture is that humans and other mammals have the form and the physiology that they have due to *having been formed in intimate contact with their microbes*. Do you see the difference? The **human microbiome**, the sum total of all microbes found on and in a normal human, is critically important to the health and functioning of its host organism. This chapter describes the relationship between the human and microorganisms, both the ones that make up the human's microbiome and the ones that are harmful.

Colonization, Infection, Disease—A Continuum

Our bodies consist of even more microbes than our own human cells. Some microbes are colonists (normal biota), some are rapidly lost (transients), and others invade the tissues. Sometimes resident biota become invaders. For the most part our resident microbiota, the human microbiome, **colonize** us for the long term, and do not cause disease. We encounter transient microbes in our environment, most of which are harmless, although occasionally they lead to **infection**, a condition in which microbes get past the host defenses, enter the tissues, and multiply. We may or may not be aware that an infection is taking place. When the cumulative effects of the infection damage or disrupt tissues and organs, the pathologic state that results is **disease**.



Source: NIH Roadmap for Medical Research website

A disease is defined as any deviation from health. There are hundreds of different human diseases, caused by such factors as infections, genetics, aging, and malfunctions of systems or organs. In this chapter we discuss only **infectious disease**—a pathologic state caused directly by microorganisms or their products.

When you consider the evolutionary time line (refer to figure 1.1) of bacteria and humans, it is quite clear that humans evolved in an environment that had long been populated by bacteria and single-celled eukaryotes. It should not be surprising, therefore, that humans actually require the microbes in and on their bodies for proper growth and development.

The extent to which this is true has been surprising even to the scientists studying it. Since 2008, a worldwide research effort has been underway that utilizes the powerful techniques of genome sequencing and "big data" tools. The American effort is called the **Human Microbiome Project (HMP)**, and there are similar projects occurring around the world. The aim has been not only to characterize the microbes living on human bodies when they are healthy but also to determine how the microbiome differs in various diseases. Previous to this

international project, scientists and clinicians mainly relied on culture techniques to determine what the "normal biota" consisted of. That meant we only knew about bacteria and fungi that we could grow in the laboratory, which vastly undercounts the actual number and variety, since many—even the majority of—microbes cannot be cultured in the laboratory, though they grow quite happily on human tissues.

Several important and surprising results have already emerged. Here are just a few examples.

- Human cells contain about 21,000 protein-encoding genes, but the microbes living in/on us contain 8 million. Many of the proteins produced by these genes are enzymes that help us digest our food and metabolize all kinds of substances that we could not otherwise use.
- We have a lot of microbes in places we used to think were sterile. One of the most striking examples is the lungs. They were previously thought to be sterile but actually seem to contain their own sparse but diverse microbiota.
- Viruses are not traditionally discussed in the context of normal biota. However, they are certainly present in healthy humans in vast quantities, both those that infect human cells and those infecting all the cells of our resident biota. For example, researchers think that there are 100 million viruses per gram of human feces (see The Microbiome feature at the end of this chapter). Throughout evolutionary history, viral infections (of cells of all types) have influenced the way cells and organisms and communities and, yes, the entire ecosystem have developed. The critical contributions of viruses are just now being rigorously studied.
- All healthy people seem to also harbor potentially dangerous pathogens in low numbers. This is a tribute to the normal biota that rarely cause disease; their presence keeps the pathogens in check.
- The makeup of the intestinal biota can influence many facets of one's overall health. As an example, differences in the gut microbiome have preliminarily been associated with risk for Crohn's disease and obesity. Though this may not seem surprising, other (preliminary) research is finding associations between the composition of the gut microbiome and heart disease, asthma, autism, diabetes, and even moods. A recent study provided evidence that the composition of the gut microbiome influenced the body's response to cancer drugs, influencing how many toxic side effects occurred. As with all revolutionary breakthroughs, some of these early results will be overturned with further study. But one thing is absolutely clear: Our microbiome has a huge influence on our physiology.
- Clearly, incoming microbes that might cause disease have to encounter the microbiome as part of their path to establishing themselves in the host. Studies are showing that the relative success of viruses such as influenza and HIV in causing disease can be greatly influenced by the composition of the host microbiomes. This has implications for future treatment strategies.

The Human Microbiome Project has shown that among healthy adults, the normal microbiota varies significantly. For instance, the microbiota on a person's right hand was found to be significantly different than that on the same person's left hand. What seemed to be more important than the exact microbial profile of any given body site was the profile of proteins,

especially the enzymatic capabilities. That profile remained stable across subjects, though the microbes that were supplying those enzymes could differ broadly. Scientists are in the process of cataloging other microorganisms besides bacteria via metagenomics—and just beginning to appreciate their numbers in the human microbiome.

The information about the human microbiome presented in this chapter reflects new findings. We will try to show you the differences between the old picture of normal biota in various organ systems and the new, emerging picture. At this point in medical history, it will be important to appreciate the transitioning view.

Acquiring the Microbiota

The human body offers a seemingly endless variety of environmental niches, with wide variations in temperature, pH, nutrients, and oxygen tension occurring from one area to another. Because the body provides such a range of habitats, it should not be surprising that the body supports a wide range of microbes. **Table 11.1** provides a breakdown of our current understanding of the microbiota living in and on a healthy host.

Table 11.1 A Shifting Understanding of Sites with Microbiota

Sites Previously Known to Harbor Normal Microbiota	
Skin and adjacent mucous membranes	External genitalia Vagina
Upper respiratory tract	External ear canal
Gastrointestinal tract, including mouth	External eye (lids, conjunctiva)
Outer portion of urethra	
Additional Sites Now Thought to Harbor At Least Some Normal Microbiota (or Their DNA)	
Lungs (lower respiratory tract)	
Bladder (and urine)	
Breast and breast milk	
Amniotic fluid and fetus	
Sites in Which DNA from Microbiota Has Been Detected	
Brain	
Bloodstream	

©Ian Hooton/Science Photo Library/Getty Images

The uppermost row contains the set of sites that microbiologists have long known to host a normal biota. The middle row presents some new sites recently found to harbor microbiota in a healthy human. The bottom row reports that two sites, the brain and the bloodstream, have both been found to contain DNA from multiple species of bacteria. Their exact role there is not entirely clear yet.

The generally antagonistic effect "good" microbes have against intruder microorganisms is called **microbial antagonism**. Normal biota exist in a steady established relationship with the host and are unlikely to be displaced by incoming microbes. This antagonistic protection is partly the result of a limited number of attachment sites in the host site, all of which are stably occupied by normal biota. This antagonism is also enabled by the chemical or physiological environment created by the resident biota, which is hostile to most other microbes. There are often members of the "normal" biota that would cause disease if they were allowed to multiply to larger numbers. Microbial antagonism is also responsible for keeping them in check.

It is important to note that hosts with compromised immune systems could very easily experience disease caused by their normal biota. Factors that weaken host defenses and increase susceptibility to infection include the following:

- old age and extreme youth (infancy, prematurity);
- genetic defects in immunity, and acquired defects in immunity (AIDS);
- surgery and organ transplants;
- underlying disease: cancer, liver malfunction, diabetes;
- chemotherapy/immunosuppressive drugs;
- physical and mental stress;
- pregnancy; and

- other infections.

Colonization of the Fetus and Newborn

Until rather recently, the uterus and its contents were thought to be sterile during embryonic and fetal development and remain essentially germ-free until just before birth **(figure 11.1)**. Many scientists now believe that the womb is not a sterile environment. Research in 2010 found that healthy newborns' stools, sampled before they have their first meal, contain a diversity of bacteria, indicating that their intestines are colonized *in utero*. These findings have been confirmed by other studies since then.

The next important source of microbiota for a newborn is its trip through the vagina. The vaginas of healthy women of child-bearing age contain a variety of bacteria. They are especially rich in *Lactobacillus* bacteria, which are capable of digesting milk, and in other species that have been found useful in protecting the baby from skin disorders and other conditions. Of course, in the United States about 30% of babies are born by cesarean section (either through medical necessity or as elective procedures). Studies have shown that babies born this way have different gut microbiomes than those born vaginally, at least initially. There is a lot of research examining the long-term effects of this difference. Some people advocate for swabbing the newborn baby with gauze containing the mother's vaginal fluids, but there is still no evidence that this produces a long-term benefit. So far there are more questions than answers about the difference in microbiomes resulting from C-sections and vaginal births.

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2. An epidemiology RN is analyzing the prevalence of infectious disease in an immunocompromised patient population. What is the difference between a true pathogen and an opportunistic pathogen?

a. *True pathogens cause a disease in the presence of immunosuppression, whereas opportunistic pathogens do not.*

b. *Opportunistic pathogens develop virulence properties within an immunocompromised host, whereas true pathogens do not.*

c. *The diseases associated with true pathogens may vary in presentation ranging from mild to severe infections, whereas opportunistic pathogens always present in severe form.*

d. *True pathogens cause disease in a healthy individual, whereas opportunistic pathogens typically cause disease in an immunocompromised host.*

Figure 11.1 **The origins of microbiota in newborns.**

©Jim Connely (ultrasound); ©Adam Gault/SPL/Getty Images (birth); ©Alik Mulikov/Shutterstock (breast feeding); ©Pixtal/SuperStock (bottle); ©Marc Romanelli/Blend Images (family) ©Kwame Zikomo/Purestock/SuperStock (with dog); ©alexmak72427/iStock/Getty Images (scar)

The baby continues to acquire resident microbiota from the environment, notably from its diet. Throughout most of evolutionary history, of course, that means human breast milk. Scientists have found that human milk contains around 600 species of bacteria and a lot of sugars that babies cannot digest. The sugars are used by healthy gut bacteria, suggesting a role for breast milk in maintaining a healthy gut microbiome in the baby. The skin, gastrointestinal tract, and portions of the respiratory and genitourinary tracts all continue to be colonized as contact continues with family members, health care personnel, the environment, and food.

11.1 LEARNING OUTCOMES—Assess Your Progress

1. Differentiate among the terms *colonization, infection*, and *disease*.
2. Enumerate the sites where normal biota is found in humans.
3. Discuss how the Human Microbiome Project is changing our understanding of normal biota.

11.2 When Colonization Leads to Disease

A microbe whose relationship with its host is parasitic and results in infection and disease is termed a pathogen. Various aspects of the host influence whether a microbe will have severe, mild, or no effects. Variation in the genes coding for components of the immune system—or even the anatomy of infection sites—is one of these factors. Gender, hormone levels, and overall health also play a role. **Pathogenicity**, then, is a broad concept that describes an organism's potential to cause disease and is used to divide pathogenic microbes into one of two groups. **True pathogens** (primary pathogens) are capable of causing disease in healthy persons with normal immune defenses. They are generally associated with a specific, recognizable disease, which may vary in severity from mild (colds) to severe (malarial) to fatal (rabies). Examples of true pathogens include the influenza virus, plague bacillus, and malarial protozoan.

Opportunistic pathogens cause disease when the host's defenses are compromised or when the pathogens become established in a part of the body that is not natural to them. Opportunists are not considered pathogenic to a normal healthy person and, unlike true pathogens, do not generally possess well-developed virulence properties. Examples of opportunistic pathogens include *Pseudomonas* species and *Candida albicans*. In practice, the distinction between true pathogens and opportunistic pathogens is becoming less useful as we gain more understanding of the many factors controlling the disease process. We'll discuss this more later.

The relative severity of the disease caused by a particular microorganism depends on the **virulence** of the microbe. Although the terms *pathogenicity* and *virulence* are often used interchangeably, *virulence* is the accurate term for describing the degree of pathogenicity. The virulence of a microbe is determined by its ability to

1. establish itself in the host, and
2. cause damage.

There is much involved in both of these steps. To establish themselves in a host, microbes must enter the host, attach firmly to host tissues, negotiate the microbiome, and survive the host defenses. To cause damage, microbes produce toxins or induce a host response that is actually injurious to the host. Any characteristic or structure of the microbe that contributes to the preceding activities is called a **virulence factor**. Virulence can be due to single or multiple factors. In some microbes, the causes of virulence are clearly established, but in others they are not.

Polymicrobial Infections

From the very earliest days of infectious disease studies, in the 1800s, scientists isolated single microorganisms to study their effects on animals or humans. Later in this chapter you will learn about Koch's postulates, a set of rules for determining the cause of an unknown infectious condition. The postulates depend on obtaining microbes in pure culture. This procedure is extremely valuable, since as you know most scientific experimentation requires isolating the variables and holding all but one constant.

This aspect of scientific rigor has probably kept us from understanding the roles that interacting microbes play in causing disease. Many scientists now believe that the majority of infections are **polymicrobial**, with contributions from more than one microbe. One classic set of infections is influenza (caused by a virus) and pneumonia (often caused by a bacterium). Influenza infection frequently leads to pneumonia. In another example, several types of skin infections are known to be caused by either *Staphylococcus* or *Streptococcus* species. In fact, researchers have found that when these two are cultivated together with another common skin resident, *Moraxella*, both staph and strep increase their transcription of virulence factors. Perhaps upon diagnosis one or the other is isolated from the skin, but it seems possible that the three of them together led to the disease symptoms.

In the next decade, many more polymicrobial causes will no doubt be discovered, which can help us look for unique prevention and treatment strategies.

The respiratory tract is the most common portal of entry.
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Step One: Becoming Established—Portals of Entry

To initiate an infection, a microbe enters the tissues of the body by a characteristic route, the **portal of entry**, usually the skin or a mucous membrane. The source of the infectious agent can be exogenous or endogenous. Organisms coming from outside the body are exogenous; organisms coming from somewhere in the same human host are considered endogenous.

The majority of pathogens have adapted to a specific portal of entry, one that provides a favorable habitat for further growth and spread. This adaptation can be so restrictive that if certain pathogens enter the "wrong" portal, they will not cause infections. For instance, inoculation of the nasal mucosa with the influenza virus is likely to give rise to the flu, but if this virus contacts only the skin, no infection will result. Occasionally, an infective agent can

utilize more than one portal. For instance, *Mycobacterium tuberculosis* enters through both the respiratory and gastrointestinal tracts, and pathogens in the genera *Streptococcus* and *Staphylococcus* have adapted to invasion through several portals of entry such as the skin, urogenital tract, and respiratory tract. **Table 11.2** outlines common portals of entry, the organisms and diseases associated with these portals, and methods of entry.

Infectious Dose

Another factor crucial to the course of an infection is the quantity of microbes in the inoculating dose. For most agents, infection will proceed only if a minimum number, called the *infectious dose* (*ID*), is present. This number has been determined experimentally for many microbes. On the low end of the scale, the ID for *Coxiella burnetii*, the causative agent of Q fever, is only a single cell, and the ID is only about 10 infectious cells in tuberculosis, giardiasis, and coccidioidomycosis. The ID is 1,000 bacteria for gonorrhea and 10,000 bacteria for typhoid fever, in contrast to 1,000,000,000 bacteria in cholera. Numbers below an infectious dose will generally not result in an infection. But if the quantity is far in excess of the ID, the onset of disease can be extremely rapid.

Step Two: Becoming Established—Attaching to the Host and Interacting with the Microbiome

Adhesion is a process by which microbes gain a more stable foothold on host tissues. Because adhesion is dependent on binding between specific molecules on both the host and pathogen, a particular pathogen is limited to only those cells (and organisms)

Medical Moment

When the Portal of Entry Is Compromised

Different portals of entry have protective mechanisms to prevent infectious agents from gaining entry. For example, the eye produces tears, which not only rinse pathogens out of the eye but also contain pathogen-fighting chemicals. The skin acts as a physical barrier, providing it is intact.

What happens when there is a breach at a portal of entry? The respiratory tract is lined with cilia, fingerlike projections that protrude from cells that sweep back and forth to move particles toward the throat so that they can be swallowed rather than remain in the respiratory tract. In *primary ciliary dyskinesia*, affected individuals lack properly functioning cilia. These individuals have frequent respiratory tract infections beginning in early childhood. Chronic respiratory infections lead to bronchiectasis, which results from damage affecting the bronchial tubes leading to the lungs. This condition affects approximately one in 16,000 individuals and is usually passed down from two parents who have the defective gene but do not have the disease themselves (autosomal recessive pattern).

Q. Cigarette smokers often have a higher rate of respiratory tract infections. Can you suggest a reason for this?

Answer appears in Appendix B.

Table 11.2 Portals of Entry and Organisms Typically Involved

Portal of Entry	Organism/Disease	How Access Is Gained
Skin	*Staphylococcus aureus, Streptococcus pyogenes, Clostridium tetani*	Via nicks, abrasions, punctures, areas of broken skin

Portal of Entry	Organism/Disease	How Access Is Gained
	Herpes simplex (type 1)	Via mucous membranes of the lips
	Helminth worms	Burrow through the skin
	Viruses, rickettsias, protozoa (i.e., malaria, West Nile virus)	Via insect bites
	Haemophilus aegyptius, Chlamydia trachomatis, Neisseria gonorrhoeae	Via the conjunctiva of the eye
Gastrointestinal tract	*Salmonella, Shigella, Vibrio, Escherichia coli*, poliovirus, hepatitis A, echovirus, rotavirus, enteric protozoans (*Giardia lamblia, Entamoeba histolytica*)	Through eating/drinking contaminated foods and fluids Via fomites (inanimate objects contaminated with the infectious organism)
Respiratory tract	Bacteria causing meningitis, influenza, measles, mumps, rubella, chickenpox, common cold, *Streptococcus pneumoniae, Klebsiella, Mycoplasma, Cryptococcus, Pneumocystis, Mycobacterium tuberculosis, Histoplasma*	Via inhalation of offending organism
Urogenital tract	HIV, *Trichomonas*, hepatitis B, syphilis, *Treponema pallidum, Neisseria gonorrhoeae, Chlamydia trachomatis*, herpes, genital warts	Enter through the skin/mucosa of penis, external genitalia, vagina/cervix, urethra; may enter through an unbroken surface or through a cut or abrasion

to which it can bind. Once attached, the pathogen is poised advantageously to invade the body compartments. Bacterial, fungal, and protozoal pathogens attach most often by mechanisms such as fimbriae (pili), surface proteins, and adhesive slimes or capsules. Viruses attach by means of specialized receptors. In addition, parasitic worms are mechanically fastened to the portal of entry by suckers, hooks, and barbs. There are many different methods in which microbes can attach themselves to host tissues. Firm attachment to host tissues is almost always a prerequisite for causing disease since the body has so many mechanisms for flushing microbes and foreign materials from its tissues.

Of course when a new microbe enters a body site, it will also encounter the resident microbiota. The microbiome is generally very stable and "settled in" in body sites and its presence can prevent newcomers from attaching or becoming established (microbial antagonism). Recently some evidence has arisen of cases where the microbiome assisted disease-causing microbes. We know one thing for sure: The microbiome is part of the host environment that incoming microbes must deal with.

Step Three: Becoming Established—Surviving Host Defenses

Microbes that are not established in a normal biota relationship in a particular body site in a host are likely to encounter resistance from host defenses when first entering, especially from certain white blood cells called **phagocytes**. These cells ordinarily engulf and destroy pathogens by means of enzymes and antimicrobial chemicals (see chapter 12).

Antiphagocytic factors are used by some pathogens to avoid phagocytes. The antiphagocytic factors of microorganisms help them to circumvent some part of the phagocytic process (see figure 11.2*b*). The most aggressive strategy involves bacteria that kill phagocytes outright. Species of both *Streptococcus* and *Staphylococcus* produce **leukocidins**, substances that are toxic to white blood cells. Some microorganisms secrete an extracellular surface layer (slime or capsule) that makes it physically difficult for the phagocyte to engulf them. *Streptococcus*

pneumoniae, Salmonella typhi, Neisseria meningitidis, and *Cryptococcus neoformans* are notable examples. Some bacteria are well adapted to survive inside phagocytes after ingestion. For instance, pathogenic species of *Legionella, Mycobacterium*, and many rickettsias are readily engulfed but are capable of avoiding destruction. The ability to survive intracellularly in phagocytes has special significance because it provides a place for the microbes to hide, grow, and be spread throughout the body.

Step Four: Causing Disease

How Virulence Factors Contribute to Tissue Damage

Virulence factors are structures or capabilities that allow a pathogen to cause infection in a host. From a microbe's perspective, they are simply adaptations it uses to invade and establish itself in the host. The effects of a pathogen's virulence factors on tissues vary greatly. Cold viruses, for example, invade and multiply but cause relatively little damage to their host. At the other end of the spectrum, pathogens such as *Clostridium tetani* or HIV severely damage or kill their host. There are three major ways that microorganisms damage their host:

1. directly through the action of enzymes or toxins (both endotoxin and exotoxins; **figure 11.2*a***),
2. indirectly by inducing the host's defenses to respond excessively or inappropriately **(figure 11.2*b*)**, and
3. epigenetic changes made to host cells by microbes **(figure 11.2*c*)**.

It is obvious that enzymes, endotoxin and exotoxins are virulence factors, but other characteristics of microbes that lead to host overreaction are also considered virulence

Salmonella bacteria attaching to intestinal epithelium.
Source: NIAID, NIH/Rocky Mountain Laboratories

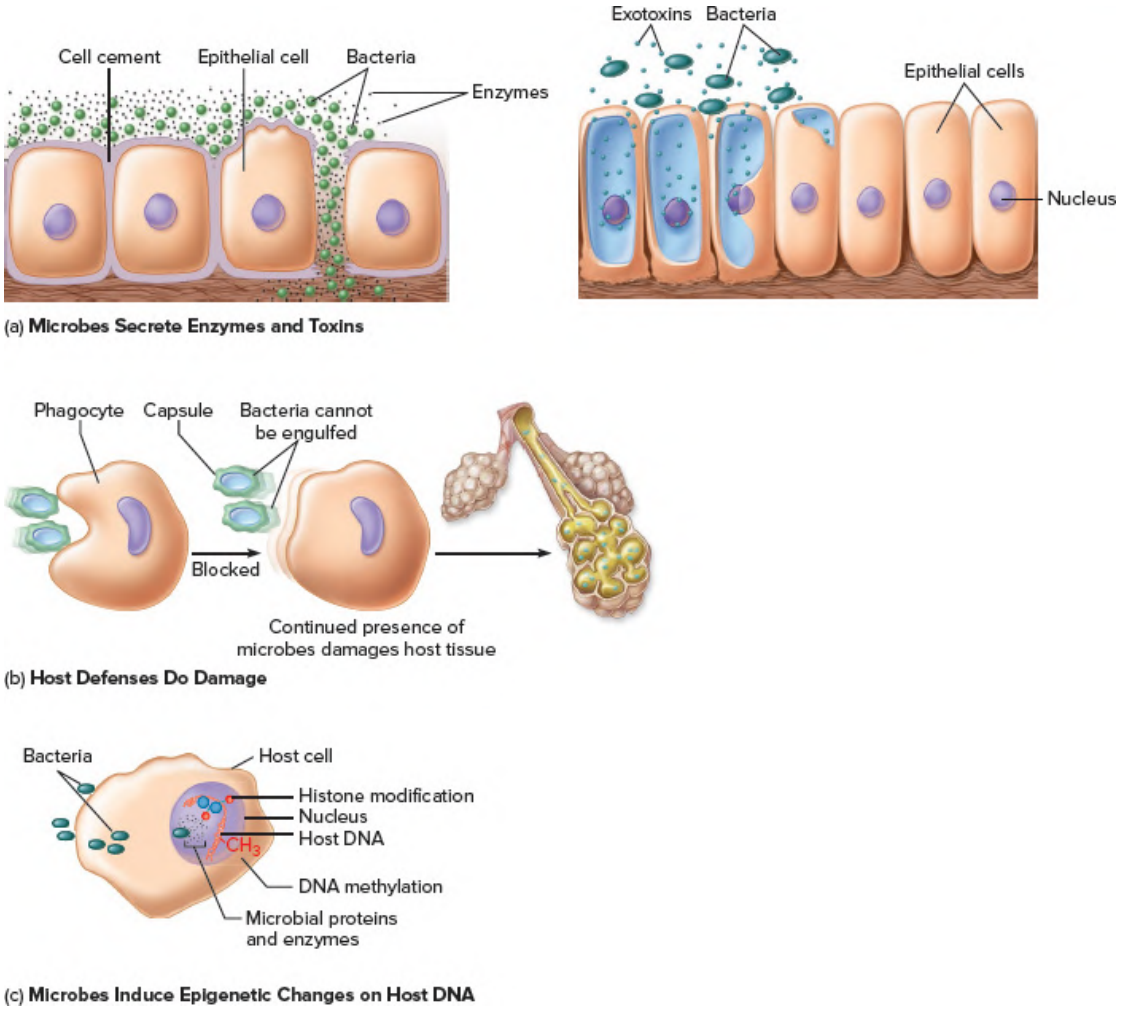


Figure 11.2 **Three ways microbes damage the host**. **(a)** Microbial enzymes and exo- and endotoxins disrupt host cell structure or connections between host cells. **(b)** Microbes evade initial host defenses, and the host continues to react to the presence of the microbe, causing (host) damage with its response. **(c)** Microbial products make epigenetic changes to the DNA and/or supporting structures, such as histones, altering the host genes that are expressed.

factors. The capsule of *Streptococcus pneumoniae* is a good example. Its presence prevents the bacterium from being cleared from the lungs by phagocytic cells, leading to a continuous influx of fluids into the lung spaces, and the condition we know as pneumonia.

1. Enzymes and Toxins

Extracellular Enzymes Many pathogenic bacteria, fungi, protozoa, and worms secrete **exoenzymes** that break down and inflict damage on tissues. Other enzymes dissolve the host's defense barriers and promote the spread of microbes to deeper tissues.

Examples of enzymes are

1. mucinase, which digests the protective coating on mucous membranes and is a factor in amoebic dysentery; and

2. hyaluronidase, which digests hyaluronic acid, the ground substance that cements animal cells together. This enzyme is an important virulence factor in staphylococci, clostridia, streptococci, and pneumococci.

Many gastrointestinal (GI) diseases are caused by bacterial toxins that affect the GI tract. These are called enterotoxins.
©Brand X Pictures

Figure 11.3 Beta-hemolysis and alpha-hemolysis by different bacteria on blood agar. Beta-hemolysis, in the lower right, results in complete clearing of the red blood cells incorporated in the agar. Alpha-hemolysis, on the lower left, refers to incomplete lysis of the red blood cells, leaving a greenish tinge to the colonies and the area surrounding them.

©McGraw-Hill Education/Lisa Burgess, photographer

Some enzymes react with components of the blood. Coagulase, an enzyme produced by pathogenic staphylococci, causes clotting of blood or plasma. By contrast, the bacterial kinases (streptokinase, staphylokinase) do just the opposite, dissolving fibrin clots and expediting the invasion of damaged tissues. In fact, one form of streptokinase is a therapy to dissolve blood clots in patients who have problems with thrombi and embolisms.

Bacterial Toxins: A Potent Source of Cellular Damage A **toxin** is a specific chemical product of microbes that is poisonous to other organisms. A toxin is named according to its specific target of action: Neurotoxins act on the nervous system; enterotoxins act on the intestine; hemotoxins lyse red blood cells; and nephrotoxins damage the kidneys.

There are two broad categories of bacterial toxins. **Exotoxins** are proteins with a strong specificity for a target cell and extremely powerful, sometimes deadly, effects. They generally affect cells by damaging the cell membrane and causing lysis or by disrupting intracellular function. **Hemolysins** (hee-mahl′-uh-sinz) are a class of bacterial exotoxin that disrupts the cell membrane of red blood cells (and some other cells, too). This damage causes the red blood cells to **hemolyze**—to burst and release hemoglobin pigment. Hemolysins that increase pathogenicity include the streptolysins of *Streptococcus pyogenes* and the alpha (α) and beta (β) toxins of *Staphylococcus aureus*. When colonies of bacteria growing on blood agar produce hemolysin, distinct zones appear around the colony. The pattern of hemolysis is often used to identify bacteria and determine their degree of virulence **(figure 11.3)**.

In contrast to the category *exotoxin*, which contains many different examples, the word *endotoxin* refers to a single substance. Endotoxin is actually a chemical called lipopolysaccharide (LPS), which is part of the outer membrane of gram-negative cell walls. Gram-negative bacteria shed these LPS molecules into tissues or into the circulation. Endotoxin differs from exotoxins because it has a variety of systemic effects on tissues and organs. Depending upon the amounts present, endotoxin can cause fever, inflammation, hemorrhage, and diarrhea. Blood infection by gram-negative bacteria such as *Salmonella, Shigella, Neisseria*

meningitidis, and *Escherichia coli* are particularly dangerous, in that it can lead to fatal endotoxic shock. **Figure 11.4** contains important information about exotoxins and endotoxin.

2. Inducing a Damaging Host Response

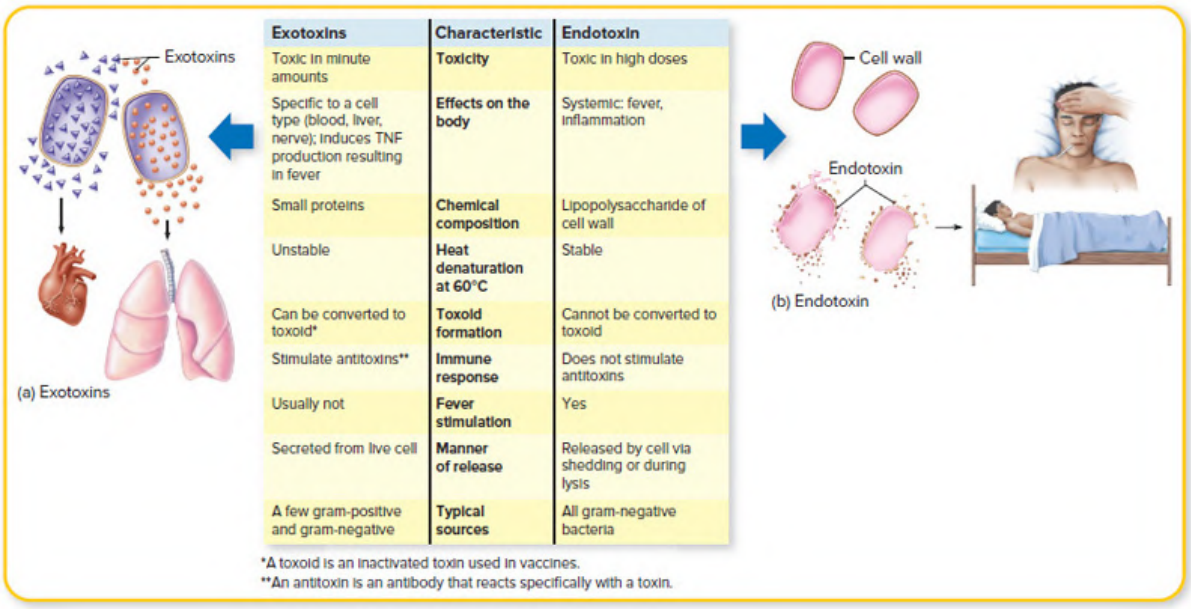
Despite the extensive discussion on direct virulence factors, such as enzymes and toxins, it is probably the case that more microbial diseases are the result of indirect damage, or the host's excessive or inappropriate response to a microorganism. This is an extremely important point because it means that pathogenicity is not a trait inherent in microorganisms but is really a consequence of the interplay between microbe and host.

3. Epigenetic Changes in Host Cells

This mechanism that microbes use to damage host cells is the most recently discovered. Microbes have been shown to shut down or activate regions of DNA in the host cell, via epigenetic processes. They can include binding to host cell histones, binding to the small RNAs used for the silencing of genes, binding to chromatin itself, and so forth. These changes can harm the host cell, or change its function in some way that favors persistence of the microbe in or on it. Some pathogens have been found to secrete proteins that interact with regions of host cell DNA that are responsible for cytoskeleton structure, and thereby cause disorganization of the cell. Sometimes these changes are passed on to new host cells, causing persistent symptoms. Some researchers speculate that this could be one source of unexplained illnesses or symptoms where no causative microbes are found.

Whether Disease Will Occur

Now that you have studied what it takes for a microbe to cause disease, we can put that in perspective with respect to a particular human host. In previous times, microbes were assigned to categories such as pathogen or nonpathogen. It is true that some microbes are very likely to cause disease (the rabies virus), and other microbes



Exotoxins	Characteristic	Endotoxin
Toxic in minute amounts	**Toxicity**	Toxic in high doses
Specific to a cell type (blood, liver, nerve); induces TNF production resulting in fever	**Effects on the body**	Systemic: fever, inflammation
Small proteins	**Chemical composition**	Lipopolysaccharide of cell wall
Unstable	**Heat denaturation at 60°C**	Stable
Can be converted to toxoid*	**Toxoid formation**	Cannot be converted to toxoid
Stimulate antitoxins**	**Immune response**	Does not stimulate antitoxins
Usually not	**Fever stimulation**	Yes
Secreted from live cell	**Manner of release**	Released by cell via shedding or during lysis
A few gram-positive and gram-negative	**Typical sources**	All gram-negative bacteria

*A toxoid is an inactivated toxin used in vaccines.
**An antitoxin is an antibody that reacts specifically with a toxin.

Figure 11.4 **The origins and effects of circulating exotoxins and endotoxin**. **(a)** Exotoxins, given off by live cells, have highly specific targets and physiological effects. **(b)** Endotoxin, given off when the cell wall of gram-negative bacteria disintegrates, has more generalized physiological effects.

are unlikely to cause disease (*Lactobacillus* bacteria in our gut). But most microbes fall somewhere in between, meaning they can cause disease under the right circumstances. In addition, the other part of the disease equation is the human host. There are many variables among humans, both more or less permanent (genetics) and temporary (fatigue or pregnancy) characteristics. When there is a decrease in a host's ability to mount an immune defense, these patients are termed *immunocompromised*. This situation can come about due to an underlying condition (that is, pregnancy or an autoimmune disease) or due to drugs being taken for another disease, such as cancer. Because of advances in treatment of many chronic diseases and cancer, many more people who are functioning in daily society are, in fact, immunocompromised. This makes them more susceptible to infections.

Microbes that don't usually cause disease in healthy people are called opportunistic pathogens. These are microbes that take advantage of immunocompromised hosts to cause disease. In practice, the distinction between "true" pathogens and opportunistic ones is becoming less useful as we gain more understanding of the infection process and also as many more people have some degree of immunocompromise. It is more useful to assess each host-microbe encounter on its own terms. In every encounter between a microbe and a host, several factors determine whether disease will result. It is why you may be the only member of your family not sickened by that "bad" potato salad that everyone ate, for example.

Figure 11.5 lays out the factors on both sides—the microbe's and the host's. Several examples are illustrated, with the likely outcomes resulting from the varying "settings" on the host and microbe sides. On the microbe side (the blue column), three factors are (1) its native virulence (for example, does it produce exotoxins?); (2) how many organisms encountered the host (how close it is to its optimal infectious dose); and (3) whether it made contact with the correct portal of entry. On the host side, important factors are (1) natural genetic variability,

which will impact how well components of our defense respond; (2) whether the host has seen the microbe before,

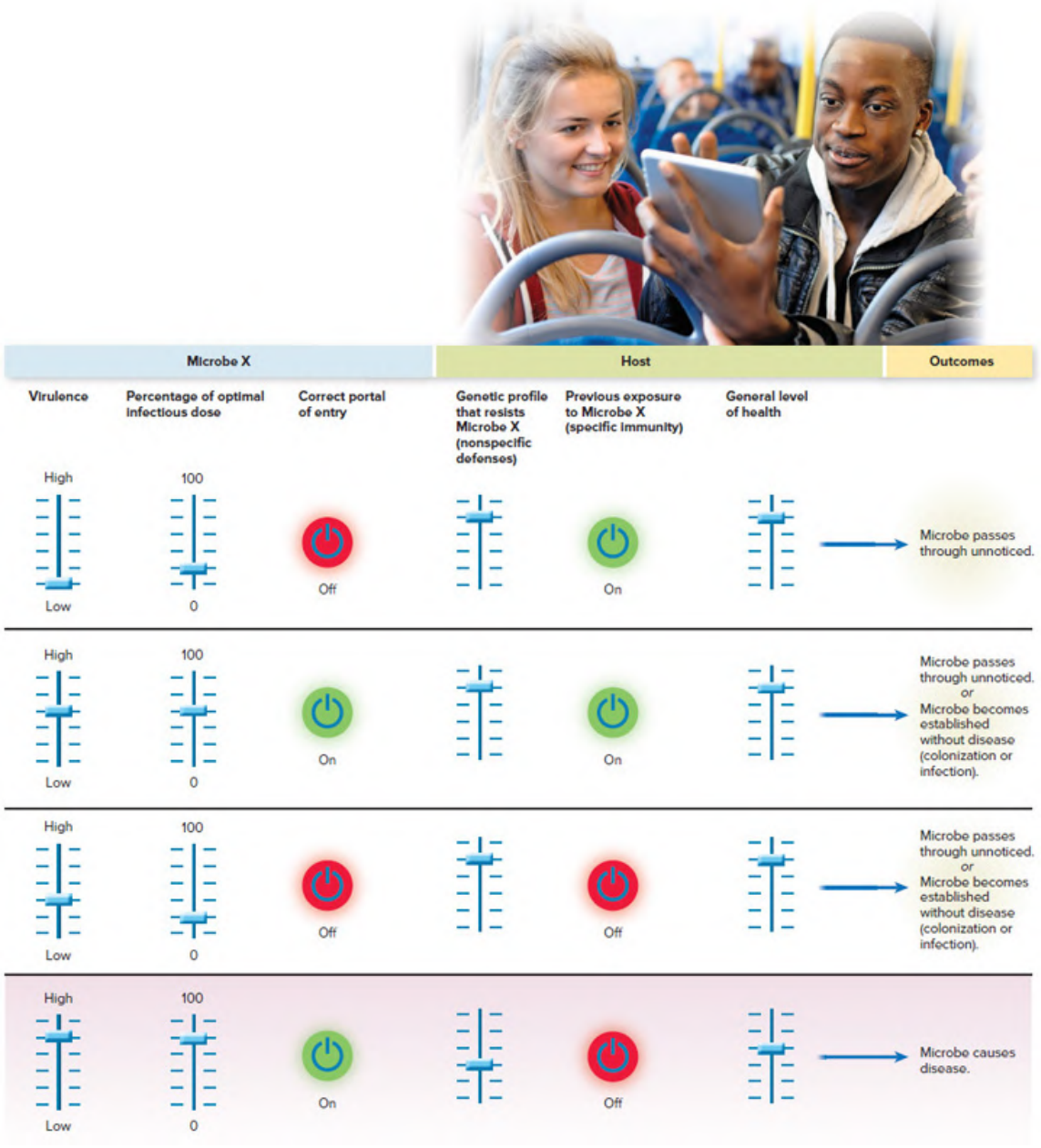


Figure 11.5 **Will disease result from an encounter between a (human) host and a microorganism?** In most cases, all of the slider bars must be in the correct ranges and the microbe's toggle switch must be in the "on" position, while the host's toggle switch must be in the "off" position in order for disease to occur. These are just a few examples and not the only options. For instance, you can see from the third row that even when the host has no specific immunity, for example, the microbe does not have enough advantages to cause disease.

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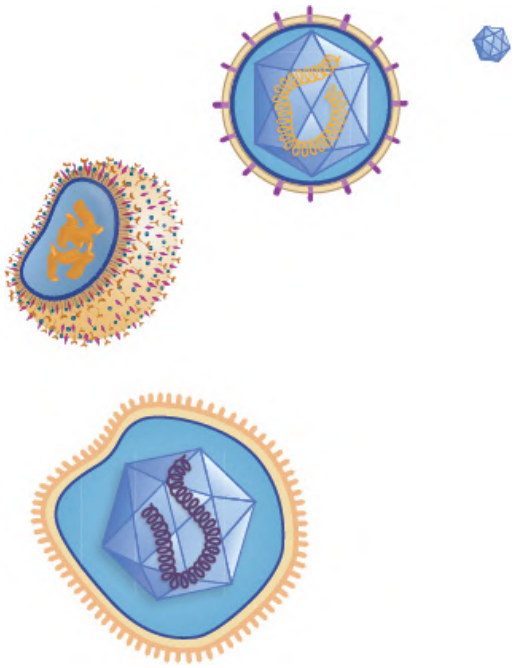
Table 11.3 Definitions of Infection Types

Type of Infection	Definition	Example
Localized infection	Microbes enter the body, remain confined to a specific tissue	Boils, warts, fungal skin infections
Systemic infection	Infection spreads to several sites and tissue fluids—usually via the bloodstream—but may travel by other means such as nerves (rabies) and cerebrospinal fluid (meningitis)	Mumps, rubella, chickenpox, AIDS, anthrax, typhoid, syphilis
Focal infection	Infectious agent spreads from a local site and is carried to other tissues	Tuberculosis, streptococcal pharyngitis
Mixed infection (polymicrobial infection)	Several agents establish themselves simultaneously at the infection site	Human bite infections, wound infections, gas gangrene
Primary infection	The initial infection	Can be any infection
Secondary infection	A second infection caused by a different microbe, which complicates a primary infection; often a result of lowered host immune defenses	Influenza complicated by pneumonia, common cold complicated by bacterial otitis media
Acute infection	Infection comes on rapidly, with severe but short-lived effects	Influenza
Chronic infection	Infection that progresses and persists over a long period of time	HIV



either through infection or vaccination; and (3) the host's general level of health. Read each row from left to right to see how one particular interaction turns out.

Let's look at row 2 as an example from the figure. In this scenario the anonymous microbe has a moderate ability to be virulent, it has encountered the host with about half of its optimal infectious dose, but it did access the correct portal of entry. The host does have a genetic profile that provides high defenses for this microbe, and has seen the microbe before, meaning that it

will have some degree of specific immunity. Lastly, the host is generally healthy. So, even though the microbe has some things going for it, it will not be likely to cause disease but will simply be transient, or, if it does become established in the host it will not cause disease. Spend some time on the other rows and you will gain an appreciation of when an infectious disease occurs or not.

Manifestations of Disease

Patterns of Infection

There is more than one way to have an infection. Clinicians use specific terms to talk about infections that are only in one location (localized infection) or in a location that is different than the initial infection (focal infection). See **table 11.3** for important terms that describe infections.

Signs and Symptoms: Warning Signals of Disease

When an infection causes pathologic changes that lead to disease, it is often accompanied by a variety of signs and symptoms. A **sign** is any objective evidence of disease as noted by an observer; a **symptom** is the subjective evidence of disease as sensed by the patient. In general, signs are more precise than symptoms, though both can have the same underlying cause. For example, an infection of the brain might present with the sign of bacteria in the spinal fluid and symptom of headache. When a disease can be identified or defined by a certain complex of signs and symptoms, it is termed a **syndrome**. Specific signs and symptoms for particular infectious diseases are covered in chapters 16 through 21.

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3. Which of the following factors is not thought to weaken host defenses?

a. *extremes in age*
b. *underlying disease states*
c. *surgery*
d. *moderate exercise*

Humans shed about 1 million skin cells—and the microbes on them—every day.
©Rubberball/Getty Images

Signs and Symptoms of Inflammation The earliest symptoms of disease usually come from the activation of the body defense process called **inflammation**. The inflammatory response includes cells and chemicals that respond nonspecifically to disruptions in the tissue. Some common symptoms of inflammation include fever, pain, soreness, and swelling. Signs of inflammation include **edema**, the accumulation of fluid in an afflicted tissue; **granulomas** and **abscesses**, walled-off collections of inflammatory cells and microbes in the tissues; and **lymphadenitis**, swollen lymph nodes.

Signs of Infection in the Blood Changes in the number of circulating white blood cells, as determined by special counts, are considered to be signs of possible infection. **Leukocytosis** (loo″-koh′-sy-toh′-sis) is an increase in the level of white blood cells, whereas **leukopenia**

(loo″-koh-pee′-nee-uh) is a decrease. Other signs of infection revolve around the occurrence of a microbe or its products in the blood. The clinical term for blood infection, **septicemia**, refers to a general state in which microorganisms are multiplying in the blood and are present in large numbers. When small numbers of bacteria or viruses are found in the blood, the correct terminology is **bacteremia**, or **viremia**, respectively which means that these microbes are present in the blood but are not necessarily multiplying.

During infection, a normal host will invariably show signs of an immune response in the form of antibodies in the serum. This fact is the basis for several serological tests used in diagnosing infectious diseases such as AIDS or syphilis. Such specific immune reactions indicate the body's attempt to develop specific immunities against pathogens. We concentrate on this role of the host defenses in chapters 12 and 13.

Infections That Go Unnoticed

It is rather common for an infection to produce no noticeable symptoms, even though the microbe is active in the host tissue. In other words, although infected, the host does not manifest the disease. An infection of this nature is known as **asymptomatic** or **subclinical** (inapparent) because the patient experiences no symptoms or disease and does not seek medical attention.

Step Five: Vacating the Host—Portals of Exit

Earlier, we introduced the idea that a parasite is considered *unsuccessful* if it does not have a mechanism for leaving its host and moving to other susceptible hosts. With few exceptions, pathogens depart by a specific avenue called the **portal of exit** (**figure 11.6**). In most cases, the pathogen is shed or released from the body through secretion, excretion, discharge, or sloughed tissue. The usually very high number of infectious agents in these materials increases the likelihood that the pathogen will reach other hosts. In many cases, the portal of exit is the same as the portal of entry, but some pathogens use a different route. As we see in the next section, the portal of exit concerns epidemiologists because it greatly influences the dissemination of infection in a population.

Figure 11.7 illustrates the five steps of disease causation by microbes.

Long-Term Infections and Long-Term Effects

The apparent recovery of the host does not always mean that the microbe has been completely removed or destroyed by the host defenses. After the initial symptoms in certain chronic infectious diseases, the infectious agent retreats into a dormant state called **latency**. Throughout this latent state, the microbe can periodically become active and produce a recurrent disease. The viral agents of herpes simplex, herpes zoster, hepatitis B, AIDS, and Epstein-Barr can persist in the host for long periods. The agents of syphilis, typhoid fever, tuberculosis, and malaria can also enter into latent stages. The person harboring a persistent infectious agent may or may not shed it during the latent stage. If it is shed, such persons are chronic carriers who serve as sources of infection for the rest of the population.

Some diseases leave **sequelae** (the medical term for consequences) in the form of long-term or permanent damage to tissues or organs. For example, meningitis can

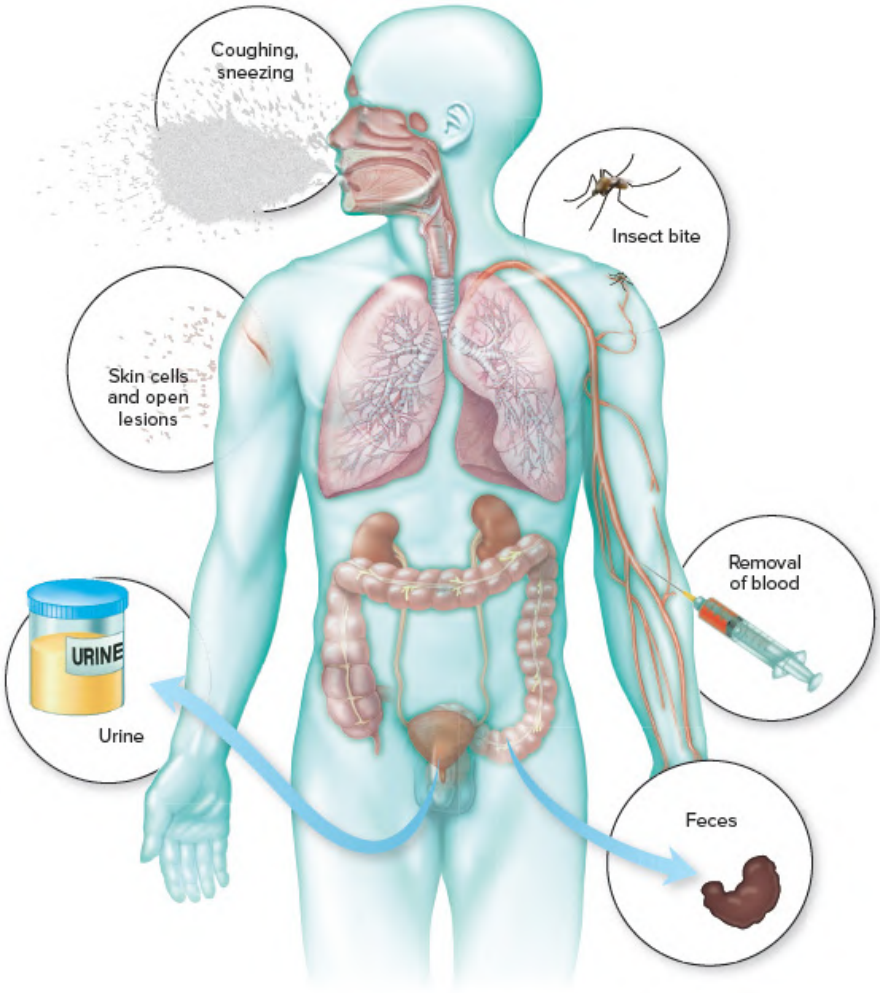

Figure 11.6 **Major portals of exit of infectious diseases**.

Medical Moment

A Gene That Protects You from the Flu

The natural genetic variability among humans is one of the reasons some people manifest disease when encountering a microbe and others do not. A recently discovered example of this is a human gene called "*IFITM3*." During the 2009–2010 swine flu pandemic, researchers were examining differences between those who became ill and those who did not. In this event the vast majority of people who were infected experienced only mild symptoms. However, a small minority of them got seriously ill and many of these died. Scientists discovered that when the protein produced by the *IFITM3* gene was present in the body in large quantities, patients were largely protected from the worst effects of the virus. Follow-up studies showed that many of the people who were sick enough to be hospitalized in that pandemic displayed a single nucleotide polymorphism (SNP) in that gene.

Q. Using the information in this article, devise a possible new antiviral treatment.

Answer in Appendix B.

result in deafness, strep throat can lead to rheumatic heart disease, Lyme disease can cause arthritis, and polio can produce paralysis.

The Course of an Infection

There are four distinct phases of infection and disease: the incubation period, the prodrome, the acute period, and the convalescent period. The **incubation period** is the time from initial contact with the infectious agent (at the portal of entry) to

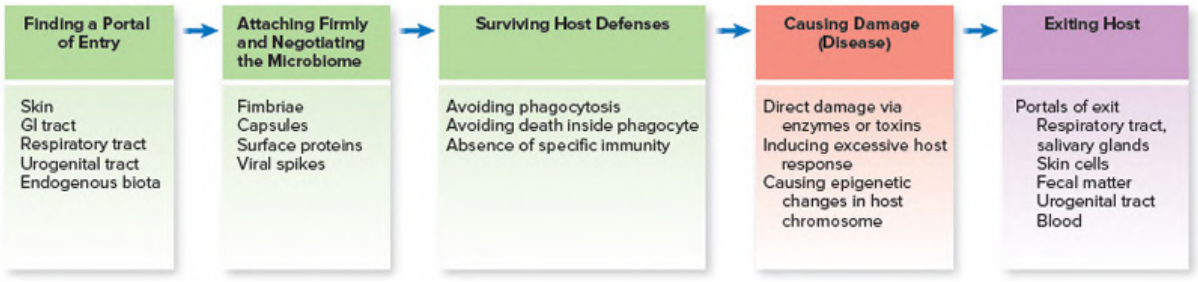

Figure 11.7 **The steps involved when a microbe causes disease in a host**.

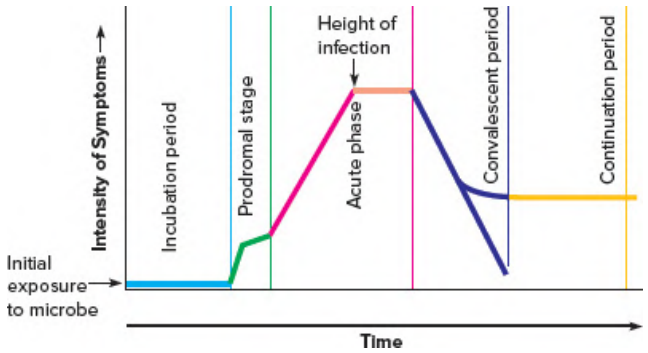

Figure 11.8 **Stages in the course of infection and disease**. The stages have different durations in different infections.

the appearance of the first symptoms. During the incubation period, the agent is multiplying at the portal of entry but has not yet caused enough damage to elicit symptoms. Although this period is relatively well defined and predictable for each microorganism, it does vary according to host resistance, degree of virulence, and distance between the target organ and the portal of entry (the farther apart, the longer the incubation period). Overall, an incubation period can range from several hours in pneumonic plague to several years in leprosy. The majority of infections, however, have incubation periods ranging between 2 and 30 days.

The earliest notable symptoms of most infections appear as a vague feeling of discomfort, such as head and muscle aches, fatigue, upset stomach, and general malaise. This short period (1–2 days) is known as the **prodromal stage**. Some diseases have very specific prodromal symptoms. Next, the infectious agent enters an **acute** phase, during which it multiplies at high levels, exhibits its greatest virulence, and becomes well established in its target tissue. This period is often marked by fever and other prominent and more specific signs and symptoms, which can include cough, rashes, diarrhea, loss of muscle control, swelling, jaundice, discharge

of exudates, or severe pain, depending on the particular infection. The length of this period is extremely variable.

As the patient begins to respond to the infection, the symptoms decline—sometimes dramatically, other times slowly. During the recovery that follows, called the **convalescence**, the patient's strength and health gradually return owing to the healing nature of the immune response. During this period, many patients stop taking their antibiotics, even though there are still pathogens in their system. Think about it—the microbes still alive at this stage of treatment are the ones in the population with the most resistance to the antibiotic. In most cases, continuing the antibiotic dosing will take care of them. But stop taking the drug now and the bacteria that are left to repopulate are the ones with the higher resistance.

These are the four phases all infectious diseases have. There is a fifth phase, which only some infections have: the **continuation** phase, in which *either* the organism lingers for months, years, or indefinitely after the patient is completely well *or* the organism is gone but symptoms continue. Typhoid fever is one disease in which the organism lingers after the patient has fully recovered. An example of a disease that lingers after the organism is no longer detectable is syphilis (in some cases). Chronic Lyme disease can also be put in that category. The stages are summarized in **figure 11.8**.

The transmissibility of the microbe during these five stages is different for each microorganism. A few agents are released mostly during incubation (measles, for example); many are released primarily during the acute period (*Shigella*); and others can be transmitted during all of these periods (hepatitis B).

Reservoirs: Where Pathogens Come From

In order for an infectious agent to continue to exist and be spread, it must have a permanent place to reside. The **reservoir** is the primary habitat in the natural world where a potential pathogen makes its home. Often it is a human or animal carrier, although soil, water, and plants are also reservoirs. The reservoir can be distinguished from the infection *transmitter*, which is the individual or object from which an infection is actually acquired. In diseases such as syphilis, the reservoir and the transmitter are the same (the human body). In the case of hepatitis A, the reservoir (a human carrier) is usually different from the mode of transmission (contaminated food). **Table 11.4** shows how reservoirs and transmission are interrelated.

Living Reservoirs

The list of both living and nonliving reservoirs is presented in table 11.4. You can probably guess that a great number of infections that affect humans have their reservoirs in other humans. Persons or animals with obvious symptomatic infection

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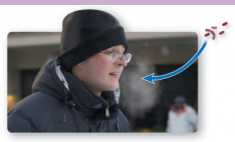
4. A patient has been diagnosed with E. coli septicemia. Which of the following characteristics are associated with endotoxins? Select all that apply.

a. *toxicity in minimal concentration*
b. *gram-negative bacteria*
c. *presence of fever*
d. *protein composition*

e. *released by cell during lysis*

Table 11.4 Reservoirs and Transmitters

Reservoirs	Transmission Examples
Living Reservoirs	
Animals (Other than humans and arthropods) Mammals, birds, reptiles, etc.	Pathogens from animals • can be directly transmitted to humans, as in the example of bats transmitting rabies to humans • can be transmitted to humans via vectors, as with fleas passing the plague from rats to people • can be transmitted through vehicles such as water, as in the case of leptospirosis, which is often transmitted from animal urine to human skin via bodies of water
Humans Actively ill	©Thinkstock/Getty Images (writing); ©Ingram Publishing (pen in mouth) A person suffering from a cold contaminates a pen, which is then picked up by a healthy person. That is indirect transmission. Alternatively, a sick person can transmit the pathogen directly by sneezing on a healthy person.
Carriers	A person who is fully recovered from his hepatitis but is still shedding hepatitis A virus in his feces may use suboptimal hand-washing technique. He contaminates food, which a healthy person ingests (indirect transmission). Carriers can also transmit through direct means, as when an incubating carrier of HIV, who does not know she is infected, transmits the virus through sexual contact.
Arthropods Biological vectors	 Source: CDC When an arthropod is the host (and reservoir) of the pathogen, it is also the mode of transmission.
Nonliving Reservoirs	
Soil **Water** **Air** **The built environment**	 ©McGraw-Hill Education/Christopher Kerrigan, photographer Some pathogens, such as the TB bacterium, can survive for long periods in nonliving reservoirs. They are then directly transmitted to humans when they come in contact with the contaminated soil, water, or air.

are obvious sources of infection, but a **carrier** is, by definition, an individual who *inconspicuously* shelters a pathogen and spreads it to others without any notice. The duration of the carrier state can be short or long term, and it is important to remember that the carrier may or may not have experienced disease due to the microbe.

Several situations can produce the carrier state. **Table 11.5** describes the various carrier states and provides examples of each.

Table 11.5 Carrier States

Carrier State	Explanation	Example
Asymptomatic carriers	Infected but show no symptoms of disease	Gonorrhea, genital herpes with no lesions, human papillomavirus
Incubating carriers	Spread the infectious agent during the incubation period	Infectious mononucleosis
Convalescent carriers	Recuperating patients without symptoms; they continue to shed viable microbes and convey the infection to others	Hepatitis A
Chronic carriers	Individuals who shelter the infectious agent for a long period after recovery because of the latency of the infectious agent	Tuberculosis, typhoid fever
Passive carriers	Medical and dental personnel who must constantly handle patient materials that are heavily contaminated with patient secretions and blood risk picking up pathogens mechanically and accidentally transferring them to other patients	Various healthcare-associated infections

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Table 11.6 Common Zoonotic Infections

Disease	Primary Animal Reservoirs
Viruses	
Rabies	Mammals
Yellow fever	Wild birds, mammals, mosquitoes
Viral fevers	Wild mammals
Hantavirus	Rodents
Influenza	Chickens, birds, swine
West Nile virus	Wild birds, mosquitoes

Bacteria	
Rocky Mountain spotted fever	Dogs, ticks
Psittacosis	Birds
Leptospirosis	Domestic animals
Anthrax	Domestic animals
Brucellosis	Cattle, sheep, pigs
Plague	Rodents, fleas
Salmonellosis	Mammals, birds, reptiles, and rodents
Tularemia	Rodents, birds, arthropods

Miscellaneous	
Ringworm	Domestic mammals
Toxoplasmosis	Cats, rodents, birds
Trypanosomiasis	Domestic and wild mammals
Trichinosis	Swine, bears
Tapeworm	Cattle, swine, fish

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5. A patient is found to have a dental abscess. Antibiotic therapy is initiated to prevent spread of the infection to other tissues. If it does spread, what type of infection is it?

a. *focal infection*
b. *primary infection*
c. *secondary infection*
d. *mixed infection*

Animals as Reservoirs and Sources Animals deserve special consideration as reservoirs of infections. The majority of animal reservoir agents are arthropods such as fleas, mosquitoes, flies, and ticks. Larger animals can also spread infection—for example, mammals (rabies), birds (psittacosis), or lizards (salmonellosis).

Many vectors and animal reservoirs spread their own infections to humans. An infection indigenous to animals but naturally transmissible to humans is a **zoonosis** (zoh″-uh-noh′-sis). In these types of infections, the human is essentially a dead-end host and does not contribute to the natural persistence of the microbe. Some zoonotic infections (rabies, for instance) can have multihost involvement, and others can have very complex cycles in the wild (see plague in chapter 18). Zoonotic spread of disease is promoted by close associations of humans with animals, and people in animal-oriented or outdoor professions are at greatest risk. At least 150 zoonoses exist worldwide; the most common ones are listed in **table 11.6**. Zoonoses make up a

full 70% of all new emerging diseases worldwide. It is worth noting that zoonotic infections are impossible to completely eradicate without also eradicating the animal reservoirs.

Nonliving Reservoirs

Microorganisms have adapted to nearly every habitat in the biosphere. They thrive in soil and water and are in the air. They also colonize what is known as “the built

Airplanes can play a role in spreading diseases—but not in the way you might think. Studies have shown that airborne diseases are *not* more easily spread in airplane cabins. However, airplanes can move sick people from one continent to another quickly and thus widen an epidemic into a pandemic.

©Tony Cordoza/Alamy Stock Photo (airplane); ©Smith Collection/Gado/Archive Photos/Getty Images (aisle); ©ColorBlind Images/Getty Images (passengers)

environment," surfaces in homes, office buildings, and structures of all kinds. Although most of these microbes are saprobic and cause little harm and considerable benefit to humans, some are opportunists and a few are regular pathogens. Because human hosts are in regular contact with these environmental sources, acquisition of pathogens from nonliving reservoirs is always a possibility.

The Transmission of Infectious Agents

Infectious diseases can be categorized on the basis of how they are acquired. A disease is **communicable** when an infected host can transmit the infectious agent to another host and establish infection in that host. (Although this terminology is standard, one must realize that it is not the disease that is communicated but the microbe. Also be aware that the word *infectious* is sometimes used interchangeably with the word *communicable*, but this is not precise usage.) The transmission of the agent can be direct or indirect, and the ease with which the disease is transmitted varies considerably from one agent to another. If the agent is highly communicable, especially through direct contact, the disease is **contagious**. Influenza and measles move readily from host to host and thus are contagious, whereas Hansen's disease (leprosy) is only weakly communicable. Because they can be spread through the population, communicable diseases are our main focus in the following sections.

In contrast, a **noncommunicable** infectious disease does *not* arise through transmission of the infectious agent from host to host. The infection and disease are acquired through some other special circumstance. Noncommunicable infections occur primarily when a compromised person is invaded by his or her own microbiota. Ear infections are good examples of this. Other instances of this are when an individual has accidental contact with a microbe that exists in a nonliving reservoir such as soil. Some examples are certain mycoses, acquired through inhalation of fungal spores, and tetanus, in which *Clostridium tetani* spores from a soiled object enter a cut or wound. Persons thus infected do not become a source of disease to others.

Patterns of Transmission in Communicable Diseases

There are many different ways that microbes can be transmitted. They are summarized in **table 11.7**. First of all, we speak of horizontal versus vertical transmission. The term *horizontal* means the disease is spread through a population from one infected individual to another. *Vertical* signifies transmission from parent to offspring via the ovum, sperm, placenta, or milk.

Within the category of horizontal transmission, we can further subdivide the mechanisms into three broad groups: direct contact, indirect routes, and vector transmission. Direct transmission involves very close contact between people. Indirect transmission occurs when an object or substance carries the agent from one person to another. A special category of indirect transmission is parenteral transmission. It refers to a puncture, in which material from the environment is deposited directly in deeper tissues. This can be intentional, in the case of contaminated needles, or unintentional, as in the case of puncture injuries. Lastly, vectors are arthropods that harbor an infectious agent and transmit it to a human.

Vectors can be further subdivided into two categories, biological and mechanical. A vector is termed biological if the microorganism lives and multiplies within the insect. Mechanical

vectors are not necessary to the life cycle of an infectious agent and merely transport it without being infected. The external body parts of these animals become contaminated when they come into physical contact with a source of pathogens. Houseflies are mechanical vectors. They feed on decaying garbage and feces, and while they are feeding, their feet and mouthparts easily become contaminated.

Table 11.7 Patterns of Transmission in Communicable Diseases

Mode of Transmission	Definition
Vertical	Transmission is from parent to offspring via the ovum, sperm, placenta, or milk
Horizontal	Disease is spread through a population from one infected individual to another
Direct (contact) transmission	Involves very close proximity or actual physical contact between two hosts Types: • Touching, kissing, sex • Droplet contact, in which fine droplets are sprayed directly upon a person during sneezing, coughing, or speaking
Indirect transmission	Infectious agent must pass from an infected host to an intermediate conveyor (a vehicle) and from there to another host Infected individuals contaminate objects, food, or air through their activities Types: • **Fomite**–inanimate object that harbors and transmits pathogens (doorknobs, telephone receivers, faucet handles) • **Vehicle**–a natural, nonliving material that can transmit infectious agents • **Air**–smaller particles evaporate and remain in the air and can be encountered by a new host; **aerosols** are suspensions of fine dust or moisture particles in the air that contain live pathogens • **Water**–some pathogens survive for long periods in water and can infect humans long after they were deposited in the water • **Soil**–microbes resistant to drying live in and can be transmitted from soil • **Food**–meats may contain pathogens with which the animal was infected; foods can also be contaminated by food handlers • **Parenteral** transmission via intentional or unintentional injection into deeper tissues (needles, knives, branches, broken glass, etc.) **Special Category: oral-fecal route**–using either vehicles or fomites. A fecal carrier with inadequate personal hygiene contaminates food during handling, and an unsuspecting person ingests it; alternatively a person touches a surface that has been contaminated with fecal material and touches his or her mouth, leading to ingestion of fecal microbes
Vector transmission	Types: • **Mechanical vector**–insect carries microbes to host on its body parts • **Biological vector**–insect injects microbes into host; part of microbe life cycle completed in insect

Contact: kissing and sex (Epstein-Barr virus, gonorrhea)

Droplets (colds, chickenpox)

©Guas/Shutterstock (kissing); Source: CDC/James Gathany (sneezing); ©silverlining56/Getty Images (opening door); ©Caia Images/Glow Images (playing soccer); ©IT Stock Free/Alamy Stock Photo (fly); ©abadonian/Getty Images (mosquito)

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Healthcare-Associated Infections

Infectious diseases that are acquired or develop during a hospital or health care facility stay are known as **healthcare-associated infections** (HAIs), also called **nosocomial** (nohz″-oh-koh′-mee-al) **infections**. This concept seems strange at first thought, because a hospital is regarded as a place to get treatment for a disease, not a place to acquire a disease. Yet it is not uncommon for a surgical patient's incision to become infected or a burn patient to develop a case of pneumonia in the clinical setting. The rate of healthcare-associated infections can be as low as 0.1% or as high as 20% of all admitted patients, depending on the clinical setting, with an average of about 4%. This adds up to about 750,000 cases in acute care hospitals every year, which result in nearly 75,000 deaths.

When you examine the circumstances, you see that a certain number of HAIs are virtually unavoidable. After all, the hospital both attracts and creates compromised patients, and it serves as a collection point for pathogens. Some patients become infected when surgical procedures or lowered defenses permit resident biota to invade their tissues. Other patients acquire infections directly or indirectly from fomites, medical equipment, other patients, medical personnel, visitors, air, and water.

The health care process itself increases the likelihood that infectious agents will be transferred from one patient to another. A 2016 study of intensive care nurses at a university hospital found that on 10% of the shifts the nurses' scrubs became contaminated with dangerous bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA). Indwelling devices such as catheters, prosthetic heart valves, grafts, drainage tubes, and tracheostomy tubes form ready portals of entry and habitats for infectious agents. Because such a high proportion of the hospital population receives antimicrobial drugs during their stay, drug-resistant microbes are selected for at a much greater rate than is the case outside the hospital.

The most common HAIs include pneumonia, gastrointestinal illness, urinary tract infections, bloodstream infections, and surgical site infections **(figure 11.9)**.

The five most common hospital pathogens, and what they usually cause, are:

Clostridium difficile (C. diff): GI infections

Staphylococcus aureus: pneumonia, surgical site infections, bloodstream infections

Klebsiella species: surgical site infections, urinary tract infections, pneumonia

Escherichia coli: urinary tract infections, surgical site infections, bloodstream infections

Enterococcus species: surgical site infections, urinary tract infections, bloodstream infections

The frequency of HAIs and the microbes responsible for them are dynamic situations. As hospitals target problem areas, the most prominent infections shift.

Other organisms hospitals are on alert for are *Acinetobacter*, a soil and water bacterium that causes outbreaks among the sickest patients in hospitals; *Burkholderia cepacia* (*B. cepacia*), another environmental bacterium that causes disease only in already-ill patients; and carbapenem-resistant Enterobacteriaceae, such as *Klebsiella* and *E. coli*. As a group these are termed "CRE." They are often resistant to most available antibiotics.

In 2016 a new organism began to worry U.S. health care officials. A multidrug-resistant yeast called *Candida auris* has been causing HAIs in several countries on four different continents, since 2009. It causes highly invasive infections with high mortality rates. Only one U.S. case was cited in the CDC alert issued in 2016, but due to its appearance over such a wide geographic area, the agency issued the alert to encourage hospitals to be on the lookout for it. This merely highlights the ever-changing nature of HAIs and the need for vigilance.

Hospitals generally employ an *infection control officer* who not only implements proper practices and procedures throughout the hospital but is also charged with

Medical Moment

Eye on Careers: Infection Control Practitioner

An infection control practitioner (ICP) holds an integral position in many hospitals and health care organizations. An ICP's biggest role is to reduce the spread of healthcare-associated infections, limiting the spread of infectious disease. Many different professionals may be designated as ICPs, including nurses, doctors, epidemiologists, or others who have taken specialized training to prepare them for this important role.

An ICP may be responsible for the following:

- tracking positive cultures to ensure treatment has been implemented;
- following up actual and potential exposures to communicable diseases;
- training and educating staff regarding infection control practices and protocols;
- communicating with government entities such as the CDC (the Centers for Disease Control and Prevention), state public health departments, workers' compensation boards, and OSHA (the Occupational Safety and Health Administration).

Q. Can you think of what type of incident might lead an ICP to get involved with the care of a health care worker in a hospital?

Answer in Appendix B.

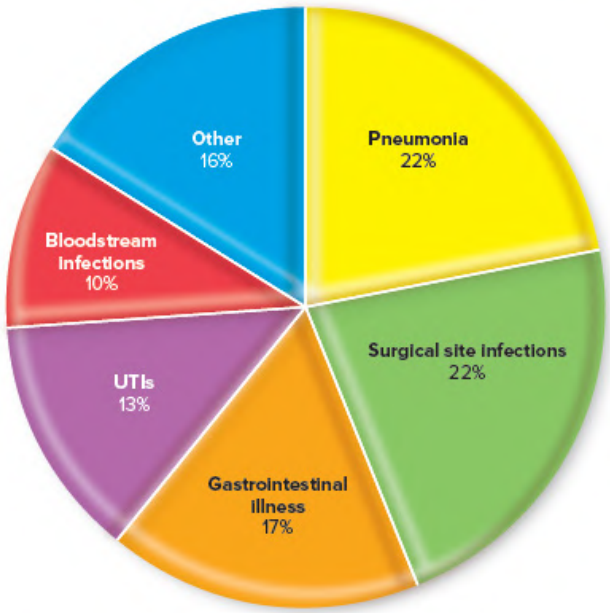


Figure 11.9 **Most common healthcare-associated infections**. Relative frequency by target area.

tracking potential outbreaks, identifying breaches in asepsis, and training other health care workers in aseptic technique. Among those most in need of this training are nurses and other caregivers whose work, by its very nature, exposes them to needlesticks, infectious secretions, blood, and physical contact with the patient. The same practices that interrupt the routes of infection in the patient can also protect the health care worker. It is for this reason that most hospitals have adopted universal precautions that recognize that all secretions from all persons in the clinical setting are potentially infectious and that transmission can occur in either direction.

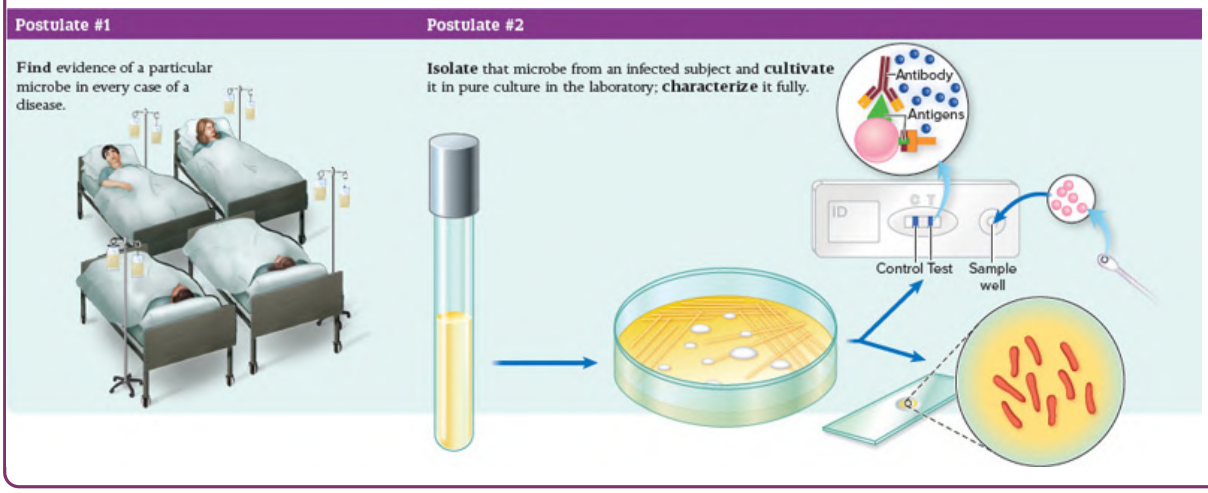
Which Agent Is the Cause? Using Koch's Postulates to Determine Etiology

An essential aim in the study of infection and disease is determining the precise **etiologic**, or causative, **agent** of a newly recognized condition. In our modern technological age, we take for granted that a certain infection is caused by a certain microbe, but such has not always been the case. More than a century ago, Robert Koch realized that in order to prove the germ theory of disease he would have to develop a standard for determining causation that would stand the test of scientific scrutiny. Out of his experimental observations on the transmission of anthrax in cows came a series of proofs, called **Koch's postulates**, that established the principal criteria for etiologic studies. **Table 11.8** demonstrates the principles of Koch's postulates.

The general idea of Koch's postulates is that you must isolate what you think the cause is, then apply it to a naive population, and then produce the same effect. This general progression is still considered the gold standard for determining that any given factor is causing—not simply correlated with—some observed condition. Many of the media reports you read about the latest "findings" are based on studies reporting correlations, not causation. Take the assertion that Facebook usage leads to shorter attention spans. Finding a causative relationship between the two things would require taking large numbers of people who had never used Facebook, measure their attention spans, and then forcing them to use Facebook for some

allotted time period. Then you would need to remeasure their attention spans. This would approach the gold standard of Koch's postulates, although you would not be able to completely hold all other variables constant in your subjects, unless you kept them all in your laboratory for the whole duration of your experiment. So you begin to see how difficult true causation is to prove.

Table 11.8 ***Koch's Postulates***



Koch's postulates are reliable for many infectious diseases, but they cannot be completely fulfilled in certain situations. For example, some infectious agents are not readily isolated or grown in the laboratory. If one cannot elicit an infection similar to that seen in humans by inoculating it into an animal, it is very difficult to prove the etiology. It is difficult to satisfy Koch's postulates for viral diseases because viruses usually have a very narrow host range. Human viruses may cause disease only in humans, or perhaps in primates, though the disease symptoms in apes will often be different. To address this, T. M. Rivers proposed modified postulates for viral infections.

One very important reason Koch's postulates are increasingly viewed with caution is the idea, presented earlier, that perhaps the majority of human infections are polymicrobial. In those infections Koch's postulates cannot be satisfied.

11.2 LEARNING OUTCOMES—Assess Your Progress

4. Differentiate between a microbe's pathogenicity and its virulence.
5. List the steps a microbe has to take to get to the point where it can cause disease.
6. Explain the significance of polymicrobial infections.
7. List several portals of entry and exit.
8. Define *infectious dose*, and explain its role in establishing infection.
9. Describe three ways microbes cause tissue damage.
10. Compare and contrast major characteristics of endotoxin and exotoxins.
11. Provide a definition of *virulence factors*.

12. Draw a diagram of the stages of disease in a human.
13. Differentiate among the various types of reservoirs, providing examples of each.
14. List several different modes of transmission of infectious agents.
15. Define *healthcare-associated infection*, and list the three most common types.
16. List Koch's postulates, and discuss when they might not be appropriate in establishing causation.

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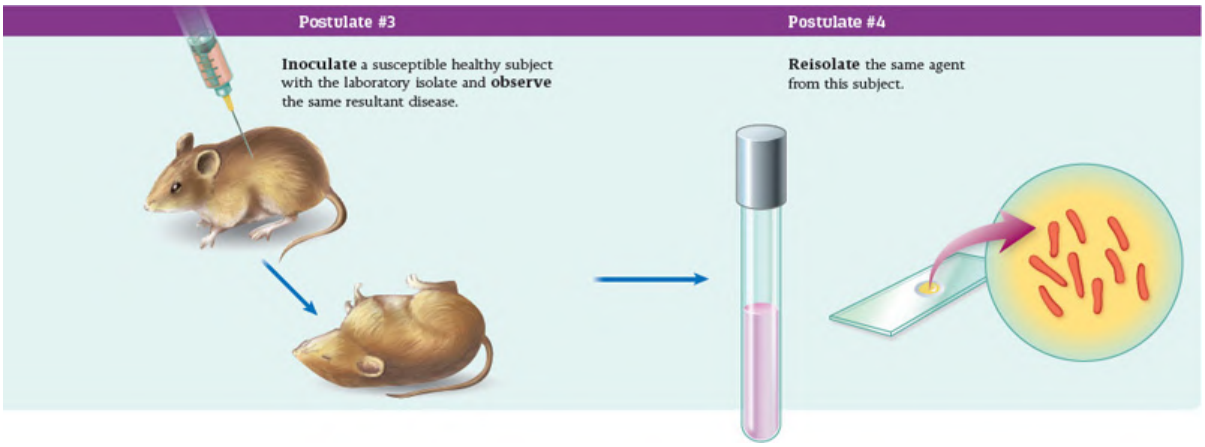
6. A client reported onset of these symptoms a few days ago: low-grade fever, arthralgia, and fatigue. The client was seen in the outpatient clinic by a health care provider, diagnosed with a bacterial infection, and prescribed a course of antibiotic therapy. At present, the client has 3 days of antibiotic treatment remaining. The client would be in which stage of infection?

a. *acute phase*
b. *convalescent period*
c. *incubation period*
d. *prodromal stage*



11.3 Epidemiology: The Study of Disease in Populations

So far, our discussion has revolved primarily around the impact of an infectious disease in a single individual. Let us now turn our attention to the effects of diseases on the community—the realm of **epidemiology**. By definition, this term involves the study of the frequency and distribution of disease and other health-related factors in defined populations. It involves many disciplines—not only microbiology but also anatomy, physiology, immunology, medicine, psychology, sociology, ecology, and statistics—and it considers all forms of disease, including heart disease, cancer, drug addiction, and mental illness.

A groundbreaking British nurse named Florence Nightingale helped to lay the foundations of modern epidemiology. She arrived in the Crimean war zone in Turkey in the mid-1850s,

where the British were fighting and dying at an astonishing rate. Estimates suggest that 20% of the soldiers there died (by contrast, 2.6% of U.S. soldiers in the Vietnam war died). Even though this was some years before the discovery of the germ theory, Nightingale understood that filth contributed to disease and instituted methods that had never been seen in military field hospitals. She insisted that separate linens and towels be used for each patient, and that the floors be cleaned and the pipes of sewage unclogged. She kept meticulous notes of what was killing the patients and was able to demonstrate that many more men died of disease than of their traumatic injuries. She used statistical analysis to convince government officials that these patterns were real. This was indeed one of the earliest forays into epidemiology—trying to understand how diseases were being transmitted and using statistics to do so.

Epidemiologists try to identify causative agents, using adaptations of Koch's postulates when the disease is not an infectious disease, or using nonexperimental types of analyses. The techniques of epidemiology are also used to track behaviors, such as exercise or smoking. The epidemiologist is a medical sleuth who collects clues on the causative agent, pathology, sources, and modes of transmission and tracks the numbers and distribution of cases of disease in the community. The outcomes of these studies help public health departments develop prevention and treatment programs and establish a basis for predictions.

Epidemiology is the study of disease in populations.
©Donald Nausbaum/Getty Images

Tracking Disease in the Population

Surveillance is an epidemiological term that basically means "keeping track of trends in nonemergency times." Weather patterns and human behaviors are two examples of things that are tracked as an aid to predicting disease patterns. Of course, an important task for surveillance involves keeping data for a large number of diseases seen by the medical community and reported to public health authorities. By law, certain **reportable**, or notifiable, **diseases** must be reported to authorities; others are reported on a voluntary basis. (For a list of reportable diseases in the United States, see **table 11.9**.)

A well-developed network of individuals and agencies at the local, district, state, national, and international levels keeps track of infectious diseases. Physicians and hospitals report all notifiable diseases that are brought to their attention. These reports are either made about individuals or in the aggregate, depending on the disease.

The Internet has revolutionized disease tracking. For example, a few years ago Google launched a service that used trending search terms ("fever," "flu symptoms") to predict flu outbreaks, theoretically faster than traditional surveillance methods. It had some flaws but other companies have improved on the concept. Most experts expect that this use of what is known as "big data" promises to speed up prediction and response time for disease outbreaks.

Epidemiological Statistics: Frequency of Cases

The **prevalence** of a disease is the total number of existing cases in a given population. It is often thought of as a snapshot and is usually reported as the percentage of the population having a particular disease at any given time. Disease **incidence** measures the number of *new* cases over a certain time period. This statistic indicates both the rate and the risk of infection. The equations used to figure these rates are

$$\text{Prevalence} = \frac{\text{Total number of cases in population}}{\text{Total number of persons in population}} \times 100 = \%$$

Example: The prevalence of smoking among adults in the United States is 17% currently.

$$\text{Incidence} = \frac{\text{Number of new cases in a designated time period}}{\text{Total number of susceptible persons}} \text{ (Usually reported per 100,000 persons)}$$

Example: The incidence of new Lyme disease cases in the United States in 2014 was 8.6 per 100,000.

Changes in incidence and prevalence are usually followed over a seasonal, yearly, and long-term basis and are helpful in predicting trends **(figure 11.10)**. Also of concern to the epidemiologist are the rates of disease with regard to sex, race, or geographic region. Figure 11.10*a* displays incidence trends over time as well as within different age groups; figure 11.10*b* shows another way of depicting epidemiological data, in a map format. Also of importance is the **mortality rate**, which measures the total number of deaths in a population due to a certain disease. Over the past century, the overall death rate from infectious diseases in the developed world has dropped, although the number of persons afflicted with infectious diseases (the **morbidity rate**) has remained relatively high.

Clearly, different infections have differing degrees of communicability in populations. They also have varying abilities to cause death. When epidemiologists keep track of mortality rates, they can use these as predictive tools. As they keep track of incidence rates in epidemic and nonepidemic situations, they can calculate a factor called the **reproductive rate** of a disease. That is also extremely useful in predicting and managing the spread of infectious disease. Please take a look at the Note near the

Table 11.9 Reportable Diseases in the United States*

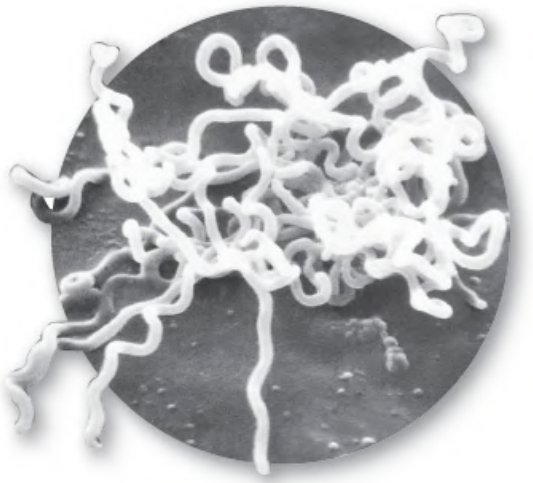
- *Anaplasma phagocytophilum*

- Anthrax
- Babesiosis
- Botulism
- Brucellosis
- California serogroup virus neuroinvasive disease
- Cancer[†]
- Chancroid
- *Chlamydia trachomatis* infections
- Cholera
- Coccidioidomycosis
- Cryptosporidiosis
- Cyclosporiasis
- Dengue fever
- Diphtheria
- Ehrlichiosis
- Encephalitis/meningitis, arboviral
  - Encephalitis/meningitis, California serogroup viral
  - Encephalitis/meningitis, eastern equine
  - Encephalitis/meningitis, Powassan
  - Encephalitis/meningitis, St. Louis
  - Encephalitis/meningitis, western equine
- Encephalitis/meningitis, West Nile
- Foodborne disease outbreak
- Giardiasis
- Gonorrhea
- *Haemophilus influenzae* invasive disease
- Hansen's disease (leprosy)
- Hantavirus pulmonary syndrome
- Hemolytic uremic syndrome
- Hepatitis, viral, acute
- Hepatitis A, acute
- Hepatitis B, acute
- Hepatitis B virus, perinatal infection
- Hepatitis C, acute
- Hepatitis, viral, chronic
- Chronic hepatitis B
- Hepatitis C virus infection (past or present)
- HIV infection
- Influenza-associated pediatric mortality
- Lead poisoning[†]
- Legionellosis
- Leptospirosis
- Listeriosis

- Lyme disease
- Malaria
- Measles
- Meningococcal disease
- Mumps
- Novel influenza A infections
- Pertussis
- Pesticide poisoning†
- Plague
- Poliomyelitis, paralytic
- Poliovirus infection
- Powassan virus diseases
- Psittacosis
- Q fever
- Rabies
  - Rabies, animal
  - Rabies, human
- Rubella
- Rubella, congenital syndrome
- Salmonellosis
- Severe acute respiratory syndrome–associated coronavirus (SARS-CoV) disease
- Shiga toxin–producing *Escherichia coli* (STEC)
- Shigellosis
- Silicosis†
- Smallpox
- Spotted fever rickettsiosis
- Streptococcal toxic shock syndrome
- *Streptococcus pneumoniae*, invasive disease
- Syphilis
- Syphilis, congenital
- Tetanus
- Toxic shock syndrome
- Trichinellosis
- Tuberculosis
- Tularemia
- Typhoid fever
- Vancomycin-intermediate *Staphylococcus aureus* (VISA)
- Vancomycin-resistant *Staphylococcus aureus* (VRSA)
- Varicella
- Vibriosis
- Viral hemorrhagic fevers
- Yellow fever
- Zika virus disease

*Reportable to the CDC; other diseases may be reportable to state departments of health.

† Diseases not of infectious origin (in case of cancer, may have been initiated by microbes—as in cervical cancer—but category includes all cancers)



The bacterium that causes syphilis, Treponema pallidum.
Source: CDC

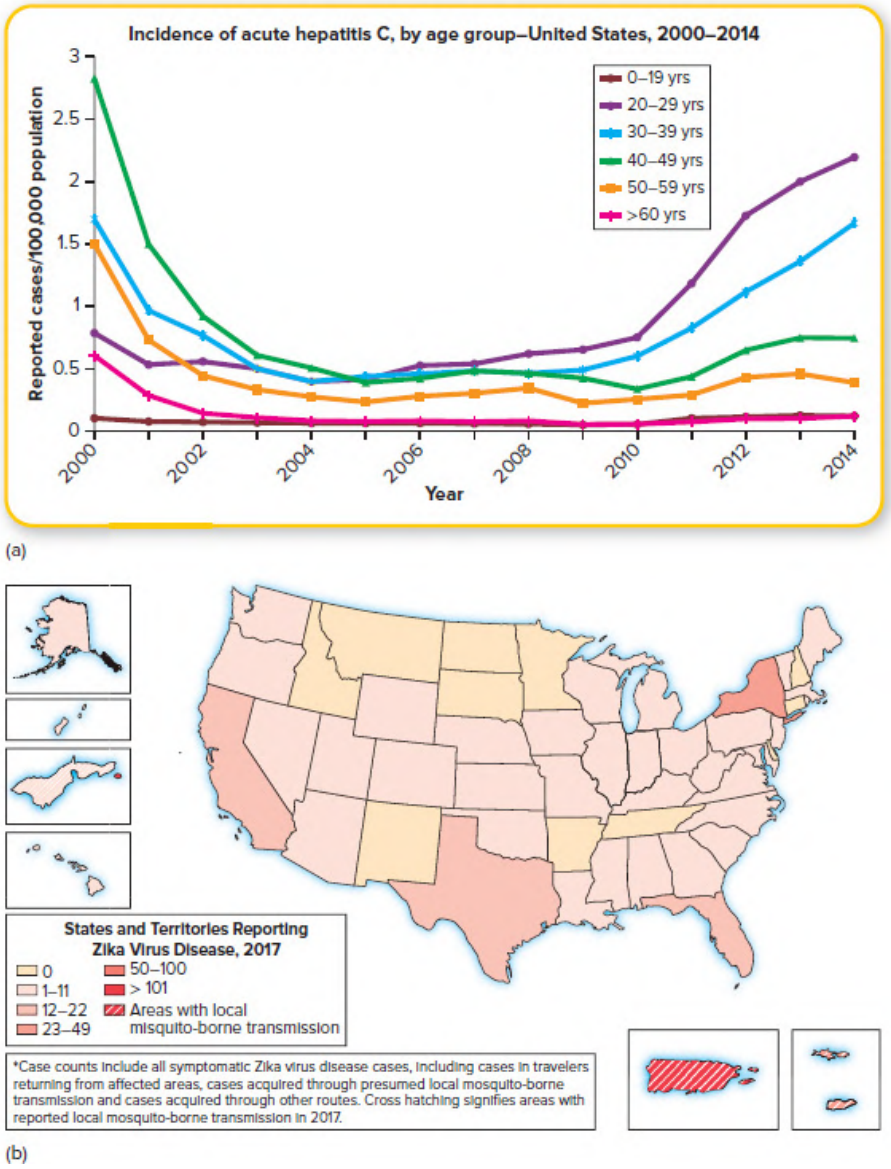


Figure 11.10 **Depictions of epidemiological data**. **(a)** A graph that displays incidence of acute hepatitis C with respect to time (2000–2014) and within different age groups; **(b)** a geographical representation of where Zika cases have occurred in the United States.
Source: CDC

end of the chapter About Epidemiology: Communicability and Deadliness in which we introduce these two important characteristics of infectious disease. In each of the disease chapters we will circle back to the two triangles introduced here.

When there is an increase in disease in a particular geographic area, it can be helpful to examine the epidemic curve—which is incidence plotted over time—to determine if the infection is a **common-source** or **propagated epidemic**. A common-source epidemic, illustrated in **figure 11.11*a***, is one in which the infectious agent was present in a single source that had widespread distribution, infecting people in a wide geographic area. When food is contaminated at a factory, it can result in common-source epidemics. A particular type of

common-source epidemic is referred to as **point-source epidemic**. It is still a common source, but usually it is a single small batch

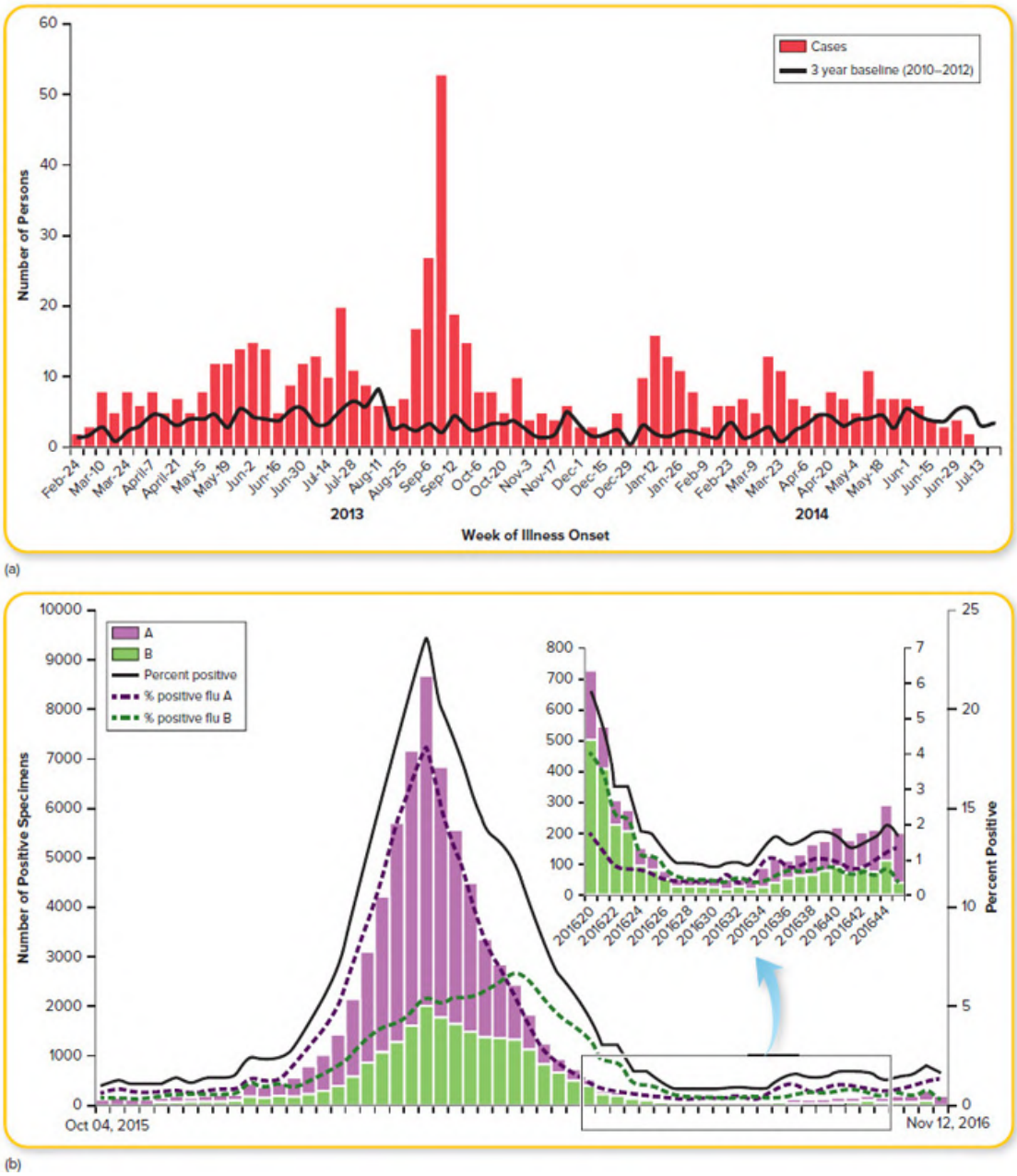


Figure 11.11 **Epidemic curves**. **(a)** A common-source epidemic curve. This illustrates a multistate outbreak of drug-resistant *Salmonella* caused by Foster Farms chicken that was contaminated. **(b)** A propagated epidemic curve, the incidence of influenza in the United States from fall of 2015 through fall of 2016.
Source: CDC

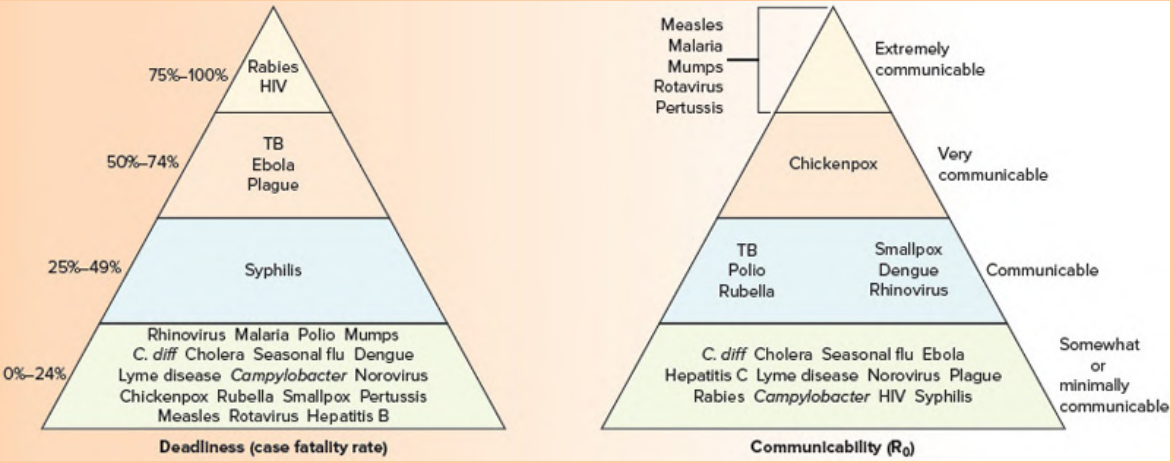
A Note About Epidemiology

There are two big descriptors of any given infectious disease—how communicable it is and how deadly it is. Epidemiologists quantify communicability by a factor called R_0 (pronounced "R-sub-zero") defined as the basic reproduction rate. It describes how many susceptible people, on average, one infected person will spread the infection to. The highly contagious measles virus has an R_0 of about 15, meaning that one infected person can spread the infection, on average, to 15 other individuals. The R_0 assumes those 15 people are unvaccinated for the microbe and have not experienced the infection, therefore having no secondary immunity. It might surprise you to learn that HIV is considered to be relatively low on the communicability scale. It has an R_0 of only about 3.4.

Deadliness is calculated via the case fatality rate (CFR): the members of persons who die of the disease within a specified time ÷ the number of persons infected. This calculation is based on persons who receive no treatment. The case fatality rate (CFR) for rabies is 100%. The CFR for cholera is about 1%. Understand that a CFR of even 1% is high—indicating that 1 of 100 infected people die.

These measures of infectious disease are approximate and can vary based on geographic location. But a general idea of R_0 and CFR can guide a lot of health care decisions and policies. Diseases with an extremely high R_0, for example, are the ones for which vaccination is most needed.

When we get to the disease chapters of this book, we will highlight key diseases in each chapter on a graph like this one. We will take both of these measures and divide them into quarters—lumping all CFRs from 0% to 25% in one quarter (at the bottom of the pyramid), and lumping R_0s into four broad categories. Practice reading the graph a little bit: Find an infection that is highly communicable but not very deadly. Now find one that is not very communicable but deadly. The good news is that none of these common infections are both highly deadly and highly communicable. And most of them are minimally communicable *and* minimally deadly.



of food or water that was contaminated, and everyone was infected at once, such as spoiled potato salad at a church picnic. That curve would have a much shorter time span on the horizontal axis. A propagated epidemic **(figure 11.11*b*)** results from an infectious agent that is communicable from person to person and therefore is sustained—propagated—over time in a population. Influenza is the classic example of this. The graph illustrates an entire year of influenza incidence in the United States. In propagated epidemics the incidence constantly increases until it peaks, then begins to decrease due to natural factors or due to control measures. Each year the influenza epidemic curve has a similar shape. The end of the epidemic is due to features of the virus, not to control measures.

An additional term, the **index case**, refers to the first patient found in an epidemiological investigation. How the cases unfurl from this case helps explain the type of epidemic it is. The index case may not turn out to be the first case—as the investigation continues, earlier cases may be found—but the index case is the case that

brought the epidemic to the attention of officials. Monitoring statistics also makes it possible to define the frequency of a disease in the population. An infectious disease that exhibits a relatively steady frequency over a long time period in a particular geographic locale is **endemic**. For example, Lyme disease is endemic to certain areas of the United States where the tick vector is found. A certain number of new cases are expected in these areas every year. When a disease is **sporadic**, occasional cases are reported at irregular intervals in random locales. Tetanus and diphtheria are reported sporadically in the United States (fewer than 50 cases a year).

When statistics indicate that the prevalence of an endemic or sporadic disease is increasing beyond what is expected for that population, the pattern is described as an **epidemic**. The spread of an epidemic across continents is a **pandemic**, as exemplified by AIDS and influenza.

11.3 LEARNING OUTCOMES—Assess Your Progress

17. Summarize the goals of epidemiology, and differentiate it from traditional medical practice.
18. Explain what is meant by a disease being "notifiable" or "reportable," and provide examples.
19. Define *incidence* and *prevalence*, and explain the difference between them.
20. Discuss the three major types of epidemics, and identify the epidemic curve associated with each.

CASE FILE WRAP-UP

©ERproductions Ltd/Blend Images LLC (health care worker); Source: CDC (aspergillosis)

Aspergillosis is an opportunistic infection, primarily affecting those who are immunocompromised. Caused by species of *Aspergillus*, a mold commonly found in the environment around us, it rarely causes disease in healthy individuals. However, those who

are immunocompromised cannot mount a natural defense to combat the fungus. The spores are inhaled and reproduce in the lungs. Focal infections may spread through the bloodstream to other body tissues, including the brain, skin, heart, kidneys, and bone. In the immunocompromised population, including recipients of stem cell transplant, aspergillosis has a high mortality rate.

The health care team initiated antifungal treatment for Cindy. She was closely monitored in the intensive care unit and after several weeks of treatment, her condition improved enough to return to the BMT unit. Her anti-fungal treatment continued for several more weeks. Cindy's white blood cell count had begun to rise and I knew this was a good sign for her ability to fight infection.

It is unknown how Cindy was exposed to *Aspergillus*. The reservoir may be indoors or outdoors as the mold is widely present in our everyday environment. *Aspergillus* may even be transmitted to a host by such common vectors as dust, food, water, or hospital devices. It is even possible that a visitor who had walked through a construction area could have introduced the spores to Cindy's hospital room. Despite the best efforts of health care workers and visitors to practice sound hand hygiene technique and appropriate isolation practices, a hospital room is never sterile.

Fecal Transplants

Clostridium difficile infection is an extreme example of what happens when the normal microbiota of the bowel is destroyed. *C. difficile* is a gram-positive organism that forms endospores. It grows slowly and can be challenging to isolate.

C. difficile is one of the most common, and most serious, healthcare-associated infections. The microbe, in its worst manifestation, causes a condition known as pseudomembranous colitis, in which pseudomembranous plaques form on the colon mucosa. Antibiotic therapy is one of the most common causes of *C. difficile*. Antibiotics so drastically alter the normal microbiota of the colon that *C. difficile* is allowed to proliferate, releasing toxins that attack the mucosa of the bowel, resulting in cramping and diarrhea that can be so severe that susceptible individuals (particularly elderly and immunosuppressed people) sometimes die from the infection.

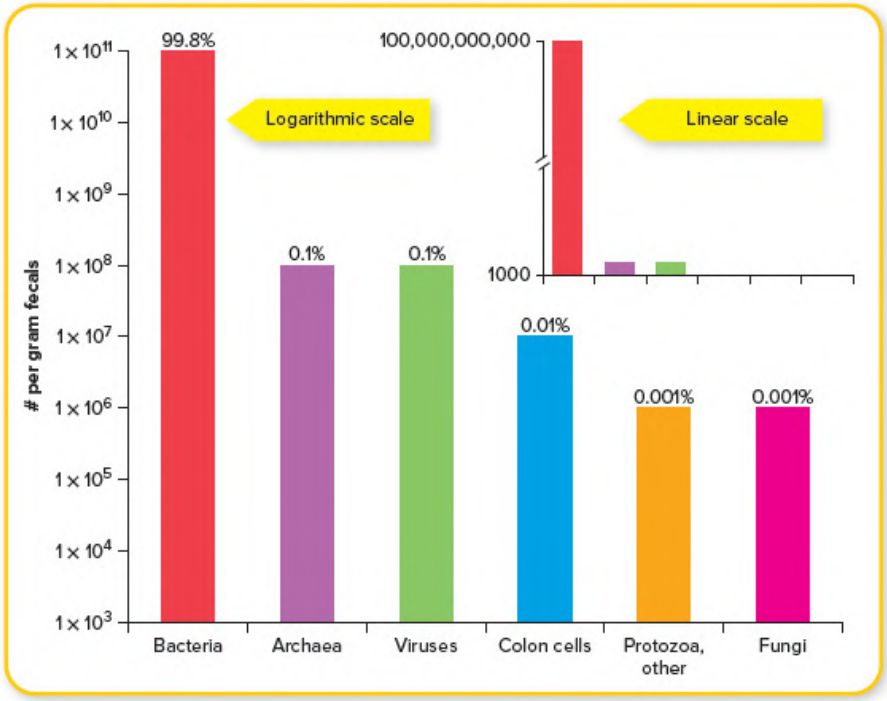
Treatment of *C. difficile* infection frequently entails potent drugs known to be effective against *C. difficile*, namely metronidazole (Flagyl), vancomycin, or fidaxomicin (Dificid). Dificid is used in more severe cases of the disease. However, relapse can still occur.

One therapy that is gaining attention is fecal transplant. The theory behind fecal transplant is to combat bacterial infection of the colon by infusing the colon with feces from a healthy person in an effort to displace the pathogenic microbes, restoring the normal balance in the colon. The treatment may sound distasteful but has proven to be effective.

Fecal transplants involve administering donor feces to individuals suffering from bacterial infections of the bowel such as *C. difficile*. The administration can be via enema, or sometimes by an epigastric tube, or even a pill. Donors are generally family members, and the donor feces are extensively tested to be sure that the donor is free of any bacterial, viral, or other harmful infections. Recipients may be administered the fecal enemas for 5 to 10 days, although sometimes one treatment is sufficient.

A study published in the *NEJM* (*New England Journal of Medicine*) in January 2013 determined a 94% cure rate in patients treated with fecal microbiota transplants, compared to only a 31% cure rate with vancomycin. The results were so overwhelmingly positive that the study was stopped early, as it was considered unethical not to offer fecal microbiota transplants to all of the study participants. In June of 2013, the FDA approved the use of fecal transplants in patients with *C. difficile* who do not respond to standard medical therapy.

Since 2011, when this became a more common practice, the assumption has been made that the cure is brought about because of the rich complement of bacteria in the healthy feces that restores health by colonizing the distressed colon. A paper from 2016 points out that bacteria are by far not the only components in transferred feces (see figure), and that scientists should pick apart the possible contributions of other components of the transplants.



Graph depicts estimates of all components in a typical fecal sample.
Source: *PLOS Biology*, 2016

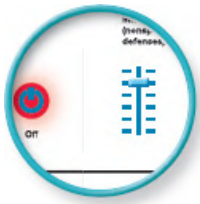
Chapter Summary

11.1 The Human Microbiome

- Humans evolved in a world where microbes were ubiquitous and therefore their development and health are closely dependent on the microbes in and on their bodies. These microbes make up the microbiome.
- Normal biota are easily cultured from the skin and in the respiratory tract, the gastrointestinal tract, the outer parts of the urethra, the vagina, the eye, and the external ear canal. Molecular techniques have also revealed normal biota to be present in the lungs, bladder, breast milk, and amniotic fluid.
- The Human Microbiome Project is finding a much wider array of normal biota than known previously.

11.2 When Colonization Leads to Disease

- The pathogenicity of a microbe refers to its ability to cause disease. Its virulence is the degree of damage it can inflict.
- The virulence of a microbe is determined by its ability to establish itself in the host and then do damage. Any characteristic or structure that enhances its ability to do these two things is called a virulence factor.
- The site at which a microorganism first contacts host tissue is called the portal of entry.
- True pathogens cause infectious disease in healthy hosts; opportunistic pathogens cause damage only when the host immune system is compromised in some way.
- The reservoir of the microbe may or may not be the same as the transmitter of the microbe.
- The infectious dose, or ID, refers to the minimum number of microbial cells required to initiate infection in the host. The ID is often influenced by quorum-sensing chemicals.
- Fimbriae and adhesive capsules allow pathogens to physically attach to host tissues.
- In order to attach to host tissue incoming microbes must successfully negotiate the resident microbiome.
- Secreted enzymes and toxins, the ability to induce injurious host responses, and epigenetic changes are the three main mechanisms utilized to damage host tissue.



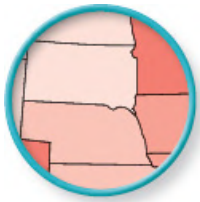
©abadonian/Getty Images

- Exotoxins and endotoxin differ in their chemical composition and tissue specificity.
- Every encounter between a susceptible host and a potential disease-causing organism does not result in disease. Multiple characteristics of the two partners come into play.
- The portal of exit by which a pathogen leaves its host is often but not always the same as the portal of entry.
- Some pathogens persist in the body in a latent state.
- There are four distinct phases of infection and disease: the incubation period, the prodrome, the acute phase, and the convalescent period. The continuation stage, present in some diseases, can be thought of as a fifth phase.

- A communicable disease can be transmitted from an infected host to others, but not all infectious diseases are communicable.
- The spread of infectious disease from person to person is called horizontal transmission. The spread from parent to offspring is called vertical transmission.
- Infectious diseases are spread by direct, indirect, or vector routes of transmission. Vehicles of indirect transmission include soil, water, food, air, and fomites (inanimate objects).
- Healthcare-associated infections are acquired in a hospital or health care facility from surgical procedures, equipment, personnel, and exposure to drug-resistant microorganisms.
- Causative agents of infectious disease may be identified according to Koch's postulates.

11.3 Epidemiology: The Study of Disease in Populations

- Epidemiology is the study of the determinants and distribution of infectious and noninfectious diseases in populations.
- Data on specific, reportable diseases are collected by local, national, and worldwide agencies.
- The prevalence of a disease is the percentage of existing cases in a given population. The disease incidence, or morbidity rate, is the number of newly infected members in a population during a specified time period.
- Outbreaks and epidemics are described as point-source or propagated based on the source of the pathogen.
- Infectious agents are categorized according to their deadliness (case fatality rate) and their communicability (R_0).



SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. The best descriptive term for the resident microbiota is **a**. commensal. **b**. parasitic. **c**. pathogenic. **d**. mutualistic.	**2**. In some circumstances, microbes can be quite virulent when they first infect a new species (such as in a zoonosis), but over decades of association with the new human host, cause milder and milder disease. Can you speculate about why this is evolutionarily advantageous to the pathogen?	**3**. Conduct research on germ-free mice. Use what you find to write a paragraph about the coevolution of microbes and humans.
Cell Structure and Function	**4**. Which of the following are virulence factors? **a**. toxins **b**. enzymes **c**. capsules **d**. all of the above	**5**. Why do you suppose specific adhesive structures, such as fimbriae, are critical to the disease-causing capabilities of many bacteria? Be thorough in your answer.	**6**. Discuss the role of endospores in ensuring the ongoing transmission of a bacterium in a population.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Metabolic Pathways	**7**. When the resident microbiota prevents the establishment of a pathogen, it is called **a**. disruption. **b**. a superinfection. **c**. a nonliving reservoir. **d**. microbial antagonism.	**8**. Correlate the stages in the course of an infectious disease with what you know about the growth of bacterial cultures.	**9**. *Helicobacter pylori*, a microbe that can cause disease in the stomach, produces an enzyme called urease that breaks down urea into ammonia and oxygen. What advantage does that confer on the bacterium?
Information Flow and Genetics	**10**. Most microbial exotoxins would be created using the process of **a**. DNA replication. **b**. protein synthesis. **c**. mutation. **d**. fatty acid synthesis.	**11**. Explain what you can about microbe-induced epigenetic changes to the host DNA.	**12**. Some of the most pathogenic *E. coli* possess an exotoxin gene originally found in *Shigella* bacteria. Construct a scenario for how and in what biological setting that gene was shared.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Microbial Systems	**13**. The _____ is the time between an encounter with a pathogen and the first symptoms. **a**. prodrome **b**. acute stage **c**. convalescence **d**. incubation period	**14**. A line of thinking called the "hygiene hypothesis" suggests that when children have more exposure to the environment (dirt, soil, animals) they have better health as adults. Why might this be?	**15**. At one time it was common practice to prescribe tetracycline for adolescents for months or years at a time to combat acne. Glance back at table 10.5 and predict what consequences that would have on the microbiome and the host.
Impact of Microorganisms	**16**. A seasonal outbreak of influenza would be what type of outbreak? **a**. point-source **b**. common-source **c**. propagated **d**. two of the above	**17**. Differentiate among colonization, infection, and disease, noting a defining characteristic of each.	**18**. In question 2, we discussed pathogens that became less virulent the longer they were associated with the human host. But there is another category of pathogen that never loses any virulence over hundreds of years of association with humans. Can you deduce what circumstances

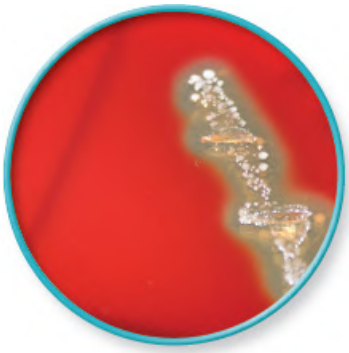
			make lower virulence of no advantage to a pathogen?
Scientific Thinking	**19**. The number of cases, including new cases as well as already existing cases, in a defined period of time is the **a**. incidence. **b**. prevalence. **c**. It could be either; need more information.	**20**. You are performing Koch's postulates on sputum specimens taken from patients with a new unexplained respiratory illness. Agar cultures of all of the sputum show an abundance of a particular kind of colony, to the exclusion even of the normal variety of normal respiratory biota. No healthy subjects' cultures have this particular kind of colony. But when you administer this bacterium in aerosol form to the proper animal model you do not get disease. What are the possible reasons for this?	**21**. One important way to critically analyze a study that finds a correlation between two factors is the *biological plausibility* of the finding. This means whether there are already known biological facts or phenomenon that might explain the correlation. Assess the biological plausibility of a correlation between the composition of the gut microbiome and one's likelihood to have irritable bowel syndrome. Do the same for a correlation between the gut microbiome and the likelihood of having the autoimmune disease rheumatoid arthritis.

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From chapter 2, figure 2.4*a***. What chemical is the organism in this illustration producing? How does this add to an organism's pathogenicity?



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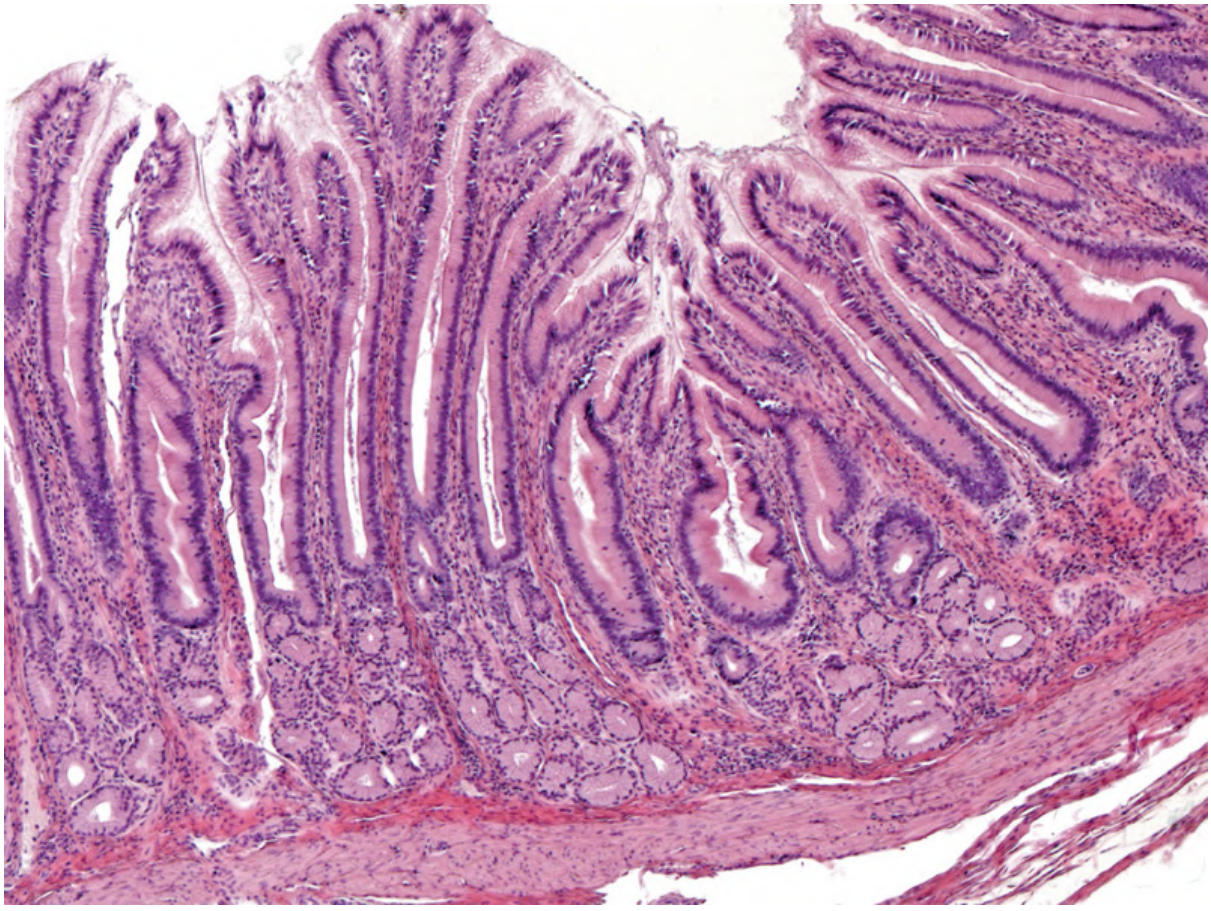
connect Resources are available through Connect, including interactive learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

12

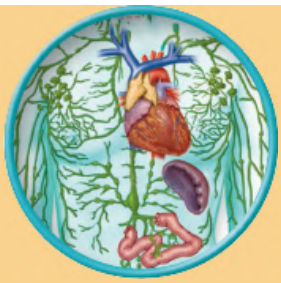
Host Defenses I

Overview and Nonspecific Defenses



©McGraw-Hill EducatioNAl Telser, photographer (tissue); ©Susumu Nishinaga/Science Source (epithelial lining); ©Pixtal/age fotostock (health care worker)

IN THIS CHAPTER…

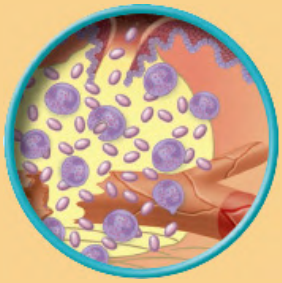


12.1 Defense Mechanisms of the Host: An Overview

1. Summarize the three lines of host defenses.
2. Define "marker" and discuss its importance in the second and third lines of defense.
3. Name the body systems that participate in immunity.
4. Describe the structure and function of the lymphatic system and its connection with the circulatory system.
5. Name three kinds of blood cells that function in nonspecific (innate) immunity.
6. Connect the mononuclear phagocyte system to innate immunity.
7. Describe how T and B lymphocytes are involved in specific (adaptive) immunity.
8. Summarize the importance of cytokines, and list one pro-inflammatory and one anti-inflammatory cytokine.

12.2 The First Line of Defense

9. Identify the three components of the first line of defense.
10. Identify the four body systems that participate in the first line of defense.
11. Describe two examples of how the normal microbiota contribute to the first line of defense.



12.3 The Second Line of Defense

12. List the four major categories of nonspecific (innate) immunity.
13. Outline the steps of phagocytosis.
14. Outline the steps of inflammation.
15. Discuss the mechanism of fever and how it helps defend the body.
16. Name four types of antimicrobial host-derived products.
17. Compose one good overview sentence about the purpose and the mode of action of the complement system, and another about the purpose and mode of action of interferon.

CASE FILE

Bacteria Cause That?

I was working in an endoscopy unit as a registered nurse when I met Natasha, a 20-year-old college student. Natasha was having a gastroscopy to determine the cause of her persistent stomach pain.

Natasha had been having episodes of epigastric pain intermittently for 5 years. Approximately every 4 to 6 weeks, she would experience intense upper abdominal pain that left her unable to engage in her usual activities, including her classes and work. She would experience what she described as intense hunger, but pain would worsen immediately after eating so she learned to avoid eating during the worst of her "attacks," which could last as long as 5 days.

She had been admitted to the emergency room after a particularly intense bout of pain. She was dehydrated and weak and her pain was constant. Lab studies revealed an elevated hemoglobin level, indicative of dehydration, and a normal white blood cell count. She had no fever. Her epigastric area was moderately tender on palpation. Her heart rate was elevated, likely as a response to the pain she was experiencing. The ER physician who examined her had admitted her to the hospital and arranged an urgent gastroscopy.

Once in the endoscopy unit, Natasha was given medications to induce conscious sedation. The back of her throat was anesthetized and the gastroscopy scope was passed down her throat. The esophagus was visualized as normal. The stomach, however, was inflamed, with evidence of prior healed ulcerations. One small unhealed lesion remained.

Just as the ER physician had expected, Natasha had a gastric ulcer. Biopsies of the inflamed area were obtained, as well as photographs of the lesion, and the scope was removed. Natasha was then given medication to reverse the effects of the sedatives. Once fully recovered, Natasha was released to go home.

A week later, Natasha had an appointment with the gastroenterologist, who told Natasha that biopsies had revealed the presence of *Helicobacter pylori* in her stomach, which had caused the ulcers. She was prescribed a combination of antibiotics and an acid reducer and was given an appointment to follow up after she had completed the therapy to confirm the efficacy of the treatment. She was also instructed to contact them again if she experienced any more episodes of stomach pain.

- How does *H. pylori* survive in the acidic environment of the stomach?
- What classic response to injury did Natasha display?

Case File Wrap-Up appears at the end of the chapter.

The skin is the largest organ of your body.
©Ingram Publishing

12.1 Defense Mechanisms of the Host: An Overview

The host defenses are a multilevel network of innate, nonspecific protections and adaptive, specific protections that are commonly referred to as the first, second, and third lines of defense **(figure 12.1)**. The interaction and cooperation of these three levels of defense normally provide complete protection against infection. The *first line of defense* includes any barrier that blocks invasion at the portal of entry. This nonspecific line of defense limits access to the internal tissues of the body. However, it is not considered a true immune response because it does not

involve recognition of a specific foreign substance but is very general in action. The *second line of defense*, also nonspecific, is a more internal system of protective cells, fluids, and processes that includes inflammation and phagocytosis. It acts rapidly at both the local and systemic levels once the first line of defense has been overcome. The highly specific *third line of defense* is acquired only as each foreign substance is encountered by white blood cells called lymphocytes. The goal of this response is to make lymphocytes that are specifically adapted to each individual invader. The reaction with each different foreign microbe produces unique protective substances and cells that can come into play if that microbe is encountered again. The third line of defense provides long-term immunity. It is discussed in detail in chapter 13. This chapter focuses on the first and second lines of defense.

Human systems are armed with various levels of defense that do not operate in a completely separate fashion. Most defenses overlap and are even redundant in some of their effects. This bombards microbial invaders with an entire assault force, making their survival unlikely. Figure 12.1 provides an overview of the three systems. The light blue box at the bottom that connects both the second and third lines of defense is a good example of how the systems overlap.

Immunology encompasses the study of all features of the body's second and third lines of defense. Although this chapter is concerned, not surprisingly, with infectious microbial agents, be aware that immunology is central to the study of fields as diverse as cancer and allergy.

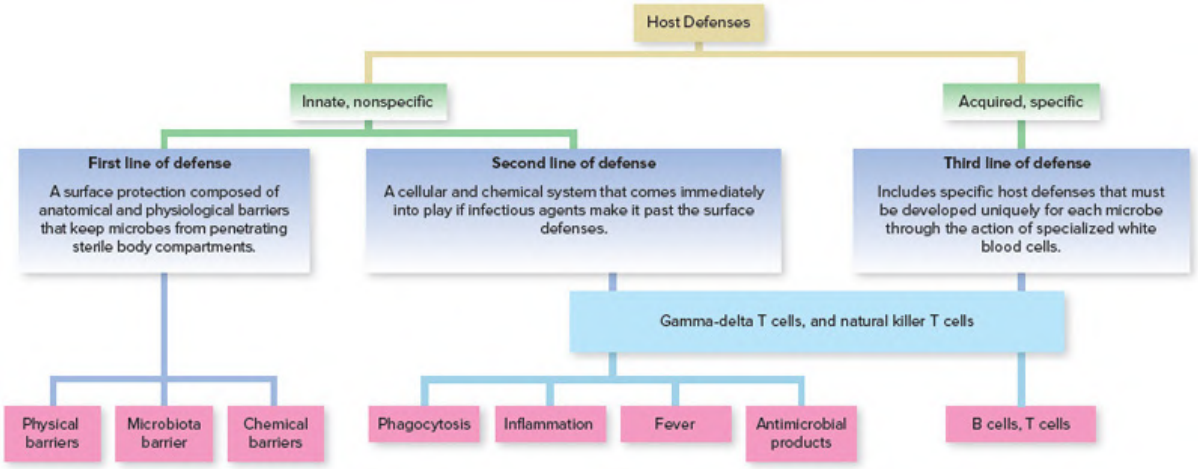


Figure 12.1 Flowchart summarizing the major components of the host defenses. Defenses are classified into one of two general categories: (1) innate and nonspecific or (2) acquired and specific. These can be further subdivided into the first, second, and third lines of defense, each being characterized by a different level and type of protection. There is also a set of cells that straddle the categories of innate and acquired defenses (light blue box).

In the body, the mandate of the immune system can be easily stated. A healthy functioning immune system is responsible for the following:

1. surveillance of the body,
2. recognition of foreign material, and
3. destruction of entities deemed to be foreign.

All cells (microbial and otherwise), as well as some particles such as pollen, display a unique mix of macromolecules on their surfaces that the immune system "senses" to determine if they

are foreign or not. These chemicals are called *antigens*. Because foreign cells or particles could potentially enter through any number of portals, the cells of the immune system constantly move about the body, searching for potential pathogens. This process is carried out primarily by white blood cells, which have been trained to recognize body cells (so-called **self**) and differentiate them from any foreign material in the body, such as an invading bacterial cell (**nonself**). The ability to evaluate cells and macromolecules as either self or nonself is central to the functioning of the immune system. While foreign substances must be recognized as a potential threat and dealt with appropriately, self cells and chemicals must not come under attack by the immune defenses. Many autoimmune disorders are a result of the immune system mistakenly attacking the body's own tissues and organs. For example, in rheumatoid arthritis, the body attacks its own joints and tissues, causing pain and loss of function.

Another word for antigens is **markers (figure 12.2)**. Markers, which generally consist of proteins and/or sugars, can be thought of as the cellular equivalent of facial characteristics in humans and allow the cells of the immune system to identify whether or not a newly discovered cell poses a threat. While cells deemed to be self are left alone, cells and other objects designated as foreign are marked for destruction by a number of methods, the most common of which is phagocytosis. Markers that many different kinds of microbes have in common are called **pathogen-associated molecular patterns (PAMPs)**. Host cells with important roles in the innate immunity of the second line of defense use **pattern recognition receptors (PRRs)** to recognize PAMPs. There is a middle ground as well. Nonself proteins that are not harmful—such as those found in food we ingest and on commensal microorganisms—are generally recognized as such and the immune system is signaled not to react.

Unlike many systems, the immune system does not exist in a single, well-defined site; rather, it is a large, complex, and diffuse network of cells and fluids that permeate every organ and tissue. It is this arrangement that promotes the surveillance and recognition processes that help screen the body for harmful substances.

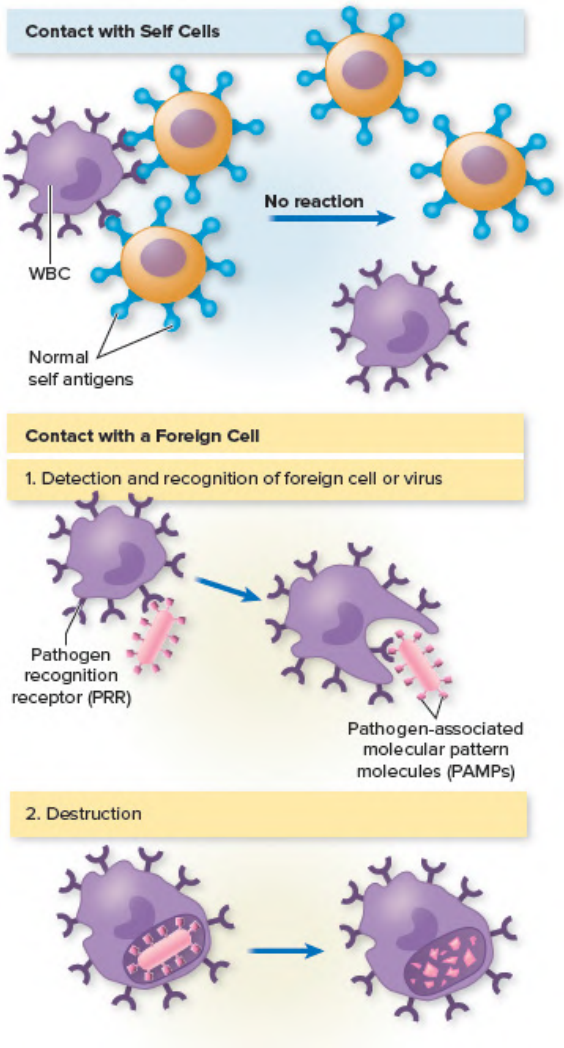


Figure 12.2 ***Search, recognize*, and *destroy* is the mandate of the immune system**. White blood cells are equipped with a very sensitive sense of "touch." As they travel through the tissues, they feel surface markers that help them determine what is self and what is not. When self markers are recognized, no response occurs. However, when nonself is detected, a reaction to destroy it is mounted.

Tissues, Organs, and Cells Participating in Immunity

The body is partitioned into several fluid-filled spaces called the intracellular, extracellular, lymphatic, cerebrospinal, and circulatory compartments. Although these compartments are physically separated, they have numerous connections. For effective immune responsiveness, the activities in one fluid compartment must be communicated to other compartments.

At the microscopic level, clusters of tissue cells are in direct contact with the **mononuclear phagocyte system (MPS)**, which is described shortly, and the extracellular fluid (ECF). Blood and lymphatic capillaries penetrate into these tissues. This close association allows cells that originate in the MPS and ECF to diffuse or migrate into the blood and lymphatics. Any products of a lymphatic reaction can be transmitted directly into the blood through the connection between these two systems; and certain cells and chemicals originating in the blood can move through the vessel walls into the extracellular spaces and migrate into the lymphatic system.

Lymphatic System Vessels and Fluids

The **lymphatic system** is a compartmentalized network of vessels, cells, and specialized accessory organs **(figure 12.3)**. It begins in the farthest reaches of the tissues

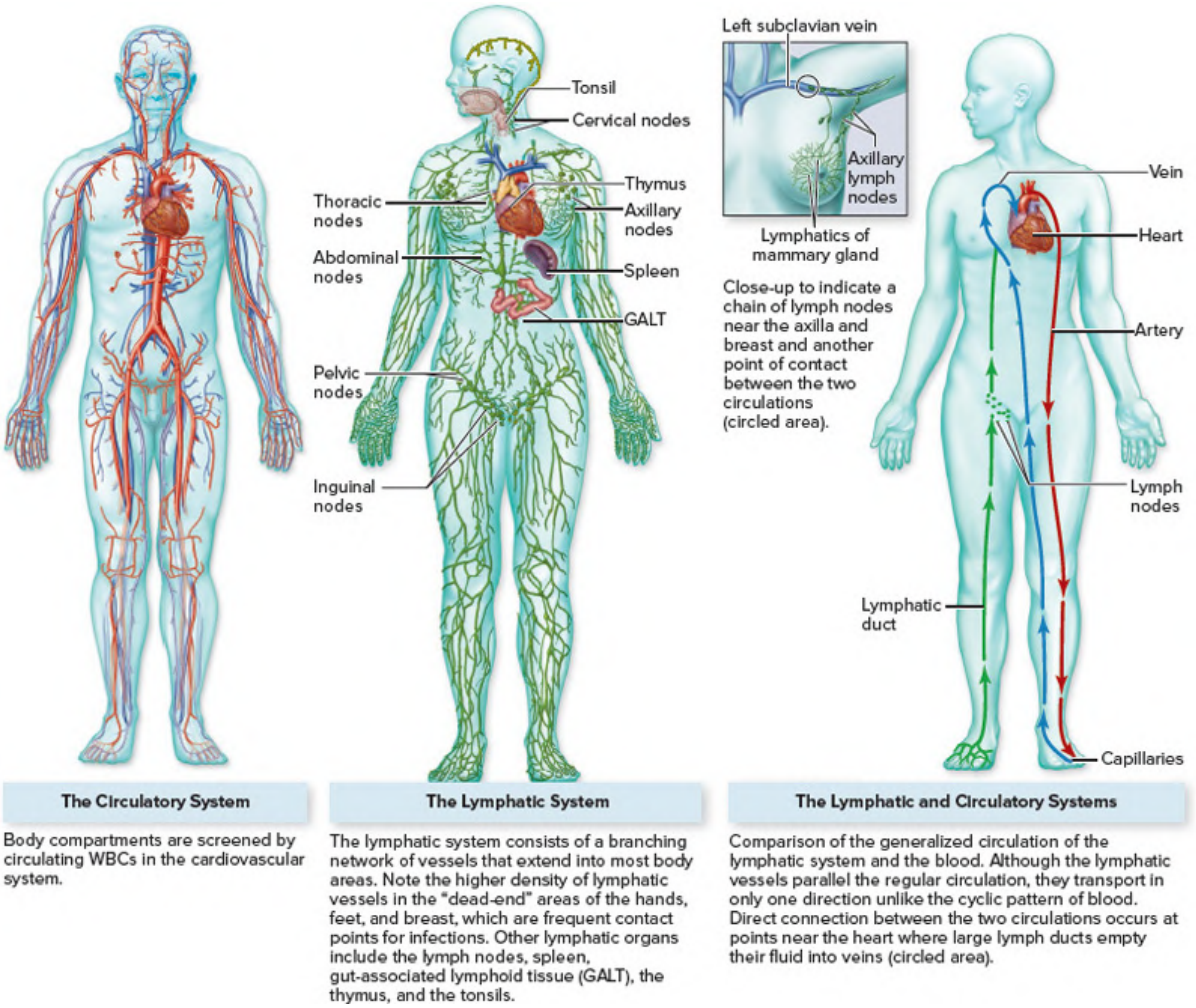
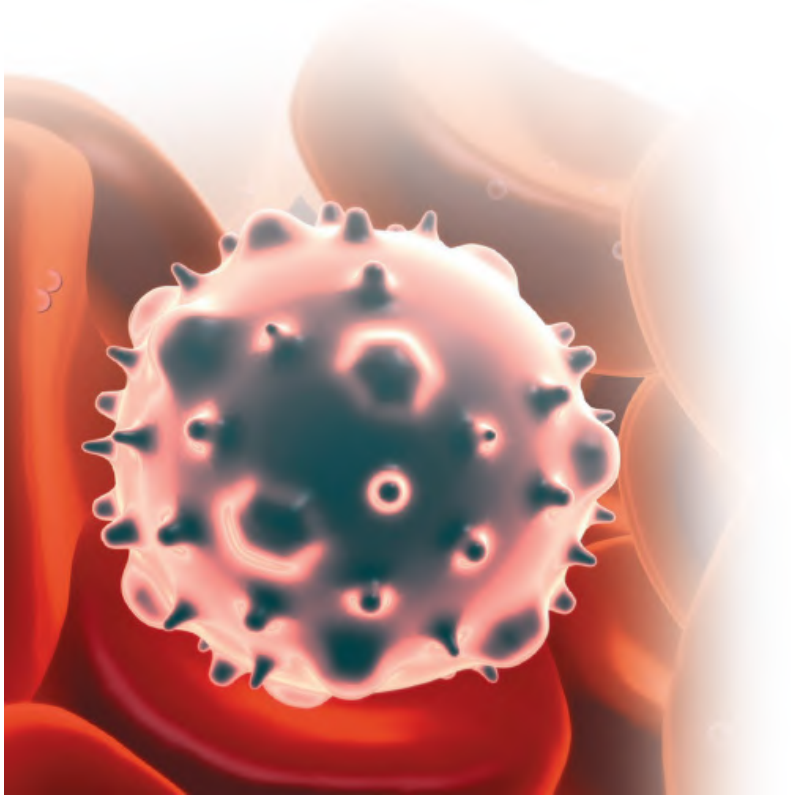


Figure 12.3 **The circulatory and lymphatic systems**.

as tiny capillaries that transport a special fluid (lymph) through an increasingly larger tributary system of vessels and filters (lymph nodes), and it leads to major vessels that drain back into the regular circulatory system. Some major functions of the lymphatic system are as follows:

1. to provide a route for the return of extracellular fluid to the circulatory system proper;
2. to act as a "drain-off" system for the inflammatory response; and
3. to render surveillance, recognition, and protection against foreign materials through a system of lymphocytes, phagocytes, and antibodies.



Drawing of red and white blood cells
©MedicalRF.com/Getty Images

Lymphatic Fluid Lymph is a plasmalike liquid carried by the lymphatic circulation. It is formed when certain blood components move out of the blood vessels into the extracellular spaces and diffuse or migrate into the lymphatic capillaries. Like blood, it transports numerous white blood cells (especially lymphocytes) and miscellaneous materials such as fats, cellular debris, and infectious agents that have gained access to the tissue spaces.

Medical Moment

Examining Lymph Nodes

Physicians routinely examine lymph nodes during a physical examination. Healthy lymph nodes feel well defined and slightly rubbery. They generally are less than 2 cm and are easily movable. The lymph nodes that can easily be felt when they are enlarged include the lymph nodes located behind the ears, along the neck, and in the armpits and the groin. Deeper lymph nodes, such as those in the chest, may be visible on an X ray or computed tomography (CT) scan.

Enlarged lymph nodes by themselves are not usually cause for concern, unless they are accompanied by other symptoms, such as fatigue, fever, weight loss, night sweats, or other constitutional symptoms. Blood tests, X rays, and biopsies may be used to determine the cause of swollen lymph nodes, particularly when other symptoms are present.

Q. A respiratory infection may lead to mild enlargement of the lymph nodes of the neck. What kind of condition(s) would you suspect if lymph nodes throughout the body were enlarged?

Answer in Appendix B.

Lymphatic Vessels The system of vessels that transports lymph is constructed along the lines of blood vessels. As the lymph is never subjected to high pressure, the lymphatic vessels appear more similar to thin-walled veins than to thicker-walled arteries. The tiniest vessels, lymphatic capillaries, accompany the blood capillaries and extend into all parts of the body except the central nervous system and certain organs such as bone, placenta, and thymus. Their thin walls are easily permeated by extracellular fluid that has escaped from the circulatory system. Lymphatic vessels are found in particularly high numbers in the hands, feet, and around the areola of the breast.

Soon you will read about the bloodstream and blood vessels. Two overriding differences between the bloodstream and the lymphatic system should be mentioned. First, because one of the main functions of the lymphatic system is returning lymph to the circulation, the flow of lymph is in one direction only, with lymph moving from the extremities toward the heart. Eventually, lymph will be returned to the bloodstream through the thoracic duct or the right lymphatic duct to the subclavian vein near the heart. The second difference concerns how lymph travels through the vessels of the lymphatic system. While blood is transported through the body by means of a dedicated pump (the heart), lymph is moved only through the contraction of the skeletal muscles through which the lymphatic ducts wend their way. This dependence on muscle movement helps to explain the swelling of the hands and feet that sometimes occurs during the night (when muscles are inactive) that clears up soon after waking.

Primary Lymphatic Organs In the lymphatic system, the sites of immune cell birth and the locations where they mature are considered **primary lymphatic organs**. Locations in the body where immune cells become activated, reside, or carry out their functions are called **secondary lymphatic organs**.

Red Bone Marrow The red bone marrow is an important intersection between the circulatory system, skeletal system, and lymphatic system. It is typically found in the internal matrix of long bones, and is the site of blood cell production. All blood cells originate in the bone marrow, including B- and T-lymphocyte precursors. After B lymphocytes begin to express markers that identify them as B cells to the rest of the body, they complete their maturation process while still in the bone marrow. Once this is complete, these B cells then migrate to secondary lymphatic organs, such as the spleen or lymph nodes, where they wait to encounter foreign antigen.

The Thymus: Site of T-Cell Maturation The **thymus** originates in the embryo as two lobes in the lower neck region that fuse into a triangular structure. Lymphocytes that originate in the bone marrow as naïve T lymphocytes migrate to the thymus to complete their maturation. Under the influence of thymic hormones, these cells develop specificity and are released into the circulation as mature T cells. The mature T cells subsequently migrate and settle in other secondary lymphoid organs, just as mature B cells do.

Secondary Lymphatic Organs One way to think of the secondary lymphatic organs is that they are where the action of immunity takes place. The lymph nodes, spleen, and various lymphoid tissues contain large numbers of T and B cells and are stationed throughout the body, ready to encounter antigen and become activated.

Lymph Nodes Lymph nodes are small, encapsulated, bean-shaped organs stationed, usually in clusters, along lymphatic channels and large blood vessels of the thoracic and abdominal cavities (see figure 12.3). Major aggregations of nodes occur in the loose connective tissue of the armpit (axillary nodes), groin (inguinal nodes), and neck (cervical nodes). Their job is to filter out materials in the lymph and to provide appropriate cells for immune reactions. In **figure 12.4**, the anatomy of a lymph node is illustrated. The outer rim of the lymph node is called the **cortex**. There are T lymphocytes in the **paracortical area**, and B lymphoctyes and macrophages

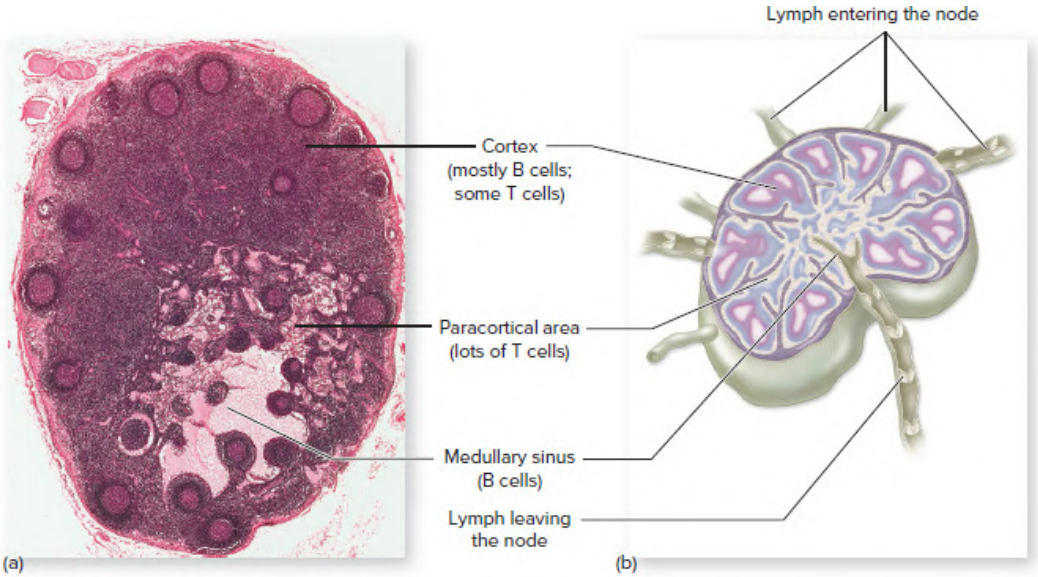


Figure 12.4 **Diagram of a lymph node**. On the left is a micrograph of a lymph node that has been cut in half. On the right is a drawing of a lymph node.
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in the **medullary sinus**. Enlargement of the lymph nodes can provide physicians with important clues as to a patient's condition. Generalized lymph node enlargement may indicate the presence of a systemic illness, while enlargement of an individual lymph node may be evidence of a localized infection. Enlargement of lymph nodes reflects the replication of many lymphocyte clones during an adaptive immune response

The Spleen The spleen is a lymphoid organ in the upper left portion of the abdominal cavity. It is somewhat similar to a lymph node except that it serves as a filter for blood instead of lymph. While the spleen's primary function is to remove worn-out red blood cells from circulation, its most important immunologic function is the filtering of pathogens from the blood and their subsequent phagocytosis by macrophages in the spleen. Although adults whose spleens have been surgically removed can live a relatively normal life, children without spleens are severely immunocompromised. The spleen also acts as a storehouse of blood that can be released in the event of hemorrhage. It can hold up to 1 cup of blood. For this reason, injury to the spleen can result in profuse bleeding.

Associated Lymphoid Tissues There are discrete bundles of lymphocytes at many sites on or just beneath the skin and mucosal surfaces all over the body. The tissues are often named for their location. For example, the **skin-associated lymphoid tissue** is called **SALT**, and the **mucosa-associated lymphoid tissue** is called **MALT**. The positioning of this diffuse system provides an effective first-strike potential against the constant influx of microbes and other foreign materials in food and air. In the pharynx, a ring of tissues called the **tonsils** provides an active source of lymphocytes. The breasts of pregnant and lactating women also become temporary sites of antibody-producing lymphoid tissues. The intestinal tract houses the best-developed collection of lymphoid tissue, called **gut-associated lymphoid tissue**, or **GALT**. Examples of GALT include the appendix, the lacteals (special lymphatic vessels stationed in each intestinal villus), and **Peyer's patches**, compact aggregations of lymphocytes in the ileum of the small intestine.

The Blood

The circulatory system consists of the heart, arteries, veins, and capillaries that circulate the blood, and the lymphatic system, which includes lymphatic vessels and lymphatic organs (lymph nodes) that circulate lymph. As you will see, these two circulations parallel, interconnect with, and complement one another.

The substance that courses through the arteries, veins, and capillaries is **whole blood**, a liquid consisting of **blood cells** suspended in **plasma**. One can visualize these two components with the naked eye when a tube of unclotted blood is allowed to sit or is spun in a centrifuge. The cells' density causes them to settle into an opaque layer at the bottom of the tube, leaving the plasma, a clear, yellowish fluid, on top. Serum is essentially the same as plasma, except it is the clear fluid from clotted blood. Serum is often used in immune testing and therapy.

Blood appears red because there are approximately 1,000× more red cells than "white" cells in this fluid.
©fStop/PunchStock

A Survey of Blood Cells The production of blood cells is called **hematopoiesis** (hee″-mat-o-poy-ee′-sis). The primary precursor of new blood cells is a pool of undifferentiated cells called pluripotential **stem cells** in the bone marrow. "Pluripotent" means that the cells are able to become any type of blood cell that is needed. These cells are produced continually in the bone marrow, and subjected to a variety of growth factors and hormones that cause them to change

over time. This process is called **differentiation**. The immature stem cells change their genetic expression, which in turn causes them to express different surface markers and respond to new signals. They become more specialized over time, eventually maturing either in the bone marrow or in other sites of the body. This is the process of blood cell development.

There are two primary lines of cells that arise from stem cells, and are still considered immature. These are (1) those that differentiate from a common myeloid cell, such as red blood cell precursors (erythroblasts), and platelet precursors (megakaryoblasts); and (2) those that differentiate from a common lymphoid precursor cell, such as precursors of many white blood cells (myeloblasts), and precursors of lymphocytes (lymphoblasts).

White blood cells **(leukocytes)** can generally be divided into two categories according to their staining patterns when viewed with a microscope: **granulocytes**, which have dark staining granules, and **agranulocytes**, which do not have granules and typically have large nuclei. The granules in granulocytes can be released to kill foreign cells or affect host tissues. **Figure 12.5** sorts out all these cells. They are vitally important to nonspecific and specific immunity.

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1. Which of the following act/acts as a filter for blood, removing worn-out red cells from circulation?
   a. *thymus*
   b. *liver*
   c. *spleen*
   d. *lymph nodes*
   e. *kidneys*

Cytokines: Critical for Cell Communication

Before we specifically address details of the first or second line of defense, we need to understand the role of some potent chemicals and proteins that influence defensive responses, and that immune cells use to communicate with one another.

Hundreds of small, active molecules are constantly being secreted to regulate, stimulate, suppress, and otherwise control the many aspects of cell development, inflammation, and immunity. These substances are the products of several types of cells, including monocytes, macrophages, lymphocytes, fibroblasts, mast cells, platelets, and the endothelial cells of blood vessels.

These cell products are generally called **cytokines**. There are dozens of cytokines that have been identified. Specific cytokines will be discussed as we cover various immune processes. **Table 12.1** provides four broad categories of cytokines based on their functions. One or two examples of each functional category are provided to demonstrate the breadth of their activities.

Medical Moment

The Tonsils

The tonsils and adenoids—secondary lymphoid organs—were very frequently removed in children until the 1970s, when the rates dropped dramatically. Before then about 90% of the surgeries were prompted by repeated infections of the organs. Today, 80% of the removals are prompted by the fact that the tissues are obstructive, causing sleep apnea and other problems.

Even though it used to be practiced almost routinely in childhood, removal of the tonsils and/or adenoids is not an innocuous procedure. Hemorrhage can occur and may be severe. In addition, there are always risks associated with general anesthetic. Postsurgical infection may complicate healing. The old adage “if it ain’t broke, don’t fix it” may apply to the routine removal of tonsils.

Q. Where exactly are the tonsils and adenoids located?

Answer in Appendix B.

The Mononuclear Phagocyte System

The tissues of the body are permeated by a support network of connective tissue fibers, or a reticulum, that interconnects nearby cells and meshes with the

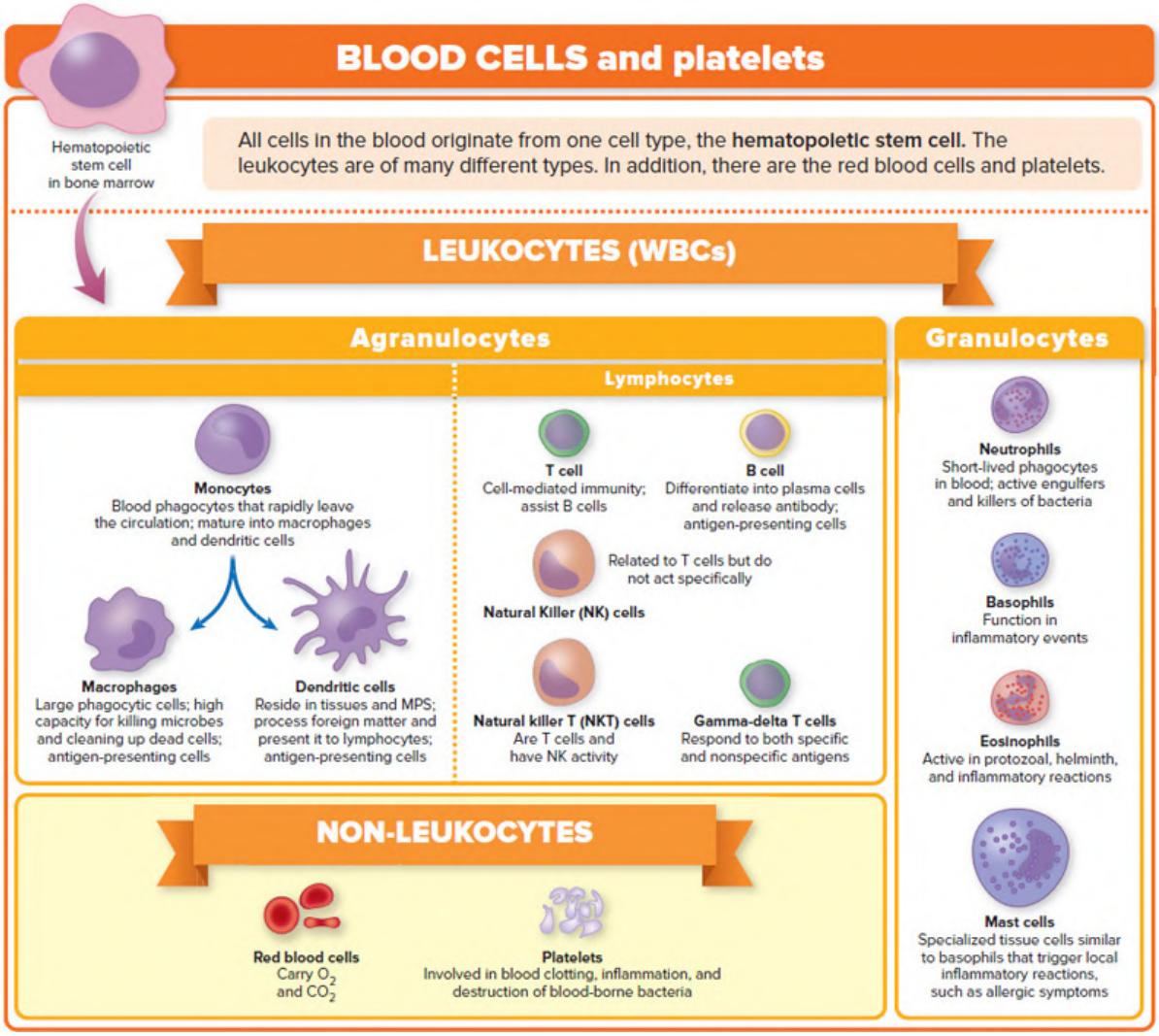

Figure 12.5 **Description of blood cells and platelets**.

massive connective tissue network surrounding all organs. The phagocytic cells enmeshed in this network are collectively called the mononuclear phagocyte system (MPS; **figure 12.6**). This

system is intrinsic to the immune function because it provides a passageway within and between tissues and organs. The MPS is found in the thymus, where important white blood cells mature, and in the lymph nodes, tonsils, spleen, and lymphoid tissue in the mucosa of the gut and respiratory tract, where most of the MPS "action" takes place.

When differentiated macrophages and dendritic cells arrive in tissues that are near portals of entry or filtration organs, they often take up residence

Table 12.1 Cytokines

	Examples	Source	Target
Pro-inflammatory cytokines, that *encourage* specific and nonspecific immune responses	Interleukin-1 (IL-1),	Macrophages, B cells, dendritic cells	B cells, T cells
	Tumor necrosis factor-β (TNF-β),	T cells	Phagocytes, tumor cells
Anti-inflammatory cytokines, that *discourage* specific and nonspecific immune responses	Interleukin-10 (IL-10)	T cells	B cells, macrophages
Vasodilators and vasoconstrictors, that can change the diameter of blood vessels or vessel permeability	Serotonin	Platelets and intestinal cells	Cells in peripheral and central nervous system
	Histamine	Mast cells and basophils	Blood vessels, sensory nerves, neutrophils
Growth factor cytokines, that regulate lymphocyte growth or activation	Interleukin-7 (IL-7)	Bone marrow cells, epithelial cells	Stem cells (stimulates growth of B and T cells)
	Erythropoietin	Endothelial cells	Stem cells (stimulates production of red blood cells)

in these tissues permanently, waiting to attack foreign intruders **(figure 12.7)**. These tissues and specialized **histiocyte** cells include the liver (Kupffer cells), lungs (alveolar macrophages), skin (Langerhans cells), brain (microglia), and others.

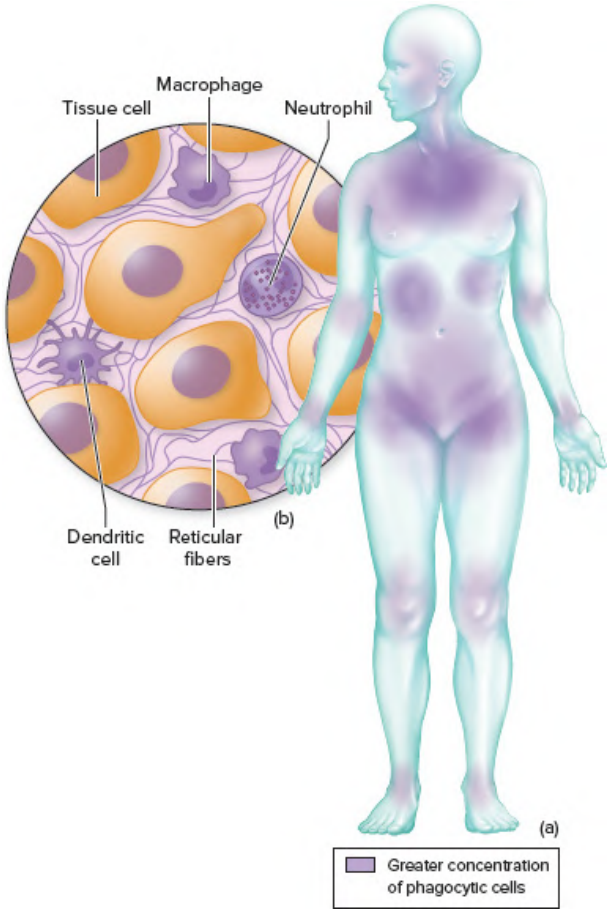

Figure 12.6 **The mononuclear phagocyte system**. **(a)** The degrees of shading in the body indicate variations in phagocyte concentration (darker = greater). **(b)** This system begins at the microscopic level with a fibrous support network (reticular fibers) enmeshing each cell. This web connects one cell to another within a tissue or organ and provides a niche for phagocytic white blood cells, which can crawl within and between tissues.

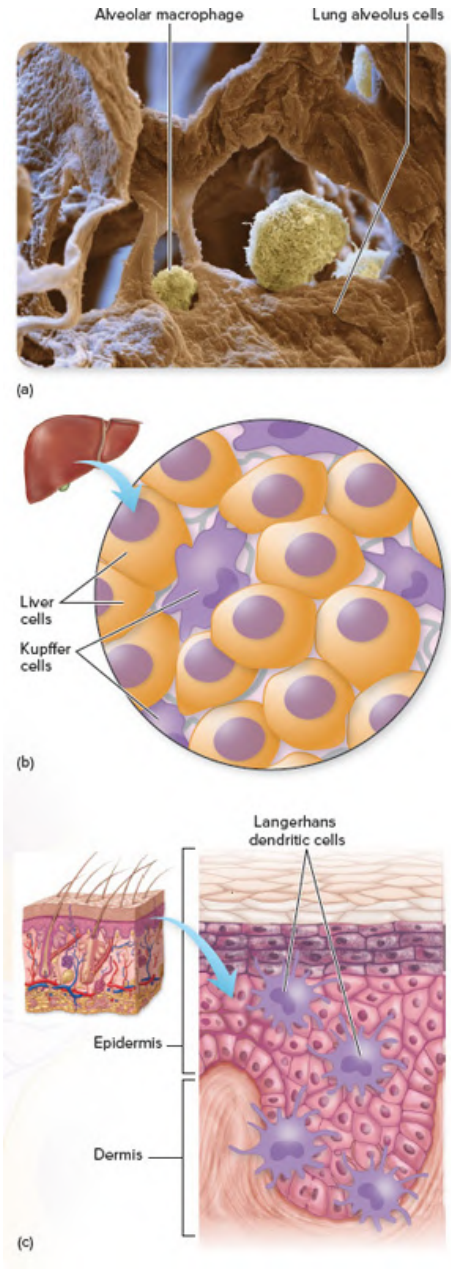

Figure 12.7 **Macrophages**. **(a)** Scanning electron micrograph of an alveolar macrophage inside a lung. **(b)** Liver tissue with Kupffer cells. **(c)** Langerhans cells deep in the epidermis.
©Eye of Science/Science Source (a)

12.1 LEARNING OUTCOMES—Defense Mechanisms of the Host: An Overview

1. Summarize the three lines of host defenses.
2. Define "marker" and discuss its importance in the second and third lines of defense.
3. Name the body systems that participate in immunity.

4. Describe the structure and function of the lymphatic system and its connection with the circulatory system.
5. Name three kinds of blood cells that function in nonspecific (innate) immunity.
6. Connect the mononuclear phagocyte system to innate immunity.
7. Describe how T and B lymphocytes are involved in specific (adaptive) immunity.
8. Summarize the importance of cytokines, and list one pro-inflammatory and one anti-inflammatory cytokine.

12.2 The First Line of Defense

The inborn, nonspecific defenses are the physical and chemical barriers that impede the entry of not only microbes but any foreign agent, whether living or not **(figure 12.8)**.

Physical or Anatomical Barriers at the Body's Surface

The skin and mucous membranes of the respiratory and digestive tracts have several built-in defenses. The outermost layer (stratum corneum) of the skin is composed of epithelial cells that have become compacted, cemented together, and impregnated with an insoluble protein, keratin. The result is a thick, tough layer that is highly impervious and waterproof. Few pathogens can penetrate this unbroken barrier, especially in regions such as the soles of the feet or the palms of the hands, where the stratum corneum is much thicker than on other parts of the body. In addition, outer layers of skin are constantly sloughing off, taking associated microbes with them. Other cutaneous barriers include hair follicles and skin glands. The hair shaft is periodically shed, and the follicle cells are **desquamated** (des′-kwuh-mayt-ud). The flushing effect of sweat glands also helps remove microbes.

The mucous membranes of the digestive, urinary, and respiratory tracts and of the eye are moist and permeable. They do provide barrier protection but without a keratinized layer. The mucous coat on the free surface of some membranes impedes the entry and attachment of bacteria. Blinking and tear production (lacrimation) flush the eye's surface with tears and rid it of irritants. The constant flow of saliva helps carry microbes into the harsh conditions of the stomach. Vomiting and defecation also evacuate noxious substances or microorganisms from the body.

The respiratory tract is constantly guarded from infection by elaborate and highly effective adaptations. Nasal hair traps larger particles. The copious flow of mucus and fluids that occurs in allergy and colds exerts a flushing action. In the respiratory tree (primarily the trachea and bronchi), a ciliated epithelium (called the ciliary escalator) moves foreign particles entrapped in mucus toward the pharynx to be removed **(figure 12.9)**. Irritation of the nasal passage reflexively initiates a sneeze, which expels a large volume of air at high velocity. Similarly, the acute sensitivity of the bronchi, trachea, and larynx to foreign matter triggers coughing, which ejects irritants.

The genitourinary tract derives partial protection via the continuous trickle of urine through the ureters and from periodic bladder emptying that flushes the urethra.

Vaginal secretions provide cleansing of the lower reproductive tract in females. Women who are postmenopausal are sometimes prone to vaginal infections due to lack of vaginal secretions

resulting from a decrease in estrogen production.

The human microbiome forms a type of structural barrier. Since humans have evolved with —and in response to—the constant presence of our microbiome, we can consider it as an integral part of our anatomy. Its presence can block the access of pathogens to epithelial surfaces and can create an unfavorable environment for pathogens by competing for limited nutrients or by altering the local pH.

New research stemming from the Human Microbiome Project has continued to highlight the importance of the normal resident biota on the development of nonspecific defenses as well as specific immunity. The presence of a robust commensal biota "trains" host defenses in such a way that commensals are kept in check and pathogens are eliminated. Evidence suggests that inflammatory bowel diseases, including Crohn's disease and ulcerative colitis, may well be a result of our overzealous attempts to free our environment of microbes and to overtreat ourselves with antibiotics. The result is an "ill-trained" gut defense system that responds inappropriately to commensal biota.

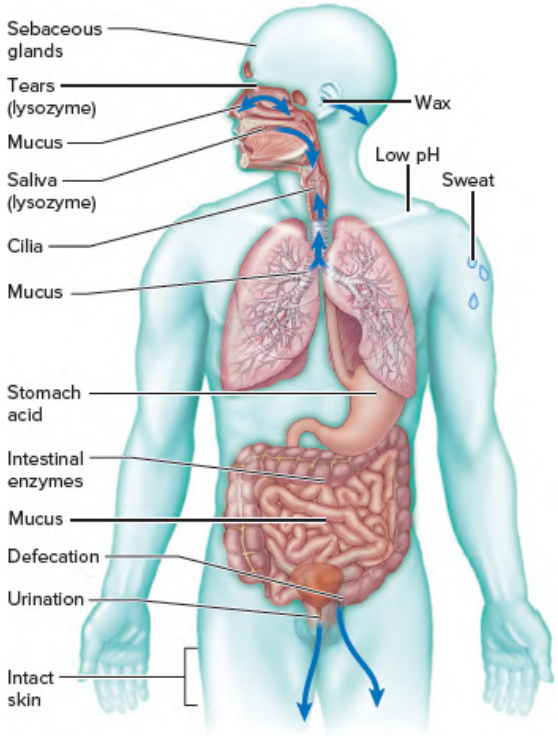

Figure 12.8 **The primary physical and chemical defense barriers. The blue arrows indicate the direction of fluid flow**.

Nonspecific Chemical Defenses

The skin and mucous membranes offer a variety of chemical defenses. Sebaceous secretions exert an antimicrobial effect, and specialized glands of the eyelids lubricate the conjunctiva with an antimicrobial secretion. An additional defense in tears and saliva is **lysozyme**, an enzyme that hydrolyzes, or breaks the glycosidic bond between neighboring sugars in the peptidoglycan layer of bacterial cell walls. The high lactic acid and electrolyte concentrations of sweat and the skin's acidic pH and fatty acid content are also inhibitory to many microbes. Likewise, the hydrochloric acid in the stomach renders protection against many pathogens that are swallowed, and the

intestine's digestive juices and bile are potentially destructive to microbes. Even semen contains an antimicrobial chemical that inhibits bacteria, and the vagina has a protective acidic pH maintained by normal biota.

The vital contribution of all types of barriers is clearly demonstrated in people who have lost them or never had them. Patients with severe skin damage due to burns are extremely susceptible to infections. Those with blockages in the salivary glands, tear ducts, intestine, and urinary tract are also at greater risk for infection. But as important as it is, the first line of defense alone is not sufficient to protect against infection. Because many pathogens find a way to circumvent the barriers by using their virulence factors (discussed in chapter 11), a whole new set of defenses—inflammation, phagocytosis, specific immune responses—are brought into play.

Tears cleanse the eyes of foreign particles and contain lysozyme.
©Andersen Ross/Blend Images

12.2 LEARNING OUTCOMES—The First Line of Defense

9. Identify the three components of the first line of defense.
10. Identify the four body systems that participate in the first line of defense.
11. Describe two examples of how the normal microbiota contribute to the first line of defense.

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2. The presence of intestinal microbiota is considered
a. *a first line of defense*.
b. *a second line of defense*.
c. *a third line of defense*.
d. *none of the above*.

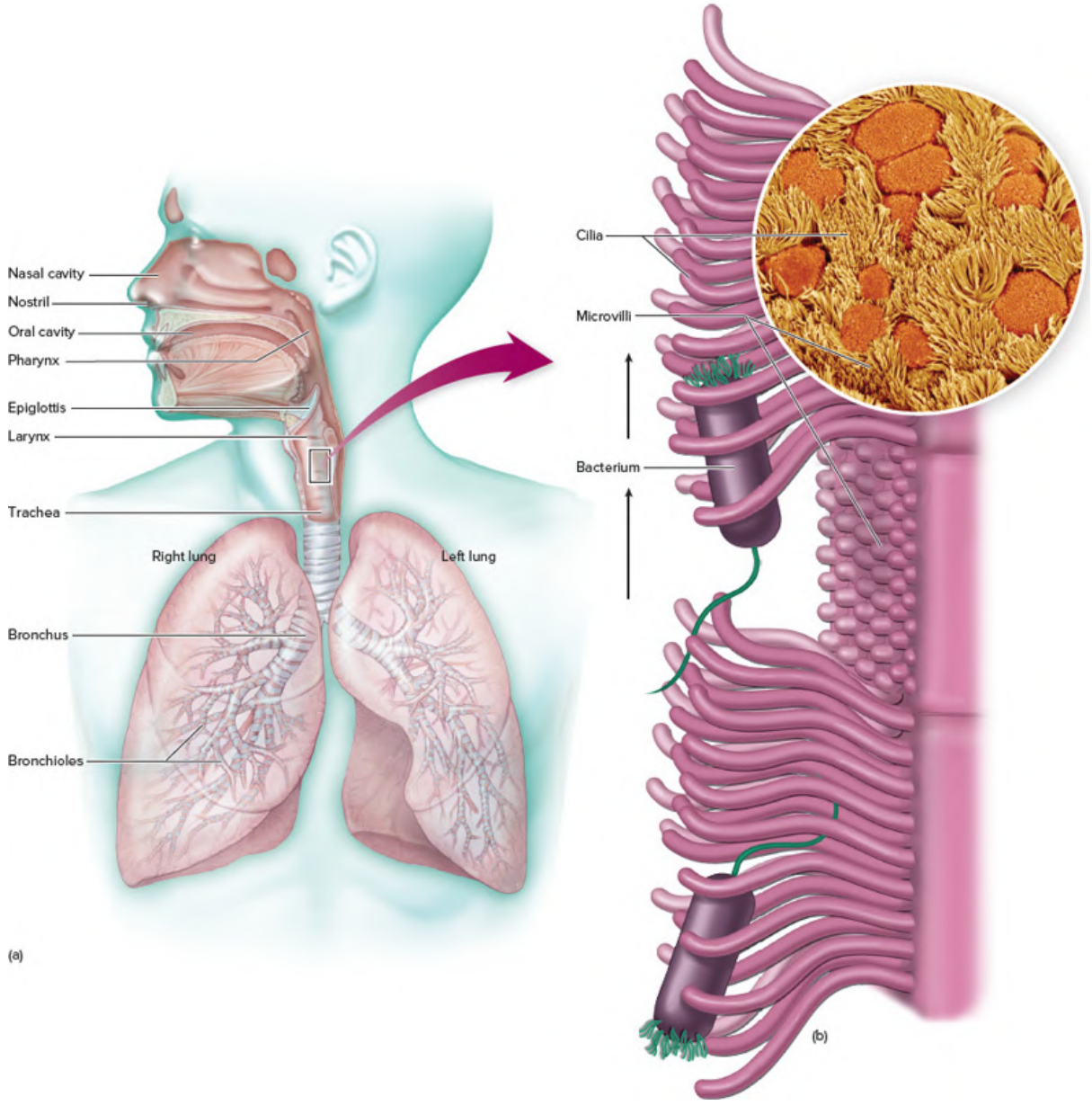


Figure 12.9 **The ciliary defense of the respiratory tree**. **(a)** The anatomy of the respiratory tract and its defenses. **(b)** The epithelial lining of the airways contains a brush border of cilia to entrap and propel particles upward toward the pharynx. In the inset you see the tracheal mucosa (5,000×).

©Susumu Nishinaga/Science Source (b) epithelial lining

©Steve Gschmeissner/Science Source

12.3 The Second Line of Defense

Now that we have introduced the principal anatomical and physiological framework of the immune system, we address some mechanisms that play important roles in host defenses: (1) phagocytosis, (2) inflammation, (3) fever, and (4) antimicrobial products. Because of the generalized nature of these defenses, they are primarily nonspecific in their effects, but they also support and interact with the specific immune responses described in chapter 13.

Phagocytosis: Cornerstone of Inflammation and Specific Immunity

By any standard, a phagocyte represents an impressive piece of living machinery, meandering through the tissues to seek, capture, and destroy a target. The general activities of phagocytes are as follows:

1. to survey the tissue compartments and discover microbes, particulate matter (dust, carbon particles, antigen-antibody complexes), and injured or dead cells;
2. to ingest and eliminate these materials; and
3. to extract immunogenic information (antigens) from foreign matter.

It is generally accepted that all cells have some capacity to engulf materials, but professional phagocytes do it for a living. The three main types of phagocytes are neutrophils, macrophages, and dendritic cells.

Neutrophils

As previously stated, **neutrophils** are general-purpose phagocytes. Neutrophils have a limited ability to phagocytose due to their rapid death when exposed to their own toxic oxygen products that also kill engulfed bacteria. Dead neutrophils accumulate quickly near sites of injury or infection and are a primary component of pus. A common sign of bacterial infection is a high neutrophil count in the blood (neutrophilia), and neutrophils are also a primary component of pus.

Monocytes Lead to Macrophages and Dendritic Cells

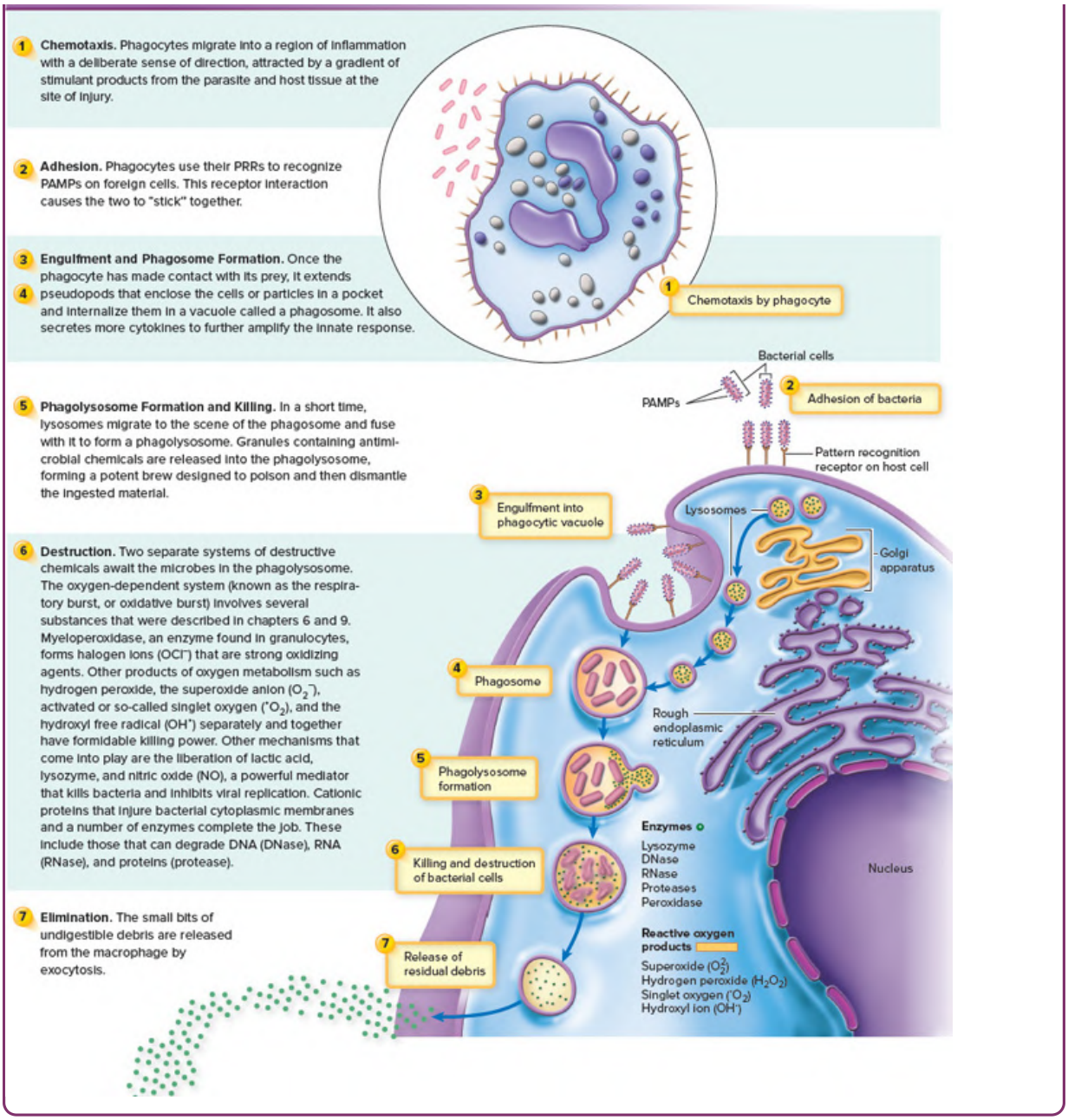
After emigrating out of the bloodstream into the tissues, **monocytes** are transformed by various inflammatory mediators into **macrophages**. This process is marked by an increase in size and by enhanced development of lysosomes and other organelles. They live much longer than

neutrophils and can consume a lot of material. Earlier we discussed specialized macrophages called histiocytes that live in a certain tissue and remain there during their life span. Remember that these are alveolar (lung) macrophages; the Kupffer cells in the liver; dendritic cells in the skin (see figure 12.7); and macrophages in the spleen, lymph nodes, bone marrow, kidney, bone, and brain. Other macrophages do not reside permanently in a particular tissue and drift nomadically throughout the MPS. Not only are macrophages dynamic scavengers, but they also process foreign substances and prepare them for reactions with B and T lymphocytes.

Mechanisms of Phagocytic Recognition, Engulfment, and Killing

The term *phagocyte* literally means "eating cell." But phagocytosis (the name for what phagocytes do) is more than just the physical process of engulfment, because phagocytes also actively attack and dismantle foreign cells with a wide array of antimicrobial substances. Phagocytosis can occur as an isolated event performed by a few phagocytic cells responding to a minor irritant in their area or as part of the orchestrated events of inflammation described in the next section. The events in phagocytosis include chemotaxis, ingestion, phagolysosome formation, destruction, and excretion. **Table 12.2** provides the details of phagocytosis.

Table 12.2 Phagocytosis



Phagocytes and other defensive cells are able to recognize some microorganisms as foreign because of signal molecules that the microbes have on their surfaces. These pathogen-associated molecular patterns (PAMPs) are molecules shared by many microorganisms—but not present in mammals—and therefore serve as "red flags" for phagocytes and other cells of innate immunity.

Bacterial PAMPs include peptidoglycan and lipopolysaccharide. Double-stranded RNA, which is found only in some viruses, is also a PAMP. On the host side, phagocytes, dendritic cells, endothelial cells, and even lymphocytes possess pattern recognition receptors (PRRs) on their surfaces that recognize and bind PAMPs. The cells possess these PRRs all the time, whether or not they have encountered PAMPs before. Many phagocytic cells of the innate immune

system contain PRRs inside their cytoplasm. These have a special name—**inflammasomes**—and they recognize microbial PAMPs as well as markers from damaged host cells once they have been phagocytosed. Recognition leads to the release of signals that initiate and regulate inflammation. (There are a lot of acronyms in immunology. Don't let them get away from you; keep up with them. If you know what all the acronyms stand for and what they do, you are halfway there in understanding host defenses!)

Figure 12.10 is an electron micrograph of a phagocyte engulfing bacteria.

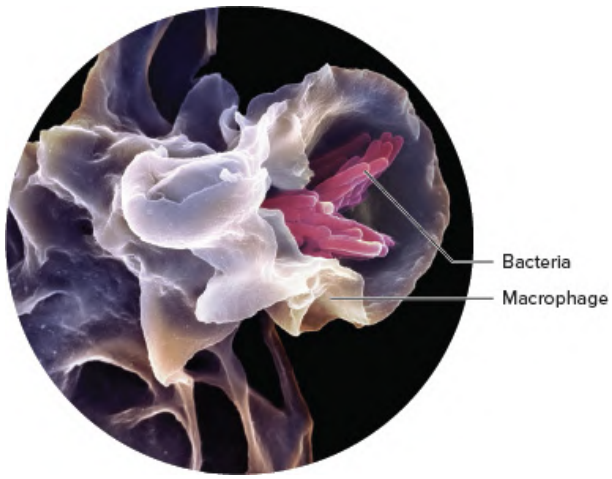


Figure 12.10 **Scanning electron micrograph of a macrophage**. This macrophage is devouring bacteria (10,000×).
©SPL/Science Source

The Inflammatory Response: A Complex Reaction to Injury

At its most general level, the inflammatory response is a reaction to any traumatic event in the tissues. It is so commonplace that all of us manifest inflammation in some way every day. It appears in the nasty flare of a cat scratch, the blistering of a burn, the painful lesion of an infection, and the symptoms of allergy. When close to our external surfaces, it is readily identifiable by a classic series of signs and symptoms characterized succinctly by four Latin terms: *rubor, calor, tumor*, and *dolor*. Rubor (redness) is caused by increased circulation and vasodilation in the injured tissues; calor (warmth) is the heat given off by the increased flow of blood; tumor (swelling) is caused by increased fluid escaping into the tissues; and dolor (pain) is caused by the stimulation of nerve endings. A fifth symptom, loss of function, has been added to give a complete picture of the effects of inflammation.

It is becoming increasingly clear that some chronic diseases, such as cardiovascular disease, can be caused by chronic inflammation. While we speak of inflammation at a local site (such as a finger), inflammation can affect an entire system—such as blood vessels, lungs, skin, the joints, and so on. Some researchers believe that the phenomenon of aging is a consequence of increasing inflammation in multiple body systems.

Factors that can elicit inflammation include trauma from infection (the primary emphasis here), tissue injury or necrosis due to physical or chemical agents, and specific immune reactions. Although the details of inflammation are very complex, its chief functions can be summarized as follows:

1. to mobilize and attract immune components to the site of the injury,
2. to set in motion mechanisms to repair tissue damage and localize and clear away harmful substances, and
3. to destroy microbes and block their further invasion.

The inflammatory response is a powerful defensive reaction, a means for the body to maintain stability and restore itself after an injury. But it has the potential to actually *cause* tissue injury, destruction, and disease.

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3. In order to assess immune function, the nurse anticipates that the physician will *first* order a
- **a.** *white blood cell (WBC) count with differential*.
- **b.** *red blood cell (RBC) count with differential*.
- **c.** *chemistry profile*.
- **d.** *coagulation profile*.

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4. A patient presents to the emergency department with a 3-day history of fever, lethargy, and respiratory symptoms. Which of the following assessment findings would indicate that the patient is experiencing an immune response? Select all that apply.
- **a.** *swollen cervical lymph nodes*
- **b.** *axillary temperature 39.0°C*
- **c.** *redness in the oropharynx*
- **d.** *sneezing*
- **e.** *large amount of nasal secretions*

The Stages of Inflammation

The process leading to inflammation is a dynamic, predictable sequence of events that can be acute, lasting from a few minutes or hours, or chronic, lasting for days, weeks, or years. Once the initial injury has occurred, a chain reaction takes place at the site of damaged tissue, summoning beneficial cells and fluids into the injured area. As an example, we will look at an injury at the microscopic level and observe the flow of major events in **table 12.3**. Be sure to examine it before moving on.

Diapedesis and Chemotaxis **Diapedesis** is the migration of WBCs out of blood vessels into tissues. It is made possible by several related characteristics of WBCs. For example, they are actively motile and readily change shape. This phenomenon is also assisted by the nature of the endothelial cells lining the venules. They contain complex adhesive receptors that capture the WBCs and participate in their transport from the venules into the extracellular spaces **(figure 12.11)**.

Another factor in the migratory habits of these WBCs is chemotaxis, or the tendency of cells to migrate in response to a specific chemical stimulus given off at a site of injury or infection. This means that cells swarm from many compartments to the site of infection and remain there to perform general and specific immune functions. These basic properties are absolutely essential for the sort of intercommunication and deployment of cells required for most immune reactions.

The Benefits of Edema and Leaky Vessels Both the secretion of fluids and the infiltration of neutrophils are physiologically beneficial activities. The influx of fluid dilutes toxic substances, and the fibrin clot can effectively trap microbes and prevent their further spread. The neutrophils that aggregate in the inflamed site are immediately involved in phagocytosing and destroying bacteria, dead tissues, and particulate matter. In some types of inflammation, accumulated phagocytes contribute to **pus**, a whitish mass of cells, liquefied cellular debris, and bacteria. Certain bacteria (streptococci, staphylococci, gonococci, and meningococci) are especially powerful attractants for neutrophils and are thus termed **pyogenic**, or pus-forming, bacteria.

The pain of a sore throat is caused by a combination of (1) damage inflicted by microbes; (2) the inflammatory response that can lead to pressure on surrounding nerves; and (3) the release of pain-inducing cytokines.
©Brand X Pictures/Punchstock

Fever

An important systemic component of inflammation—and innate immunity in general—is fever, defined as an abnormally elevated body temperature. Although fever is very common in infection, it is also associated with certain allergies, cancers, and other organic illnesses. Fevers whose causes are unknown are called fevers of unknown origin, or FUO.

The body temperature is normally maintained by a control center in the hypothalamus region of the brain. This thermostat regulates the body's heat production and heat loss and sets the core temperature at around 37°C (98.6°F) with slight fluctuations during a daily cycle. Fever is initiated when circulating substances called **pyrogens** (py′-roh-jenz) reset the hypothalamic thermostat to a higher setting. This change signals the musculature to increase heat production and peripheral arterioles to decrease heat loss through vasoconstriction. Fevers range

Table 12.3 Inflammation

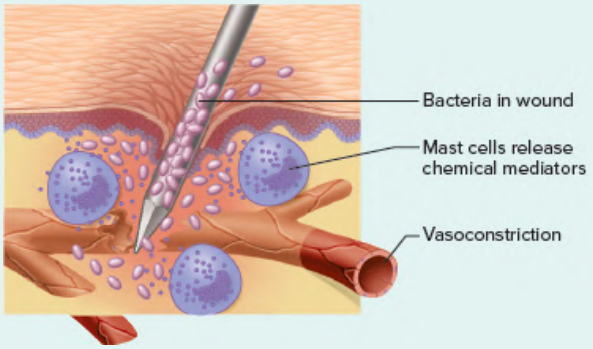
1 Injury/Immediate Reactions

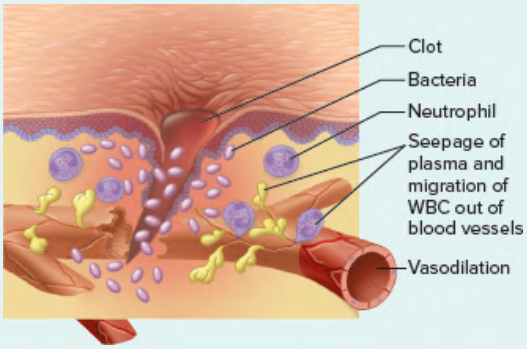
Following an injury, early changes occur in the vasculature (arterioles, capillaries, venules) in the vicinity of the damaged tissue. These changes are controlled by nervous stimulation, **chemical mediators**, and **cytokines** released by blood cells, tissue cells, and platelets in the injured area. Some mediators are **vasoactive**—that is, they affect the endothelial cells and smooth muscle cells of blood vessels, and others are **chemotactic factors**, also called **chemokines**, that affect white blood cells.

2 Vascular Reactions

In quick succession, the blood vessels in the vicinity dilate (widen), then constrict, and then dilate again. The wide-narrow-wide sequence is thought to be for the purpose of flushing irritants (such as bacteria) away from the area, then the narrowing is an attempt to stem blood leaving the blood vessels, followed by a long-term dilation to bring helpful blood components to the site. The overall effect of vasodilation is to increase the flow of blood into the area, which facilitates the influx of immune components and also causes redness and warmth.

Some vasoactive substances cause the endothelial cells in the walls of postcapillary venules to contract and form gaps through which blood-borne components exude into the extracellular spaces. The fluid part that escapes is called the **exudate**.





3 Edema and Pus Formation

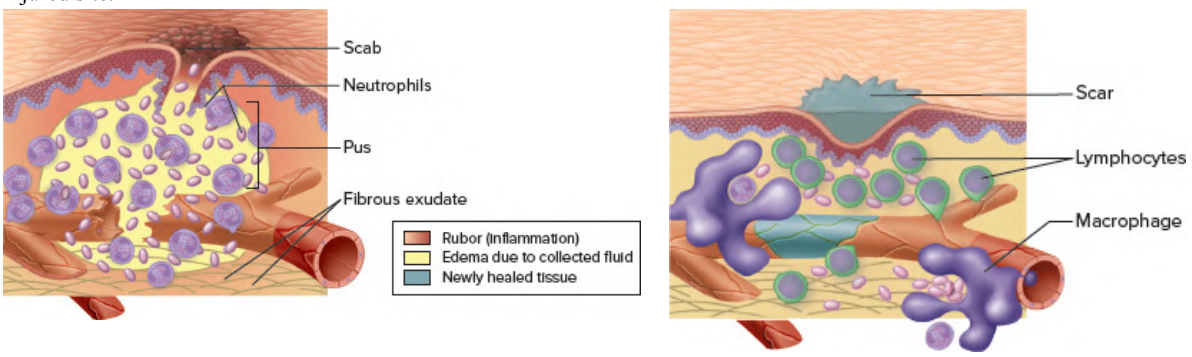
Accumulation of this fluid in the tissues gives rise to local swelling and hardness called edema. The fluid contains varying amounts of plasma proteins, such as globulins, albumin, the clotting protein

4 Resolution/Scar Formation

Repair is the last step and results either in complete resolution to healthy tissue, or in formation of scar tissue, depending on the tissue type and the extent of the

fibrinogen, blood cells, and cellular debris. Depending on its content, the fluid may be clear (called **serous**), or it may contain red blood cells or pus. Pus is composed mainly of white blood cells and the debris generated by phagocytosis. In some types of edema, the fibrinogen is converted to fibrin threads that enmesh the injury site. Within an hour, multitudes of neutrophils responding chemotactically to special signaling molecules converge on the injured site.

damage. Note here that macrophages are pictured leaving the blood vessels in a process called **diapedesis** (dye″-ah-puh-dee′-sis). Meanwhile, differentiated stem cells in the area begin to divide and repopulate the damaged site with new cells to replace those that were damaged.



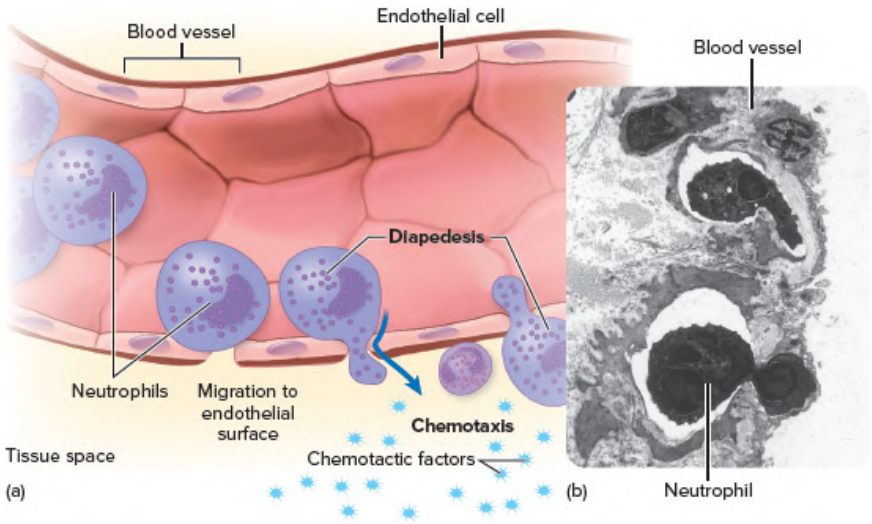


Figure 12.11 **Diapedesis and chemotaxis of leukocytes**. **(a)** View of a venule depicts white blood cells squeezing themselves between spaces in the blood vessel wall via diapedesis. This process, shown in cross section, indicates how the pool of leukocytes adheres to the endothelial wall. From this site, they are poised to migrate out of the vessel into the tissue space. **(b)** This micrograph captures neutrophils in the process of diapedesis.
Courtesy Steve Kunkel (b)

in severity from low grade (37.7°C to 38.3°C, or 100°F to 101°F) to high (40.0°C to 41.1°C, or 104°F to 106°F). Fevers above 104°F to 105°F should be treated to prevent serious damage to host tissues. Pyrogens are described as **exogenous** (coming from outside the body) or **endogenous** (originating internally). Exogenous pyrogens are products of infectious agents such as viruses, bacteria, protozoa, and fungi. One well-characterized exogenous pyrogen is endotoxin, the lipopolysaccharide found in the cell walls of gram-negative bacteria. Blood, blood products, vaccines, or injectable solutions can also contain exogenous pyrogens. Endogenous pyrogens are released by monocytes, neutrophils, and macrophages during the process of

phagocytosis and appear to be a natural part of the immune response. Two potent pyrogens released by macrophages are the cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF).

NCLEX® PREP

5. An RN is providing care for a patient following joint replacement surgery. The patient develops a fever in the first 24 hours postoperatively. What is the priority intervention?

a. *Collect blood cultures from patient's peripheral IV catheters.*
b. *Administer antipyretic medication.*
c. *Apply ice packs to the patient's surgical site.*
d. *Administer IV bolus fluids in anticipation of the patient's increased metabolic demands.*
e. *Notify the provider managing the patient's care.*

Benefits of Fever

Aside from its practical and medical importance as a sign of disease, increased body temperature has additional benefits:

- Fever inhibits multiplication of temperature-sensitive microorganisms such as the poliovirus, cold viruses, herpes zoster virus, and systemic and subcutaneous fungal pathogens.
- Fever impedes the nutrition of bacteria by reducing the availability of iron.
- Fever increases metabolism and stimulates immune reactions and naturally protective physiological processes. It speeds up hematopoiesis, phagocytosis, and specific immune reactions and helps specific lymphocytes home in on sites of infection.

Thousands of years ago, people realized that fever was part of an innate protective response. Hippocrates offered the idea that it was the body's attempt to burn off a noxious agent. Sir Thomas Sydenham wrote in the seventh century: "Why, fever itself is Nature's instrument!" So widely held was the view that fever could be therapeutic that pyretotherapy (treating disease by inducing an intermittent fever) was once used to treat syphilis, gonorrhea, leishmaniasis (a protozoal infection), and cancer. This attitude fell out of favor when drugs for relieving fever (aspirin) first came into use in the early 1900s, and an adverse view of fever began to dominate.

Treatment of Fever

With this revised perspective on fever, whether to suppress it or not can be a difficult decision. Some advocates feel that a slight to moderate fever in an otherwise healthy person should be allowed to run its course, in light of its potential benefits and minimal side effects. Side effects of fever include tachycardia (rapid heart rate), tachypnea (elevated respiratory rate), and, in some individuals, a lowering of seizure threshold. Therefore, all medical experts do agree that high and prolonged fevers or fevers in patients with cardiovascular disease, head trauma, seizures, or respiratory ailments are risky and should be treated immediately with fever-reducing drugs.

©Science Photo Library/Getty Images

Antimicrobial Products

In this section we will examine a broad group of products the host produces to battle microbial infections. We will look at four main categories: **interferons (IFN)**, which are best known for targeting viruses, but can also target bacteria and even tumor cells; **complement**, which targets membranes of pathogens and also of pathogen-infected host cells; **antimicrobial peptides**, which can directly kill all manner of microbes; and **restriction factors**, which inhibit the multiplication of viruses in host cells.

Interferons

Interferons are small proteins produced naturally by certain white blood and tissue cells. Although the interferon system was originally thought to be directed exclusively against viruses, it is now known to be involved also in defenses against other microbes and in immune regulation and intercommunication. Three major types are *interferons alpha* and *beta*, which are produced by many cells, including lymphocytes, fibroblasts, and macrophages; and *interferon gamma*, a product of T cells.

Their biological activities are extensive. In all cases, interferons bind to cell surfaces and induce changes in genetic expression, but the exact results vary. In addition to antiviral effects discussed in the next section, all three IFNs can inhibit the expression of cancer genes and have tumor suppressor effects.

Characteristics of Interferons When viruses, and sometimes other microbes or their component parts, bind to the receptors on a host cell, a signal is sent to the nucleus that directs the cell to synthesize interferons. After transcribing and translating the interferon genes, newly synthesized interferon molecules are rapidly secreted by the cell into the extracellular space, where they bind to other host cells. When interferons bind to the second cell, *that* cell produces proteins that inhibit viral multiplication. These proteins use a variety of mechanisms, such as degrading the viral RNA or preventing the translation of viral proteins **(figure 12.12)**. Interferons can also induce the production of other proteins that combat infection, such as an enzyme that produces reactive oxygen chemicals that damage pathogens. Interferons are not microbe-specific, so their synthesis in response to one type of microbe will also protect against other types. Because these proteins are inhibitors of viruses, they have been used as a valuable treatment for a number of virus infections.

Complement

The immune system has another versatile system called complement that, like inflammation and phagocytosis, is brought into play at several levels. The complement system, named for its property of "complementing" immune reactions, consists of

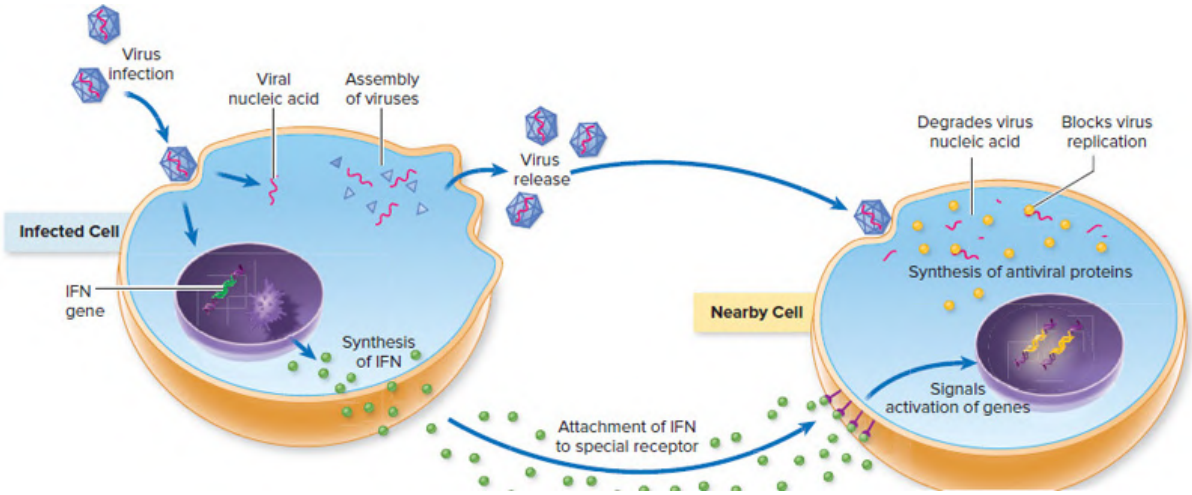


Figure 12.12 **The antiviral activity of interferons**. When a cell is infected by a virus (and sometimes other microbes), its nucleus is triggered to transcribe and translate the interferon (IFN) genes. Interferons diffuse out of the cell and bind to IFN receptors on nearby uninfected cells, where they induce production of proteins that eliminate genes from foreign organisms. Note that the original cell is not protected by IFNs and that IFNs do not prevent viruses (or other organisms) from invading the protected cells.

over 30 different blood proteins that work together to primarily destroy bacteria, but can have effects on viruses, other parasites, or nearby cells.

The concept of a cascade reaction is helpful in understanding how complement functions. A cascade reaction is a sequential physiological response like that of blood clotting, in which the first substance in a chemical series activates the next substance, which activates the next, and so on, until a desired end product is reached.

Four Stages of the Complement Cascade In general, the complement system goes through four stages during its deployment:

- initiation,
- activation and cascade,
- polymerization, and
- membrane attack.

There are multiple ways in which the complement cascade can be initiated. When we study the third line of defense we will learn about antibodies, which are host cell proteins that attach to the surfaces of pathogens. Complement action can be initiated by binding to the antibodies that are already bound to microbes. That scenario is called the **classical complement pathway**. Another important way that the complement cascade is initiated is called the **alternative complement pathway**. It does not require antibody to get started, but instead is initiated simply by the presence of foreign cell antigens. Since we have not yet studied antibodies we will illustrate complement activity using the alternative pathway **(figure 12.13)**.

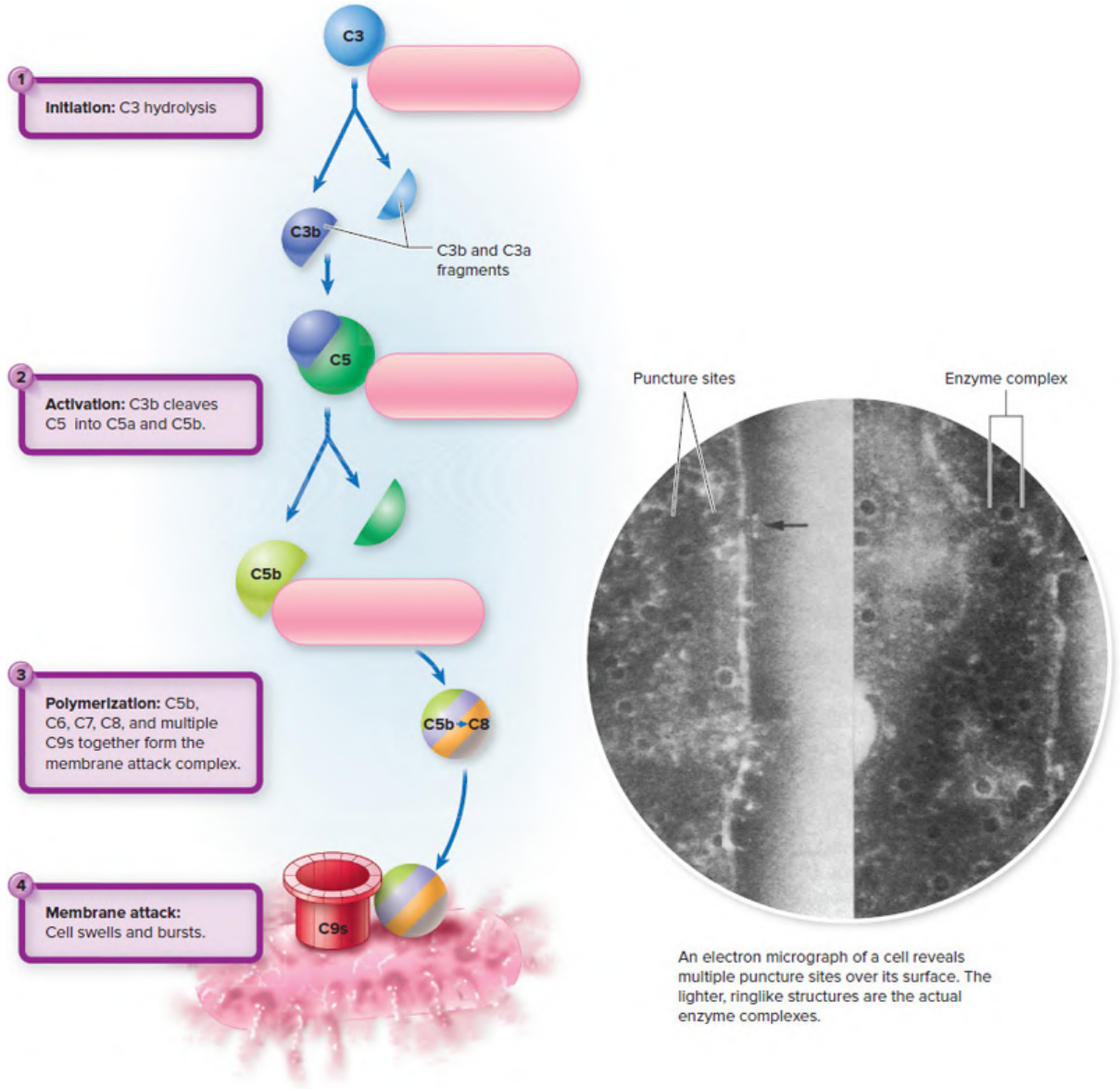


Figure 12.13 **Steps in the alternative complement pathway at a single site**. All complement pathways function in a similar way, but the details differ. The alternative pathway is illustrated here.
©Sucharit Bhakdi (micrograph)

The complement cascade can involve more than 30 different proteins. They are usually referred to as "C1," "C5," etc., and may be further divided, as in "C3b." Their numbered names do not necessarily correspond with the order in which they act.

Initiation In the alternative pathway, a C3 protein, either free or bound to a pathogen membrane, is hydrolyzed into two fragments, C3b and C3a (see figure 12.13).

Activation and Cascade The next steps involve further enzymatic action. The C3b protein cleaves the protein C5 into C5a and C5b.

Polymerization The C5b fragment is now free to form a complex with C6, C7, and C8. This complex is called the **membrane attack complex (MAC)**.

Membrane Attack The MAC is positioned on the offending cell's membrane and forms pores in the membrane. This causes the membrane to lose its structural integrity, which leads to inappropriate flow of water and ions in and out of the cell, which eventually leads to the lysis of the cell.

The C3 molecule is readily available in tissues and the lymphatic system, often being left over from previous immune responses. Since it acts nonspecifically, it can react right away to new pathogens it may encounter. This also accounts for the fact that complement activation can go awry, and lead to autoimmune dysfunction in hosts. The activation of complement by any means provides a strong linkage between the second line of defense and the third (specific) line of defense. It plays a role in regulating specific immunity as well. Binding of individual complement proteins, even when they do not result in a membrane attack complex, can help attract host antibodies. These antibodies can bind to the pathogen more easily, making them an easy target for phagocytes to identify them and eat them.

The classical pathway is activated by the presence of antibodies on cell surfaces, and begins with "earlier" complement proteins, starting with one named C1. There are other ways besides the classical and alternative pathways to initiate complement activity, but these two are the most well-studied.

Antimicrobial Peptides

Antimicrobial peptides are short proteins, of between 12 and 50 amino acids, that have the capability of inserting themselves into bacterial membranes **(figure 12.14)**. One type of peptide, called a defensin, inserts itself into cell membranes of bacteria, causing the membrane to fold around it. When a few defensin molecules gather in one area, they can make a pore in the membrane. If enough of these are formed, the cell could lyse. There are many other examples of antimicrobial peptides that have similar mechanisms for killing microbes, and some are even effective against eukaryotes or viruses. Because they will be effective against large groups of microorganisms, they are part of the innate immune system, but like other proteins in this section, they can have more far-reaching effects on other types of cells in the immune system as well. Many researchers are trying to turn these antimicrobial peptides into practical use as therapeutic drugs, while others are using computer programs to try to design synthetic peptides that will have as good an effect as these naturally-occurring ones.

Host Restriction Factors

Some host cells and immune cells make molecules that can limit the ability of viruses to replicate once they are inside a host cell. These proteins and nucleic acids can bind to certain parts of the virus, prevent synthesis of new virus parts,

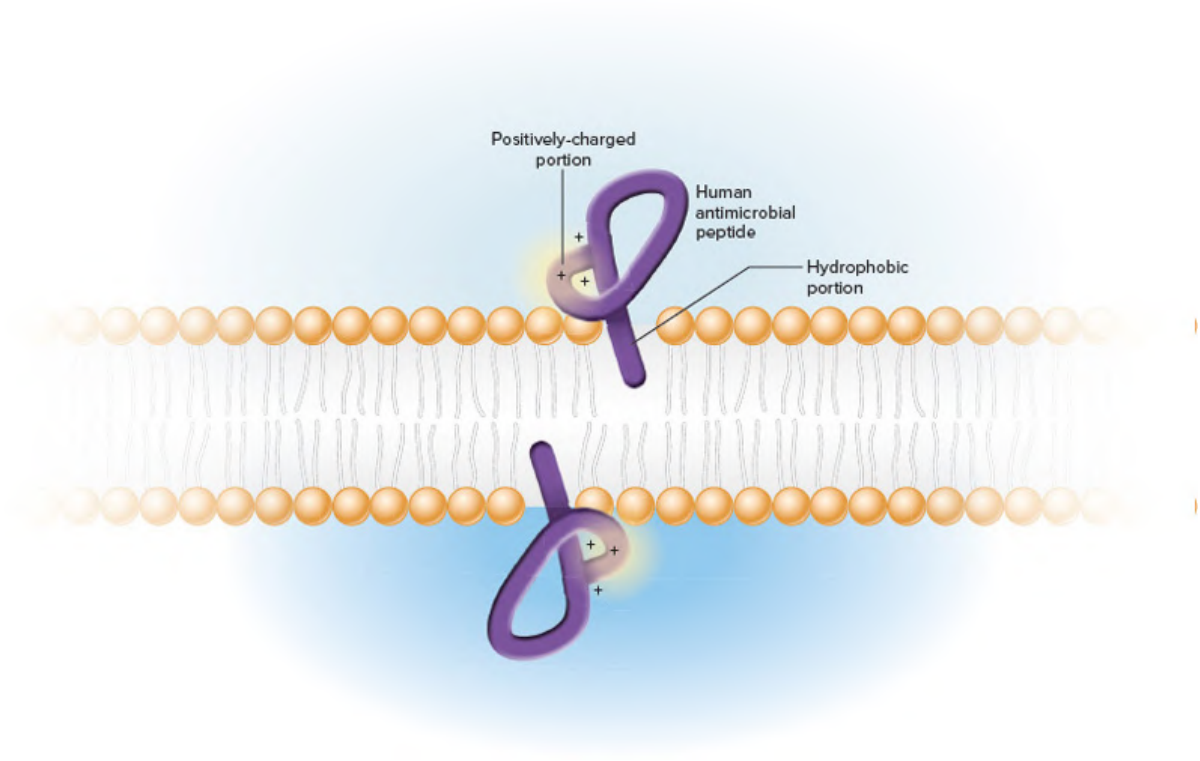


Figure 12.14 **Antimicrobial peptides**. The cytoplasmic membrane is shown (white and yellow). The purple antimicrobial peptides insert themselves in the membrane and disrupt its integrity.

prevent assembly of a new virus, or prevent virus release from host cells. These are collectively called *restriction factors*. One well-characterized set of restriction factors restricts the replication of retroviruses (such as HIV) by spontaneously changing cytosines to uracils, and utilizing miRNAs that selectively target the viral DNA.

These apparently ancient nonspecific defensive strategies are the subject of active research in the hopes that new antiviral medications can be developed, or that understanding them might aid in vaccine development.

12.3 LEARNING OUTCOMES—The Second Line of Defense

12. List the four major categories of nonspecific (innate) immunity.
13. Outline the steps of phagocytosis.
14. Outline the steps of inflammation.
15. Discuss the mechanism of fever and how it helps defend the body.
16. Name four types of antimicrobial host-derived products.
17. Compose one good overview sentence about the purpose and the mode of action of the complement system, and another about the purpose and mode of action of interferon.

CASE FILE WRAP-UP

©Pixtal/age fotostock (health care worker); ©McGraw-Hill EducatioNAl Telser, photographer (tissue)

Helicobacter pylori is a gram-negative bacterium found in the stomach. It is estimated that more than half of the world's population harbors this bacterium in their upper gastrointestinal tract. However, most people are asymptomatic and may never know that they carry the microorganism. Symptoms occur in about 20% of infected people. Symptoms may include stomach pain, bloating, belching, and nausea.

The stomach's acidic environment is usually very efficient at killing microorganisms that are swallowed. How does *H. pylori* survive and thrive in this harsh environment? *H. pylori* uses its flagella to burrow into the mucous lining of the stomach until it reaches a more neutral area. In a process known as chemotaxis, *H. pylori* can sense areas of acidity and can move to more hospitable areas. It also has the ability to neutralize acid in its immediate environment by using urease to break down urea into ammonia and carbon dioxide. The ammonia neutralizes harmful stomach acid.

An inflammatory response by the body tries to combat the invasion by *H. pylori*. A significant part of this response is for the stomach to produce even more acid. Unfortunately, the extra acid sometimes damages the lining of the stomach and the duodenum, causing ulcerations (ulcers).

©McGraw-Hill Education/Mark Dierker, photographer

The Microbiome

Are We Treating Autoimmune Diseases Incorrectly?

Chronic inflammatory diseases are extremely common in the developed world. A subcategory of these is autoimmune disease, which has long been thought to be the result of a disregulated host immune response in which we mount an immune response directly against self cells. Examples of autoimmune disease include rheumatoid arthritis, multiple sclerosis, fibromyalgia, and systemic lupus erythematosus.

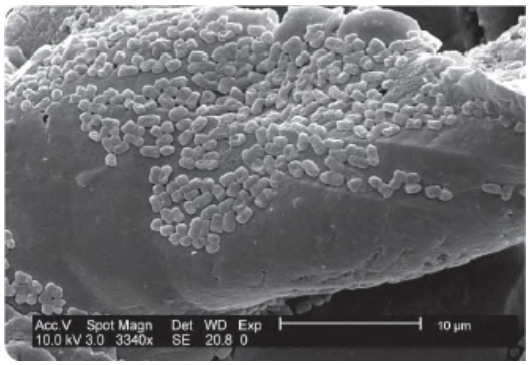
Since the immense influence of the human microbiome has been discovered, some researchers are pinning the cause of autoimmune diseases on microbes themselves. In fact, there is an institute in California, called the Autoimmunity Research Foundation, dedicated to studying the role of microbes in autoimmunity.

Here is the reasoning: Researchers favoring the microbe theory point out that autoimmunity was discovered and its causes studied during the first half of the 1900s, clearly long before we knew about the importance of the microbiome. Now that we know that our microbiome contains over 9 million genes (compared to just 20,500 human genes) and that they all have the potential to interact with each other, we have to consider the impact that the microbiome—and the potential pathogens it contains—has on our physiology. As we have seen throughout this book, many things can change the composition of the microbiome, and that change has been associated with disease.

The researchers describe a process they call *successive infection* that starts with human cells being infected by one or more pathogens "hiding" in the microbiome. They have demonstrated that the microbial proteins and enzymes alter the physiology of the infected host cells, so that they struggle to produce the correct cytokines and antimicrobial substances. This slows down the nonspecific immune response, which has a snowball effect by allowing additional microbes to invade (successive infections), and a chronic low-grade inflammation develops in various organs or locations.

Why is it important to know whether it is microbial crippling of our immune response or a genetic predisposition to inappropriate immunity that causes autoimmune diseases? Because it determines whether treatment is aimed at (a) dampening the immune response or (b) revving up the immune response to stop the snowball effect. For decades, these diseases have been treated with immunosuppressive drugs. The researchers supporting the new approach have begun treating patients with these disorders with immune-stimulating drugs and are claiming success. Currently, this approach is considered an alternative—even fringe—treatment, but it is a good illustration of the scientific process and the uncertainties along the way.

Source: *Autoimmune Review*, February 3, 2009.



Normal biota *E. coli* on a human surface
Source: CDC/Janice Carr

Chapter Summary

12.1 Defense Mechanisms of the Host: An Overview

- The interconnecting network of host protection against microbial invasion is organized into three lines of defense.
- The immune system operates as a surveillance system that discriminates between the host's self identity markers and the nonself identity markers of foreign cells.

- The immune system is a network of vessels, organs, and cells positioned throughout the body. It is composed of primary and secondary lymphatic organs.
- Primary organs are the thymus and the red blood marrow; secondary organs are the lymphatic vessels, lymph nodes, spleen, lymphoid tissues such as GALT, MALT, and SALT, and the tonsils.
- The lymphatic system returns tissue fluid to general circulation, concentrates and processes foreign invaders, and initiates the specific immune response. It is closely integrated with the circulatory system.
- The blood contains both specific and nonspecific defenses. Nonspecific cellular defenses include the granulocytes, macrophages, and dendritic cells. The two major components of the specific immune response are the T lymphocytes, which provide specific cell-mediated immunity, and the B lymphocytes, which produce specific antibody-mediated immunity.
- The mononuclear phagocyte system consists of cells distributed throughout the body's network of connective tissue fibers. They stand ready to attack and ingest microbes that have managed to bypass the first line of defense.
- Cytokines are molecules produced by cells of the immune system that regulate, stimulate, suppress, and influence cell development, inflammation, and immunity.

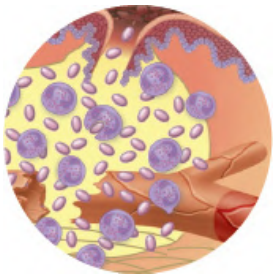
12.2 The First Line of Defense

- The first line of defense is made up of physical and chemical barriers. The normal microbiota add to this barrier function.
- Mucous membranes and the skin are physical barriers. Sweat, lysozymes, fatty acids, and pH levels are typical chemical barriers.

©Susumu Nishinaga/Science Source

12.3 The Second Line of Defense

- Nonspecific immune reactions are generalized responses to invasion, regardless of the type. These include phagocytosis, inflammation, fever, and an array of antimicrobial products.
- Phagocytosis is accomplished by macrophages along with neutrophils and a few other cell types.



- The four symptoms of inflammation are rubor (redness), calor (heat), tumor (swelling), and dolor (pain). Loss of function often accompanies these.
- Fever is another component of nonspecific immunity. It is caused by both endogenous and exogenous pyrogens. Fever increases the rapidity of the host immune responses and reduces the viability of many microbial invaders.
- There are four main types of antimicrobial products in the second line of defense: interferons, the complement cascade, antimicrobial peptides, and host restriction factors.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's Taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. A microorganism carries _____ markers and a B cell carries _____ markers. **a**. self, nonself **b**. nonself, self **c**. self, self **d**. nonself, non*self*	**2**. Explain why the presence of lysozyme in human fluids such as tears and saliva is clear evidence of the coevolution of humans and microbes.	**3**. The pediatrician you work for has just recommended to the mother of a child with a cold and fever of 100°F *not* to treat the fever with children's Tylenol®. When the doctor leaves the room, the mother asks you to explain his recommendation. Use an evolutionary perspective to construct an answer for her.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Cell Structure and Function	**4**. Which of the following cells are lymphocytes? **a**. macrophages **b**. neutrophils **c**. red blood cells **d**. B cells	**5**. In what way is a phagocyte a tiny container of disinfectants?	**6**. Conduct research on the NK cells and NKT cells and describe distinguishing characteristics.
Metabolic Pathways	**7**. Cytokines are secreted by which cells? **a**. macrophages **b**. B cells **c**. T cells **d**. all of these	**8**. Explain the sequence of physiological events leading to fever.	**9**. Explain the physiological events that lead to each of the four cardinal signs of inflammation.
Information Flow and Genetics	**10**. The initial reaction to the presence of viruses in a human cell is the production of _____ by that cell. **a**. complement **b**. interferon **c**. antiviral protein **d**. fever	**11**. If the synthesis of antiviral proteins begins after exposure of the host cell to interferons, what kind of genes are the antiviral protein genes likely to be? **a**. constitutive **b**. repressed **c**. induced **d**. all of these	**12**. If scientists were going to recreate host cell restriction factors in the lab so that they could be used as antiviral drugs, how would they go about that?

Microbial Systems	**13**. An example of an exogenous pyrogen is **a**. interleukin-1.	**14**. Why do you think that the intestines	**15**. A less diverse gut microbiome has been linked to a reduction in healthy immune

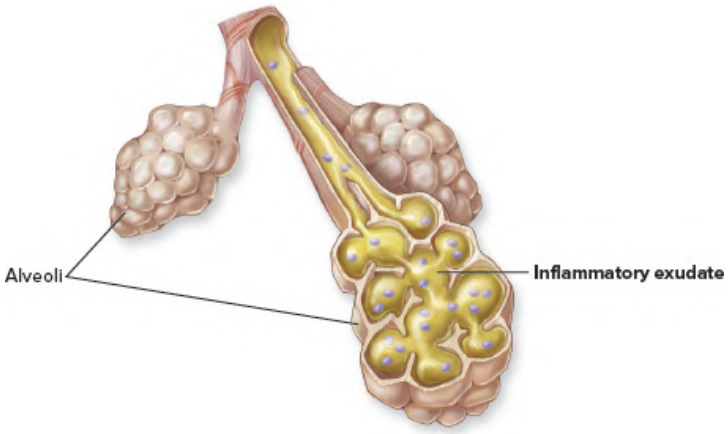
	b. endotoxin. **c**. complement. **d**. interferon.	have one of the body's most well-developed sets of lymphoid tissues?	responses. Why might that be?
Impact of Microorganisms	**16**. The normal microbiota is part of the _____ line of defense. **a**. first **b**. second **c**. third **d**. None of these is correct.	**17**. People who smoke have higher rates of respiratory infections. Explain why this might be, invoking aspects of the first line of defense.	**18**. Recent studies suggest that even healthy blood contains a microbiome, most of which exists inside the white blood cells. Why are there microorganisms and/or their DNA there rather than in the red blood cells; and what might their role be?
Scientific Thinking	**19**. Figure 12.3 demonstrates that lymphatic fluid flows **a**. toward the heart. **b**. away from the heart. **c**. both toward and away from the heart. **d**. None of these is correct.	**20**. Suggest some reasons for so much redundancy and interactivity of immune responses.	**21**. There have been (controversial) attempts in the past to treat HIV+ people by infecting them with the malarial parasite. One of the reasons it was controversial is that it was ethically questionable for physicians to knowingly infect patients with a potentially serious organism (the malaria protozoan). Why did scientists think that a malaria infection might wipe out HIV in a patient's body?

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From chapter 11, figure 11.2**. Relate specific events in inflammation to the symptoms of pneumonia pictured in this drawing.



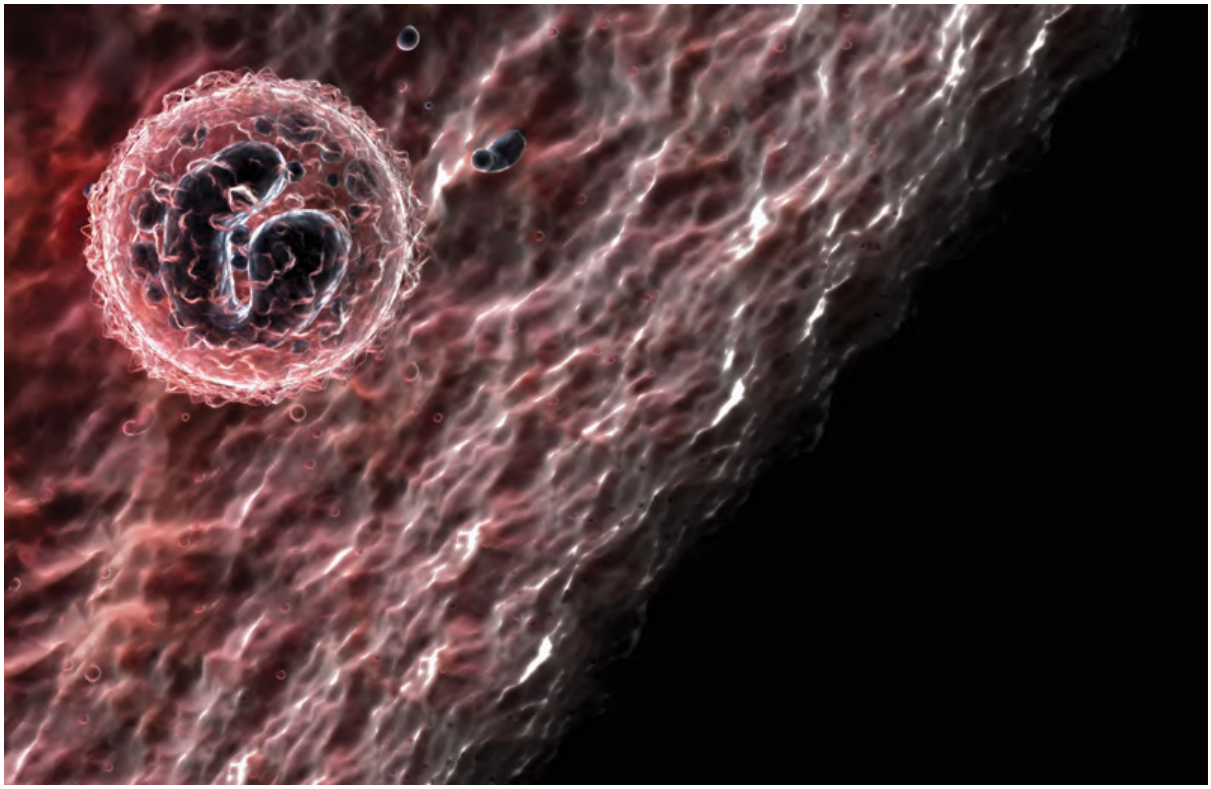
connect Resources are available through Connect, including interactive learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

13

Host Defenses II

Specific Immunity and Immunization



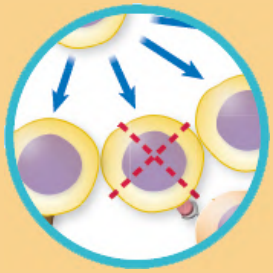
©MedicalRF.com/Getty Images (monocytes); ©O. Dimier/PhotoAlto (health care worker); ©Steve Gschmeissner/Science Source (cytotoxic T cell)

IN THIS CHAPTER…

13.1 Specific Immunity: The Third and Final Line of Defense

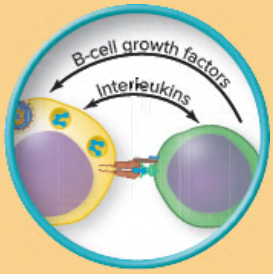
1. Describe how the third line of defense is different from the other two.

2. Compare the terms *antigen, immunogen*, and *epitope*.
3. List the four stages of a specific immune response.
4. Discuss the role of cell markers in the immune response.
5. Describe the major histocompatibility complex in two sentences.



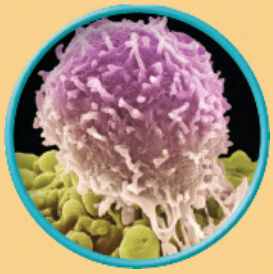
13.2 Stage I: The Development of Lymphocyte Diversity

6. Summarize the maturation process of both B cells and T cells.
7. Draw a diagram showing how lymphocytes are capable of responding to nearly any epitope imaginable.
8. Describe the structures of the B-cell receptor and the T-cell receptor.
9. Outline the processes of clonal deletion and clonal selection.



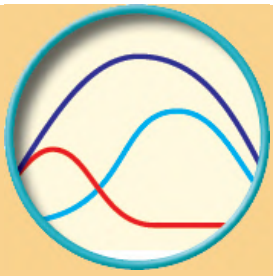
13.3 Stage II: Presentation of Antigens

10. List characteristics of antigens that optimize their immunogenicity.
11. Describe how the immune system responds to alloantigens, superantigens, and allergens.
12. List the types of cells that can act as antigen-presenting cells.



13.4 Stages III and IV: T-Cell Response

13. Describe the main differences between T helper cells and T cytotoxic cells.
14. Explain how naive T cells become sensitized to an antigen.
15. Note the similarities and differences between gamma-delta T cells and the other T cells.



13.5 Stages III and IV: B-Cell Response

16. Diagram the steps in B-cell activation, including all types of cells produced.
17. Make a detailed drawing of an antibody molecule.
18. Explain the various end results of antibody binding to an antigen.
19. List the five types of antibodies and important facts about each.
20. Draw and label a graph—with time on the horizontal axis—that shows the development of the primary and secondary immune responses.

13.6 Specific Immunity and Vaccination

21. List and define the four different descriptors of specific immune states.
22. Discuss the qualities of an effective vaccine.
23. Name the two major categories of vaccines and then the subcategories under each.
24. Explain the principle of herd immunity and the risks that unfold when it is not maintained.

CASE FILE

When More Immune Response Is Not Better

As an RN in the pediatric intensive care unit, I cared for children with a wide range of conditions and diseases. One day, I was assigned to admit a patient from the emergency department. Trevor was a 15-year-old male who was previously healthy. Of note, he reported that he had respiratory symptoms 2 weeks prior that had resolved. Trevor presented to my unit with fever, lethargy, and an unusual skin rash that had begun 3 days ago.

When I assessed Trevor, I found him to be drowsy but responsive. He followed commands appropriately and answered questions. I noted that his eyes had a yellow tint. His heart rate was higher than normal and he had a fever. A generalized rash was present across his upper chest, neck, and back. I noted areas of bruising on his legs and arms.

Because we were concerned that Trevor's symptoms may indicate an infection, we maintained isolation precautions in the room. I gave him medication for his fever and monitored his vital signs closely. I administered an intravenous fluid bolus to counteract dehydration. I collected blood samples and sent them to the laboratory.

When Trevor's laboratory results returned, they were quite altered. The white blood cell, red blood cell, and platelet counts were low. His liver function was altered. I assumed this explained the yellow tint to his sclera, known as jaundice. Labs assessing his ability to appropriately form blood clots were abnormal, likely explaining his bruising. The medical team consulted hematology specialists and ordered additional labs for workup. The patient's ferritin level came back extremely elevated.

Based on the laboratory values and Trevor's clinical presentation, a preliminary diagnosis of hemophagocytic lymphohistiocytosis (HLH) was made. He was scheduled for a bone marrow biopsy for confirmation. Intravenous steroids were ordered and the medical team discussed the treatment plan, focused upon suppressing Trevor's immune system.

- What is hemophagocytic lymphohistiocytosis (HLH)?
- How did Trevor acquire HLH?

Case File Wrap-Up appears at the end of the chapter.

13.1 Specific Immunity: The Third and Final Line of Defense

When host barriers and nonspecific defenses fail to control an infectious agent, a person with a normally functioning immune system has a mechanism to resist the pathogen—the third, specific line of immunity. This sort of immunity is not innate but adaptive; it is acquired only after an immunizing event such as an infection.

Acquired specific immunity is the product of a dual system that we have previously mentioned —the B and T lymphocytes. During development, these lymphocytes undergo a selective process that prepares them for reacting only to one specific antigen or immunogen. During this time **immunocompetence**, the ability of the body to react with countless foreign substances, develops. An infant is born with the theoretical potential to react to an extraordinary array of different substances.

Antigens are molecules that can be seen and identified by the immune system. They may or may not provoke an immune response after being "sensed" by the immune system. If they do provoke a response they can be called **immunogens**. These molecules are usually protein or polysaccharide molecules on or inside all cells and viruses, including our own. (Environmental chemicals can also be antigens. These are covered in chapter 14.) In fact, any exposed or released protein or polysaccharide is potentially an antigen, even those on our own cells. For reasons we discuss later, our own antigens do not usually evoke a response from our own immune systems.

A lymphocyte's capacity to discriminate differences in molecular shape is so fine that it recognizes and responds to only a portion of the antigen molecule. This molecular fragment, called the **epitope**, is the primary signal that the molecule is foreign.

In chapter 12, we discussed pathogen-associated molecular patterns (PAMPs) that stimulate responses by phagocytic cells during an innate defense response. While PAMPs are molecules shared by many types of microbes that stimulate a nonspecific response, antigens are highly individual and stimulate specific immunity.

To the left you will see six characteristics that make specific immunity unique. Two of the most important are **specificity** and **memory**. Unlike mechanisms such as anatomical barriers or phagocytosis, acquired immunity is specific. In general, the antibodies produced during an infection against the chickenpox virus will function against that virus and not against the measles virus. The property of memory refers to the rapid mobilization of lymphocytes that have been programmed to "recall" their first engagement with the invader and rush to the attack once again.

The elegance and complexity of immune function are largely due to lymphocytes working closely together with phagocytes. To simplify and clarify the network of immunologic development and interaction, we present it here as a series of stages, with each stage covered in a separate section **(figure 13.1)**. The principal stages are as follows:

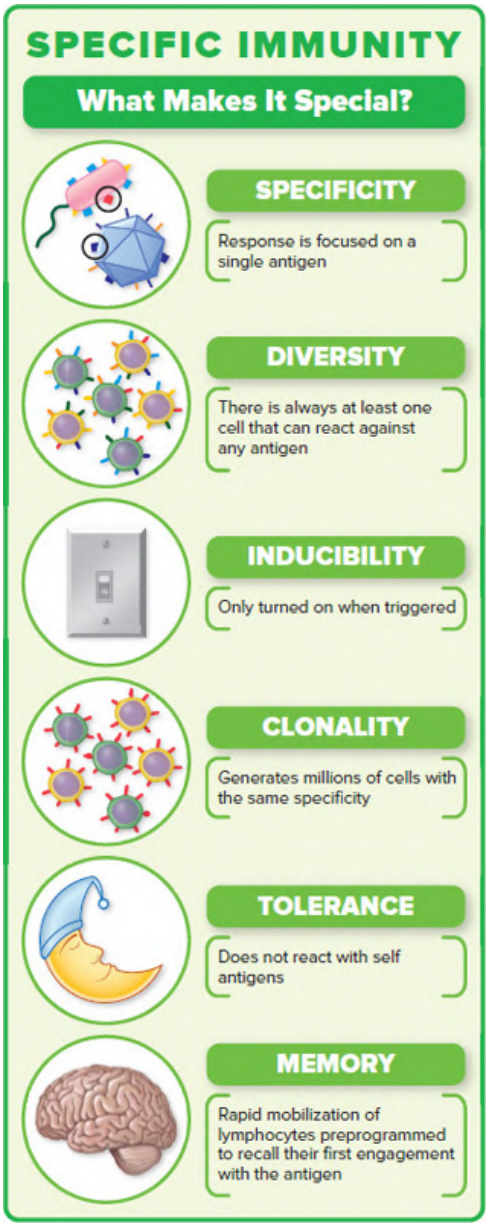
I. Lymphocyte development and clonal deletion;
II. Presentation of antigen and clonal selection;
III. The challenge of B and T lymphocytes by antigens;
IV. T-lymphocyte response: cell-mediated immunity; and B-lymphocyte response: the production and activities of antibodies.

We will give an overview here and spend the rest of the chapter filling in the details.

A Brief Overview of the Immune Response

Lymphocyte Development

Although all lymphocytes arise from the same basic stem cell type, at some point in development they diverge into two distinct types. Final development of B cells occurs in specialized bone marrow sites, and that of T cells occurs in the thymus. Both cell types subsequently migrate to separate areas in the lymphoid organs (for instance, nodes and spleen). B and T cells constantly recirculate through the circulatory system and lymphatics, migrating into and out of the lymphoid organs.



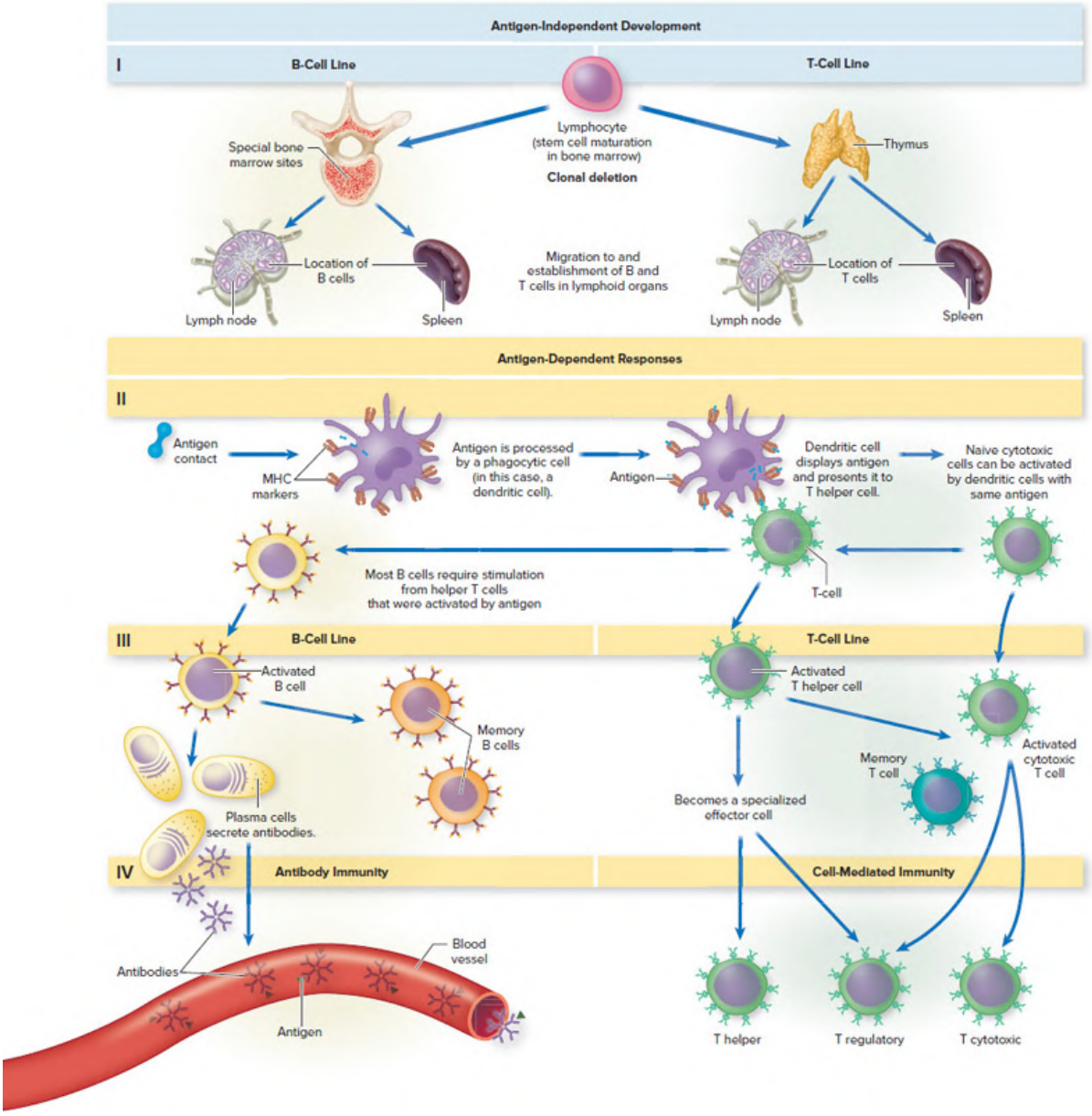


Figure 13.1 **Overview of the stages of lymphocyte development and function**. **I**. Development of B- and T-lymphocyte specificity and migration to lymphoid organs. **II**. Dendritic cell displays antigen and presents it to naive T helper cell. **III**. Lymphocyte activation, clonal expansion, and formation of memory B and T cells. **IV**. End result of lymphocyte activation. Left-hand side: antibody release; right-hand side: cell-mediated immunity.

Entrance and Presentation of Antigens and Clonal Selection

When foreign cells, such as pathogens (carrying antigens), cross the first line of defense and enter the tissue, resident phagocytes migrate to the site. Tissue macrophages ingest the pathogen and induce an inflammatory response in the tissue if appropriate. Tissue dendritic cells ingest the antigen and migrate to the nearest lymphoid organ (often the draining lymph nodes). Here they process and present antigen to T and B lymphocytes. In most cases, the response of B cells also requires the additional assistance of special classes of T cells called T helper cells. One special class of T cells,

called gamma-delta T cells, can be activated quickly by PAMPs, as seen in the nonspecific response, or by specific antigens as seen here.

The Role of Markers and Receptors in Presentation and Activation All cells—both foreign cells and "self" cells—have a variety of different markers on their surfaces, each type playing a distinct and significant role in detection, recognition, and cell communication. Cell markers play important roles in the immune response, serving to activate different components of immunity. Because of their importance in the immune response, we concentrate here on the major markers of lymphocytes and macrophages.

The Major Histocompatibility Complex One set of genes that codes for human cell markers or receptors is the **major histocompatibility complex (MHC)**. This gene complex gives rise to a series of glycoproteins (called MHC molecules) found on all cells except red blood cells. The MHC is also known as the human leukocyte antigen (HLA) system. This marker set plays a vital role in recognition of self by the immune system and in rejection of foreign tissue.

Three classes of MHC genes have been identified:

1. Class I genes code for markers that appear on all nucleated cells. They display unique characteristics of self and allow for the recognition of self molecules and the regulation of immune reactions. The system is rather complicated in its details, but in general, each human being inherits a particular combination of class I MHC (HLA) genes in a relatively predictable fashion.
2. Class II MHC genes also code for immune regulatory markers. These markers are found on macrophages, dendritic cells, and B cells and are involved in presenting antigens to T cells during cooperative immune reactions.
3. Class III MHC genes encode proteins involved with the complement system, among others. We'll focus on classes I and II in this chapter. **Figure 13.2** shows these two types of markers.

CD Molecules Other markers that are important in immunity are particular CD molecules. *CD* stands for "cluster of differentiation," and it is just a naming scheme for many of the cell surface molecules. Well over 300 CD molecules have been named. Many CD molecules, or CDs for short, are involved in the immune response. We discuss some of the most important CDs, including CD3, CD4, and CD8, in the following sections.

Lymphocyte Receptors Lymphocyte markers are frequently called receptors, a name that emphasizes that their major role is to "accept" or "grasp" antigens in some form. B cells have receptors that bind antigens, and T cells have receptors that bind antigens that have been processed and complexed with MHC molecules on the presenting cell surface. **Figure 13.3** illustrates the surfaces of B and T cells and their antigen receptors. There are potentially millions and even billions of unique types of antigens. The many sources of antigens include microorganisms as well as an endless array of chemical compounds in the environment. We will soon see how T and B cells recognize so many different antigens.

Genes are the source of immunologic diversity.
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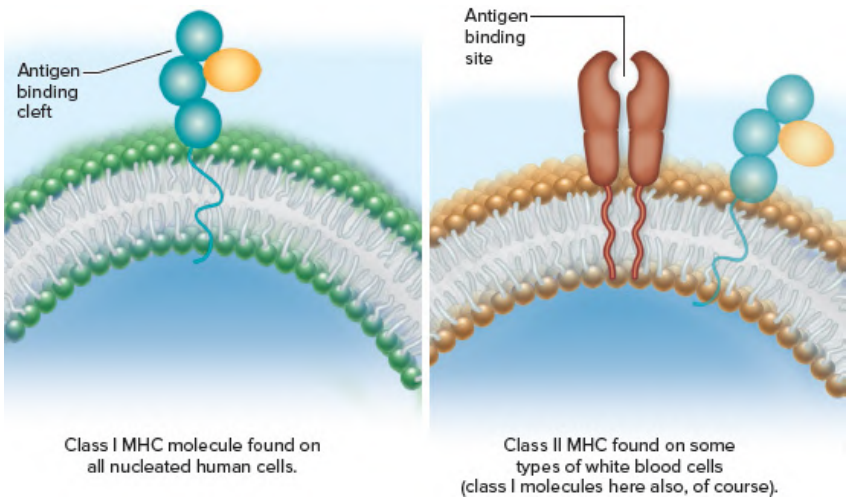

Figure 13.2 **Classes I and II of molecules of the human major histocompatibility complex**.

Challenging B and T Cells with Antigen

When challenged by antigen, both B cells and T cells *proliferate* and *differentiate*. The multiplication of a particular lymphocyte creates a clone, or group of genetically identical cells, some of which are memory cells that will ensure future reactiveness against that antigen. Because the B-cell and T-cell responses differ significantly from this point on in the sequence, they are summarized separately.

How T Cells Respond to Antigen

T-cell types and responses are extremely varied. When activated (sensitized) by antigen, a T cell gives rise to a variety of different cell types with different roles in the immune response. They generally fall into three categories:

1. helper T cells that activate macrophages, assist B-cell processes, and help activate cytotoxic T cells;
2. regulatory T cells that control the T-cell response by secreting anti-inflammatory cytokines or preventing proliferation; and
3. cytotoxic T cells that lead to the destruction of infected host cells and other "foreign" cells.

Although T cells secrete cytokines that help destroy pathogens and regulate immune responses, they do not produce antibodies.

How B Cells Respond to Antigen: Release of Antibodies

When a B cell is activated, or sensitized, by an antigen, it divides, giving rise to plasma cells, each with the same reactive profile. Plasma cells release antibodies into the tissue and blood. When these antibodies attach to the antigen for which they are specific, the antigen is marked for destruction or neutralization.

13.1 LEARNING OUTCOMES—Assess Your Progress

1. Describe how the third line of defense is different from the other two.
2. Compare the terms *antigen, immunogen*, and *epitope*.

3. List the four stages of a specific immune response.
4. Discuss the role of cell markers in the immune response.
5. Describe the major histocompatibility complex in two sentences.

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1. The major histocompatibility complex (MHC) is a gene complex that gives rise to a series of glycoproteins (MHC molecules) found on all cells *except*
   a. *red blood cells*.
   b. *white blood cells*.
   c. *plasma cells*.
   d. *B cells*.

13.2 Stage I: The Development of Lymphocyte Diversity

Specific Events in T-Cell Development

The maturation of most T cells and the development of their specific receptors are directed by the thymus and its hormones **(table 13.1)**. Other T cells reach full maturity in the gastrointestinal tract. In addition to the antigen-specific T-cell receptor, all mature T lymphocytes express coreceptors called CD3. CD3 molecules surround the T-cell receptor and assist in binding. T cells also express either a CD4 or a CD8 coreceptor (see figure 13.3). CD4 is an accessory receptor protein mostly found on T helper cells that helps the T-cell receptor bind to MHC class II molecules. CD8 is mostly found on cytotoxic T cells, and it helps bind MHC class I molecules. Like B cells, T cells also constantly circulate between the lymphatic

Medical Moment

The Thymus

The thymus is a small gland situated partly in the neck and partly in the thorax, behind the sternum. It weighs about 15 grams at birth, is largest at around puberty, and then atrophies slowly thereafter.

What is the main function of the thymus? Within the thymus, thymocytes (hematopoietic precursors from the bone marrow) mature into T cells. Once the T cells have become fully mature, they travel from the thymus and become the stock of circulating T cells responsible for many aspects of immunity. What happens when the thymus malfunctions, or when it is not working properly due to underlying disease? The following conditions may occur:

- **DiGeorge syndrome**. This rare genetic condition results in thymus deficiency. Patients with the syndrome may present with severe immunodeficiency.
- **Severe combined immunodeficiency (SCID) syndromes**. These are a group of genetic disorders resulting from defective hematopoietic precursor cells, causing a deficiency of both B lymphocytes and T lymphocytes.
- **Cancer**. Thymomas, tumors that originate from thymic epithelial cells, and thymic lymphomas, tumors originating from thymocytes, are the primary tumors affecting the thymus.

Q. Which of these three conditions would you consider to be most life-threatening, and why?

Answer in Appendix B.

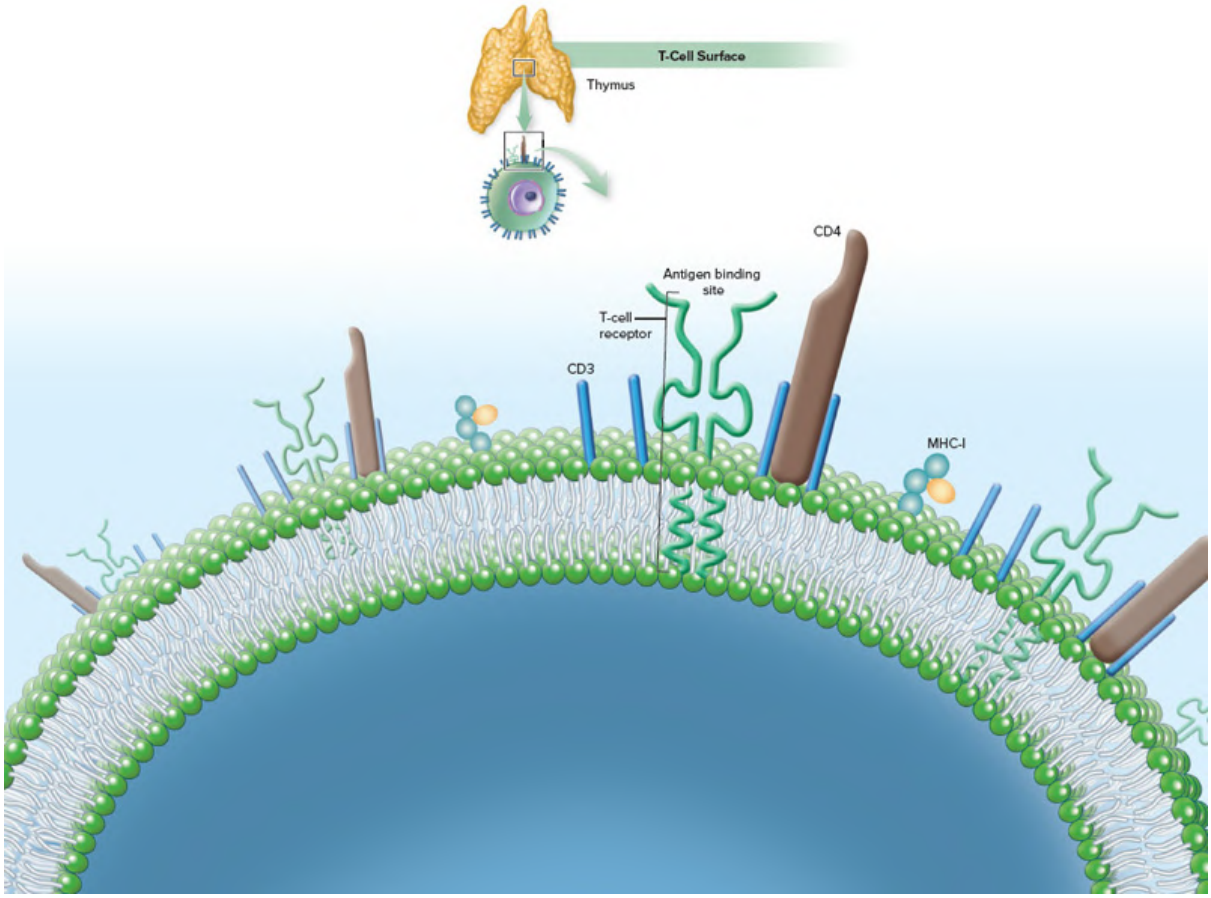


Figure 13.3 **The surfaces of T cells and B cells.**

and general circulatory systems, migrating to specific T-cell areas of the lymph nodes and spleen. It has been estimated that more than 10^9 T cells pass between the lymphatic and general circulations per day.

Specific Events in B-Cell Development

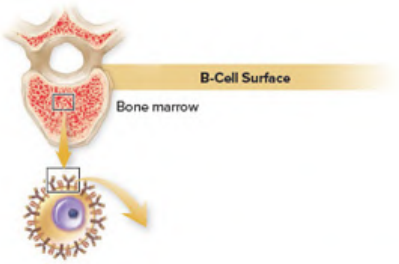
B cells develop in the bone marrow. As a result of gene modification and selection, hundreds of millions of distinct B cells develop. These naive lymphocytes circulate through the blood, "homing" to specific sites in the lymph nodes, spleen, and other lymphoid tissue, where they adhere to specific binding molecules. Here they will come into contact with antigens throughout life.

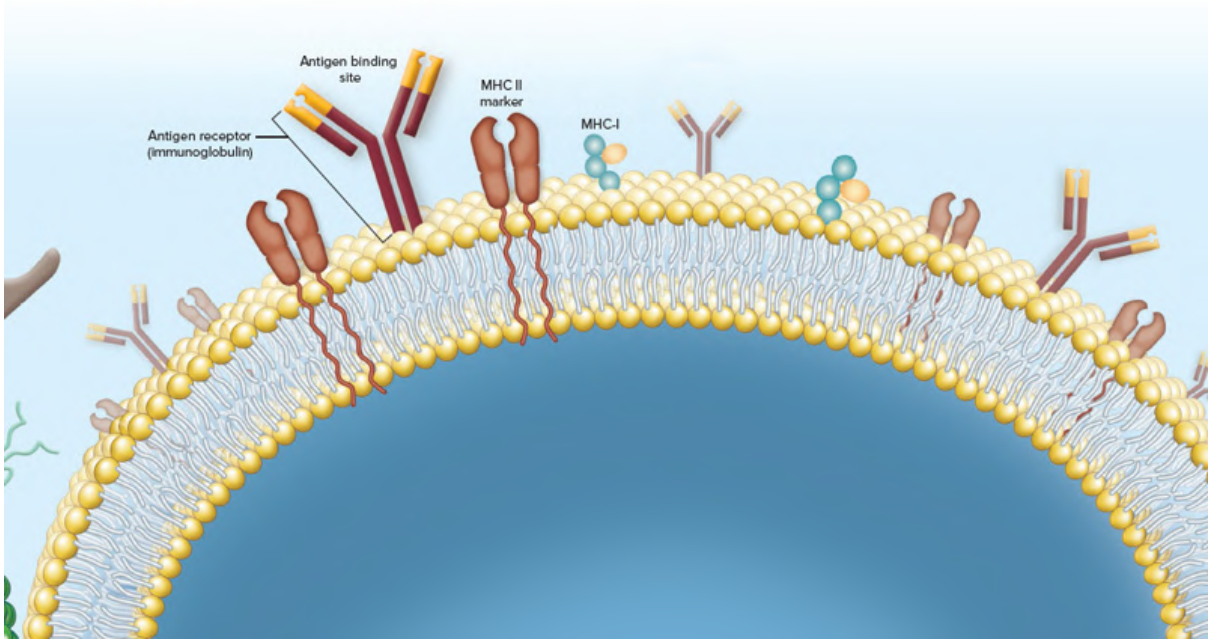
Table 13.1 Contrasting Properties of B Cells and T Cells

	B Cells	T Cells
Site of Maturation	Bone marrow	Thymus
Specific Surface Markers	Immunoglobulin	T-cell receptor Several CD molecules
Circulation in Blood	Low numbers	High numbers
Receptors for Antigen	B-cell receptor (immunoglobulin)	T-cell receptor
Distribution in Lymphatic Organs	Cortex (in follicles)	Paracortical sites (interior to the follicles)

	B Cells	T Cells
Require Antigen Presented with MHC	No	Yes*
Product of Antigenic Stimulation	Plasma cells and memory cells	Several types of activated T cells and memory cells
General Functions	Production of antibodies to inactivate, neutralize, target antigens	Cells activated to help other immune cells; suppress or kill abnormal cells; mediate hypersensitivity; synthesize cytokines

*Gamma-delta T cells can be activated differently.





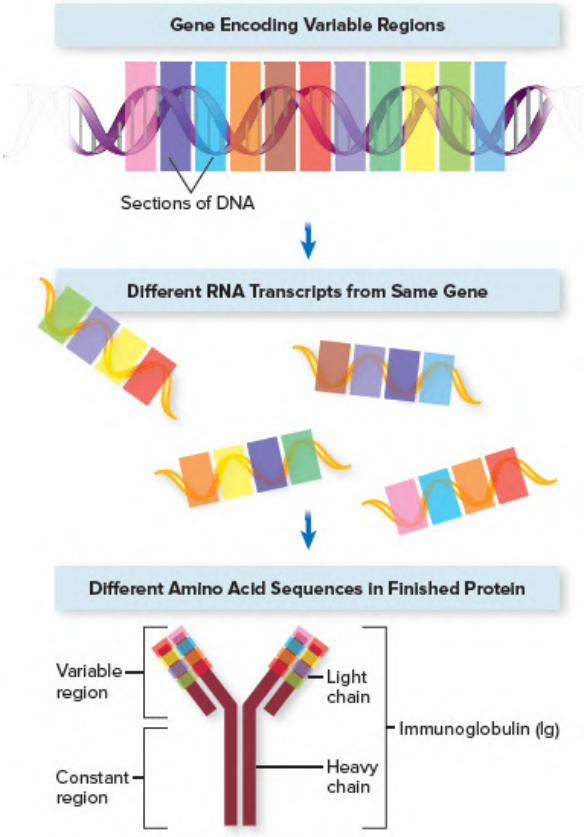


Figure 13.4 **The mechanism behind antibody variability**. The genes coding for the variable regions of antibody molecules have multiple different sections along their lengths. As a result of alternative splicing, very different RNA transcripts are created from the same original gene. When those transcripts are translated, the resulting protein will have extremely variable amino acid sequences—and therefore extremely variable shapes.

Building Immunologic Diversity

By the time T and B cells reach the lymphoid tissues, each one is already equipped to respond to a unique antigen. This amazing specificity is generated by extensive rearrangements of more than 500 different gene segments that code for the antigen receptors—which are antibody molecules—on the T and B cells **(figure 13.4)**. In time, every possible recombination occurs, leading to a huge assortment of lymphocytes. It is estimated that each human produces antibodies with 10 trillion different specificities. While these DNA recombinations enable the recognition of a huge array of antigens (this is called the repertoire), keep in mind that a single naive lymphocyte can only recognize one antigen with its receptors.

The Specific B-Cell Receptor: An Immunoglobulin Molecule

In the case of B lymphocytes, the receptor genes that undergo the recombination described are genes coding for **immunoglobulin** (im″-yoo-noh-glahb′-yoo-lin) **(Ig)** synthesis. Immunoglobulins are large glycoprotein molecules that serve as the antigen receptors of B cells and, when secreted, as antibodies (see figure 13.3).

The immunoglobulin molecule is a conglomerate of four protein chains. Two of them are called light chains, and two of them are called heavy chains (see figure 13.4). The ends of the forks formed by the light and heavy chains contain pockets, called the **antigen binding sites**. These sites are highly variable in shape to fit a wide range of antigens. This extreme versatility is due to **variable**

(V) regions in antigen binding sites, where amino acid composition is highly varied from one clone of B lymphocytes to another, as a result of the gene rearrangements just discussed. The remainder of the light chains and heavy chains consist of constant (C) regions, whose amino acid content does not vary greatly from one antibody to another.

T-Cell Receptors

The T-cell receptor for antigen belongs to the same protein family as the B-cell receptor. It is similar to the B-cell receptor in being formed by genetic modification, having variable and constant regions, being inserted into the membrane, and having an antigen binding site formed from two parallel polypeptide chains (see figure 13.3). Unlike the immunoglobulins, the T-cell receptor is relatively small and is never secreted. Various other receptors and markers that are not antigen-specific are described in a later section.

Clonal Deletion and Selection

Table 13.2 illustrates the mechanism by which the exactly correct B or T cell is activated by any incoming antigen. This process is called **clonal selection**. After activation, the B or T cell multiplies rapidly in a process called clonal expansion. Two important features of clonal selection are that (1) lymphocyte specificity is preprogrammed, existing in the genetic makeup before an antigen has ever entered the tissues; and (2) each genetically distinct lymphocyte expresses only a single specificity and can react to that chemical epitope.

One potentially problematic outcome of random genetic assortment is the development of clones of lymphocytes able to react to *self*. This outcome can lead to severe damage if the immune system actually perceives self molecules as foreign and mounts a harmful response against the host's tissues. Any such clones are destroyed during development through **clonal *deletion*** *(step 1 in the table)*. The removal of such potentially harmful clones is the basis of immune tolerance or tolerance to self. Since humans are exposed to many new antigenic substances during their lifetimes, T cells and B cells in the periphery of the body have mechanisms for *not* reacting to harmless antigens. Some diseases (autoimmunity) are thought to be caused by the loss of immune tolerance through the survival of certain "forbidden clones" or failure of these other systems (see chapter 14).

Medical Moment

Blood Exposure in Neonates

The neonatal population must be handled very carefully with respect to exposure to blood products. If they require frequent lab sampling or procedures they may require blood product transfusions. Blood banks develop practices to limit infant exposure to donor blood. Many centers dispense blood from a single donor in small amounts, or aliquots, for the duration of the patient's stay.

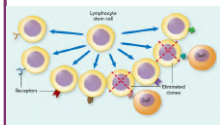
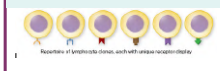
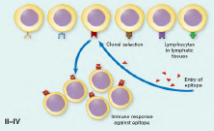
Even foreign proteins that are introduced for the baby's benefit, such as blood products, stimulate the immune system. Antigens present on red blood cells activate the immune response in the neonate. Immediately after harmless transfusion, lymphocyte counts increase.

Along with risk for blood transfusion complications, blood products may transmit infectious disease. Using consistent donor products in neonates limits these risks inherent to blood transfusion, while also reducing the patient's antigen exposure.

Q. What process is responsible for the increase in numbers of lymphocytes?

Answer in Appendix B.

Table 13.2 Clonal Deletion and Clonal Selection of B and T Cells

	Each genetically unique line of lymphocytes arising from extensive recombinations of surface proteins is termed a **clone**. This proliferative stage of lymphocyte development does not require the actual presence of foreign antigens. At the same time, any lymphoctyes that develop a specificity for self molecules and could be harmful are eliminated from the pool of cells. This is called **clonal deletion** and leads to **immune tolerance**.
	The specificity for a single epitope is programmed into the lymphocyte and is set for the life of a given cell. The end result is an enormous pool of mature but naive lymphocytes that are ready to further differentiate under the influence of certain organs and immune stimuli.
	When any epitope enters the immune surveillance system, it encounters specific lymphocytes ready to recognize it. Such contact stimulates activation of that clone, leading to genetic changes that cause it to differentiate into an effector cell. Mitotic divisions then expand it into a larger population of lymphocytes, all bearing the same specificity.

13.2 LEARNING OUTCOMES—Assess Your Progress

6. Summarize the maturation process of both B cells and T cells.
7. Draw a diagram showing how lymphocytes are capable of responding to nearly any epitope imaginable.
8. Describe the structures of the B-cell receptor and the T-cell receptor.
9. Outline the processes of clonal deletion and clonal selection.

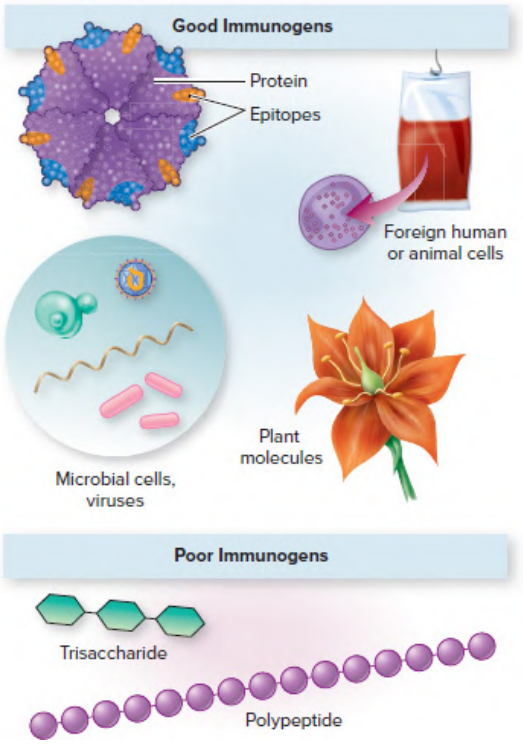


Figure 13.5 **A comparison of good immunogens and poor immunogens**. Top: Good immunogens are large and complex. Bottom: Small molecules and linear molecules are less likely to be good immunogens.

13.3 Stage II: Presentation of Antigens

Entrance of Antigens

To be perceived as an antigen or immunogen, a substance must meet certain requirements in foreignness, shape, size, and accessibility. One important characteristic of an antigen is that it be perceived as foreign, meaning that it is not a normal constituent of the body. Whole microbes or their parts, cells, or substances that arise from other humans, animals, plants, and various molecules all possess this quality of foreignness and thus are potentially antigenic to the immune system of an individual. Molecules of complex composition such as proteins and protein-containing compounds prove to be more immunogenic than repetitious polymers composed of a single type of unit. Most materials that serve as antigens fall into these chemical categories:

- proteins and polypeptides (enzymes, cell surface structures, exotoxins);
- lipoproteins (cell membranes);
- glycoproteins (blood cell markers);
- nucleoproteins (DNA complexed to proteins but not pure DNA); and
- polysaccharides (certain bacterial capsules) and lipopolysaccharides.

"Good" Antigens

Antigens can be varying degrees of immunogenic. Characteristics of good antigens (provoking a strong response) are (a) their chemical composition; (b) their context—meaning what types of cytokines are present; and (c) their size. We can generalize that large antigens are better than small antigens. However, large size alone is not sufficient for antigenicity; glycogen, a polymer of glucose

with a highly repetitious structure, has a molecular weight over 100,000 Daltons and is not normally antigenic, whereas insulin, a protein with a molecular weight of 6,000 Daltons, can be antigenic **(figure 13.5)**. Note that this aspect of size relates to the ability to stimulate immune "wakefulness." Remember that it is the smaller epitope to which the awakened immune cells bind, initiating the cascade of effects to follow.

Small foreign molecules that are too small by themselves to stimulate the immune response are termed **haptens**. However, if such an incomplete antigen is linked to a larger carrier molecule, the combined molecule can develop immunogenicity. The carrier group contributes to the size of the complex and enhances the proper spatial orientation of the determinative group, while the hapten serves as the epitope **(figure 13.6)**.

Haptens include such molecules as drugs, metals, and ordinarily innocuous household, industrial, and environmental chemicals. Many haptens inappropriately develop antigenicity in the body by combining with large carrier molecules such as serum proteins (see allergy in chapter 14).

Because each human being is genetically and biochemically unique (except for identical twins), the proteins and other molecules of one person can be antigenic to another. **Alloantigens** are cell surface markers and molecules that occur in some members of the same species but not in others. Alloantigens are the basis for an individual's blood group and major histocompatibility profile, and they are responsible for incompatibilities that can occur in blood transfusion or organ grafting.

Some bacterial toxins, which belong to a group of immunogens called **superantigens**, are potent stimuli for T cells. Their presence in an infection activates

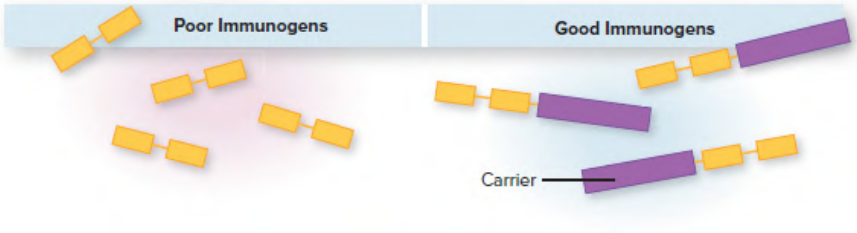


Figure 13.6 **Haptens**. Some small molecules are poor immunogens but can be made more immunogenic by combining them with a carrier.

T cells at a rate 100 times greater than ordinary antigens. The result can be an overwhelming release of cytokines and cell death. Such diseases as toxic shock syndrome and certain autoimmune diseases are associated with this class of antigens.

Antigens that evoke allergic reactions, called **allergens**, are characterized in detail in chapter 14.

Antigen Processing and Presentation

In most immune reactions, the antigen must be formally presented to lymphocytes by cells called **antigen-presenting cells (APCs)**. Three different cells can serve as APCs: macrophages, B cells, and **dendritic** (den′-drih-tik) **cells**. Dendritic cells are the most potent and versatile of the APCs. Antigen-presenting cells grab the antigen-carrying microbe and ingest it. They degrade it and pass its antigens back out onto their membranes, complexed either with MHC-I or MHC-II markers. After processing is complete, the antigen is bound to the MHC receptor and moved to the surface of the APC so that it will be readily accessible to T lymphocytes during presentation. **Table 13.3** illustrates how antigen is presented to T cells. The activated T cell that is the product of this reaction is central to all of T-cell immunity and most of B-cell immunity.

Most antigens must be presented first to T cells, even though they will eventually activate both the T-cell and B-cell systems. However, a few antigens can trigger a response directly from B lymphocytes without the cooperation of APCs or T helper cells. These are called T-cell-independent antigens. They are usually simple molecules such

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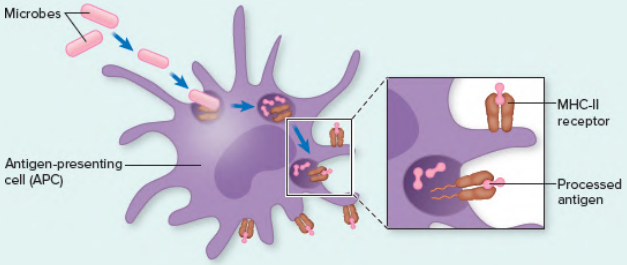
2. In reviewing a client's history, the nurse notes that the client's blood type is O-negative. The client's blood type is based on the presence or absence of
- **a.** *haptens*.
- **b.** *alloantigens*.
- **c.** *immunogens*.
- **d.** *lipoproteins*.

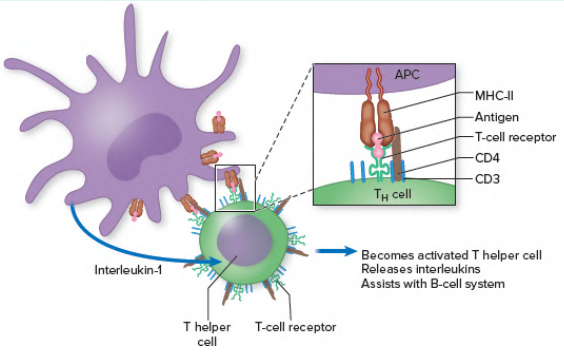
Table 13.3 How Antigen Is Presented to T Cells



Antigen-presenting cells (APCs) engulf a microbe, take it into intracellular vesicles, and degrade it into smaller peptides or pieces. The antigen pieces complexed with MHC-II receptors are transported to the APC membrane (inset). From this surface location the antigens are presented to a T helper cell, which is specific for the antigen being presented. (Note that in some cases the antigen is complexed with MHC-I receptors, not MHC-II.)

First, the MHC-II antigen on the APC binds to the T-cell receptor.

Next, a coreceptor on the T cell (typically CD4) hooks itself to a position on the MHC-II receptor. This combination ensures the simultaneous recognition of the antigen (nonself) and the MHC receptor (self). Once identification has occurred, the APC activates this T helper (T_H) cell. The APC also secretes **interleukin-1 (IL-1)**. The T_H cell, in turn, produces **IL-2**, which is a growth factor for the T helper cells and cytotoxic T cells. These T helper cells can then help activate B cells.



as carbohydrates with many repeating and invariable determinant groups. Examples include lipopolysaccharide from the cell wall of *Escherichia coli*, polysaccharide from the capsule of *Streptococcus pneumoniae*, and molecules from rabies and Epstein-Barr virus. Because so few antigens are of this type, most B-cell reactions require T helper cells.

13.3 LEARNING OUTCOMES—Assess Your Progress

10. List characteristics of antigens that optimize their immunogenicity.
11. Describe how the immune system responds to alloantigens and superantigens.
12. List the types of cells that can act as antigen-presenting cells.

A Note About Epitopes and Antigens

While up to now we have been calling the immunogenic substance "the antigen," it is more precisely termed the epitope. You could say, for instance, "the antigenic portion of the protein on a microbe is the epitope." You will also note that in practice, clinicians, and even other parts of this book, use the word "antigen" when the precise term is "epitope." You will know, however, that the part of the molecule that is actually recognized by the immune system is the epitope. This means, also, that every epitope can be recognized by B- and T-cell receptors that were formed during genetic reassortment. The particular tertiary structure and shape of this determinant must conform like a key to the receptor "lock" of the lymphocyte, which then responds to it. Certain amino acids accessible at the surface of proteins or protruding carbohydrate side chains are typical examples. Many foreign cells and molecules are very complex antigenically, with numerous component parts, each of which will elicit a separate and different lymphocyte response. Examples of these multiple, or *mosaic*, antigens include bacterial cells containing cell wall; membrane; and flagellar, capsular, and toxin antigens, as well as viruses. T-cell antigen receptors recognize these small pieces of antigens—epitopes—in combination with MHC molecules.

13.4 Stages III and IV: T-Cell Response

T-cell reactions are among the most complex and diverse in the immune system and involve several subsets of T cells, whose particular actions are dictated by the APCs that activate them. We refer to T cells as "restricted"—that is, they require some type of MHC (self) recognition before they can be activated. T cells produce cytokines with a spectrum of biological effects. They also are influenced by cytokines secreted by other cells.

The end result of T-cell stimulation is the mobilization of other T cells, B cells, and phagocytes.

The Activation of T Cells and Their Differentiation into Subsets

A T cell is initially sensitized when an antigen/MHC complex comes in contact with its receptors. Activated T cells then begin to divide, and they differentiate into one of the subsets of effector cells (cells that actually perform the ultimate action of the system) and memory cells that will be available to mount an immediate response upon subsequent contact **(table 13.4)**. Memory T cells are some of the longest-lived blood cells known.

T Helper (T_H) Cells

The first thing you will notice about the T-cell response is the central role of a type of T cell called helper cells **(table 13.5)**. There are many different types of T helper cells and they all bear the CD4 marker. T helper cells are critical in regulating immune reactions to antigens, including those of B cells and other T cells. They are also involved in activating macrophages. They do this directly by receptor contact and indirectly by releasing cytokines like interferon gamma (IFNγ). Some T helper cells secrete

Table 13.4 Characteristics of Subsets of T-Cell Types in the Classic T-Cell Response

Types	Co-receptors on T cell	Functions/Important Features
T helper cell 1 (T_H1)	CD4	Activates the cell-mediated immunity pathway; secretes tumor necrosis factor and interferon gamma; also responsible for delayed hypersensitivity (allergy occurring several hours or days after contact); secretes IL-2

Types	Co-receptors on T cell	Functions/Important Features
T helper cell 2 (T_H2)	CD4	Can activate macrophages to expel helminths or protozoans, phagocytose extracellular antigens; contributes to type 1 (allergic) hypersensitivity; can encourage tumor development
T helper cell 17 (T_H17)	CD4	Promotes inflammation
T follicular helper cell	CD4, CD40L	Drives B-cell proliferation, and aids B cells in antibody class switching
T regulatory cell (T_{reg})	CD25, CD4	Controls specific immune response; prevents autoimmunity; can contribute to cancer progression
T cytotoxic cell (T_C)	CD8	Destroys a target foreign cell by lysis; important in destruction of complex microbes, cancer cells, virus-infected cells; graft rejection; requires MHC-I for function
Gamma-delta T cells	—	React specifically and nonspecifically; responsive to lipid antigens

interleukin-2, which stimulates the primary growth and activation of many types of T cells, including cytotoxic T cells. Some T helper cells secrete interleukins-4, -5, and -6, which stimulate various activities of B cells. T helper cells are the most prevalent type of T cell in the blood and lymphoid organs, making up about 65% of this population. The severe depression of this class of T cells (with CD4 receptors) by HIV is what largely accounts for the pathology of AIDS.

The details of the T-cell reaction to antigen can seem complex. Spend some time with table 13.5 and the mechanism will start to be clear.

Cytotoxic T (T_C) Cells: Cells That Kill Other Cells

Cytotoxic T cells have one job: to destroy other cells. Target cells that T_C cells can destroy include the following:

- Virally infected cells. Cytotoxic cells recognize these because of telltale virus peptides expressed on their surface. Cytotoxic defenses are an essential protection against viruses. They also recognize and target cells carrying intracellular bacteria.
- Cancer cells. T cells constantly survey the tissues and immediately attack any abnormal cells they encounter **(figure 13.7)**. The importance of this function is clearly demonstrated in the susceptibility of T-cell-deficient people to cancer (see chapter 14).
- Cells from other animals and humans. Cytotoxic cell-mediated immunity is the most important factor in graft rejection. In this instance, the T_C cells attack the foreign tissues that have been implanted into a recipient's body.

Keep in mind that there are always T helper cells and T cytotoxic cells in any given immune response. This idea is termed **cross-presentation**.

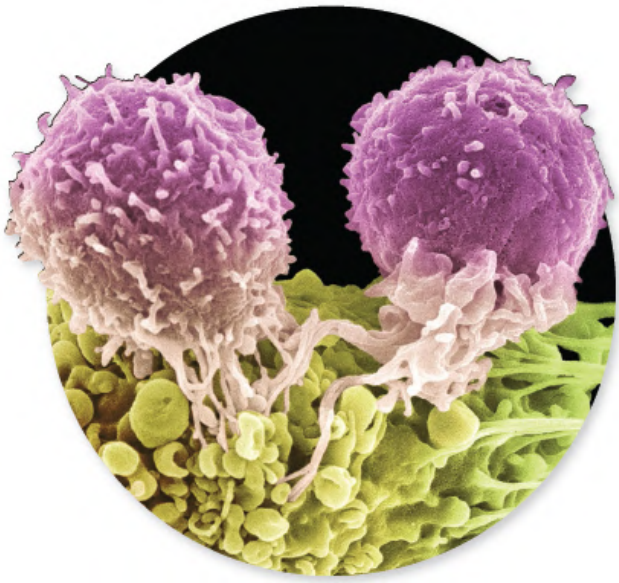


Figure 13.7 T cells attacking a cancer cell. We've been showing drawings of the markers and receptors bringing immune cells and foreign cells together. That is how these T cells (pink) bound to this cancer cell.
©Steve Gschmeissner/Science Source

Gamma-Delta T Cells

The subcategory of T cells called gamma-delta T cells is distinct from other T cells. They do have T-cell receptors that are rearranged to recognize a wide range of antigens, but they frequently respond to certain kinds of PAMPs on microorganisms the way white blood cells (WBCs) in the nonspecific system do. This allows them to respond more quickly. However, they still produce memory cells when they are activated. For these reasons, they are considered a bridge between the nonspecific and specific immune responses. They are particularly responsive to certain types of phospholipids and can recognize and react against tumor cells.

Additional Cells with Orders to Kill

Natural killer (NK) cells are a type of lymphocyte related to T cells that lack specificity for antigens. They circulate through the spleen, blood, and lungs, and are probably the first killer cells to attack cancer cells and virus-infected cells. They destroy such cells by similar mechanisms as T cells. They are not considered part of specific cell-mediated immunity, although their activities are acutely sensitive to cytokines such as interleukin-12 and interferon. Finally, there is a hybrid kind of cell that is part killer cell and part T cell, with T-cell receptors for antigen and the ability to release large amounts of cytokines very quickly, leading to cell death. These cells are called natural killer T cells, or NKT cells. Because they have T-cell receptors but respond very quickly, they are considered to be another important link between nonspecific and specific immunity.

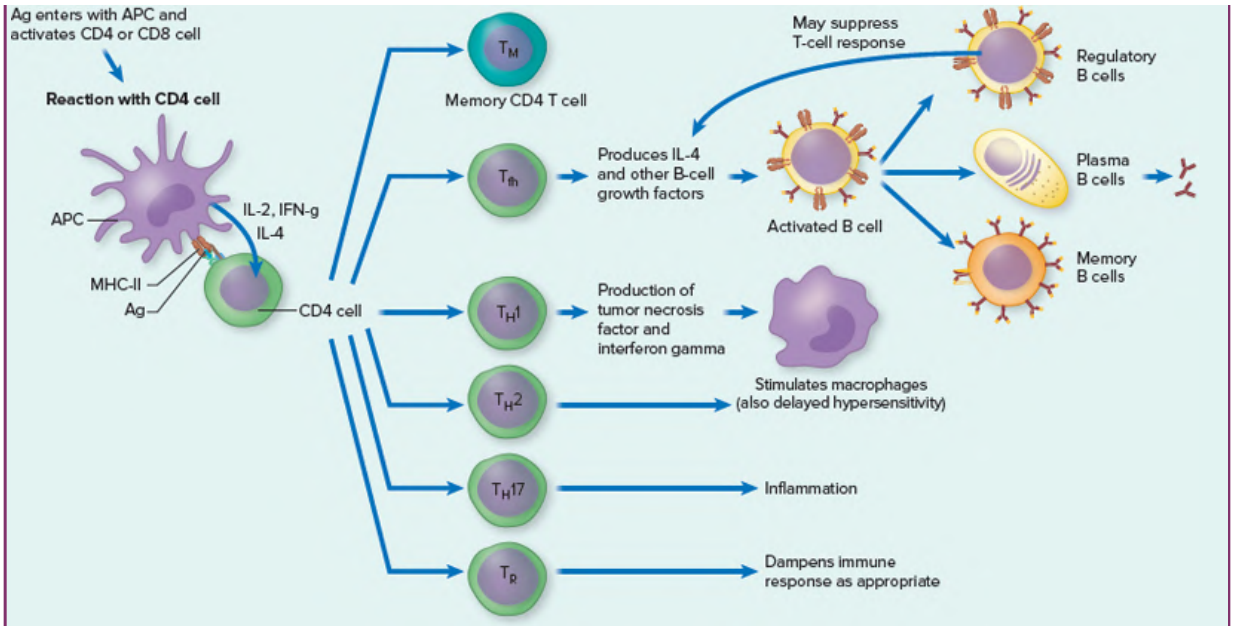
As you can see, the T-cell system is very complex. In summary, T cells differentiate into five different types of cells (and also memory cells), each of which contributes to the orchestrated immune response, under the influence of a multitude of cytokines.

Fish—and, in fact, all jawed vertebrates—share many features of the specific immune system.
©Photographer's Choice/Getty Images

13.4 LEARNING OUTCOMES—Assess Your Progress

13. Describe the main differences between T helper cells and T cytotoxic cells.
14. Explain how naive T cells become sensitized to an antigen.
15. Note the similarities and differences between gamma-delta T cells and the other T cells.

Table 13.5 T-Cell Activation



When T helper (CD4) cells are stimulated by antigen/MHC complex, they differentiate into either T helper 1 (T_H1) cells, T helper 2 (T_H2) cells, T helper 17 (T_H17) cells, T follicular helper (T_{fh}) cells, or T regulatory cells (T_{reg}) depending on what type of cytokines the antigen-presenting cells secrete.

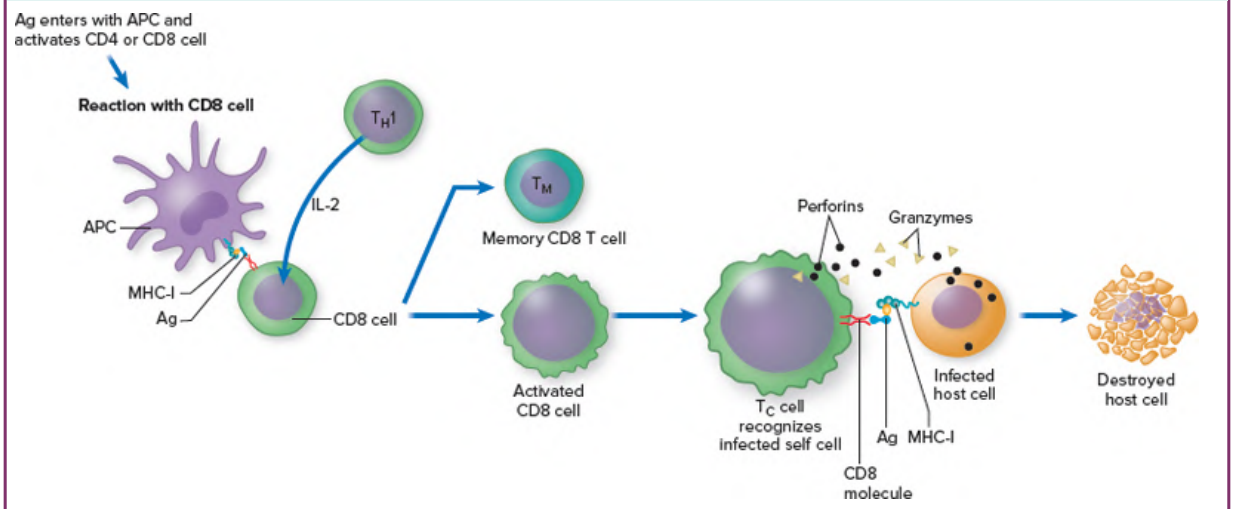
A T_H1 cell will activate phagocytic cells to be better at inducing inflammation.

The job of T_H2 cells is to enhance the antibody response. One of their important roles is to respond to extracellular microbes, helminths, and allergens.

T_{fh} cells aid in B-cell differentiation.

T_H17 cells are so named because they secrete interleukin-17, which leads to the production of other cytokines that promote inflammation. Inflammation is useful, of course, but when excessive or inappropriate may lead to inflammatory diseases such as Crohn's disease or psoriasis. T_H17 may be critical to these conditions.

T_{reg} cells are also broadly in the T_H class, in that they also carry CD4 markers. But they are usually put in their own category. They act to control the inflammatory process, to prevent autoimmunity, and to make sure the immune response doesn't inappropriately target normal biota.



For a CD8 **killer T cell** to become activated, it must recognize a foreign peptide complexed with self MHC-I and mount a direct attack upon the target cell. After activation, the T_C cell severely injures the target cell. This process involves the secretion of **perforins** and **granzymes**. Perforins are proteins that can punch holes in the membranes of target cells. Granzymes are enzymes

that attack proteins of target cells. The action of the perforins causes ions to leak out of target cells and creates a passageway for granzymes to enter. These events are usually followed by targeted cell death through a process called *apoptosis*.

13.5 Stages III and IV: B-Cell Response

At the same time that the T-cell system is being activated by antigen, B cells are being stimulated as well. The immunologic activation of most B cells also requires a series of events. **Table 13.6** contains the details.

Products of B Lymphocytes: Antibody Structure and Functions

Earlier we saw how a basic immunoglobulin (Ig) molecule can have so many different variable regions. Let us view this structure once again, using an IgG molecule as a model. There are two functionally distinct segments called *fragments*. The two "arms" that bind antigen are termed *antigen binding fragments (Fabs)*, and the rest of the molecule is the crystallizable fragment (Fc), so called because it was the first to be crystallized in pure form. The basic immunoglobulin molecule is a composite of four polypeptide chains: a pair of identical heavy (H) chains and a pair of identical light (L) chains **(figure 13.8)**. One light chain is bonded to one heavy chain, and the two heavy chains are bonded to one another with disulfide bonds, creating a symmetrical, Y-shaped arrangement.

The end of each Fab fragment (consisting of the variable regions of the heavy and light chains) folds into a groove that will accommodate one epitope. The presence of a special region at the site of attachment between the Fab and Fc fragments allows swiveling of the Fab fragments. In this way, they can change their angle to accommodate nearby antigen sites that vary slightly in distance and position. Figure 13.8 shows two views of antibody structure.

Don't Give Up!

Specific immunity is very complex. Don't be overwhelmed with all the illustrations and tables in this chapter. Seeing the processes with the use of drawings is better than just reading about them, but they are still intimidating. Even scientists studying immunology get overwhelmed. Look at the end results of each process and think about what is important. Ask your instructor what the key concepts are for T-cell activation, B-cell activation, and so on. Just don't ask them "Will it be on the test?"!

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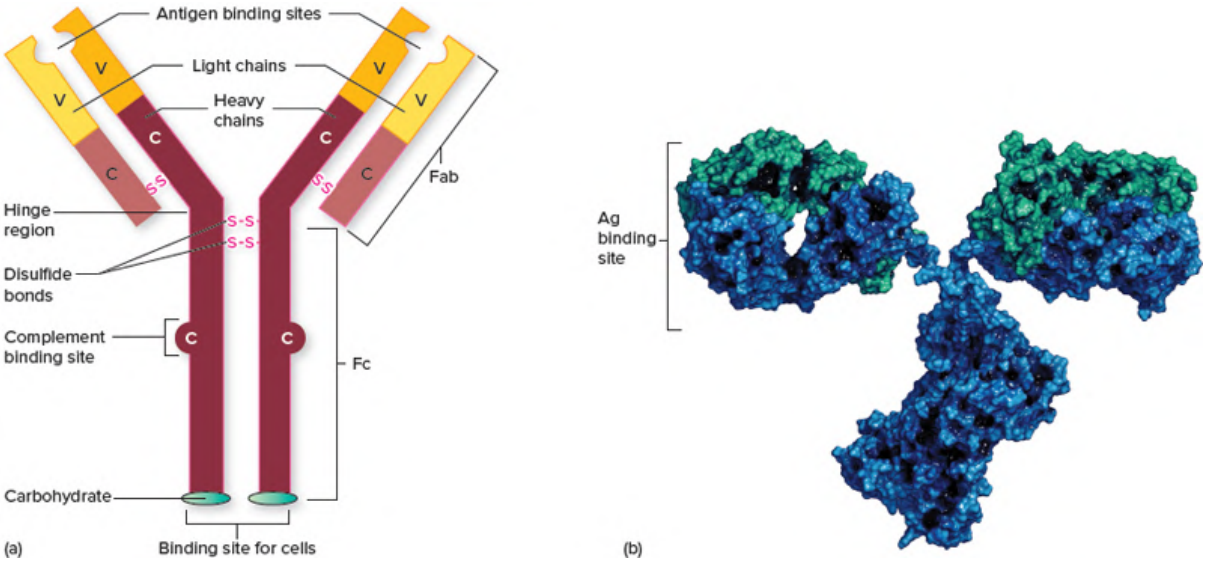


Figure 13.8 **Antibody structure**. **(a)** Diagrammatic view of IgG depicts the principal functional areas (Fabs and Fc) of the molecule. Each Fab contains a hypervariable region (V) and a constant, nonvariable region (C). **(b)** Realistic model of immunoglobulin shows the tertiary and quaternary structure achieved by additional intrachain and interchain bonds. ©molekuul_be/Shutterstock (b)

Table 13.6 B-Cell Activation

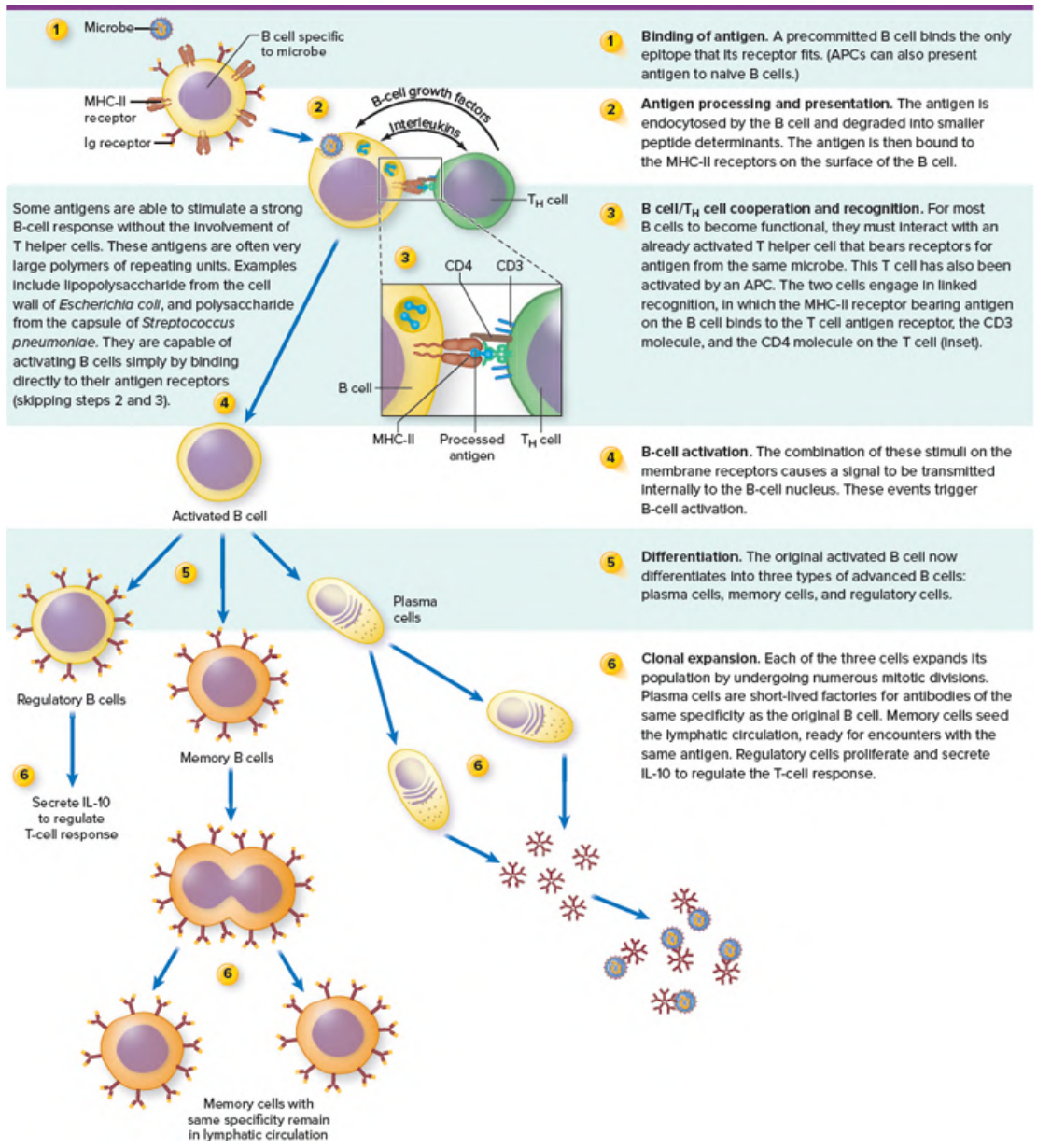
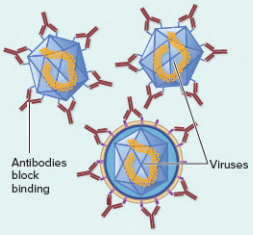
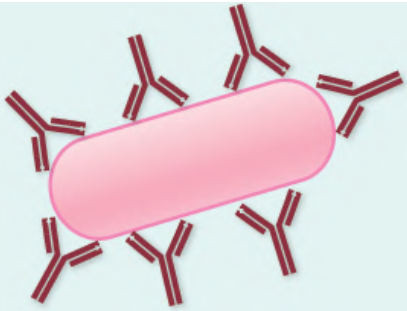
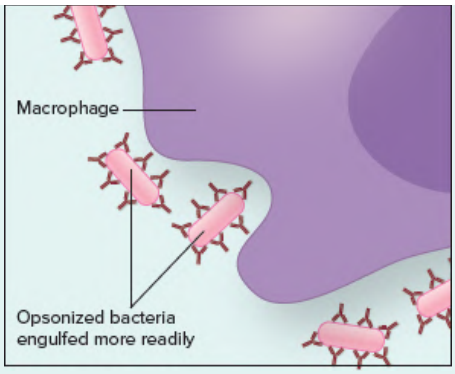


Table 13.7 Summary of Antibody Functions



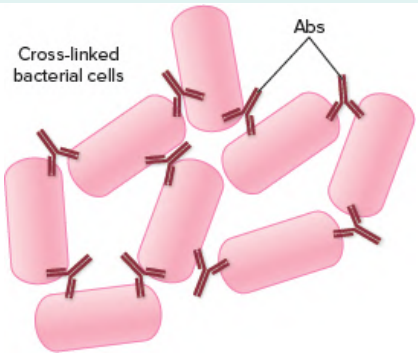


Antibodies coat the surface of a bacterium, preventing its normal function and reproduction in various ways.

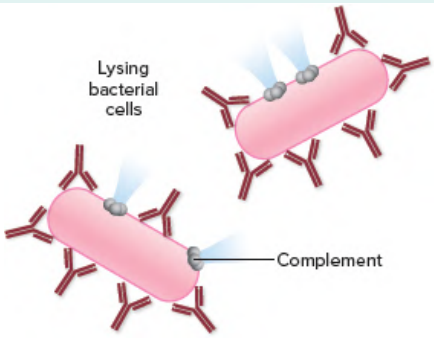


Antibodies called opsonins stimulate **opsonization** (ahp″-son-uh-zaz′-shun), a process that makes microbes more readily recognized by phagocytes, which dispose of them. Opsonization has been likened to putting handles on a slippery object to provide phagocytes a better grip.

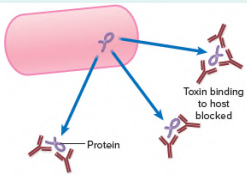
In **neutralization** reactions, antibodies fill the surface receptors on a virus or the active site on a microbial enzyme to prevent it from attaching normally.



The capacity for antibodies to aggregate, or **agglutinate**, antigens is the consequence of their cross-linking cells or particles into large clumps. Agglutination renders microbes immobile and enhances their phagocytosis. This is a principle behind certain immune tests discussed in chapter 15.



The interaction of an antibody with complement can result in the specific rupturing of cells and some viruses.



An **antitoxin** is a special type of antibody that neutralizes bacterial exotoxins.

Antibody-Antigen Interactions and the Function of the Fab

The site on the antibody where the epitope binds is composed of a *hypervariable region*, whose amino acid content can be extremely varied. The specificity of antigen binding sites for antigens is very similar to enzymes and substrates. Because the specificity of the two Fab sites is identical, an Ig molecule can bind epitope on the same cell or on two separate cells and thereby link them.

The principal activity of an antibody is to immobilize, call attention to, or neutralize the antigen for which it was formed. Follow along in **table 13.7** to see how each of these works.

Functions of the Fc Fragment

Although the Fab fragments bind antigen, the Fc fragment has a different binding function. In most classes of immunoglobulin, the Fc end can bind to receptors on the membranes of cells, such as macrophages, neutrophils, eosinophils, mast cells, basophils, and lymphocytes. The effect of an antibody's Fc end binding to a cell depends upon that cell's role. In the case of opsonization, the

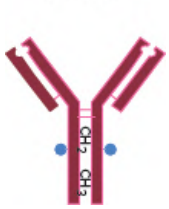
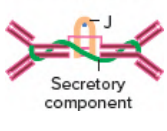
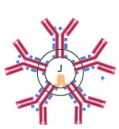
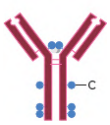
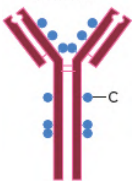
attachment of antibody to foreign cells and viruses is followed by the binding of the Fc end to phagocytes. The Fc end of the antibody of allergy (IgE) binds to basophils and mast cells, which causes the release of allergic mediators such as histamine.

The Classes of Immunoglobulins

Immunoglobulins exist as structural and functional classes called *isotypes*. The classes are differentiated with shorthand names (Ig, followed by a letter: IgG, IgA, IgM, IgD, IgE). Complete descriptions are found in **table 13.8**.

IgA is worth investigating a bit more. The two forms of IgA are (1) a monomer that circulates in small amounts in the blood and (2) a dimer that is a significant component of the mucous and serous secretions of the salivary glands, intestine, nasal membrane, breast, lung, and genitourinary tract. The dimer, called secretory IgA, is formed by two monomers held together by a J chain. To facilitate the transport of IgA

Table 13.8 Characteristics of the Immunoglobulin (Ig) Classes

	IgG	IgA	IgM	IgD	IgE
	Monomer 	Dimer, Monomer 	Pentamer 	Monomer 	Monomer 
Number of Antigen Binding Sites	2	4, 2	10	2	2
Molecular Weight	150,000	170,000–385,000	900,000	180,000	200,000
Percentage of Total Antibody in Serum	80%	13%	6%	1%	0.002%
Average Half-Life in Serum (Days)	23	6	5	3	2.5
Crosses Placenta?	Yes	No	No	No	No
Fixes Complement?	Yes	No	Yes	No	No
Fc Binds to	Phagocytes				Mast cells and basophils
Biological Function	Monomer produced by plasma cells in a primary response and by memory cells responding the second time to a given antigenic stimulus; most prevalent antibody circulating throughout the tissue fluids and blood; neutralizes toxins, opsonizes, fixes complement	Dimer is secretory antibody on mucous membranes; monomer in small quantities in blood	Produced at first response to antigen; can serve as B-cell receptor	Receptor on B cells; triggering molecule for B-cell activation	Antibody of allergy; worm infections; mediates anaphylaxis, asthma, etc.

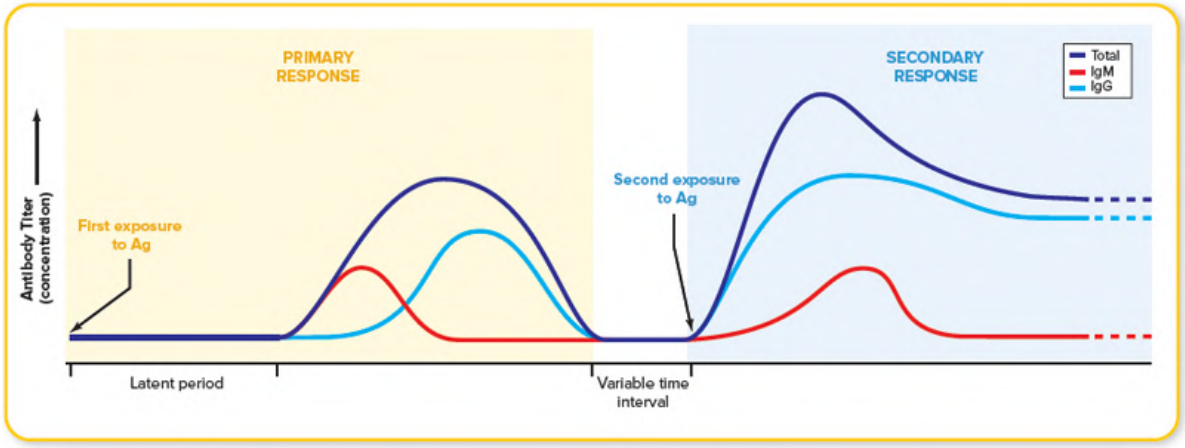
across membranes, a secretory piece is later added. IgA coats the surface of mucous membranes and also is suspended in saliva, tears, colostrum, and mucus. It provides the most important specific local immunity to enteric, respiratory, and genitourinary pathogens. During lactation, the breast becomes a site for the proliferation of lymphocytes that produce IgA. The very earliest secretion of the breast, a thin, yellow milk called **colostrum**, is very high in IgA. These antibodies form a protective coating in the gastrointestinal tract of a nursing infant that guards against infection by a number of enteric pathogens (*Escherichia coli, Salmonella*, poliovirus, rotavirus). This is one of the reasons that new mothers are encouraged to nurse their newborns, at least for a few weeks, to take advantage of the immune properties of this first milk. Protection at this level is especially critical because an infant's own IgA and natural intestinal barriers are not yet well developed. As with immunity *in utero*, the necessary antibodies will be donated only if the mother herself has active immunity to the microbe through a prior infection or vaccination.

Monitoring Antibody Production over Time: Primary and Secondary Responses to Antigens

We can learn a great deal about how the immune system reacts to an antigen by studying the levels of antibodies in serum over time. This level is expressed quantitatively as the **titer** (ty′-tur), or concentration of antibodies. **Table 13.9** illustrates one of the most important features of specific immunity: memory.

©Blend Images/Getty Images

Table 13.9 Primary and Secondary Response to Antigens



Upon the first exposure to an antigen, the system undergoes a **primary response**. The earliest part of this response, the *latent period*, is marked by a lack of antibodies for that antigen, but much activity is occurring. During this time, the antigen is being concentrated in lymphoid tissue and is being processed by the correct clones of B lymphocytes. As plasma cells synthesize antibodies, the serum titer increases to a certain plateau and then tapers off to a low level over a few weeks or months. Early in the primary response, most of the antibodies are the IgM type, which is the first class to be secreted by plasma cells. Later, the class of the antibodies (but not their specificity) is switched to IgG or some other class (IgA or IgE). After the initial response, there is no activity, but memory cells of the same specificity are seeded throughout the lymphatic system.

When the immune system is exposed again to the same immunogen within weeks, months, or even years, a **secondary response** occurs. The rate of antibody synthesis, the peak titer, and the length of antibody persistence are greatly increased over the primary response. The speed and intensity seen in this response are attributable to the memory B cells that were formed during the primary response. The secondary response is also called the **anamnestic response** (from the Greek word for "memory"). The advantage of this response is evident: It provides a quick and potent strike against subsequent exposures to infectious agents.

In certain settings, the oral polio vaccine is dispensed onto a sugar cube to make it palatable to children.
©Image Source/Getty Images

It is a well-accepted principle that memory B and T cells are only created from clones activated by a specific antigen. This provides a much quicker and more effective response on the second exposure and all exposures afterward. But researchers are now investigating a phenomenon that has been suspected for some time and confirmed in rigorous studies. It seems that exposure to a particular antigen can result in memory cells that will recognize antigens that are *chemically related* to it, even if those antigens have not been seen by the host. This might explain the well-known phenomenon, seen most clearly in developing countries, that vaccines against one disease can provide some protection against others. In Africa, for example, vaccinating against measles also cuts deaths from pneumonia, sepsis, and diarrhea by a third.

This realization upsets the long-held view that memory only exists because of specific exposures, but it makes sense if we consider how activation of specific immunity occurs, via recognition of epitopes, small pieces of macromolecules on the surfaces of microbes. If other microbes share those chemical signatures (epitopes), memory cells will react against them as well. This is a promising development, because it could result in using nonpathogenic microbes in vaccines to protect against more dangerous ones.

13.5 LEARNING OUTCOMES—Assess Your Progress

16. Diagram the steps in B-cell activation, including all types of cells produced.
17. Make a detailed drawing of an antibody molecule.
18. Explain the various end results of antibody binding to an antigen.
19. List the five types of antibodies and important facts about each.
20. Draw and label a graph—with time on the horizontal axis—that shows the development of the primary and secondary immune responses.

13.6 Specific Immunity and Vaccination

Specific immunity in humans and other mammals is categorized using two different sets of criteria, which, when combined, result in four specific descriptors of the immune state. Immunity can either be natural or artificial. Also, it can be either active or passive.

Natural immunity encompasses any immunity that is acquired during the normal biological experiences of an individual rather than through medical intervention.

Artificial immunity is protection from infection obtained through medical procedures. This type of immunity is induced by immunization with vaccines and immune serum.

Active immunity occurs when an individual receives an immune stimulus (antigen) that activates the B and T cells, causing the body to produce immune substances such as antibodies. Active immunity is marked by several characteristics: (1) It creates a memory that renders the person ready for quick action upon reexposure to that same antigen; (2) it requires several days to develop; and (3) it lasts for a relatively long time, sometimes for life. Active immunity can be stimulated by natural or artificial means.

Passive immunity occurs when an individual receives immune substances (usually antibodies) that were produced actively in the body of another human or animal donor. The recipient is protected for a short time, even though he or she has not had prior exposure to the antigen. It is characterized by (1) lack of memory for the original antigen; (2) lack of production of new antibodies against that disease; (3) immediate onset of protection; and (4) short-term effectiveness, because antibodies have a limited period of function and, ultimately, the recipient's body disposes of them. Passive immunity can also be natural or artificial in origin.

Table 13.10 illustrates the various possible combinations of acquired immunities.

Table 13.10 The Four Types of Acquired Immunity

Natural Immunity is acquired through the normal life experiences of a human and is not induced through medical means.

Natural Immunity is acquired through the normal life experiences of a human and is not induced through medical means.

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Active
After recovering from infectious disease, a person will generally be actively resistant to reinfection for a period that varies according to the disease. In the case of childhood viral infections such as measles, mumps, and rubella, this natural active stimulus provides nearly lifelong immunity. Other diseases result in a less extended immunity of a few months to years (such as pneumococcal pneumonia and shigellosis), and reinfection is possible. Even a subclinical infection can stimulate natural active immunity. This probably accounts for the fact that some people are immune to an infectious agent without ever having been noticeably infected with or vaccinated for it.

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Passive
Natural, passively acquired immunity occurs only as a result of the prenatal and postnatal mother-child relationship. During fetal life, IgG antibodies circulating in the maternal bloodstream are small enough to pass or be actively transported across the placenta. This natural mechanism provides an infant with a mixture of many maternal antibodies that can protect it for the first few critical months outside the womb, while its own immune system is gradually developing active immunity. Depending on the microbe, passive protection lasts anywhere from a few months to a year.

Another source of natural passive immunity comes to the baby by way of the mother's milk. Although the human infant acquires 99% of natural passive immunity *in utero* and only about 1% through nursing, the milk-borne antibodies provide a special type of intestinal protection that is not available from transplacental antibodies.

Artificial Immunity is that produced purposefully through medical procedures.

Natural Immunity is acquired through the normal life experiences of a human and is not induced through medical means.

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Active
Vaccination exposes a person to a specially prepared microbial (antigenic) stimulus, which then triggers the immune system to produce antibodies and lymphocytes to protect the person upon future exposure to that microbe. As with natural active immunity, the degree and length of protection vary. Vaccines are also being developed for threats to human health that do not involve microbes at all (such as prostate cancer and heroin addiction).

©Dave Moyer

Passive
Passive immunotherapy involves a preparation that contains specific antibodies against a particular infectious agent. Pooled human serum from donor blood (gamma globulin) and immune serum globulins containing high quantities of antibodies are frequently used.

Immunization: A Lively History

The basic notion of immunization has existed for thousands of years. It probably stemmed from the observation that persons who had recovered from certain communicable diseases rarely got a second case. Undoubtedly, the earliest crude attempts involved bringing a susceptible person into contact with a diseased person or animal. The first recorded attempt at immunization occurred in sixth-century China. It consisted of drying and grinding up smallpox scabs and blowing them with a straw into the nostrils of vulnerable family members. By the 10th century, this practice had changed to the deliberate inoculation of dried pus from the smallpox pustules of one

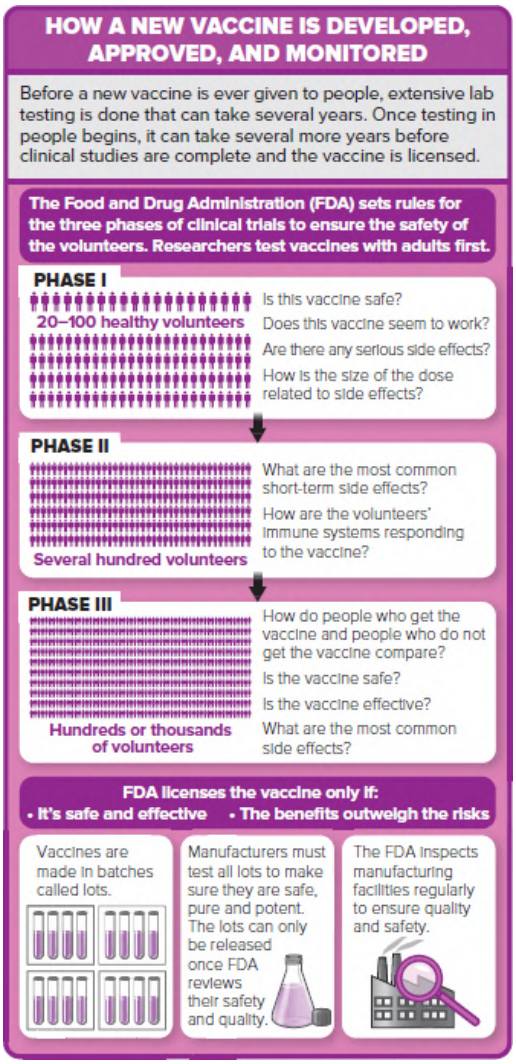
patient into the arm of a healthy person, a technique later called **variolation** (variola is the name for the smallpox virus). This method was used in parts of the Far East for centuries before it was brought to England in 1721.

Although the principles of the technique had some merit, unfortunately many recipients and their contacts died of smallpox. This outcome vividly demonstrates a cardinal rule for a workable vaccine: It must contain an antigen that will provide protection but not cause the disease.

Eventually, this human experimentation paved the way for the first really effective vaccine, developed by the English physician Edward Jenner in 1796. Jenner's work gave rise to the words **vaccine** and *vaccination* (from L., *vacca*, "cow"), which now apply to any immunity obtained by inoculation with selected antigens. Jenner was inspired by the case of a dairymaid who had been infected by a pustular infection called cowpox. This related virus afflicts cattle but causes a milder condition in humans. She explained that she and other milkmaids had remained free of smallpox. Other residents of the region expressed a similar confidence in the cross-protection of cowpox. To test the effectiveness of this new vaccine, Jenner prepared material from human cowpox lesions and inoculated a young boy. When challenged 2 months later with an injection of crusts from a smallpox patient, the boy proved immune.

Jenner's discovery—that a less pathogenic agent could confer protection against a more pathogenic one—is especially remarkable in view of the fact that microscopy was still in its infancy and the nature of viruses was unknown. At first, the use of the vaccine was regarded with some fear and skepticism **(figure 13.9)**. When Jenner's method proved successful and word of its significance spread, it was eventually adopted in many other countries. In 1980, the World Health Organization declared that smallpox had been eradicated.

Figure 13.9 **Ridicule of the new idea of vaccination**. This cartoon appeared during Edward Jenner's advocacy for vaccination against smallpox.
Source: Library of Congress [LC-USZC4-3147]



Passive Immunization

The only natural forms of passive immunization occur as (1) a fetus develops and encounters selected antibodies that are able to cross the placental barrier, and (2) a newborn nurses and receives IgA in breast milk that is secreted at birth and for a short time afterward.

The first attempts at artificial passive immunization involved the transfusion of horse serum containing antitoxins to prevent tetanus and to treat patients exposed to diphtheria. Since then, antisera from animals have been replaced with products of human origin that function with various degrees of specificity. Intravenous immunoglobulin (IVIG), sometimes called *gamma globulin*, contains immunoglobulin extracted from the pooled blood of human donors. The method of processing IVIG concentrates the antibodies to increase potency and eliminates potential pathogens (such as hepatitis B and HIV). It is most useful in patients who have a diminished ability to mount their own immune response.

A preparation called specific immune globulin (SIG) is derived from a more defined group of donors. Companies that prepare SIG obtain serum from patients who are convalescing and in a hyperimmune state after such infections as pertussis, tetanus, chickenpox, and hepatitis B. These globulins are preferable to ISG because they contain higher titers of specific antibodies obtained

from a smaller pool of patients. Although donated immunities only last a relatively short time, they act immediately and can protect patients for whom no other useful medication or vaccine exists.

Artificial Active Immunity: Vaccination

The basic principle behind vaccination is to stimulate a primary response and a memory response that primes the immune system for future exposure to a virulent pathogen. If this pathogen enters the body, the immune response will be immediate, powerful, and sustained.

Vaccines have profoundly reduced the prevalence and impact of many infectious diseases that were once common and often deadly. Initially, the emphasis was on immunizing babies and children against formerly common childhood diseases, like measles, mumps, and rubella. Recent years have seen an additional push to immunize adolescents and adults against conditions such as human papillomavirus (HPV), *Streptococcus pneumoniae*, and shingles. Vaccines are also being developed for threats to human health that do not involve microbes at all.

In this section, we survey the principles of vaccine preparation and important considerations surrounding vaccine indication and safety. (Vaccines are also given specific consideration in later chapters on infectious diseases and organ systems.)

Principles of Vaccine Preparation

In natural immunity, an infectious agent stimulates a relatively long-term protective response. In artificial active immunity, the objective is to obtain this same response with a modified version of the microbe or its components. Qualities of an effective vaccine are as follows:

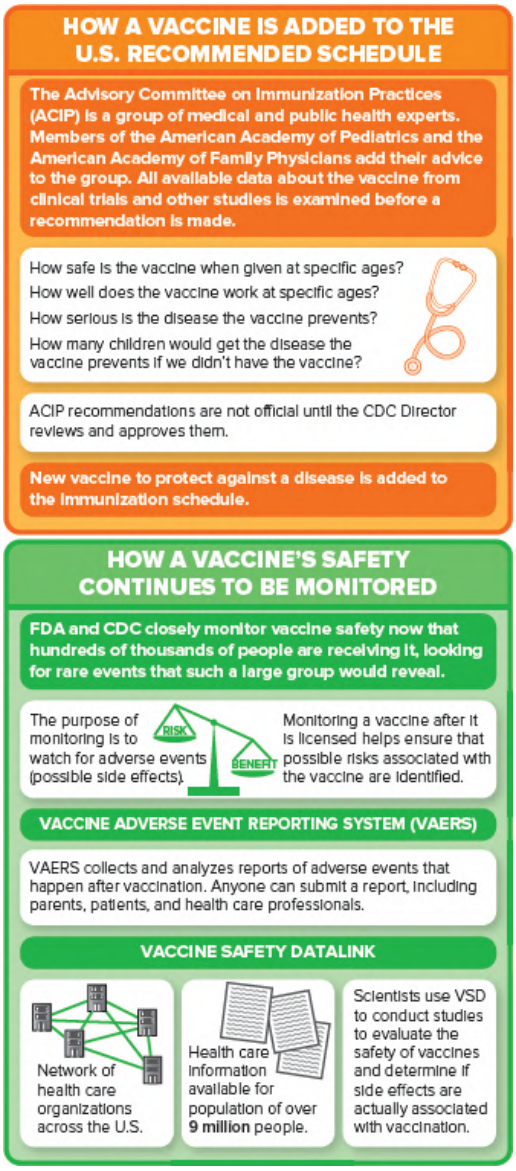
- It should protect against exposure to natural, wild forms of the pathogen.
- It should have a low level of adverse side effects or toxicity and not cause harm.
- It should stimulate both antibody (B-cell) response and cell-mediated (T-cell) response.
- It should have long-term, lasting effects (produce memory).
- It should not require numerous doses or boosters.
- It should be inexpensive, have a relatively long shelf life, and be easy to administer.

Vaccine preparations can be broadly categorized as either whole-organism or part-of-organism preparations. These categories also have subcategories:

1. Whole cells or viruses
    a. live, attenuated microbial cells or viruses
    b. killed cells or inactivated viruses
2. Part-of-organism preparations: antigenic molecules derived from bacterial cells or viruses (subunits)
    a. subunits derived from cultures of cells or viruses
    b. subunits chemically synthesized to mimic natural molecules found on pathogens
    c. subunits manufactured via genetic engineering
    d. subunits conjugated with proteins (often from other microbes) to make them more immunogenic. These are called **conjugated vaccines**.

These categories are also shown in **table 13.11**.

Note: As of 2008, there were no vaccines used in the United States that consisted of killed whole bacteria. The last two available were those for cholera and plague.



Adapted from the Centers for Disease Control and Prevention

Development of New Vaccines

Despite considerable successes, dozens of bacterial, viral, protozoal, and fungal diseases still remain without a functional vaccine. At the present time, no reliable vaccines are available for HIV/AIDS, various diarrheal diseases, respiratory diseases, and worm infections that affect over 200 million people per year worldwide. Worse than that, most existing vaccines are out of reach for much of the world's population.

DNA vaccines are one promising new approach to immunization. The technique in these formulations is very similar to gene therapy as described in chapter 8, except in this case, microbial

(not human) DNA is inserted into a plasmid vector

Table 13.11 Types of Vaccines

Whole-Cell Vaccines

Whole cells or viruses are very effective immunogens, since they are so large and complex. Depending on the vaccine, these are either killed or attenuated.

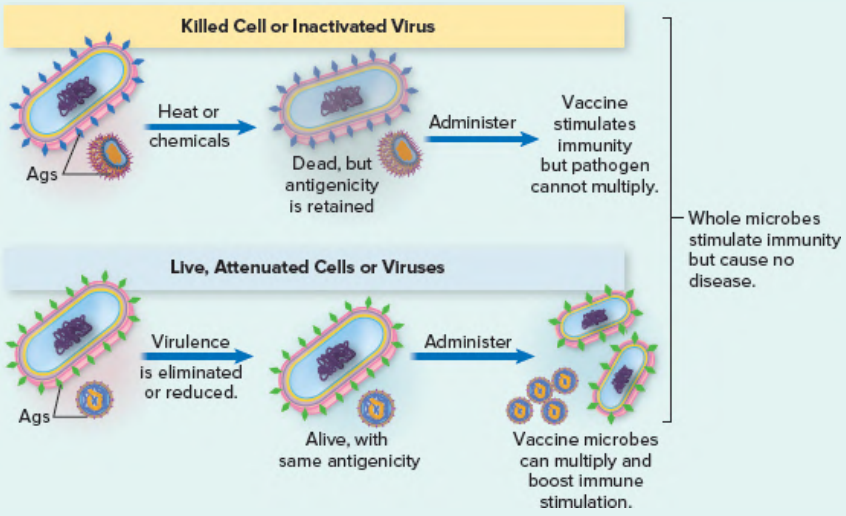
Killed vaccines (viruses are termed "inactivated" instead of "killed") are prepared by cultivating the desired strain or strains of a bacterium or virus and treating them with chemicals, radiation, heat, or some other agent that does not destroy antigenicity. The hepatitis A vaccine and three forms of the influenza vaccine contain inactivated viruses. Because the microbe does not multiply, killed vaccines often require a larger dose and more boosters to be effective.

Live attenuated vaccines contain live microbes whose virulence has been *attenuated*, or lessened/eliminated. This is usually achieved by modifying the growth conditions or manipulating microbial genes in a way that eliminates virulence factors. Vaccines for measles, mumps, polio (Sabin), and rubella contain live, nonvirulent viruses.

The advantages of live preparations are as follows:

1. Viable microorganisms can multiply and produce infection (but not disease) like the natural organism.
2. They confer long-lasting protection.
3. They usually require fewer doses and boosters than other types of vaccines.
4. They are particularly effective at inducing cell-mediated immunity.

Disadvantages of using live microbes in vaccines are that they require special storage facilities and can conceivably mutate back to become virulent again.

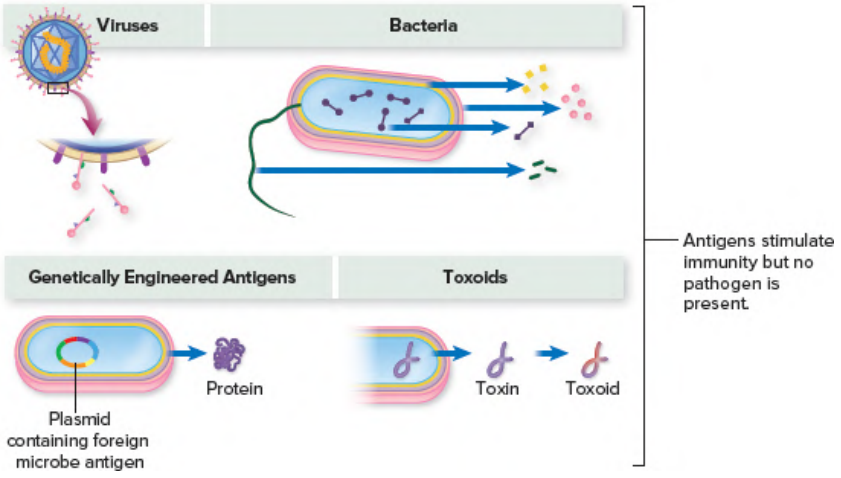


Subunit Vaccines (Parts of Organisms)

Whole-Cell Vaccines

If the exact epitopes that stimulate immunity are known, it is possible to produce a vaccine based on a selected component of a microorganism. These vaccines for bacteria are called **subunit vaccines**. The antigens used in these vaccines may be taken from cultures of the microbes, produced by genetic engineering or synthesized chemically.

Examples of component antigens currently in use are the capsules of the pneumococcus and meningococcus, the protein surface antigen of anthrax, and the surface proteins of hepatitis B virus. A special type of vaccine is the **toxoid**, which consists of a purified bacterial exotoxin that has been chemically denatured. By eliciting the production of antitoxins that can neutralize the natural toxin, toxoid vaccines provide protection against diseases such as diphtheria, tetanus, and pertussis.



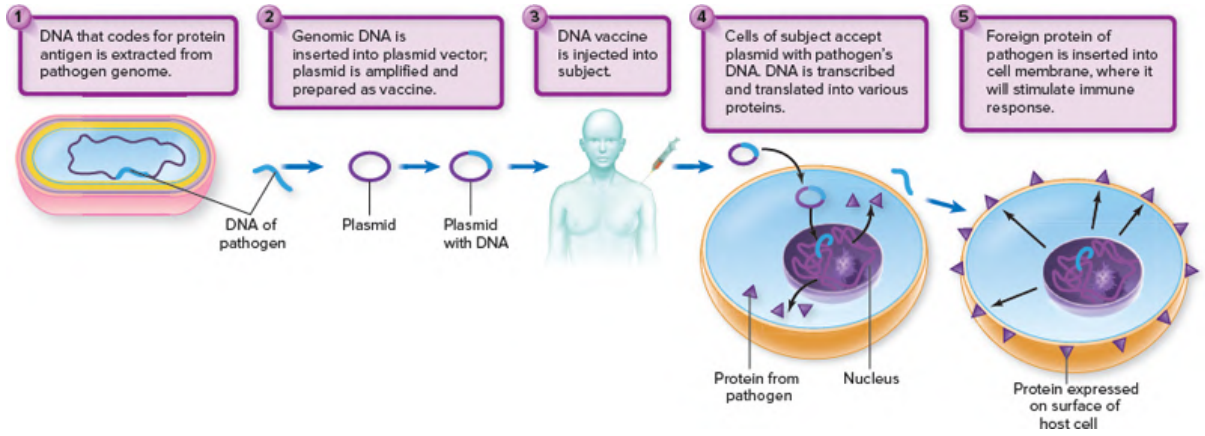


Figure 13.10 **DNA vaccine preparation**. DNA vaccines contain part of the pathogen's DNA, which is used to "infect" a recipient's cells. Processing of the DNA leads to production of an antigen protein that can stimulate a specific response against that pathogen.

and inoculated into a recipient **(figure 13.10)**. The expectation is that the human cells will take up some of the plasmids and express the microbial DNA in the form of proteins. Because these proteins are foreign, they will be recognized during immune surveillance and cause B and T cells to be sensitized and form memory cells.

Vaccination strategies are now under intense investigation for the prevention and treatment of noninfectious diseases. One of the best examples is vaccination for Alzheimer's disease. Researchers are examining whether administering a peptide that is found in the brain plaques of

Alzheimer's patients as a vaccine can lead to prevention of the disease. The principle is the same as with vaccines against microbial infection. If T and B memory cells that recognize these disease-inducing proteins can be created in a host via vaccination, once the proteins begin to form in the patient the memory response eliminates them before they cause damage.

Immunotherapy as a treatment for cancer has been in use now for some years. In these situations, T cells or dendritic cells are removed from the patient. In the laboratory the cells are sensitized to known cancer antigens. These activated cells are then injected back into the patients, in the hopes that the actual tumor antigens match the ones used for activation in the lab, and the patient's own immune system will attack the tumor.

Route of Administration and Side Effects of Vaccines

Most vaccines are injected by subcutaneous, intramuscular, or intradermal routes. One type of the influenza vaccine comes in the form of a nasal spray. Oral (or nasal) vaccines are available for only a few diseases, but they have some distinct advantages. An oral or nasal dose of a vaccine can stimulate protection (IgA) on the mucous membrane of the portal of entry. Oral and nasal vaccines are also easier to give than injections, and are more readily accepted.

Some vaccines require the addition of a special binding substance, or **adjuvant** (ad′-joo-vunt). An adjuvant is any compound that enhances immunogenicity and prolongs antigen retention at the injection site. The adjuvant precipitates the antigen and holds it in the tissues so that it will be released gradually. Its gradual release presumably facilitates contact with antigen-presenting cells and lymphocytes. The most common adjuvant is alum (aluminum hydroxide salts).

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3. Which characteristic is associated with passive artificial immunity?
- **a.** *long-term protection duration*
- **b.** *requires several days to develop protection*
- **c.** *immediate protection*
- **d.** *creation of memory in response to antigen*

For several years various research groups have been working on developing a vaccine delivery system using a patch. This would eliminate the need for sterile conditions and refrigeration, and of course, would be more comfortable to receive. One of these began clinical trials in 2017. It delivers the antigen using hundreds of microscopic needles—which cannot be felt—on the surface of the patch.

In recent years a significant "anti-vaccine" movement has taken shape among citizens of first-world countries. This is a science text, so we present important science facts about vaccines, and how they come to be recommended. Vaccines must go through many years of trials in experimental animals and human volunteers before they are licensed for general use. Even after they have been approved, like all therapeutic products, they are not without complications. The most common of these are local reactions at the injection site, fever, allergies, and other adverse outcomes. Some patients experience allergic reactions to the medium used to grow the vaccine organism (eggs or tissue culture) rather than to vaccine antigens. More serious reactions are extremely rare (fewer than 1 case in 220,000) but they can occur, and are intensively studied by scientists. All of the risks are detailed in materials provided to you by your doctor before the vaccination occurs. When known or

suspected adverse effects have been detected, vaccines are altered or withdrawn. Several years ago, the whole-cell pertussis vaccine was replaced by the acellular capsule (aP) form when it was associated with adverse neurological effects. Vaccine companies have also phased out certain preservatives, such as thimerosal, that are thought to cause allergies and other potential side effects.

Some people have attempted to link childhood vaccinations to later development of diabetes, asthma, and, most prominently, autism. However, the original 1998 scientific paper that suggested that vaccines might be responsible for autism was entirely discredited, and the principal author's medical license was revoked after authorities found the research and its claims fraudulent.

In 2011, the Institute of Medicine, an independent nonprofit agency of the widely respected National Academies of Science, published the results of its comprehensive examination of more than 1,000 independent research articles about childhood vaccines and stated unequivocally that the MMR vaccine does not cause autism, and that very few adverse health events were associated with vaccines. In 2013 they conducted another review of the science examining whether the schedule of childhood immunizations was safe and effective. They found that it was. In contrast, the price of not being vaccinated has become painfully clear. Outbreaks of measles, mumps, diphtheria, polio, typhoid fever, and whooping cough have popped up all over this country in college dormitories, in antivaccination religious communities, and in airplanes.

These outbreaks are often attributed to a decrease in the level of herd immunity, a phenomenon in which a certain percentage of the population is vaccinated, which means that the microbe is unable to maintain its circulation through the population. Think about it—this means that getting vaccinated serves the common good as well as your individual good. At a time when most of the world's population is clamoring for vaccines, some in the developed world are refusing vaccination, essentially relying on others' willingness to be vaccinated to keep them and their children safe.

Some have speculated that vaccination has done too good of a job—at least in terms of being so effective for so long that many young parents have no memory of the prevaccination era and don't appreciate the much greater risk of not vaccinating compared to vaccinating. In the decade before measles vaccination began, 3 to 4 million cases occurred each year in the United States. Typically, 300 to 400 children died annually and 1,000 more were chronically disabled due to measles encephalitis. Put simply, childhood vaccines save the lives of 2.5 million children a year (worldwide), according to UNICEF.

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Professionals involved in giving vaccinations must understand their inherent risks but also realize that the risks from the infectious disease almost always outweigh the chance of an adverse vaccine reaction.

13.6 LEARNING OUTCOMES—Assess Your Progress

21. List and define the four different descriptors of specific immune states.
22. Discuss the qualities of an effective vaccine.
23. Name the two major categories of vaccines and then the subcategories under each.
24. Explain the principle of herd immunity and the risks that unfold when it is not maintained.

CASE FILE WRAP-UP

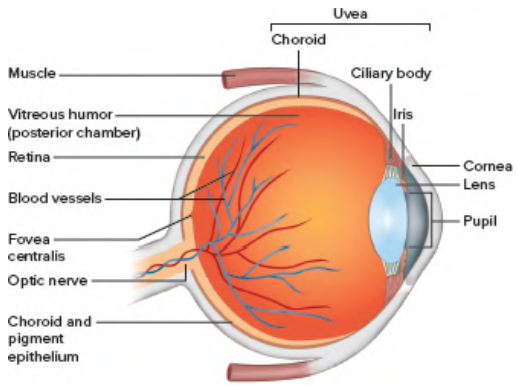
©O. Dimier/PhotoAlto (health care worker); ©MedicalRF.com/Getty Images (monocytes)

Hemophagocytic lymphohistiocytosis (HLH) is a disease affecting the immune system. Often a genetic condition, HLH has an autosomal recessive inheritance pattern. However, in cases such as Trevor's, HLH may occur spontaneously. In response to a virus, the body's macrophages and T cells can become overactive. These overactive immune cells destroy other blood cells: red blood cells, white blood cells, and platelets. Not technically an autoimmune disorder, HLH is best understood as an overstimulated immune system. Further destruction to body cells and tissues is caused by the excessive release of cytokines, causing inflammation. Though the exact cause of Trevor's disease is unknown, it is possible his respiratory viral infection triggered the inappropriate immune response.

Untreated, HLH is fatal. Rapid diagnosis and initiation of treatment is essential for increasing the patient's likelihood of survival. Medical treatment is concentrated on suppressing the body's overactive immune response. Often patients are treated with steroids and chemotherapy. Due to the suppression of the body's immune response with these medications, the patient must be carefully monitored and treated for signs of infection. The only cure for inherited or recurrent HLH is stem cell transplantation.

The Microbiome

Gut Bacteria Cause Blindness?



There is a condition well known to ophthalmologists called autoimmune uveitis (yoo-vee-eye'-tis). You can see from the illustration that the uvea is a central part of the eye. In autoimmune uveitis, T cells inappropriately attack retinal tissue in the uvea. This condition is responsible for up to 15% of severe vision problems and blindness in the developed world.

Scientists have struggled to understand this disease, for the following reason: The eye is a site of immunological privilege. This means that the blood vessels in the eye are less permeable and that there is no direct lymphatic vessel drainage. This prevents inflammatory products and immune cells from causing damage to the delicate eye tissue. But in autoimmune uveitis, T cells that recognize eye antigens cross through the blood vessel barrier and mount an immune response in the eye. So not only do the T cells breach the protected area, they do so after being sensitized to eye antigens that don't circulate in the lymphatic system. How does that happen?

A 2015 study conducted by researchers at the United States National Eye Institute, using a mouse model, found that T-cell activity in the mouse intestines increased just prior to the onset of uveitis. The intestines also contained increased levels of a cytokine released by T cells. The hypothesis is that T cells are activated by antigens from bacteria in the gut microbiome, and that they then mistakenly react with a similar protein in the eye.

This is an example of very interesting research—that is very preliminary. The study is suggestive of a cause, but is not proof. For example, researchers did not identify the exact substance that produces the cross reaction with eye antigens. However, a 2017 study showed a similar pathway to age-related macular degeneration. In that study, particular members of the gut microbiomes were correlated with the occurrence of macular degeneration. Further studies will be required to move these associations from "correlated with the disease" to "causes the disease."

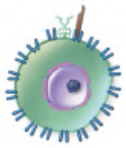
Chapter Summary

13.1 Specific Immunity: The Third and Final Line of Defense

- The surfaces of all cell membranes contain protein markers or receptors. These function in identification, communication, and cell development. Some are also self identity markers.
- Human identity markers are genetically determined by major histocompatibility complexes (MHCs).
- Lymphocytes respond to a specific portion of an antigen called the epitope. A given microorganism has many such epitopes.
- Acquired specific immunity consists of four stages:
  - **Stage I**. Lymphocytes originate in hematopoietic tissue but go on to diverge into two distinct types: B cells and T cells.
  - **Stage II**. Antigen-presenting cells detect invading pathogens and present these antigens to lymphocytes, which recognize the antigen and initiate the specific immune response.
  - **Stage III**. Lymphocytes proliferate, producing clones of progeny that include groups of responder cells, regulator cells, and memory cells.
  - **Stage IV**. Activated T lymphocytes (one of three subtypes) regulate and participate directly in the specific immune responses. Activated B lymphocytes become plasma cells that produce and secrete large quantities of antibodies. Regulatory B cells are also produced.

13.2 Stage I: The Development of Lymphocyte Diversity

- Immature lymphocytes released from hematopoietic tissue migrate to one of two sites for further development. B cells mature in the stromal cells of the bone marrow. T cells mature in the thymus.
- During development, both B and T cells develop millions of genetically different clones. Together these clones possess enough genetic variability to respond to many millions of different antigens.
- Clones that react to self antigens are deleted during development.
- Binding of antigen to a particular clone is called clonal selection. That clone is exclusively amplified in a process called clonal expansion.



13.3 Stage II: Presentation of Antigens

- Antigens are molecules that can be seen and identified by immune system cells. They are called immunogens if they actually produce an adaptive immune response.
- Antigen-presenting cells (APCs), such as dendritic cells and macrophages, engulf and process foreign antigen and bind the epitope to MHC class II molecules on their cell surface for presentation to CD4 T lymphocytes.

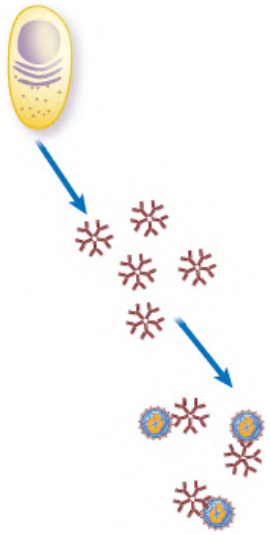
13.4 Stages III and IV: T-Cell Response

- The three main classes of T cells are T helper cells, T regulatory cells, and T cytotoxic cells. Each subset of T cell produces a distinct set of cytokines that stimulate lymphocytes or destroy foreign cells.
- Gamma-delta T cells have T-cell receptors but can respond in a nonspecific manner.

- Natural killer T (NKT) cells contain natural killer (NK) markers and T-cell markers. Natural killer cells are not themselves specific, but they participate in specific immune responses.

13.5 Stages III and IV: B-Cell Response

- B cells produce five classes of antibody: IgM, IgG, IgA, IgD, and IgE.
- Antibodies bind physically to the specific portion of the antigen (the epitope) that stimulates their production, thereby immobilizing the antigen and enabling it to be destroyed by other components of the immune system.
- The memory response means that the second exposure to antigen provokes a much faster and more vigorous response than the first.



13.6 Specific Immunity and Vaccination

- Active immunity means that your body produces antibodies to a disease agent. If you contract the disease, you can develop natural active immunity. If you are vaccinated, your body will produce artificial active immunity.
- In passive immunity, you receive antibodies from another person. Natural passive immunity comes from the mother. Artificial passive immunity is administered medically.
- Artificial passive immunity usually involves administration of antiserum. Antibodies collected from donors (human or otherwise) are injected into people who need protection immediately.
- Artificial active agents are vaccines that provoke a protective immune response in the recipient but do not cause the actual disease.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. A single bacterium has _____ epitope(s). **a**. *a specific* **b**. *multiple* **c**. *MHC* **d**. *clonal*	**2**. Would you suspect that the nonspecific or the specific arms of the immune response evolved first in mammals? Explain your answer.	**3**. Provide an explanation to refute the following statement: Humans cannot develop specific immunity to a novel biological agent created in a laboratory.
Cell Structure and Function	**4**. The primary B-cell receptor is **a**. IgD. **b**. IgA. **c**. IgE. **d**. IgG.	**5**. Name three antigen-presenting cells, and what other functions each of them has.	**6**. Major histocompatibility molecules are critical for specific immunity. But they are named after another major function they have. How does histocompatibility work?
Metabolic Pathways	**7**. In humans, B cells mature in the ___ and T cells mature in the ____. **a**. GALT; liver **b**. bursa; thymus **c**. bone marrow; thymus **d**. lymph nodes; spleen	**8**. Explain how the memory response is the cornerstone of vaccination.	**9**. Conduct research on clonal deletion and write a paragraph that explains it to a typical high school biology student.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Information Flow and Genetics	**10**. Which of the following cells is capable of specifically responding to a nearly infinite number of epitopes? **a**. B and T cells **b**. Plasma cells **c**. T cytotoxic cells **d**. All of these	**11**. Is antibody diversity generated at the DNA or RNA level? Explain.	**12**. In order for gene rearrangement of antigen receptors to be successful, the DNA sequence needs to remain in-frame after the rearrangement occurs. Explain what "in-frame" means and why it would be necessary in the generation of diversity in antigen receptors.
Microbial Systems	**13**. Some microbial products can activate B cells without the assistance of T cells. Which of the following can do this? **a**. Capsule of *S. pneumoniae* **b**. Lipopolysaccharide **c**. Some viral capsids **d**. All of these	**14**. Explain why it is useful that antibodies have two antigen-binding arms.	**15**. Using the details of T-cell activation, suggest a reason why they are so critical in recognizing and destroying virally-infected host cells.

Impact of Microorganisms	**16**. A vaccine that contains parts of viruses is called **a**. acellular. **b**. recombinant. **c**. subunit. **d**. attenuated.	**17**. Explain how herd immunity works to protect the spread of infectious disease. Include two ways that people can be "immune" to infection.	**18**. Conduct research and discuss one current example illustrating how low herd immunity within a population has led to

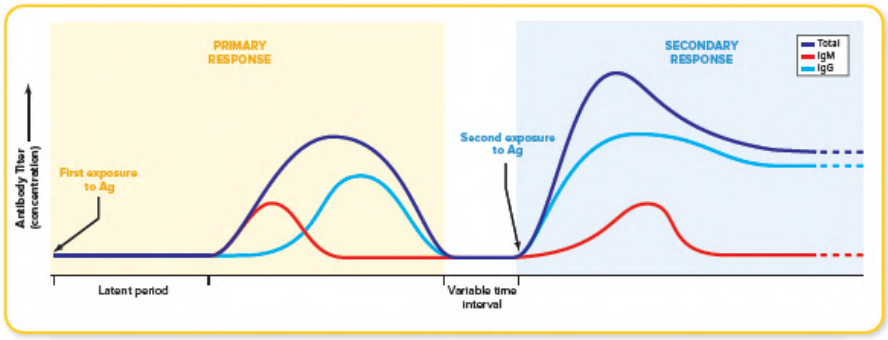
			localized disease outbreaks in the United States.
Scientific Thinking	**19.** If you draw a blood sample from a patient to determine whether he or she has a herpes simplex infection, and the patient displays a large amount of IgG against the virus but low levels of IgM, what do you conclude? **a.** The patient is newly infected. **b.** The patient has had the infection for a while. **c.** The patient is not infected. **d.** It is impossible to draw conclusions.	**20.** Chronic lymphocytic leukemia leads to the production of cancerous B cells, and treatment often involves bone marrow transplantation. Based upon your knowledge of lymphocyte development, explain how this procedure can lead to therapeutic effects.	**21.** Scientists have been developing a vaccine to prevent addiction to heroin and other opioids. Sketch out a scenario for how it might work.

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From table 13.9**. In this figure describing primary and secondary responses to antigen, indicate where a vaccination might be most effective, and also indicate where natural infection would play a role.

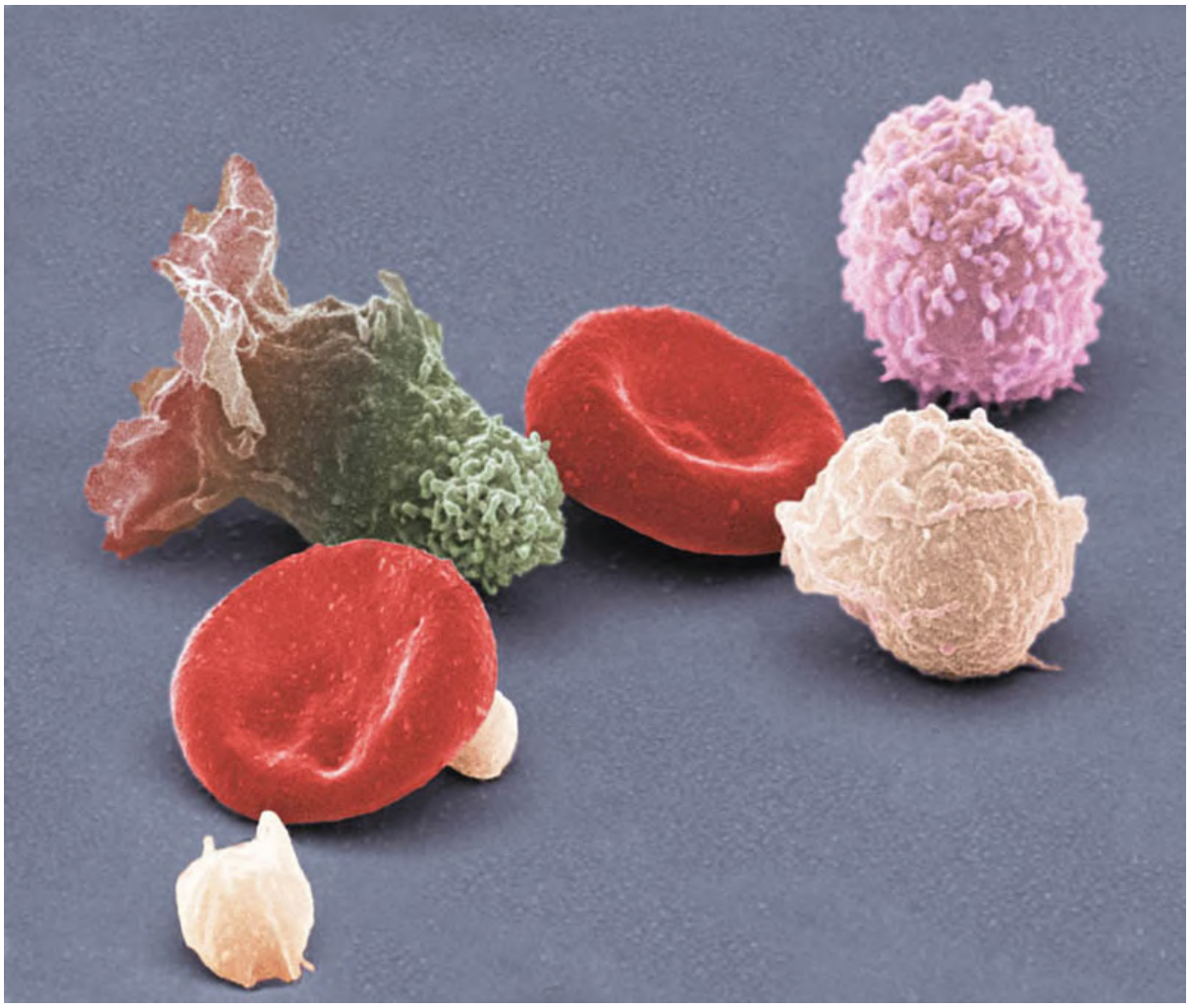


connect Resources are available through Connect, including interactive learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

14

Disorders in Immunity



©Steve Gschmeissner/Science Source (blood cells); ©Pixtal/age fotostock (sneeze); ©Southern Illinois University/Science Source (type I reaction); ©Koshi Johnson/Medical Images (type III reaction); ©Aaron Roeth Photography (autoimmunity); ©McGraw-Hill Education/Christopher Kerrigan, photographer (hyposensitivity); ©McGraw-Hill Education/Rick Brady, photographer (health care worker)

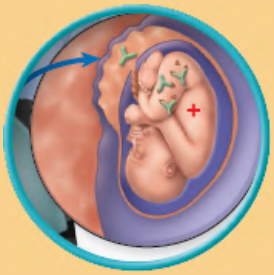
IN THIS CHAPTER…

14.1 The Immune Response: A Two-Sided Coin

1. Define *immunopathology*, and describe the two major categories of immune dysfunction.
2. Identify the four major categories of hypersensitivities, or overreactions to antigens.

14.2 Type I Allergic Reactions: Atopy and Anaphylaxis

3. Summarize genetic and environmental factors that influence allergy development.
4. Identify three conditions caused by IgE-mediated allergic reactions.
5. Identify the two clinical forms of anaphylaxis, explaining why one is more often fatal than the other.
6. List the three main ways to prevent or short-circuit type I allergic reactions.

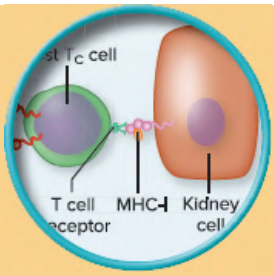


14.3 Type II Hypersensitivities: Reactions That Lyse Foreign Cells

7. List the three immune components causing cell lysis in type II hypersensitivity reactions.
8. Explain the role of Rh factor in hemolytic disease development and how it is prevented in newborns.

14.4 Type III Hypersensitivities: Immune Complex Reactions

9. Identify commonalities and differences between type II and type III hypersensitivities.



14.5 Type IV Hypersensitivities: Cell-Mediated (Delayed) Reactions

10. Describe one example of a type IV delayed hypersensitivity reaction.
11. List four classes of grafts, and explain how host versus graft and graft versus host diseases develop.

14.6 An Inappropriate Response to Self: Autoimmunity

12. List at least three autoimmune diseases and the most important immunologic features in them.

14.7 Immunodeficiency Diseases: Hyposensitivity of the Immune System

13. Distinguish between primary and secondary immunodeficiencies, explaining how each develops.

CASE FILE

A Body Attacking Itself

A 57-year-old woman was admitted to the acute care unit where I was working as an RN. Her admitting diagnosis was rheumatoid arthritis (RA). Her past medical history was significant for gastric ulcers and insulin-dependent diabetes. She had been seen by her primary care physician in the clinic, who decided to admit her after examining her and listening to her history of symptoms.

This patient was unique in that her symptoms had begun quite suddenly 2 days prior, rather than insidiously as is usually the case in RA. She had awoken with moderately severe pain in her knees, ankles, hands, and feet. She also complained of extreme fatigue and stated that she felt as though she had the flu. She had a low-grade fever on admission. Her other vital signs were normal. Her hands, knees, and ankles were mildly swollen and were warm to the touch. She had difficulty moving due to joint pain and was having difficulty managing at home.

Blood work was ordered, including a complete blood count (CBC), chemistry panel, anticyclic citrullinated peptide (anti-CPP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), and C-reactive protein. X rays were also ordered of the affected joints. Test results indicated that the patient was mildly anemic. The ESR was elevated and the patient was positive for both rheumatoid factor and anti-CPP. X rays showed soft tissue swelling around the affected joints, but little destruction of cartilage or damage to the bone was seen, which was not surprising considering the patient had had symptoms for such a short time.

Given that the patient had a history of gastrointestinal (GI) bleeding and had brittle diabetes, the physician started the patient on Enbrel (etanercept), a tumor necrosis factor (TNF) inhibitor. I questioned why the patient was not started on a steroid, as is usually the case. The physician explained that starting the patient on anti-inflammatory drugs might have precipitated another stomach ulcer, and steroids such as prednisone are known to affect blood glucose levels; therefore, the physician believed the patient would do better on a TNF inhibitor such as Enbrel. Before starting the patient on the drug, the physician screened her for hepatitis and tuberculosis. All of her screening tests were negative, and within 2 weeks the patient was in remission and feeling great.

- How is tumor necrosis factor significant in rheumatoid arthritis?
- How do TNF inhibitors reduce inflammation?

Case File Wrap-Up appears at the end of the chapter.

14.1 The Immune Response: A Two-Sided Coin

Humans possess a powerful and necessary system of defense. However, by its very nature, it also carries the potential to cause injury and disease. In most instances, a defect in immune function results in irritating but nondebilitating symptoms such as those of hay fever and dermatitis. But abnormal immune functions are also actively involved in debilitating or life-threatening diseases such as asthma, anaphylaxis, diabetes, rheumatoid arthritis, and graft rejection.

The majority of our previous discussions of the immune response have centered around its numerous beneficial effects. In this chapter, we survey **immunopathology**, disease states associated with overreactivity or underreactivity of the immune response **(figure 14.1)**. Overreactivity (also called hypersensitivity) takes the forms of allergy and autoimmunity. In these conditions, the tissues are innocent bystanders attacked by immune components that can't distinguish one's own tissues from foreign material. In immunodeficiency or **hyposensitivity diseases**, immune function is incompletely developed, is suppressed, or has been destroyed. We will start the chapter with a discussion of hypersensitivity, and then discuss hyposensitivities.

Hypersensitivity: Four Types

Hypersensitivity reactions are classified into four major categories: type I ("common" allergy and anaphylaxis), type II (IgG- and IgM-mediated cell damage), type III (immune complex), and type IV (delayed hypersensitivity) **(table 14.1)**. In general, types I, II, and III involve a B-cell–immunoglobulin response, and type IV involves a T-cell response. The antigens that elicit these reactions can be exogenous, originating from outside the body (microbes, pollen grains, and foreign cells and proteins), or endogenous, arising from self tissue (autoimmunities).

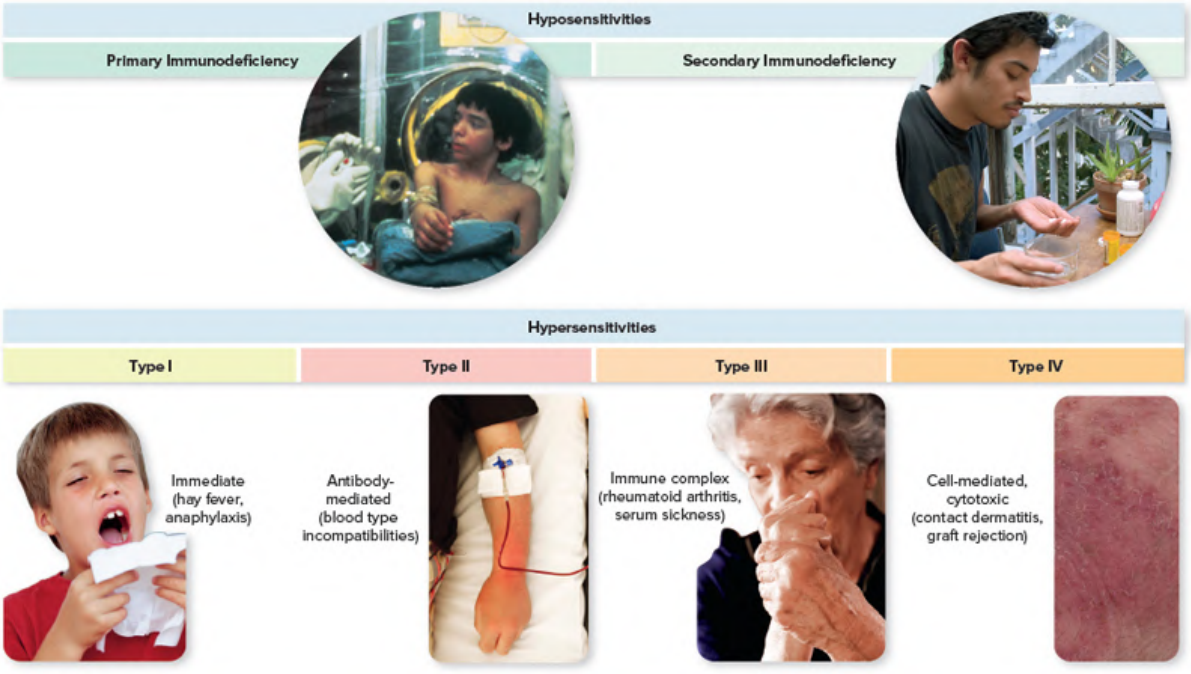


Figure 14.1 Overview of disorders of the immune system. Just as the system of T cells and B cells provides necessary protection against infection and disease, the same system can cause serious and debilitating conditions by overreacting

(hypersensitivity) or underreacting (hyposensitivity) to immune stimuli.

Courtesy Baylor College of Medicine, Public Affairs (primary); ©McGraw-Hill Education/Christopher Kerrigan, photographer (secondary); ©Pixtal/age fotostock (type I); ©Roc Canals Photography/Getty Images (type II); ©Dynamic Graphics/JupiterImages (type III); ©BW Folsom/Shutterstock (type IV)

Table 14.1 Hypersensitivity States

Type		Systems and Mechanisms Involved	Examples
I	Immediate hypersensitivity	IgE-mediated; involves mast cells, basophils, and allergic mediators	Anaphylaxis, allergies such as hay fever, asthma
II	Antibody-mediated	IgG, IgM antibodies act upon cells with complement and cause cell lysis; includes some autoimmune diseases	Blood group incompatibility; pernicious anemia; myasthenia gravis
III	Immune complex-mediated	Antibody-mediated inflammation; circulating IgG complexes deposited in basement membranes of target organs; includes some autoimmune diseases	Systemic lupus erythematosus; rheumatoid arthritis; serum sickness; rheumatic fever
IV	T-cell-mediated	Delayed hypersensitivity and cytotoxic reactions in tissues; includes some autoimmune diseases	Infection reactions; contact dermatitis; graft rejection

One of the reasons allergies are easily mistaken for infections is that both involve tissue damage and thus trigger the inflammatory response, as described in chapter 12. Many symptoms and signs of inflammation (redness, heat, skin eruptions, edema, and granuloma) are prominent features of allergies.

14.1 LEARNING OUTCOMES—Assess Your Progress

1. Define *immunopathology*, and identify the two major categories of immune dysfunction.
2. Identify the four major categories of hypersensitivities, or overreactions to antigens.

Asthma is a form of type I hypersensitivity.
©Science Photo Library/Getty Images

14.2 Type I Allergic Reactions: Atopy and Anaphylaxis

The term *allergy* refers to an exaggerated immune response that is manifested by inflammation. Allergic individuals are acutely sensitive to repeated contact with antigens, called *allergens*, which do not noticeably affect nonallergic individuals. All type I reactions share a similar physiological mechanism, are immediate in onset, and are associated with exposure to specific antigens. However, there are two levels of severity: **Atopy** is any chronic local allergy such as hay fever or asthma; **anaphylaxis** (an″-uh-fih-lax′-us) is a systemic, sometimes fatal reaction that involves airway obstruction and circulatory collapse.

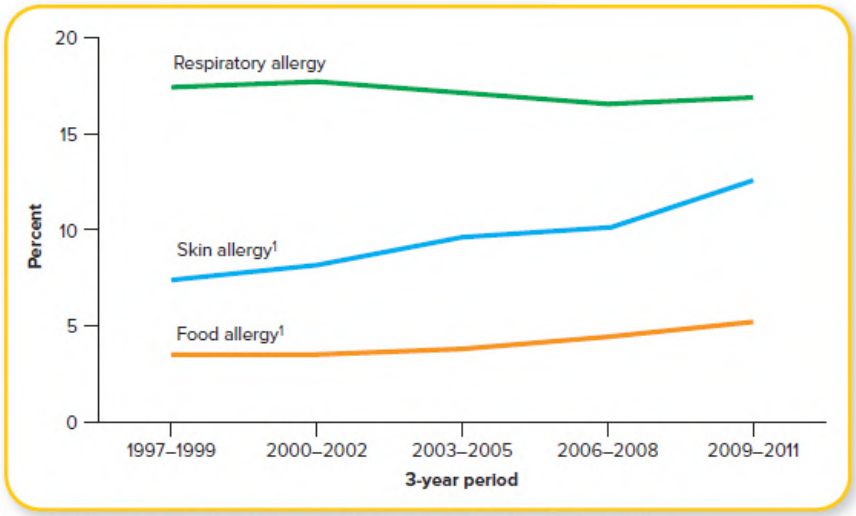
Although the general effects of hypersensitivity are detrimental, we must be aware that it involves the very same types of immune reactions as those at work in protective immunities. Based upon this fact, *all* humans have the potential to develop allergies under particular circumstances.

Who Is Affected?

In the United States, nearly half of the population is affected by airborne allergens, such as dust, pollen, and mold. The incidence of some allergies in children has been increasing over the last 20 years **(figure 14.2)**. The majority of type I allergies are relatively mild, but certain forms such as asthma and some food allergies may require hospitalization and can cause death especially in the

youngest patients. In some individuals, atopic allergies last for a lifetime; others "outgrow" them; still others suddenly develop them later in life.

A predisposition to allergies seems to "run in families," that is, have a strong familial association. Be aware that the part that is hereditary is a *generalized susceptibility*, not the allergy to a specific substance. For example, a parent who is allergic to ragweed pollen can have a child who is allergic to cat hair. The prospect of a child's developing



[1]Significant increasing linear trend for food and skin allergy from 1997–1999 to 2009–2011.
Source: CDC/NCHS, Health Data Interactive, National Health Interview Survey.

Figure 14.2 Allergies in U.S. children ages 0 to 17 from 1997 to 2011. There have been statistically significant increases in both skin and food allergies over this time period.
Source: CDC

atopic allergy is at least 25% if one parent exhibits symptoms, and increases to nearly 50% if grandparents or siblings are also afflicted. The actual basis for atopy seems to be a genetic program that favors allergic antibody (IgE) production, increased reactivity of mast cells, and increased susceptibility of target tissue to allergic mediators.

The "hygiene hypothesis" provides one possible explanation for an environmental component to allergy development. This hypothesis suggests that the industrialized world has created a very hygienic environment, supplemented with antimicrobial products of all kinds and very well insulated homes, and that this has been bad for our immune systems. It seems that our immune systems need to be "trained" by interaction with microbes as we develop. In fact, children who grow up on farms have been found to have lower incidences of several types of allergies. Also, researchers have found that the combination of being delivered by cesarean section and a maternal history of allergy elevates the risk that a child will be allergic to foods by a factor of eight. As we saw in chapter 11, delivery by cesarean section keeps the baby from being exposed to vaginal and stool bacteria. Additional work has shown that babies need to be exposed to commensal bacteria in order for the IgA system to develop normally.

Another possible factor affecting allergy development appears to be related to breast feeding. Newborn babies that are breast fed exclusively for the first 4 months of life have a lower risk of asthma and eczema, especially if they have a family history of allergy. This is thought to come from the presence of cytokines and growth factors in human milk that act on the baby's gut mucosa to

induce tolerance, rather than reactivity, to allergens. New information from the Human Microbiome Project reveals that nearly 600 species of bacteria can be transferred to infants through breast milk. Combined with data from other studies showing that a disruption of microbial populations in the gut may influence the development of asthma, it is clear that these organisms play an important role in the development of tolerance to foreign antigens.

Could allergies reflect some beneficial evolutionary adaptation? Most allergy sufferers would answer with a resounding "No!" Why would humans and other mammals evolve an allergic response that causes suffering, tissue damage, and even death? One possible explanation may be that the components involved in an allergic response exist to defend against helminthic worms and other multicellular human parasites. It is only relatively recently in our evolutionary history that developed countries have seen dramatically fewer infections with these parasites. One hypothesis is that the part of the immune system that fights helminthic worms is left idle in a population that has recently been "scrubbed" of these parasites, and goes awry.

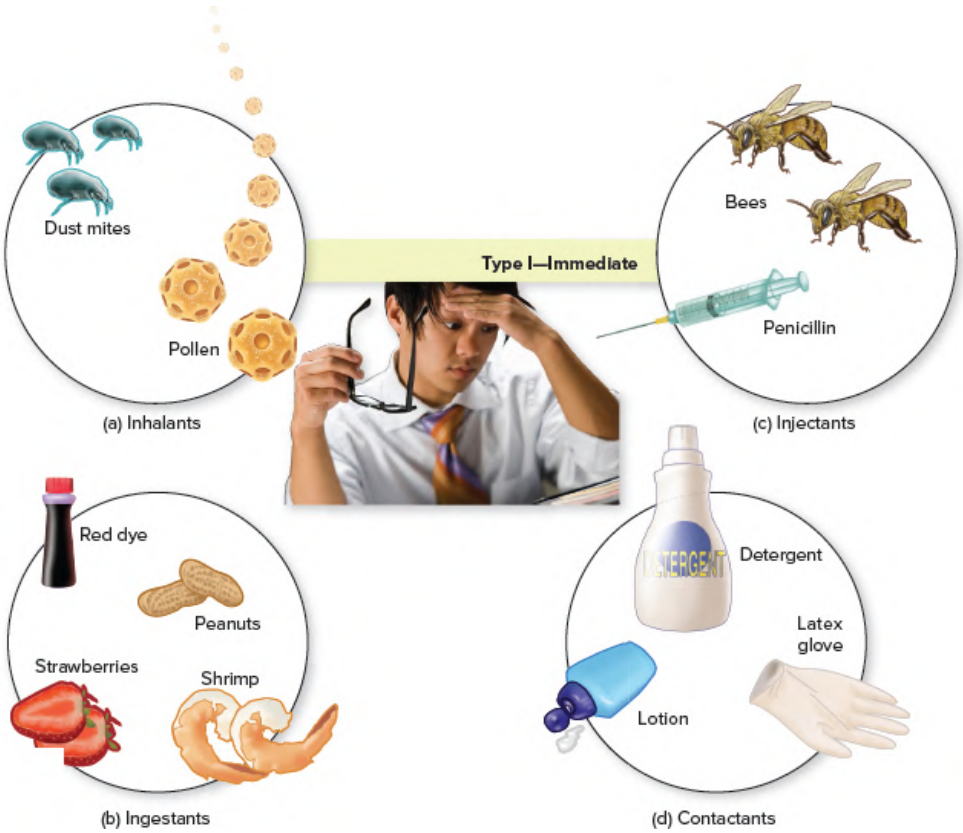


Figure 14.3 **Common allergens, classified by portal of entry**. **(a)** Common inhalants, or airborne environmental allergens, include pollen and insect parts. **(b)** Common ingestants, allergens that enter by mouth. **(c)** Common injectants, allergens that enter via the parenteral route. **(d)** Common contactants, allergens that enter through the skin.
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NCLEX® PREP

1. During a visit to his physician, a patient is asked about his allergies. The patient reports a generalized, raised rash following a dose of an intravenous antibiotic. This is an example of what type of reaction?

a. *immediate hypersensitivity*
b. *antibody-mediated hypersensitivity*
c. *immune complex hypersensitivity*
d. *cell-mediated hypersensitivity*
e. *side effect*

The Nature of Allergens and Their Portals of Entry

As with all antigens, allergens have certain immunogenic characteristics. Proteins are more allergenic than carbohydrates, fats, or nucleic acids. Some allergens are haptens, nonprotein substances with a molecular weight of less than 1,000 that can form complexes with carrier molecules in the body (shown in figure 13.6). Organic and inorganic chemicals found in industrial and household products, cosmetics, food, and drugs are commonly this type of allergen.

Allergens typically enter through epithelial portals in the respiratory tract, gastrointestinal tract, and skin **(figure 14.3)**. The mucosal surfaces of the gut and respiratory system present a thin, moist surface that is normally quite penetrable. The dry, tough keratin coating of skin is less permeable, but access still occurs through tiny breaks, glands, and hair follicles.

Airborne environmental allergens such as pollen, house dust, dander (shed skin scales), or fungal spores are termed *inhalants* (figure 14.3*a*). Each geographic region harbors a particular combination of airborne substances that varies with the season and humidity. Pollen is given off seasonally by trees and other flowering plants, while mold spores are released throughout the year. Airborne animal hair and dander, feathers, and the saliva of dogs and cats are common sources of allergens. The component of house dust that appears to account for most dust allergies is not soil or other debris but the decomposed bodies and feces of tiny mites that commonly live in this dust.

Allergens can be *ingestants, injectants*, or *contactants*. Ingestants often cause food allergies (figure 14.3*b*). Injectant allergies are triggered by drugs, vaccines, or hymenopteran (bee) venom (figure 14.3*c*). Contactants are allergens that enter through the

©Jupiterimages/Photos.com/Alamy Stock Photo

skin (figure 14.3*d*). Many contact allergies are of the type IV (delayed) variety, discussed later in this chapter. It is also possible to be exposed to certain allergens, penicillin among them, during sexual intercourse due to the presence of allergens in the semen.

Mechanisms of Type I Allergy: Sensitization and Provocation

In general, type I allergies develop in stages. **Figure 14.4** tells the whole story. You see that it begins with the initial encounter with allergen, and that sets up the conditions for the allergy to manifest on subsequent encounters.

The Role of Mast Cells and Basophils

Mast cells and basophils play an important role in allergy for the following reasons:

1. Their ubiquitous location in tissues. Mast cells are located in the connective tissue of virtually all organs, but there are particularly high concentrations in the lungs, skin, gastrointestinal tract, and genitourinary tract. Basophils circulate in the blood but migrate readily into tissues.
2. Their capacity to bind IgE during sensitization (figure 14.4*a*) and *degranulate*. Each cell carries 30,000 to 100,000 cell receptors, which trigger the release of inflammatory cytokines from cytoplasmic granules (secretory vesicles) when bound by IgE that has been stimulated by the allergen.

The symptoms of allergy are not caused by the direct action of allergen on tissues, but rather by the physiological effects of mast-cell-derived allergic mediators on target

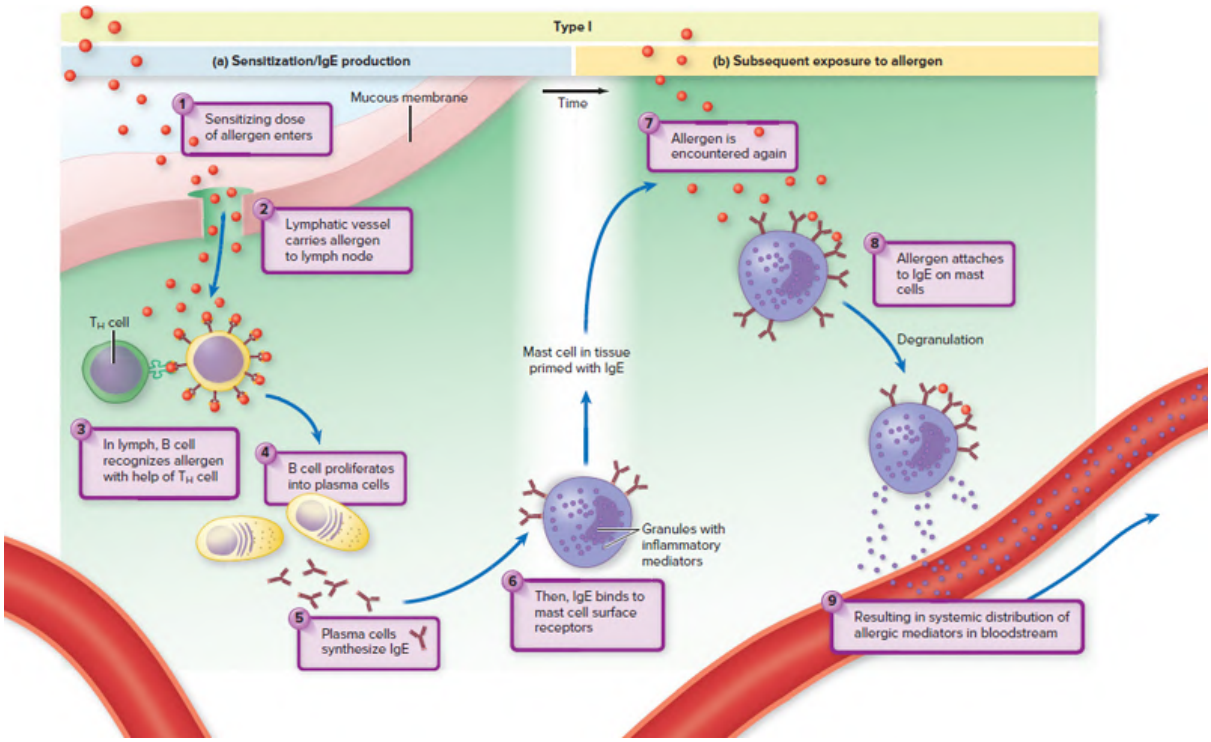


Figure 14.4 **A schematic view of cellular reactions during the type I allergic response**. **(a)** Sensitization (initial contact with sensitizing dose), 1–6. **(b)** Provocation (later contacts with provocative dose), 7–9. **(c)** The spectrum of reactions to inflammatory cytokines released by mast cells and the common symptoms they elicit in target tissues and organs. ©Ingram Publishing (headache); ©robeo/Getty Images (hives); ©Brand X Pictures (stomachache); ©Science Photo Library (asthma); ©Ingram Publishing (blowing nose)

organs. In recent years researchers have also pinpointed a role for T-cell participation in allergic reactions to certain substances. This is not surprising since the orchestration of B-cell responses is

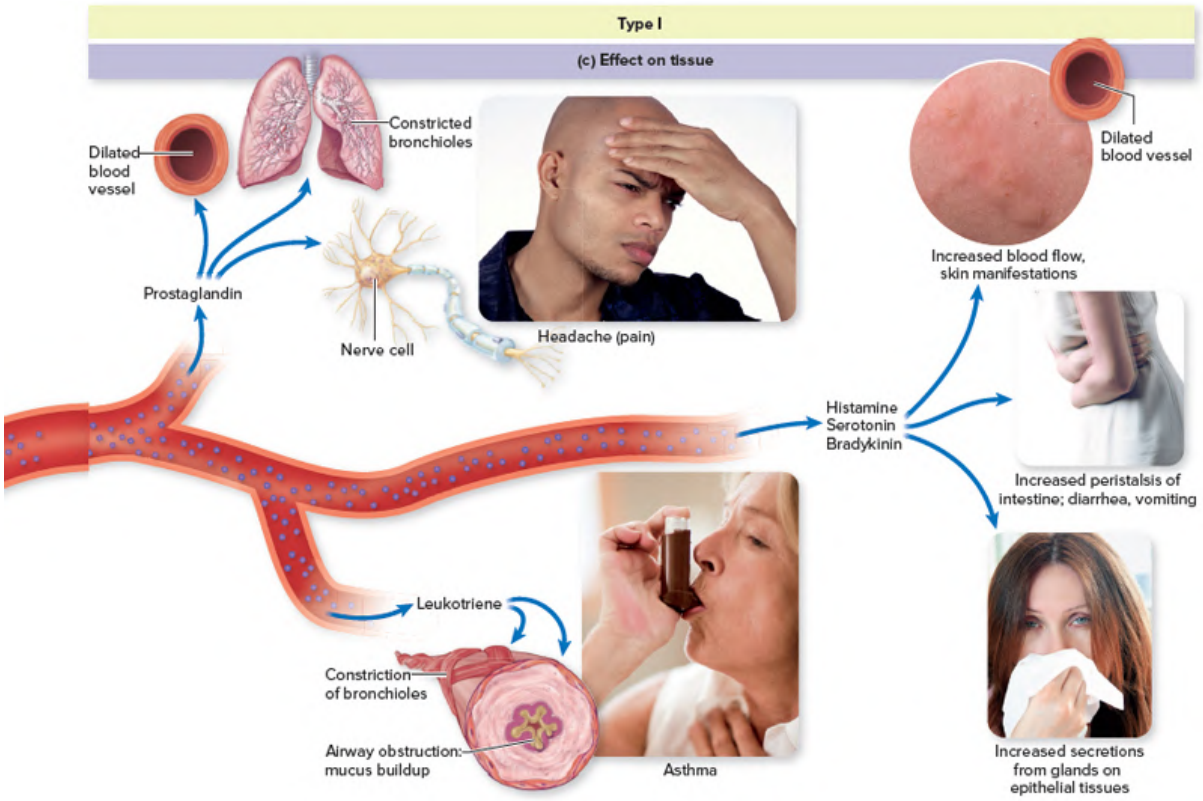
usually influenced by T cells. So, as in most biological phenomenon, it is more complicated than it appears on the surface. In this summary we will focus on the role of IgE.

Cytokines, Target Organs, and Allergic Symptoms

Numerous substances involved in mediating allergy have been identified. The principal chemical mediators produced by mast cells and basophils are histamine, serotonin, leukotriene, platelet-activating factor, prostaglandins, and bradykinin (figure 14.4*c*). These inflammatory cytokines, acting alone or in combination, account for the tremendous scope of allergic symptoms.

Histamine, the most profuse and fastest-acting allergic mediator, is a potent stimulator of secretory glands and smooth muscle. Histamine's actions on smooth muscle vary with location. It *constricts* the smooth muscle layers of the small bronchi and intestine, thereby causing labored breathing and increased intestinal motility. In contrast, histamine *relaxes* vascular smooth muscle and dilates arterioles and venules, resulting in *wheal-and-flare* reactions in the skin and pruritus (itching). Histamine can also stimulate eosinophils to release inflammatory cytokines, escalating the symptoms.

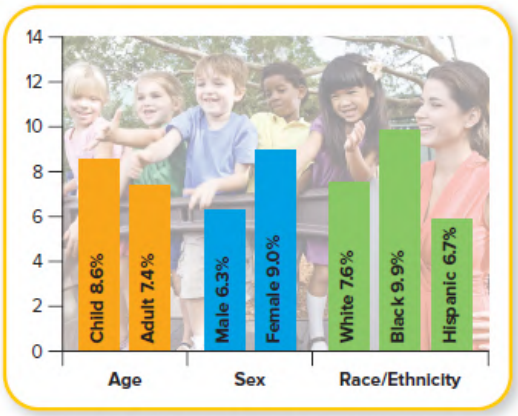
In allergic reactions, **bradykinin** causes prolonged smooth muscle contraction of the bronchioles, dilation of peripheral arterioles, increased capillary permeability, and increased mucus secretion. Although the exact role of **serotonin** in human allergy is uncertain, its effects appear to complement those of histamine and bradykinin.



Leukotriene (loo″-koh-try′-een) is known as the "slow-reacting substance of anaphylaxis" for its property of inducing gradual contraction of smooth muscle. This type of leukotriene is

responsible for the prolonged bronchospasm, vascular permeability, and mucus secretion of the asthmatic individual. Other leukotrienes stimulate the activities of polymorphonuclear leukocytes, or granulocytes, which play a role in various immune functions (see chapter 12).

Prostaglandins are a group of powerful inflammatory agents. Normally, these substances regulate smooth muscle contraction (e.g., they stimulate uterine contractions during delivery). In allergic reactions, they are responsible for vasodilation, increased vascular permeability, increased sensitivity to pain, and bronchoconstriction. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and ibuprofen, work by preventing the actions of prostaglandins.



Source: National Health Interview Survey, National Center for Health Statistics, Centers for Disease Control and Prevention

Figure 14.5 Asthma prevalence in the United States. Data are from 2015 and are displayed with respect to age, gender, and race/ethnicity.

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IgE- and Mast-Cell-Mediated Allergic Conditions

The mechanisms just described lead to the development of hay fever, allergic asthma, food allergy, drug allergy, and eczema. This section covers the main characteristics of these conditions.

Atopic Diseases

Hay fever is a generic term for allergic rhinitis, a seasonal reaction to inhaled plant pollen or molds, or a chronic, year-round reaction to a wide spectrum of airborne allergens or inhalants. The targets are typically respiratory membranes, and the symptoms include nasal congestion; sneezing; coughing; profuse mucus secretion; itchy, red, and teary eyes; and mild bronchoconstriction.

Asthma is a respiratory disease characterized by episodes of impaired breathing due to severe bronchoconstriction. The airways of asthmatic people are exquisitely responsive to minute amounts of inhalants, ingestants, or other stimuli, such as infectious agents. The symptoms of asthma range from occasional, annoying bouts of difficult breathing to fatal suffocation. Labored breathing, shortness of breath, wheezing, cough, and ventilatory **rales** are present to one degree or another. The respiratory tract of an asthmatic person is chronically inflamed and severely overreactive to allergic mediators, especially leukotrienes and serotonin from pulmonary mast cells. Upon activation of the allergic response, natural killer (NK) T cells are recruited and activated, adding to the cytokine storm brewing in the lungs. In the United States it has become apparent that certain groups of children have a higher rate of asthma, and thus higher death rates from it, than others

(figure 14.5). Since 2012 there has been an interagency effort by the U.S. government to reduce the excessively high rate of asthma in African-American children.

Atopic dermatitis is an intensely itchy inflammatory condition of the skin, sometimes also called **eczema**. Sensitization occurs through ingestion, inhalation, and, occasionally, skin contact with allergens. It usually begins in infancy with reddened, weeping, encrusted skin lesions on the face, scalp, neck, and inner surfaces of the limbs and trunk that may progress to a dry, scaly, thickened skin condition in adulthood **(figure 14.6)**. The itchy, painful lesions cause considerable discomfort, and they are predisposed to secondary bacterial infections.

Figure 14.6 **Atopic dermatitis, or eczema**. **(a)** Vesicular, weepy, encrusted lesions are typical in afflicted infants. **(b)** In adulthood, lesions are more likely to be dry, scaly, and thickened.
©Dr. P. Marazzi/Science Source (a); ©Biophoto Associates/Science Source (b)

Food Allergy

The most common food allergens come from peanuts, fish, cow's milk, eggs, shellfish, and soybeans. Although the mode of entry is intestinal, food allergies can also affect the skin and respiratory tract. Gastrointestinal symptoms include vomiting, diarrhea, and abdominal pain. Other manifestations of food allergies include hives, rhinitis, asthma, and occasionally, anaphylaxis. Classic food hypersensitivity involves IgE and degranulation of mast cells, but not all reactions involve

this mechanism. (Do not confuse food allergy with food *intolerance*. Many people are lactose intolerant, for example, due to a deficiency in the enzyme that degrades the milk sugar.) Egg allergies, in particular, must be considered when vaccinating individuals, due to the presence of egg protein in many vaccine preparations.

Drug Allergy

Modern chemotherapy has been responsible for many medical advances. Unfortunately, it has also been hampered by the fact that drugs are foreign compounds capable of stimulating allergic reactions. In fact, allergy to drugs is one of the most common side effects of treatment (occurring in 5% to 10% of hospitalized patients). Depending on the allergen, route of entry, and individual sensitivities, virtually any tissue of the body can be affected, and reactions range from a rash **(figure 14.7)** to fatal anaphylaxis. Compounds implicated most often are antibiotics (penicillin), synthetic antimicrobials (sulfa drugs), aspirin, opiates, and contrast dye used in X rays. The actual allergen is not the intact drug itself but a hapten produced when the liver processes the drug.

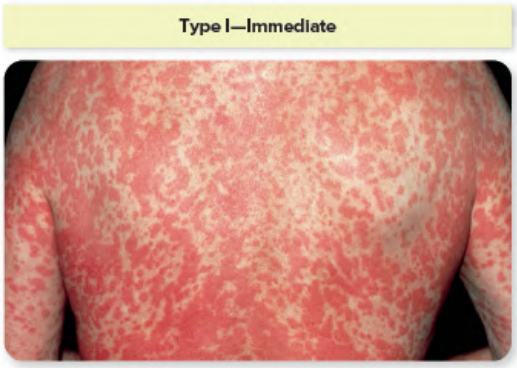

Figure 14.7 **Drug allergy reaction**. A typical rash that develops in an allergic reaction to an antibiotic.
©Dr. P. Marazzi/Science Source

Anaphylaxis: An Overpowering IgE-Mediated Allergic Reaction

The term *anaphylaxis*, or anaphylactic shock, refers to a swift reaction to allergens. Two clinical types of anaphylaxis are seen in humans. *Cutaneous anaphylaxis* is the wheal-and-flare inflammatory reaction to the local injection of allergen. *Systemic anaphylaxis*, on the other hand, is characterized by sudden respiratory and circulatory disruption that can be fatal within minutes. The allergen type and route of entry causing anaphylaxis vary, though bee stings and injection of antibiotics or serum are the most common causes. Bee venom is a complex material containing several allergens and enzymes that can create a sensitivity that can last for decades after exposure.

The underlying physiological events in systemic anaphylaxis parallel those of atopy, but the concentration of chemical mediators and the strength of the response are greatly amplified. The immune system of a sensitized person exposed to a provocative dose of allergen responds with a sudden, massive release of chemicals into the tissues and blood, which act rapidly on the target organs. Anaphylactic persons have been known to die within 15 minutes from complete airway blockage.

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2. Anaphylaxis is characterized by which of the following clinical manifestations? Select all that apply.
- **a.** *circulatory disruption (tachycardia, low blood pressure)*
- **b.** *swelling of the lips, tongue, or throat*
- **c.** *loss of consciousness*
- **d.** *skin wheal and erythema*
- **e.** *itching*

Diagnosis of Allergy

Because allergy mimics infection and other conditions, it is important to determine if a person is actually allergic and to identify the specific allergen or allergens. Allergy testing can be done on blood samples, or directly on the skin.

Blood Testing

The most widely used blood test is a radioallergosorbent test (RAST), which measures levels of IgE to specific allergens. A new test that can distinguish whether a patient has experienced an allergic attack measures elevated blood levels of tryptase, an enzyme released by mast cells that increases during an allergic response. Several types of specific *in vitro* tests can determine the allergic potential of a patient's blood sample. A differential blood cell count can reveal high levels of basophils and eosinophils, indicating allergy. The leukocyte histamine-release test measures the amount of histamine released from the patient's basophils when exposed to a specific allergen.

Skin Testing

A tried and true *in vivo* method to detect precise atopic or anaphylactic sensitivities is skin testing. With this technique, a patient's skin is injected, scratched, or pricked with a small amount of a pure allergen extract. There are hundreds of

these allergen extracts containing common airborne allergens and more unusual allergens (mule dander, theater dust, bird feathers). Unfortunately, skin tests for food allergies using food extracts are unreliable in most cases. In patients with numerous allergies, the allergist maps the skin on the inner aspect of the forearms or back and injects the allergens intradermally according to this predetermined pattern **(figure 14.8*a*)**. Approximately 20 minutes after antigenic challenge, each site is appraised for a wheal response indicative of histamine release. The diameter of the wheal is measured and rated on a scale of 0 (no reaction) to 4 (greater than 15 mm) **(figure 14.8*b*)**.

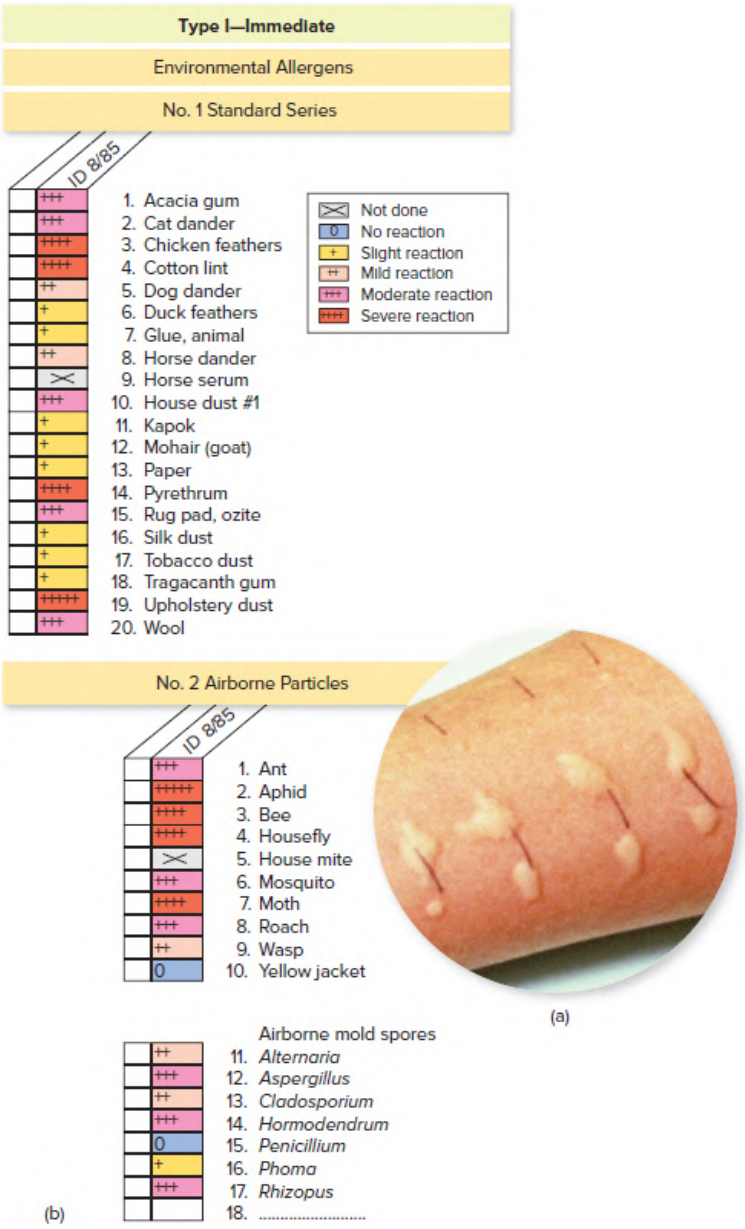

Figure 14.8 A method for conducting an allergy skin test. The forearm (or back) is mapped and then injected with a selection of allergen extracts. The allergist must be very aware of the potential of anaphylactic attacks triggered by these injections. **(a)** Close-up of skin wheals showing a number of positive reactions (dark lines are measurer's marks). **(b)** An actual skin test record for some common environmental allergens [not related to **(a)**].

©Southern Illinois University/Science Source (a)

Treatment and Prevention of Allergy

In general, the methods of treating and preventing type I allergy involve the following **(figure 14.9):**

1. taking drugs that block the action of lymphocytes, mast cells, or chemical mediators;
2. avoiding the allergen, although this may be very difficult in many instances; and

3. desensitization: controlled exposure to the antigen through ingestion, sublingual absorption, or injection to reset the allergic reaction.

1. Taking Drugs to Block Allergy

The aim of antiallergy medication is to block the progress of the allergic response somewhere along the route between IgE production and the appearance of symptoms. Oral anti-inflammatory drugs such as corticosteroids inhibit the activity of lymphocytes and thereby reduce the production of IgE, but they also have dangerous side effects and should not be taken for prolonged periods. Some drugs block the degranulation of mast cells and reduce the levels of inflammatory cytokines (Cromolyn). Asthma and rhinitis sufferers can find relief from a monoclonal antibody that binds to IgE in such a way that it is unable to dock with mast cells. Therefore no degranulation takes place (omalizumab [Xolair®]).

Widely used medications for preventing symptoms of atopic allergy are **antihistamines**, the active ingredients in most over-the-counter allergy-control drugs. Antihistamines interfere with histamine activity by binding to histamine receptors on target organs. Other drugs that relieve inflammatory symptoms are aspirin and acetaminophen, which reduce pain by interfering with prostaglandin, and theophylline, a bronchodilator that reverses spasms in the respiratory smooth muscles. Persons who suffer from anaphylactic attacks are urged to carry at all times injectable or aerosolized epinephrine (adrenaline) and an identification tag indicating their sensitivity. Epinephrine reverses constriction of the airways and slows the release of allergic mediators. Although epinephrine works quickly and well, it has a very short half-life. It is very common to require more than one dose in anaphylactic reactions. Injectable epinephrine buys the individual time to get to a hospital for continuing treatment.

2. Avoiding the Allergen

It is obvious that if you experience a negative health effect when exposed to a substance, then the first goal should be to avoid it. It is the most basic of all preventive measures and is practiced daily by millions of people.

3. Desensitization

In practice avoiding an allergen can be very difficult, as you may have noticed if you're ever on a plane and the flight crew announces that there will be no peanuts available because someone in the cabin has a peanut allergy. But the field of allergy

medicine is changing rapidly. Desensitization, in the form of "allergy shots," has been used for decades. It involves injecting specific amounts of the allergen under the skin.

The allergen preparations contain pure, preserved suspensions of plant antigens, venoms, dust mites, dander, and molds (but so far, desensitization to foods has not proved very effective). The immunologic basis of this treatment is open to differences in interpretation. One hypothesis suggests that injected allergens stimulate the formation of allergen-specific IgG—**blocking antibodies**—that can remove allergen from the system before it can bind to IgE. It is also possible that allergen delivered in this fashion combines with the IgE itself and takes it from circulation.

Researchers are now looking at desensitization treatments that are delivered sublingually (under the tongue) and orally, in order to present the allergen through a mucosal surface, hoping to trigger IgA and IgG responses that would have a blocking effect on the allergic response.

Evidence also suggests that if children are allergic to fresh milk, having them consume it in small amounts in the form of baked goods (in which milk is an ingredient) can help desensitize them. As you know, heating proteins denatures them, changing their physical form. It is possible that the changed presentation of epitopes increases the IgG response—which blocks the antigen binding to the IgE on mast cells, and thus blocks degranulation.

Peanut allergies are one of the scariest for parents, as children can develop anaphylactic symptoms quickly from this allergy. Recent research has shown that "oral immunotherapy," a treatment in which children were given increasing amounts of peanut protein daily, can effectively cure the peanut allergy in a majority of children. Again, this suggests that altering the way the antigen is presented (in this case, the dose; in the previous example, the physical form) can correct an inappropriate response.

There are also a great many clinical trials directed at more generalized parts of the immune system so those with multiple allergies could be helped with a single treatment. Probiotics have been shown to be helpful in some cases, and are still under investigation.

And if you remember, at the beginning of the chapter we inferred that the development of allergies might be the result of the sudden (in evolutionary time) disappearance of worm and protozoan pathogens in the developed world. The parts of immunity, such as IgE, which participate in allergy, are the components that naturally react to helminths and larger microbes. So for several years now scientists (and some amateur allergy sufferers!) have experimented with using deliberate infections with *Trichuris suis*, a whipworm whose natural host is pigs. The worms can establish a brief colonization in humans without causing symptoms, during which time the immune system responds, and in some cases, seems to reset itself so that it stops responding to the inappropriate antigen—the allergen. This approach is still experimental.

Figure 14.9 supplies a summary of ways to interfere with allergy symptoms.

14.2 LEARNING OUTCOMES—Assess Your Progress

3. Summarize genetic and environmental factors that influence allergy development.
4. Identify three conditions caused by IgE-mediated allergic reactions.
5. Identify the two clinical forms of anaphylaxis, explaining why one is more often fatal than the other.
6. List the three main ways to prevent or short-circuit type I allergic reactions.

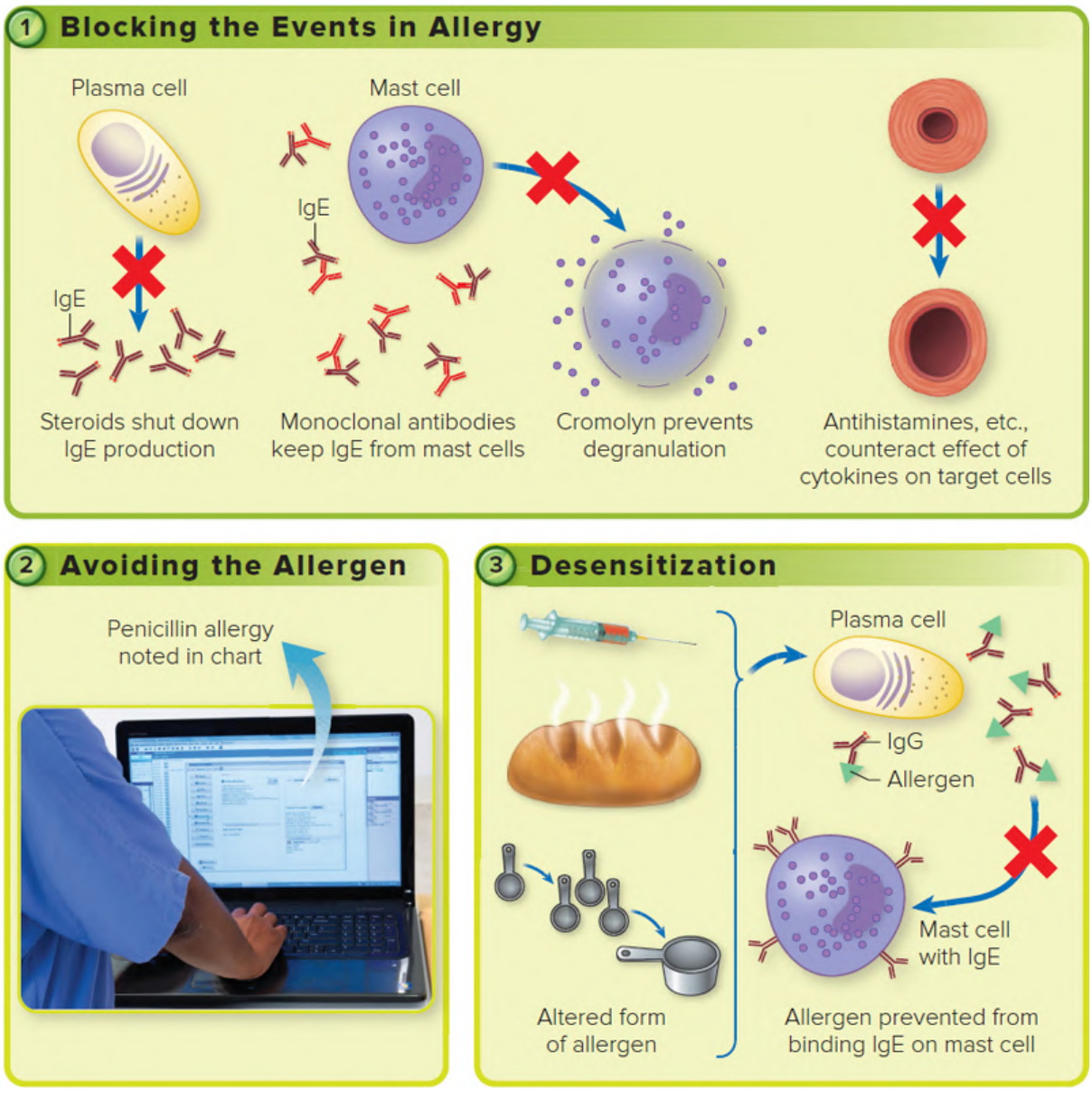

Figure 14.9 **Strategies for combating allergy attacks**.
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14.3 Type II Hypersensitivities: Reactions That Lyse Foreign Cells

The diseases termed type II hypersensitivities are a complex group of syndromes that involve complement-assisted destruction (lysis) of foreign cells by antibodies (IgG and IgM) directed against those cells' surface antigens. This category includes transfusion reactions and some types of autoimmunities (discussed in a later section). The cells targeted for destruction are often red blood cells, but other cells can be involved.

Chapters 12 and 13 described the functions of unique surface markers on cell membranes. Ordinarily, these molecules play essential roles in transport, recognition, and development, but they

become medically important when the tissues of one person are placed into the body of another person. Blood transfusions and organ donations introduce **alloantigens** (molecules that differ in the same species) on donor cells that are recognized by the lymphocytes of the recipient. These reactions are not really immune dysfunctions the way that allergy and autoimmunity are. The immune system is in fact working normally, but it is not equipped to distinguish between the desirable foreign cells of a transplanted tissue and the undesirable ones of a microbe.

The Rh Factor and Its Clinical Importance

We are all aware of the need to match blood types when transfusing blood into a person. Those blood types are matched on the basis of the A, B, and O antigens on the surfaces of red blood cells, as well as another antigen, called the **Rh factor** (or D antigen). Because the Rh factor can cause disease in natural circumstances (a normal pregnancy), we will use it to illustrate type II hypersensitivities. This factor was first discovered in experiments exploring the genetic relationships among animals. Rabbits inoculated with the red blood cells (RBCs) of rhesus monkeys produced an antibody to an antigen on the rhesus blood cells. That antibody also reacted to a similar antigen on human blood cells. Further tests showed that the antigen that both monkeys and humans have on their blood cells (termed *Rh* for "rhesus") was present in about 85% of humans and absent in the other 15%. (These percentages vary in different ethnic groups.) The details of Rh inheritance are complicated, but in simplest terms, a person's Rh type results from a combination of two possible alleles—a dominant one that codes for the factor and a recessive one that does not. The "+" or "−" that appears after a blood type (i.e., O+) reflects the Rh status of the person. Unlike with the ABO antigens where antibodies are preformed against foreign blood antigens, the only ways one can develop antibodies against this factor are through exposure to a fetus's antigen while pregnant or through blood transfusion. Although the Rh factor should be matched for a transfusion to avoid this situation, it is acceptable to transfuse Rh– blood if the Rh type is not known.

Hemolytic Disease of the Newborn and Rh Incompatibility

The potential for placental sensitization occurs when a mother is Rh− and her unborn child is Rh+. The mother's immune system detects the foreign Rh factors on the fetal RBCs and is sensitized to them by producing antibodies and memory B cells. The first Rh+ child is usually not affected because the process begins so late in pregnancy that the child is born before maternal sensitization is completed. However, the mother's immune system has been strongly primed for a second contact with this factor in a subsequent pregnancy **(figure 14.10*a*)**.

In the next pregnancy with an Rh+ fetus, fetal blood cells escape into the maternal circulation late in pregnancy and elicit a memory response. Maternal anti-Rh antibodies then cross the placenta into the fetal circulation, where they affix to fetal RBCs and cause complement-mediated lysis. The outcome is a potentially fatal **hemolytic disease of the newborn (HDN)**, characterized by severe anemia and jaundice. It is also called *erythroblastosis fetalis* (eh-rith″-roh-blas-toh′-sis fee-tal′-is), reflecting the release of immature nucleated RBCs (erythroblasts) into the blood to compensate for destroyed RBCs.

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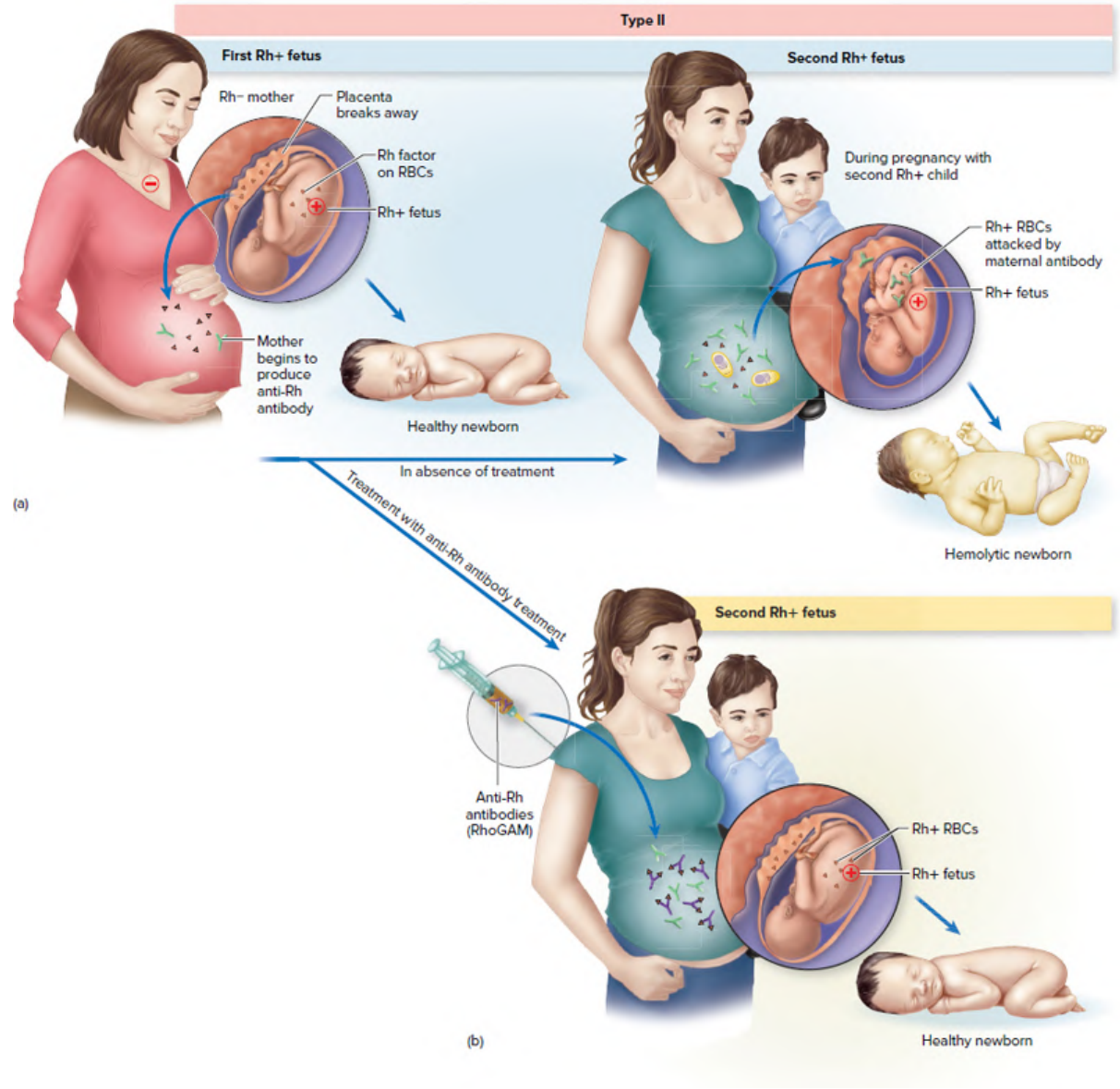


Figure 14.10 **Development and control of Rh incompatibility**. **(a)** A naturally occurring blood cell incompatibility results when an Rh+ fetus develops within an Rh− mother. Initial sensitization of the maternal immune system occurs when fetal blood passes the placental barrier. In most cases, the fetus develops normally. However, a subsequent pregnancy with an Rh+ fetus results in a severe fetal hemolysis characterized by jaundice. **(b)** Control of incompatibility: Anti-Rh antibody (RhoGAM) can be administered to Rh− mothers during pregnancy to help bind, inactivate, and remove any Rh factor that may be transferred from the fetus. In some cases, RhoGAM is administered before sensitization occurs.

Maternal-fetal incompatibilities are also possible in the ABO blood group, but adverse reactions occur less frequently than with Rh sensitization because the antibodies to these blood group antigens are IgM rather than IgG and are unable to cross the placenta in large numbers. In fact, the maternal-fetal relationship is a fascinating instance of foreign tissue not being rejected, despite the extensive potential for contact.

Preventing Hemolytic Disease of the Newborn

Once sensitization of the mother to Rh factor has occurred, all other Rh+ fetuses will be at risk for hemolytic disease of the newborn. Prevention requires a careful family history of an Rh− pregnant woman. If the father is also Rh−, the child will be Rh− and free of risk; but if the father is Rh+, there is a possibility that the fetus may be Rh+. In this case, the mother must be passively immunized with antiserum containing antibodies against the Rh factor. This antiserum is called RhoGAM. It is the immunoglobulin fraction of human anti-Rh serum, prepared from pooled human sera. This antiserum, injected at 28 to 32 weeks and again immediately after delivery, reacts with any fetal RBCs that have escaped into the maternal circulation, thereby preventing the sensitization of the mother's immune system to Rh factor **(figure 14.10*b*)**. Anti-Rh antibody must be given with each pregnancy that involves an Rh+ fetus, but it is ineffective if the mother has been previously sensitized.

14.3 LEARNING OUTCOMES—Assess Your Progress

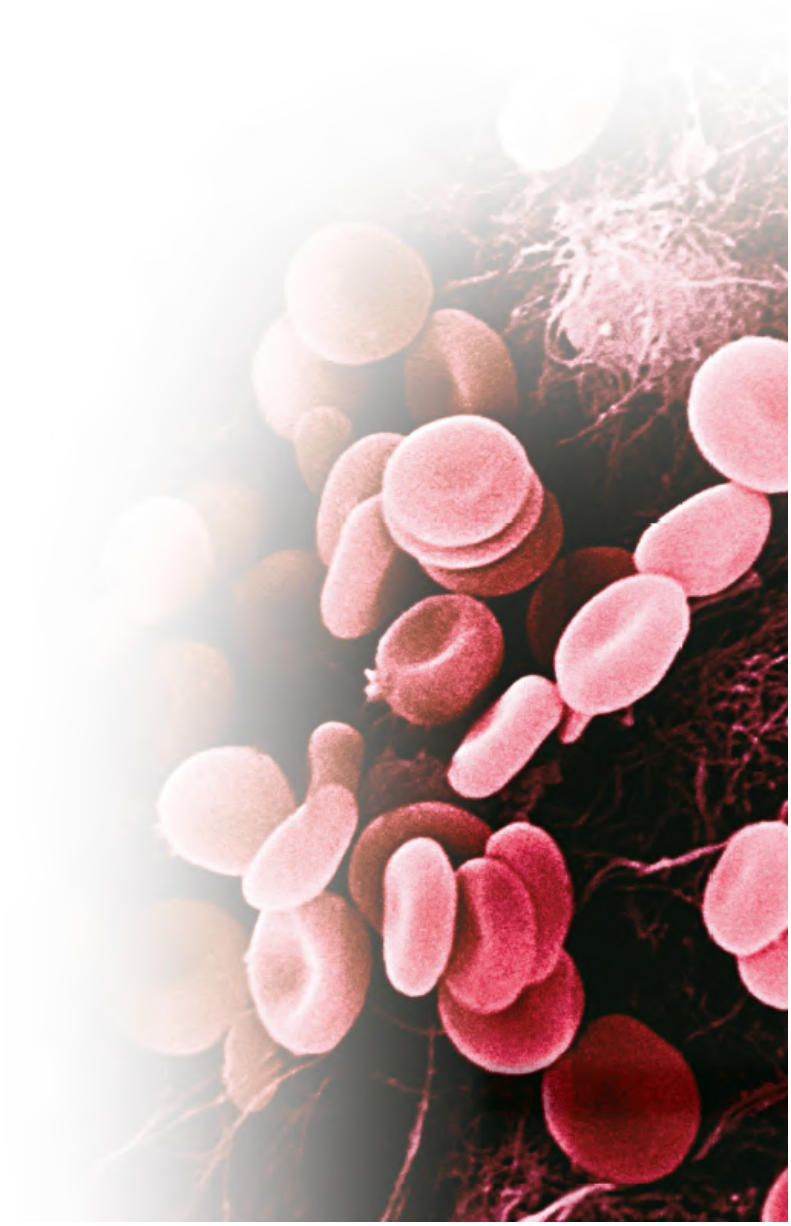
7. List the three immune components causing cell lysis in type II hypersensitivity reactions.
8. Explain the role of Rh factor in hemolytic disease development and how it is prevented in newborns.

14.4 Type III Hypersensitivities: Immune Complex Reactions

Type III hypersensitivity involves the reaction of soluble antigen with antibody and the deposition of the resulting complexes in various tissues of the body. It is similar to type II, because it involves the production of IgG and IgM antibodies after repeated exposure to antigens and the activation of complement. Type III differs from type II because the antigens that provoke it are not attached to the surface of a cell. The interaction of these antigens with antibodies produces free-floating complexes that can be deposited in the tissues, causing an **immune complex reaction**, or disease.

Mechanisms of Immune Complex Disease

After initial exposure to a large amount of antigen, the immune system produces large quantities of antibodies that circulate in the fluid compartments. When this antigen enters the system a second time, it reacts with the antibodies to form antigen-antibody complexes. These complexes recruit inflammatory components such as complement and neutrophils, which would ordinarily eliminate Ag-Ab complexes as part of the normal immune response. In an immune complex disease, however, there is an excess of antigen and small amounts of antibody. This causes cross linking of multiple antigens with the scarce antibodies, and the complexes can then deposit in the **basement membranes** of epithelial tissues and become inaccessible. In response to these events, neutrophils release lysosomal granules that digest tissues and cause a destructive inflammatory condition.



Red blood cells
©Image Source/Getty Images

Types of Immune Complex Disease

In the early days of using antiserums as treatments for infectious diseases, hypersensitivity reactions to serum were common. In addition to anaphylaxis, two syndromes were identified, the **Arthus reaction** and **serum sickness**, associated with certain types of passive immunization (especially with animal serum).

Serum sickness and the Arthus reaction are like anaphylaxis in that all three of them require sensitization, which leads to preformed antibodies. Characteristics that set serum sickness and the Arthus reaction apart from anaphylaxis are as follows:

1. they depend on IgG, IgM, or IgA (precipitating antibodies) rather than IgE;
2. they require large doses of antigen (not a minuscule dose as in anaphylaxis); and
3. their symptoms are delayed (a few hours to days).

The Arthus reaction is a *localized* dermal injury due to inflamed blood vessels in the vicinity of any injected antigen. Serum sickness, however, is a *systemic* injury initiated by antigen-antibody complexes that circulate in the blood and settle into membranes at various sites.

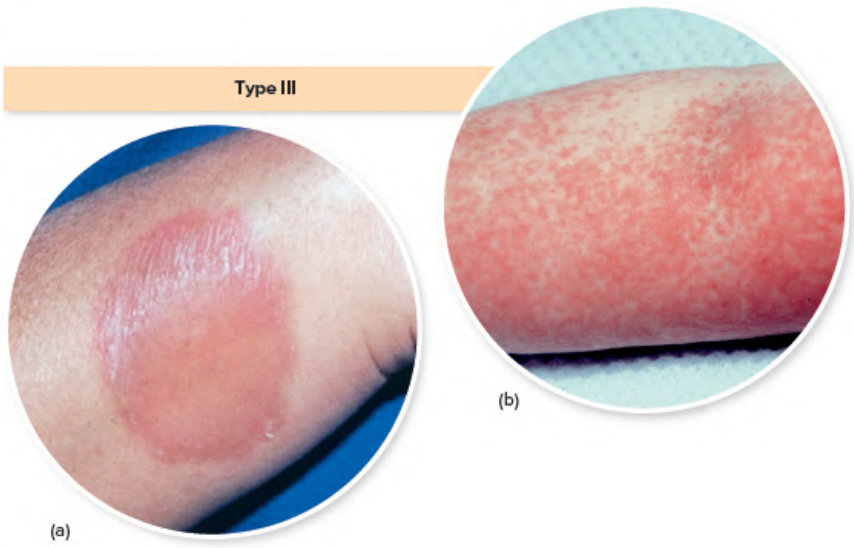


Figure 14.11 **Typical presentation of the Arthus reaction (a) and serum sickness (b), two immune complex diseases.**
©Koshi Johnson/Medical Images (a); Courtesy Gary P. Wiliams, M.D. (b)

The Arthus Reaction

The Arthus reaction is usually an acute response to a second injection of drugs or vaccines (boosters) at the same site as the first injection. In a few hours, the area becomes red, hot to the touch, swollen, and very painful **(figure 14.11*a*)**. These symptoms are mainly due to the destruction of tissues in and around the blood vessels and the release of histamine from mast cells and basophils. Although the reaction is usually self-limiting and rapidly cleared, intravascular blood clotting can occasionally cause necrosis and loss of tissue.

Serum Sickness

Serum sickness was named for a condition that appeared in soldiers after repeated injections of horse serum to treat tetanus, though it is also caused by injections of animal hormones and drugs. The immune complexes enter the circulation; are carried throughout the body; and are eventually deposited in blood vessels of the kidney, heart, skin, and joints. The condition can become chronic, causing symptoms such as enlarged lymph nodes, rashes, painful joints, swelling, fever, and renal dysfunction **(figure 14.11*b*)**.

Medical Moment

Patch Testing

Patch testing is an alternative to skin testing for allergies. In patch testing, allergens are introduced onto a patch, which is then secured to the skin. Patch testing is useful for diagnosing delayed hypersensitivity reactions. It is also useful for determining whether a particular substance is causing contact dermatitis, or irritation of the skin caused by an allergic reaction. Patch testing can be used to detect allergy to hair dyes, fragrances, latex, resins, and metals such as silver.

The patches are worn on the skin (usually the back) for 48 hours, at which time the patches are removed and the skin is assessed for irritation. During patch testing, it is important to avoid bathing, showering, or activities that may result in heavy perspiration (i.e., working outdoors or exercising).

Q. Which cells of the immune system do you think are responsible for processing the antigen presented in a patch test?

Answer in Appendix B.

14.4 LEARNING OUTCOMES—Assess Your Progress

9. Identify commonalities and differences between type II and type III hypersensitivities.

14.5 Type IV Hypersensitivities: Cell-Mediated (Delayed) Reactions

The adverse immune responses we have covered so far are explained primarily by B-cell involvement and antibodies. Type IV hypersensitivity is different; it involves primarily the T-cell branch of the immune system. In general, type IV diseases result when T cells respond to antigens displayed on self tissues or transplanted foreign cells. Type IV immune dysfunction has traditionally been known as delayed hypersensitivity because the symptoms arise one to several days following the second contact with an antigen.

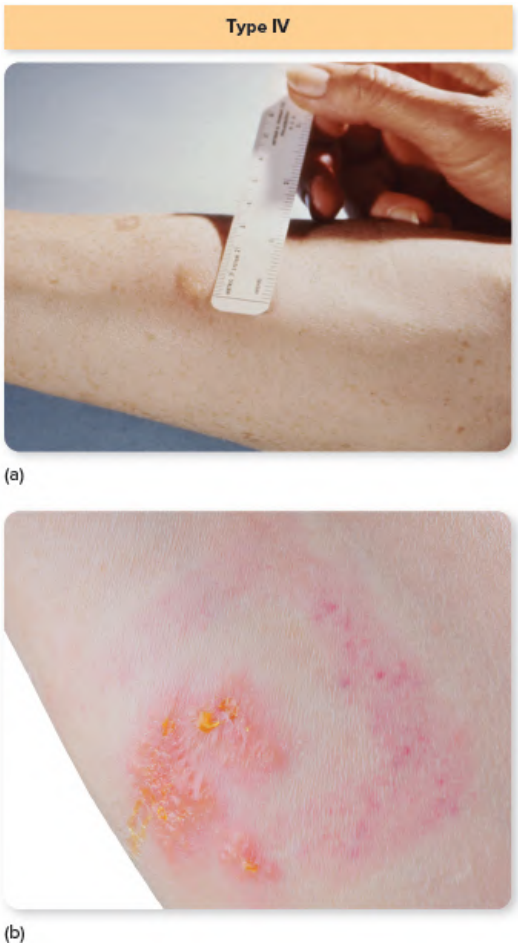


Figure 14.12 **Type IV delayed reactions**. **(a)** Positive tuberculin test. Intradermal injection of tuberculin extract in a person sensitized to tuberculosis yields a slightly raised red bump greater than 10 mm in diameter. **(b)** Contact dermatitis from poison oak, showing various stages of involvement: blister, scales, and thickened patches.
Source: CDC/Donald Kopanoff (a); ©carroteater/Shutterstock (b)

Infectious Allergy

A classic example of delayed-type hypersensitivity occurs when a person sensitized by tuberculosis infection is injected with an extract (tuberculin) of the bacterium *Mycobacterium tuberculosis*. The so-called tuberculin reaction is an acute skin inflammation at the injection site appearing within 24 to 48 hours. This has been the mainstay diagnostic tool for TB infection for decades, and still is. This form of hypersensitivity arises from time-consuming cellular events involving a specific class of T cells (T_H1) and their release of cytokines that recruit various inflammatory cells such as macrophages, neutrophils, and eosinophils. The buildup of fluid and cells at the site gives rise to a red bump **(figure 14.12*a*)**.

Contact Dermatitis

The most common delayed allergic reaction, contact dermatitis, is caused by exposure to resins in poison ivy or poison oak, to simple haptens in household and personal articles (jewelry, cosmetics, elasticized undergarments), and to certain drugs. Like immediate atopic dermatitis, the reaction to these allergens requires a sensitizing dose followed by a provocative dose. The allergen first

penetrates the outer skin layers, is processed by Langerhans cells (skin dendritic cells), and presented to T cells. When subsequent exposures attract lymphocytes and macrophages to this area, these cells release enzymes and inflammatory cytokines that damage the epidermis in the immediate vicinity. This response accounts for the intensely itchy papules and blisters that are the early symptoms **(figure 14.12*b*)**. As healing progresses, the epidermis is replaced by a thick, keratinized layer.

T Cells and Their Role in Organ Transplantation

Transplantation or grafting of organs and tissues is a common medical procedure. Although it is life-saving, this technique is plagued by the natural tendency of lymphocytes to seek out foreign antigens and mount a campaign to destroy them. The bulk of the damage that occurs in graft rejections can be attributed to cytotoxic T-cell action.

The Genetic and Biochemical Basis for Graft Rejection

In chapter 13, we learned that the genes and markers in major histocompatibility (MHC or HLA) classes I and II are extremely important in recognizing self and in regulating the immune response. Although the cells of each person can exhibit variability in the pattern of these cell surface molecules, the pattern is identical in different cells of the same person. Similarity is seen among related siblings and parents, but the more distant the relationship, the less likely that the MHC genes and markers will be alike. When donor tissue (a graft) displays surface molecules of a different MHC class, the T cells of the recipient (called the host) will recognize its foreignness and react against it.

T-Cell-Mediated Recognition of Foreign MHC Receptors

Host Rejection of Graft When the cytotoxic T cells of a host recognize foreign class I MHC markers on the surface of grafted cells, they release interleukin-2 as part of a general immune mobilization **(figure 14.13)**. Antigen-specific helper and cytotoxic T cells bind to the grafted tissue and secrete lymphokines that begin the rejection process within 2 weeks of transplantation. Antibodies formed against the transplanted tissue contribute to the damage, resulting in the destruction of the vascular supply and death of the graft.

Graft Rejection of Host Graft incompatibility is a two-way phenomenon. Some grafted tissues (especially bone marrow) contain an indigenous population

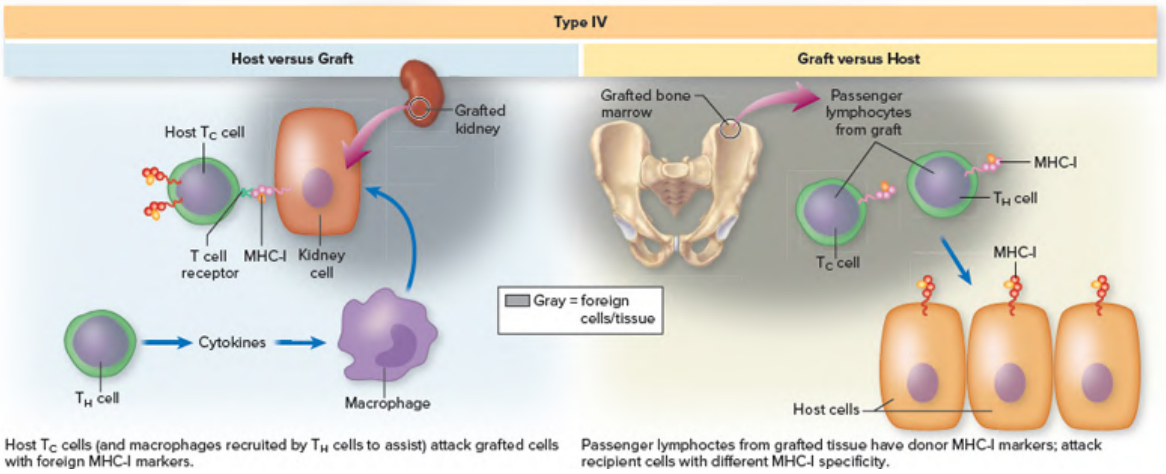


Figure 14.13 **Development of incompatible tissue graft reactions**.

called passenger lymphocytes (figure 14.13). This makes it quite possible for the graft to reject the host, causing **graft versus host disease (GVHD)**. Because any host tissue bearing MHC markers foreign to the graft can be attacked, the effects of GVHD are widely systemic and toxic. A papular, peeling skin rash is the most common symptom though other organs are also affected. GVHD typically occurs within 100 to 300 days of the graft.

Classes of Grafts

Grafts are generally classified according to the genetic relationship between the donor and the recipient **(figure 14.14)**. Tissue transplanted from one site on an individual's body to another site on his or her body is known as an **autograft**. Typical examples are skin replacement in burn repair and the use of a vein to fashion a coronary artery bypass. In an **isograft**, tissue from an identical twin is used. Because isografts do not contain foreign antigens, they are not rejected. **Allografts**, the most common type of grafts, are exchanges between genetically different individuals belonging to the same species (two humans). A close genetic correlation is sought for most allograft transplants (see next section). A **xenograft** is a tissue exchange between individuals of different species.

Identical twins are the only people with exactly matching tissue antigens.
©Rubberball/Getty Images

Types of Transplants

Over 30,000 people receive transplants each year in the United States, while over 119,000 people are on a waiting list for one. Transplantation can be performed with every major organ, including parts of the brain and even faces. But it most often involves the kidney, liver, heart, skin, coronary artery, cornea, and bone marrow. Kidney transplants are by far the most common in the United States. The sources of organs and tissues are live donors, the recently deceased, and fetal tissues.

In the past decade, advancements in transplantation science have expanded the possibilities for treatment and survival. Fetal tissues have been used in the treatment of diabetes and Parkinson disease, while parents have successfully donated portions of their organs to help save their children suffering from the effects of cystic fibrosis or liver disease. Recent advances in stem cell technology have made it possible to isolate stem cells more efficiently

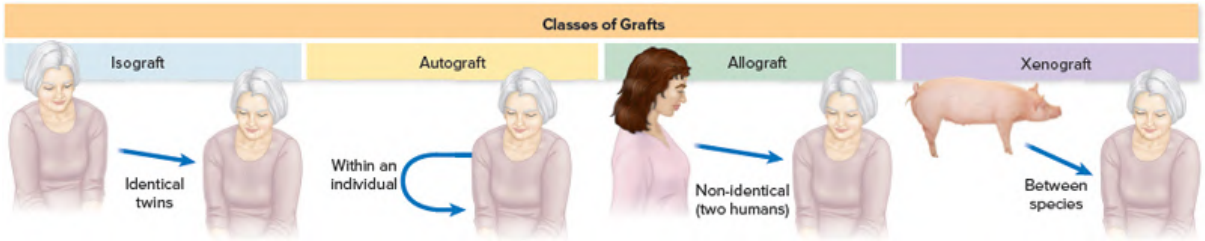


Figure 14.14 **Four classes of tissue grafts**.
©iStockphoto/Getty Images

from blood donors, and the use of umbilical cord blood cells has furthered progress in this area of science. Though many hurdles still exist, scientists are using genetic engineering technology to

develop an ample supply of immunologically compatible, safe tissues for xenotransplantation.

Bone marrow transplantation is used in patients with immune deficiencies, aplastic anemia, leukemia and other cancers, and radiation damage. Before closely matched donor marrow can be infused, the patient is pretreated with chemotherapy and whole-body irradiation to destroy the person's own blood stem cells, preventing rejection of the new marrow cells. The donor marrow cells are then dripped intravenously into the circulatory system, and the new cells settle automatically into the appropriate bone marrow regions. However, because donor lymphoid cells can still cause GVHD, antirejection drugs may be necessary. Interestingly, after transplantation, a recipient's blood type may change to the blood type of the donor.

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3. A patient receives a kidney transplantation from a nonrelated human donor. Which of the following terms properly describes the tissue graft?

a. *autograph*
b. *allograft*
c. *isograft*
d. *xenograft*

14.5 LEARNING OUTCOMES—Assess Your Progress

10. Describe one example of a type IV delayed hypersensitivity reaction.
11. List four classes of grafts, and explain how host versus graft and graft versus host diseases develop.

14.6 An Inappropriate Response to Self: Autoimmunity

The immune diseases we have covered so far are all caused by foreign antigens. In the case of autoimmunity, individuals actually develop hypersensitivity to themselves. This pathologic process accounts for **autoimmune diseases**, in which **autoantibodies**, T cells, and, in some cases, both, mount an abnormal attack against self antigens. The scope of autoimmune disease includes *systemic*, involving several major organs, or *organ-specific* reactions, involving only one organ or tissue. There are more than 80 different autoimmune diseases, together affecting up to 23 million Americans. Many of these diseases result in catastrophic consequences. For example, people with rheumatoid arthritis have a 60% increased risk of death from cardiovascular disease. Some major diseases, their targets, and basic pathology are presented in **table 14.2**.

Organ transplants have saved hundreds of thousands of lives.
©Getty Images/OJO Images

Table 14.2 Selected Autoimmune Diseases

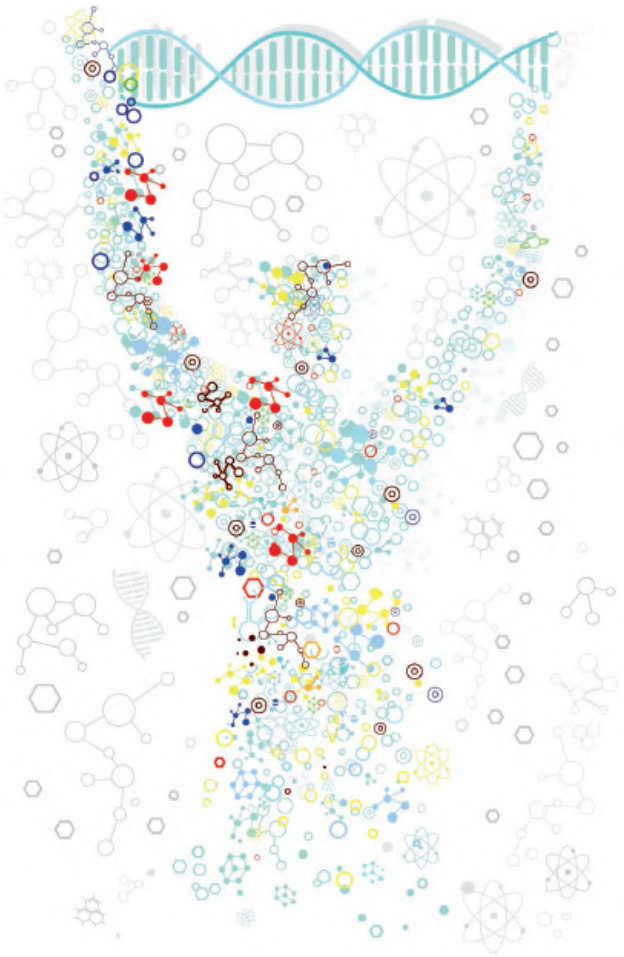
Disease	Target	Type of Hypersensitivity	Characteristics
Systemic lupus erythematosus (SLE)	Systemic	III	Inflammation of many organs; antibodies against red and white blood cells, platelets, clotting factors, nucleus DNA
Rheumatoid arthritis and ankylosing spondylitis	Systemic	II, III, and IV	Vasculitis; frequent target is joint lining; antibodies against other antibodies (rheumatoid factor), T-cell cytokine damage
Graves' disease	Thyroid	II	Antibodies against thyroid-stimulating hormone receptors
Myasthenia gravis	Muscle	II	Antibodies against the acetylcholine receptors on the nerve-muscle junction alter function
Type 1 diabetes	Pancreas	IV	T cells attack insulin-producing cells
Multiple sclerosis	Myelin	II and IV	T cells and antibodies sensitized to myelin sheath destroy neurons

Possible Causes of Autoimmune Disease

The root cause of autoimmune disease is still a frustrating mystery for the most part. We present some factors that influence—or may influence—the development of autoimmunity.

Genetics Susceptibility can be influenced strongly by genetics and gender. Cases cluster in families, and even unaffected members tend to develop the autoantibodies for that disease. Studies show particular genes in the class I and II major histocompatibility complex coincide with certain autoimmune diseases. For example, autoimmune joint diseases such as rheumatoid arthritis and ankylosing spondylitis are more common in persons with the B-27 HLA type. With the expansion of genomic technology and the screening of whole genomes, many novel genes have recently been found to play a role in the pathway to autoimmunity. Some research suggests that autoimmune symptoms are a side effect of the body's immune response to cancer that is developing in the body (and often does not come to fruition, though the autoimmune disease remains).

A moderate, regulated amount of autoimmunity is probably required to dispose of old cells and cellular debris. Disease apparently arises when this regulatory or recognition apparatus goes awry. Sometimes the processes go awry due to genetic irregularities or inherent errors in the host's physiological processes. In a large subset of cases, however, microbes are behind the malfunctioning.



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Molecular Mimicry This is a process in which microbial antigens bearing molecular determinants similar to human cells induce the formation of antibodies that can cross-react with normal tissues. This is one possible explanation for the pathology of rheumatic fever. Similarly, T cells primed to react with streptococcal surface proteins also appear to react with keratin cells in the skin, causing them to proliferate. For this reason, psoriasis patients often report flare-ups after a strep throat infection.

Infection Autoimmune disorders such as type 1 diabetes and multiple sclerosis are possibly triggered by *viral infection*. Viruses can noticeably alter cell receptors, thereby causing immune cells to attack the tissues bearing viral receptors.

The Gut Microbiome Research has shown us the importance of the gut microbiome. We have also seen that the composition of the gut microbiome has changed over the last 50 years, due to the use of antibiotics, less exposure to the outdoors, and an altered, more artificial, diet. Many scientists connect the rise in autoimmune diseases over the same time period to this phenomenon. One direction these studies are going is the idea that a healthy microbiome is fundamentally important to training our immune system what to react against and what to tolerate. Drastic changes in the microbiome would be expected to disrupt this training process.

There have also been specific connections drawn between members of the microbiome and autoimmunity. For example, mice prone to rheumatoid arthritis have a much higher percentage of a genus of bacterium called *Prevotella* than those not

Figure 14.15 **Common autoimmune diseases**. **(a)** Systemic lupus erythematosus. One symptom is a prominent rash across the bridge of the nose and on the cheeks. These papules and blotches can also occur on the chest and limbs. **(b)** Rheumatoid arthritis commonly targets the synovial membrane of joints. Over time, chronic inflammation causes thickening of this membrane, erosion of the articular cartilage, and fusion of the joint. These effects severely limit motion and can eventually swell and distort the joints.

©ISM/CID/Medical Images (a); ©Aaron Roeth Photography (b)

prone to it. Other scientists are getting ever more specific. It was noticed that a substance from a member of a healthy microbiome, called polysaccharide A (PSA) protects mammals from multiple sclerosis symptoms, and it is being developed as a therapeutic treatment. Many researchers predict

that altering the microbiome, or using chemicals from a healthy microbiome, will be a major treatment strategy for autoimmune diseases within 10 to 15 years.

Examples of Autoimmune Disease

Systemic Autoimmunities

One of the most severe chronic autoimmune diseases is systemic lupus erythematosus (SLE, or lupus). This name originated from the characteristic butterfly-shaped rash that drapes across the nose and cheeks **(figure 14.15*a*)**, as ancient physicians thought the rash resembled a wolf bite on the face (*lupus* is Latin for "wolf"). Although the manifestations of the disease vary considerably, all SLE patients produce autoantibodies against a variety of targets, including organs and tissues or intracellular materials, such as the nucleoprotein of the nucleus and mitochondria. It is not known how such a generalized loss of self-tolerance arises, though viral infection and loss of normal immune response suppression are suspected.

Rheumatoid arthritis, another systemic autoimmune disease, causes progressive, debilitating damage to the joints and at times to the lungs, eyes, skin, and nervous system **(figure 14.15*b*)**. In the joint form of the disease, autoantibodies form immune complexes that bind to the synovial membrane of the joints, which activates phagocytes to release cytokines. Chronic inflammation develops and leads to scar tissue and joint destruction. These cytokines (i.e., tumor necrosis factor [TNF]) can then trigger additional type IV delayed hypersensitivity responses. An IgM antibody, called rheumatoid factor (RF), is present in some, but not all, cases. Treatment has recently involved the targeting of TNF or TNF-mediated pathways, but new drugs, targeting other immune system components, are now appearing. This is a different disease than osteoarthritis, which is caused by the wearing down of cartilage between bones and is a more or less natural consequence of aging.

Autoimmunities of the Endocrine Glands

The underlying cause of **Graves' disease** is the attachment of autoantibodies to receptors on the thyroxin-secreting follicle cells of the thyroid gland. The abnormal stimulation of these cells causes the overproduction of this hormone and the symptoms of hyperthyroidism, which affect nearly every body system.

Type 1 diabetes is another condition that may be a result of autoimmunity. Insulin, secreted by the beta cells in the pancreas, regulates the utilization of glucose

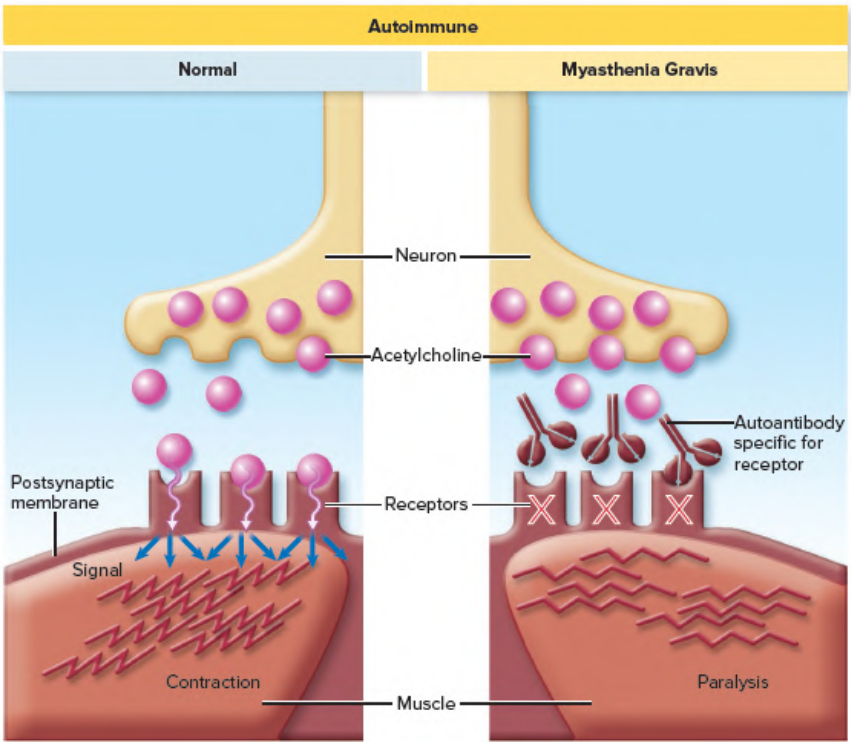

Figure 14.16 Mechanism for involvement of autoantibodies in myasthenia gravis. Antibodies developed against receptors on the postsynaptic membrane block them so that acetylcholine cannot bind and muscle contraction is inhibited.

by cells. Molecular mimicry has been implicated in the sensitization of cytotoxic T cells in type 1 diabetes, which leads to the lysis of beta cells. The reduced amount of insulin underlies the symptoms of this disease.

Neuromuscular Autoimmunities

Myasthenia gravis is a syndrome caused by autoantibodies binding to the receptors for acetylcholine, a chemical required to transmit a nerve impulse across the synaptic junction to a muscle **(figure 14.16)**. The first effects are usually felt in the muscles of the eyes and throat, but the syndrome eventually progresses to complete loss of skeletal muscle function and death. Current treatment usually includes immunosuppressive drugs and therapy to remove the autoantibodies from the circulation.

Multiple sclerosis (MS) is a paralyzing neuromuscular disease associated with lesions in the insulating myelin sheath that surrounds neurons in the white matter of the central nervous system. T-cell and autoantibody-induced damage severely compromise the capacity of neurons to send impulses, resulting in muscular weakness and tremors, difficulties in speech and vision, and paralysis. Data suggest a possible association between infection with human herpesvirus 6 and the onset of disease. Immunosuppressants like cortisone and interferon beta alleviate symptoms, and the disease can be treated with monoclonal antibody therapy toward certain T-cell antigens.

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14.6 LEARNING OUTCOMES—Assess Your Progress

12. List at least three autoimmune diseases and the most important immunologic features in them.

Table 14.3 General Categories of Immunodeficiency Diseases with Selected Examples

Primary Immune Deficiencies (Genetic)	Secondary Immune Deficiencies (Acquired)
B-cell defects (low levels of B cells and antibodies) Agammaglobulinemia (X-linked, non-sex-linked) Hypogammaglobulinemia Selective immunoglobulin deficiencies **T-cell defects (lack of all classes of T cells)** Thymic aplasia (DiGeorge syndrome)	**From natural causes** Infections (AIDS) or cancers Nutrition deficiencies Stress Pregnancy Aging

Primary Immune Deficiencies (Genetic)	Secondary Immune Deficiencies (Acquired)
Combined B-cell and T-cell defects (usually caused by lack or abnormality of lymphoid stem cell) Severe combined immunodeficiency (SCID) disease Adenosine deaminase (ADA) deficiency **Complement defects** Lacking one of C components Hereditary angioedema associated with rheumatoid diseases	**From immunosuppressive agents** Irradiation Severe burns Steroids (cortisones) Immunosuppressive drugs Removal of spleen

14.7 Immunodeficiency Diseases: Hyposensitivity of the Immune System

Occasionally, errors occur in the development of the immune system, and a person is born with or develops weakened immune responses, called **immunodeficiencies**. The most obvious consequences of immunodeficiencies are recurrent, overwhelming infections, often with opportunistic microbes. Immunodeficiencies fall into two general categories: *primary diseases*, present at birth (congenital) and usually stemming from genetic errors, and *secondary diseases*, acquired after birth and caused by natural or artificial agents **(table 14.3)**.

Medical Moment

Hand Washing

The importance of hand washing cannot be overemphasized, particularly when caring for patients who have compromised immune systems. Such a simple step can be lifesaving for patients who are unable to fight off infection. Recognizing this fact, hospitals have made a conscious and concentrated effort to increase the number of hand washing stations available to increase compliance among health care workers.

Alcohol-based hand sanitizers have provided an additional option of hand antisepsis. Hand sanitizer should be applied liberally to the palm of one hand. The hands should then be rubbed together, dispersing the sanitizer over the surfaces of both hands, including the fingers and wrists. When hands are visibly soiled, clean running water and soap should be used.

Q. Using hand sanitizers is considered by many to be more effective than hand washing (except when hands are visibly soiled). Can you think of a reason why that might be?

Answer in Appendix B.

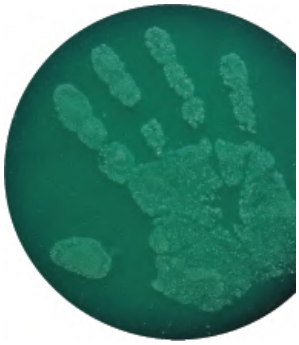
Primary Immunodeficiency Diseases

Primary deficiencies affect both specific immunities and less-specific ones such as phagocytosis. Consult **figure 14.17** to survey the places in the normal sequential development of lymphocytes, where defects can occur, and the possible consequences. In many cases, the deficiency is due to an inherited abnormality, though the exact nature of the abnormality is not known for a number of diseases. In some deficiencies, the lymphocyte in question is completely absent or is present at very low levels. In other cases, lymphocytes are present but do not function normally. Because the development of B cells and T cells diverges at some point, an individual can lack one or both cell lines. It must be emphasized, however, that some deficiencies affect other cell functions as well.

Clinical Deficiencies in B-Cell Development or Expression

Genetic deficiencies in B cells usually result in abnormal immunoglobulin expression. In some instances, only certain immunoglobulin classes are absent; in others, the levels of all types of immunoglobulin (Ig) are reduced.

The term **agammaglobulinemia** literally means the absence of gamma globulin, the component of serum that contains immunoglobulins. Because it is very rare for Ig to be completely absent, some physicians prefer the term **hypogammaglobulinemia**. The symptoms of recurrent, serious bacterial infections usually appear about 6 months after birth. The most common infection sites are the lungs, sinuses, meninges, and blood. Many Ig-deficient patients can have recurrent infections with viruses and protozoa, as well. The current treatment for this condition is passive immunotherapy with immune serum globulin.



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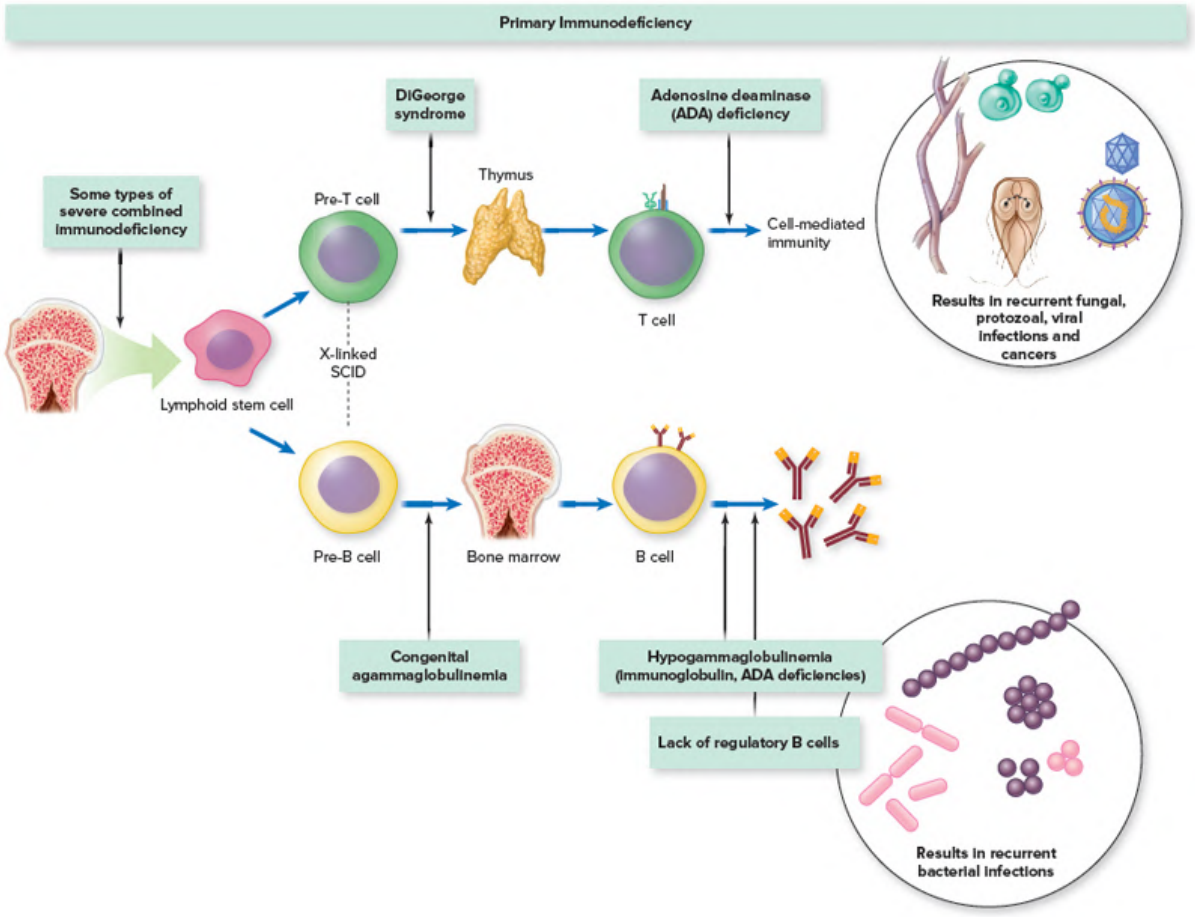

Figure 14.17 **The stages of development and the functions of B cells and T cells, whose failure causes immunodeficiencies**. Where the black arrows for each condition occur, no further development takes place from that point forward.

AIDS is a secondary immunodeficiency disease.
©McGraw-Hill Education/Christopher Kerrigan, photographer

The lack of one particular class of immunoglobulin is a relatively common condition, though its underlying genetic mechanisms are not clear. IgA deficiency is the most prevalent form, where patients have normal quantities of B cells and other immunoglobulins but are unable to synthesize IgA. Consequently, they lack protection against local microbial invasion of the mucous membranes and suffer recurrent respiratory and gastrointestinal infections.

Clinical Deficiencies in T-Cell Development or Expression

Due to T-cells' critical role in immune defenses, a genetic defect in T-cell development results in a broad spectrum of disease, including severe opportunistic infections and cancer. In fact, a dysfunctional

T-cell line is usually more devastating than a defective B-cell line because T helper cells are required to assist in most specific immune reactions.

Abnormal Development of the Thymus The most severe of the T-cell deficiencies involves the congenital absence or immaturity of the thymus. Thymic aplasia, or **DiGeorge syndrome**, makes children highly susceptible to persistent infections by fungi, protozoa, and viruses. Vaccinations using live, attenuated microbes pose a danger, and common childhood infections such as chickenpox can be overwhelming and fatal in these children. Patients typically have reduced antibody levels as well.

Severe Combined Immunodeficiencies: Dysfunction in B and T Cells

Severe combined immunodeficiencies (SCIDs) are the most serious and potentially lethal forms of immunodeficiency disease because they involve dysfunction in both lymphocyte systems. Some SCIDs are due to the complete absence of the lymphocyte stem cell in the marrow; others are attributable to the dysfunction of B cells and T cells later in development. Infants with SCID usually manifest the T-cell deficiencies within days after birth by developing candidiasis, sepsis, pneumonia, or systemic viral infections.

In the two most common forms, Swiss-type agammaglobulinemia and thymic alymphoplasia, genetic defects in the development of the lymphoid cell line result in extremely low numbers of all lymphocyte types and poorly developed humoral and cellular immunity. A rare form of SCID, called **adenosine deaminase (ADA) deficiency**, is caused by an autosomal recessive defect in the metabolism of adenosine. In this case, lymphocytes develop but a metabolic product builds up abnormally and selectively destroys them. A small number of SCID cases are due to a developmental defect in receptors on B and T cells.

Because of their profound lack of specific adaptive immunities, SCID children require the most rigorous kinds of aseptic techniques to protect them from opportunistic infections. Aside from life in a sterile plastic bubble, exemplified by David Vetter, remembered as the "Boy in the Bubble" **(figure 14.18*a*)**, the only serious option for their longtime survival is total replacement or correction of dysfunctional lymphoid cells. Some infants can benefit from fetal liver or stem cell grafts, though transplantation is complicated by graft versus host disease. A more lasting treatment for both X-linked and ADA types of SCID is gene therapy—insertion of normal genes to replace the defective genes (see chapter 8).

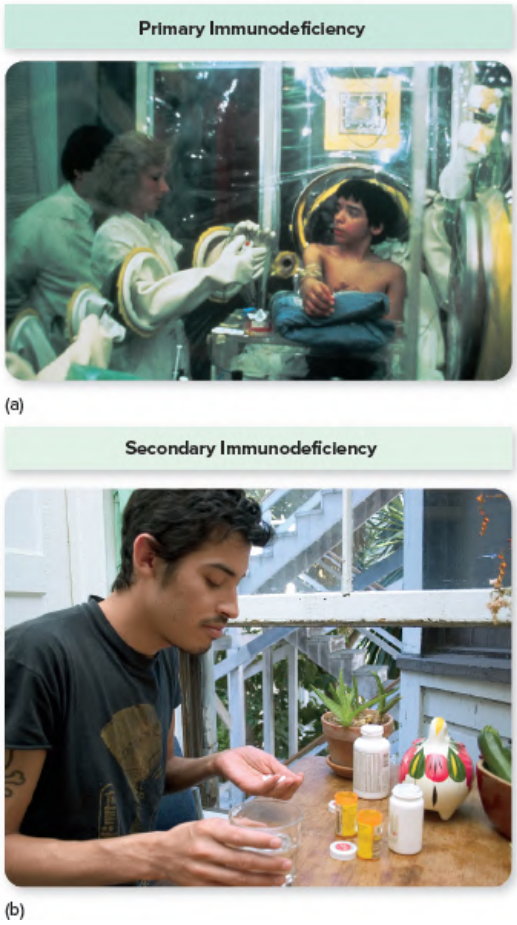

Figure 14.18 **The two types of immunodeficiency**. **(a)** David Vetter, who lived from 1971 to his death in 1984 in a sterile bubble. **(b)** An AIDS patient.
Courtesy Baylor College of Medicine, Public Affairs (a); ©McGraw-Hill Education/Christopher Kerrigan, photographer (b)

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4. AIDS, an infection-induced immunodeficiency, affects
- **a.** *T helper cells*.
- **b.** *antigen-presenting cells*.
- **c.** *red blood cells*.
- **d.** *both a and b*.
- **e.** *both b and c*.

Secondary Immunodeficiency Diseases

Secondary acquired deficiencies in B cells and T cells are caused by one of four agents:

1. infection,
2. noninfectious metabolic disease,
3. chemotherapy, or
4. radiation.

One well-recognized infection-induced immunodeficiency is **AIDS** (see chapter 18). This syndrome is caused when several types of immune cells, including T helper cells, monocytes, macrophages, and antigen-presenting cells, are infected by the human immunodeficiency virus (HIV). It is generally thought that the depletion of T helper cells and functional impairment of immune responses ultimately account for the cancers and opportunistic infections associated with this disease **(figure 14.18*b*)**. Other infections that can deplete immunities are measles, leprosy, and malaria.

Cancers that target the bone marrow or lymphoid organs can be responsible for extreme malfunction of both humoral and cellular immunity. In leukemia, cancer cells outnumber normal cells, displacing them from bone marrow and blood. Plasma cell tumors produce large amounts of nonfunctional antibodies, while thymus tumors cause severe T-cell deficiencies.

An ironic outcome of lifesaving medical procedures is the possible suppression of a patient's immune system. Drugs that prevent graft rejection or decrease the symptoms of rheumatoid arthritis can likewise suppress beneficial immune responses, while radiation and anticancer drugs are damaging to the bone marrow and other body cells.

It has become apparent in recent studies that some cancers are able to suppress the immune response, which allows the malignant cells to proliferate unchecked. Recent advances in cancer treatments sometimes involve reversing this suppression so that the immune system can do its job —destroying cells that have cancer markers (antigens) on them.

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14.7 LEARNING OUTCOMES—Assess Your Progress

13. Distinguish between primary and secondary immunodeficiencies, explaining how each develops.

CASE FILE WRAP-UP

©McGraw-Hill Education/Rick Brady, photographer (health care worker); ©Steve Gschmeissner/Science Source (blood cells)

Tumor necrosis factor (TNF) is found in higher amounts in the synovial fluid (the fluid that bathes the joints) of patients with rheumatoid arthritis (RA). TNF inhibitors such as Enbrel (etanercept) have been shown to reduce inflammation in RA patients.

Etanercept is a pharmaceutical that is composed of a genetically-engineered receptor for human tumor necrosis factor, fused to the Fc portion of the immunoglobulin IgG. How does it work? The circulating TNF in the body binds to the drug instead of to the natural receptor in the body. This reduces the effective concentration of TNF acting in the body and reduces symptoms due to TNF.

The patient in the case file opener had a past medical history of gastric ulcers and diabetes, which would make it risky for her to take NSAIDs (nonsteroidal anti-inflammatory drugs, such as ibuprofen) or many of the other drugs that are often the initial treatment for RA. Steroids may have been effective, but steroids can alter blood glucose. The patient was screened for hepatitis and tuberculosis because patients taking Enbrel may become more susceptible to infection. Patients who are taking etanercept must be cautioned to report signs of infection and stop taking the drug when they have an infection.

The Microbiome

Asthma and the Airway—and Gut—Microbiome

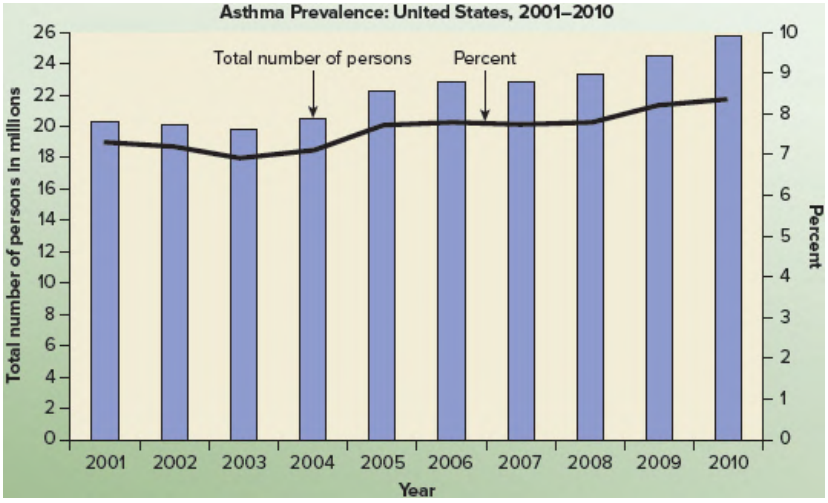
Asthma is considered a form of allergy, an inappropriate antibody response to epitopes that should not provoke a reaction. It affects more than 25 million people in the United States, 7 million of them children under the age of 18. Scientists are not sure what causes it. They have

found factors associated with it, but these can be as varied as poor living conditions, exposure to roaches, and too *little* exposure to microbes.

Current research is showing that a disrupted microbiome in the airway is nearly always found in people who have asthma. This is surely not unexpected. But the gut microbiome also seems to be important. That gut microbiome is showing up everywhere! Scientists have been trying to tell us for years that the gut is almost like a second nervous system, and this asthma research bears that out.

Researchers looked at 319 babies in Canada and found a subgroup of 22 with a high risk of asthma. These babies were missing four types of bacteria that were present in healthy babies. The researchers concluded that the lack of these four bacterial groups put babies at a greatly increased risk for asthma. Importantly, they were able to determine the sequence of events because they sampled the babies before any evidence of disease. If they had sampled them at the same time they categorized them as having asthma or not, it would be impossible to determine if the lack of bacteria led to asthma, or if asthma somehow caused the absence of these bacteria. They have not yet teased out why the babies were missing those bacteria. They are investigating exposures such as cesarean birth, early antibiotic treatment, and breast-feeding.

Not only does the gut microbiome regulate and influence digestive functions, but it also is important in training the immune system to react appropriately throughout the body. Since the gut is the portal of entry for so many environmental microbes (through eating and drinking), it seems logical that it would be the "training center" for the body's defenses, saying: "Don't react to this; it is just a harmless bacterium coming in with this bite of carrot."



Chapter Summary

14.1 The Immune Response: A Two-Sided Coin

- Immunopathology is the study of diseases associated with overactivity and underactivity of the immune response, including allergies, autoimmunity, transplantation and transfusion reactions, immunodeficiency diseases, and cancer.

- There are four categories of hypersensitivity reactions: type I (allergy and anaphylaxis), type II (complement, IgG- and IgM-mediated tissue destruction), type III (immune complex reactions), and type IV (delayed hypersensitivity reactions).

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14.2 Type I Allergic Reactions: Atopy and Anaphylaxis

- Antigens that trigger hypersensitivity reactions are allergens, which are either exogenous (originate outside the host) or endogenous (involve the host's own tissue).
- Atopy is a chronic, local allergy, whereas anaphylaxis is a systemic, potentially fatal allergic response. Both result from excessive IgE production in response to exogenous antigens.
- Type I allergens include inhalants, ingestants, injectants, and contactants; potential portals of entry are the skin, respiratory tract, gastrointestinal tract, and genitourinary tract.
- Type I hypersensitivities are set up by a sensitizing dose of allergen and expressed when a second provocative dose triggers the allergic response.
- The primary participants in type I hypersensitivities are IgE, basophils, mast cells, and mediators of the inflammatory response.

- Allergies are diagnosed using skin tests and blood tests that assay for specific immune cells, IgE production, or local reactions to allergens.
- Allergies are treated by medications that interrupt the allergic response at certain points. Allergic reactions can often be prevented by desensitization therapy.

14.3 Type II Hypersensitivities: Reactions That Lyse Foreign Cells

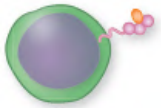
- Type II hypersensitivities are complement-assisted reactions that occur when preformed antibodies (IgG or IgM) react with foreign cell-bound antigens, leading to membrane attack complex formation and lysis.
- Hemolytic disease of the newborn (erythroblastosis fetalis) is a type II hypersensitivity that occurs when Rh– mothers are initially sensitized to RBCs from a firstborn Rh+ baby. When carrying a second Rh+ fetus, maternal anti-Rh antibodies can cross the placenta, causing hemolysis of the fetal Rh+ RBCs.

14.4 Type III Hypersensitivities: Immune Complex Reactions

- Type III hypersensitivity reactions occur when large quantities of antigen react with host antibody to form insoluble immune complexes that settle in tissue cell membranes, causing chronic destructive inflammation.
- Like type II reactions, type III hypersensitivities involve the production of IgG and IgM and the activation of complement; they differ in that the antigen recognized in these reactions is soluble.
- The mediators of type III hypersensitivity reactions include soluble IgA, IgG, or IgM, and agents of the inflammatory response.
- Localized (Arthus) and systemic (serum sickness) reactions are two forms of type III hypersensitivities.

14.5 Type IV Hypersensitivities: Cell-Mediated (Delayed) Reactions

- Type IV or delayed hypersensitivity reactions, like the tuberculin reaction and transplant reactions (host rejection and GVHD), occur when cytotoxic T cells attack either transplanted foreign cells or self tissue.
- The four classes of transplants or grafts are categorized by the degree of MHC similarity between graft and host. From most to least similar, these are autografts, isografts, allografts, and xenografts.



14.6 An Inappropriate Response to Self: Autoimmunity

- Autoimmune reactions occur when autoantibodies or host T cells mount an abnormal attack against self antigens. The reasons are unclear, but may be influenced by molecular mimicry, viral infection, or the composition of the gut microbiome.
- Examples of autoimmune diseases include systemic lupus erythematosus, rheumatoid arthritis, diabetes mellitus, myasthenia gravis, and multiple sclerosis.

14.7 Immunodeficiency Diseases: Hyposensitivity of the Immune System

- Immunodeficiency diseases occur when the immune response is reduced or absent.
- Primary immune diseases are genetically induced deficiencies of B cells, T cells, the thymus gland, or combinations of these. SCIDs are the most severe due to the loss of both humoral and cell-mediated immunity.
- Secondary immune diseases are caused by infection (i.e., AIDS), organic disease, chemotherapy, or radiation.
- Some malignancies lead to the suppression of normal immune response, allowing cells to proliferate abnormally.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. Allergy and atopy might have evolved in human populations that have had a "sudden" decrease in **a**. vaccinations. **b**. exposure to vitamin D. **c**. exposure to helminth infections. **d**. intake of processed sugar.	**2**. Can you think of a reason why humans have evolved to mount an immune response to their own fetuses if they have a particular set of foreign antigens (as in the case of erythroblastosis fetalis)?	**3**. Do you suppose that if we continue to transplant allografts into human patients for thousands of years that the immune system will evolve to not react to those foreign antigens? Provide support for your argument.
Cell Structure and Function	**4**. Molecular mimicry is when _____ on host cells resemble _____ on pathogens causing the host to mount an immune response to host tissues. (Same word for both blanks.) **a**. antibodies **b**. markers **c**. antigens **d**. two of the above	**5**. What feature of antibodies makes them particularly suited for forming complexes with antigen, as in type III hypersensitivities?	**6**. An autoimmune disease called antiphospholipid syndrome manifests as thrombosis, or the formation of blood clots. It is characterized by the presence of antibodies directed against phospholipids. Connect the sign (elevated antibodies) to the symptom (thrombosis).

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Metabolic Pathways	**7**. Which substance is most likely to be allergenic? **a**. protein **b**. fat **c**. carbohydrate **d**. nucleic acid	**8**. One of the strategies for preventing type I hypersensitivity is to induce plasma cells to secrete IgG instead of IgE. Why would that block allergy symptoms?	**9**. Epi-pens are devices used to instantly inject a person with epinephrine in the event of an anaphylactic exposure. What does epinephrine do to stop anaphylaxis? Predict what would happen if too much epinephrine was administered.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Information Flow and Genetics	**10**. What percent of the human population is positive for the Rh antigen? **a**. 50% **b**. 65% **c**. 85% **d**. 99%	**11**. Steroids administered to stop an allergic response act in several ways. One of them is to shut down the synthesis of IgE by plasma cells. Identify some intracellular host macromolecules that may be bound (obstructed) by steroid molecules to result in this outcome.	**12**. A very small number of people have a condition called vibratory urticaria. In this condition, physical vibrations (bumpy bus rides, jackhammers, running) can cause skin rashes. Scientists have found a genetic mutation in a single gene for a protein that stabilizes the surface of immune cells. You take it from here: What would a mutation in that protein possibly lead to?

Microbial Systems	**13**. The hygiene hypothesis suggests that **a**. there are still too many microorganisms in the environment. **b**. we may need more contact with antimicrobials as our systems mature	**14**. Probiotic supplements are sometimes promoted to alleviate the symptoms of hypersensitivity diseases such as rheumatoid arthritis and eczema. Trace	**15**. Recent research shows that asthmatic patients have a much higher load of bacteria in their lower airways than do healthy

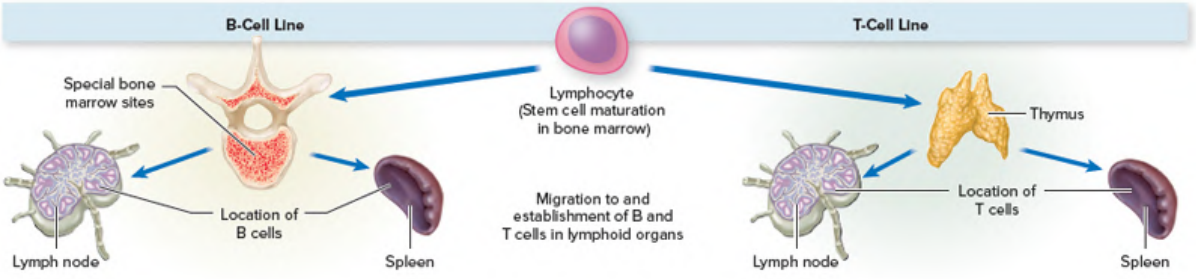
	c. there are not enough microbes on farms. **d**. we may need more contact with microbes as our systems mature.	the path to biological plausibility for that claim, if any.	subjects. How could that contribute to what we see in the pathology of asthma?
Impact of Microorganisms	**16**. Which statement is true of autoimmunity? **a**. It involves misshapen antibodies. **b**. It refers to "automatic immunity." **c**. It often manifests as types II, III, and IV hypersensitivities. **d**. It has an acute course and then usually resolves itself.	**17**. It is often said that the normal microbiota of a human "trains" the immune system to react to foreign antigens and not react to self antigens. Use what you know about clonal deletion and clonal selection to suggest how this might happen.	**18**. Investigate the link between *Streptococcus pyogenes* infection and some types of obsessive-compulsive disorder. Identify similarities with some concepts in this chapter.
Scientific Thinking	**19**. Which disease would be most similar to AIDS in its pathology? **a**. X-linked agammaglobulinemia **b**. SCID **c**. ADA deficiency **d**. myasthenia gravis	**20**. Do you think people with B-cell deficiencies can be successfully vaccinated against various microbes? Why or why not?	**21**. The chapter tells us that the gut microbiome of mice prone to rheumatoid arthritis (RA) have a higher percentage of the bacterial genus *Prevotella* than mice that are not prone to RA. Does this prove that high *Prevotella* populations in the gut cause RA? Elaborate.

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. As you learned in **chapter 13**, B and T cells originate in tissues outside of the lymphatic system. With this in mind, provide at least one example of how an abnormality in these areas (i.e., in the bone marrow or thymus) can lead to immune deficiency.

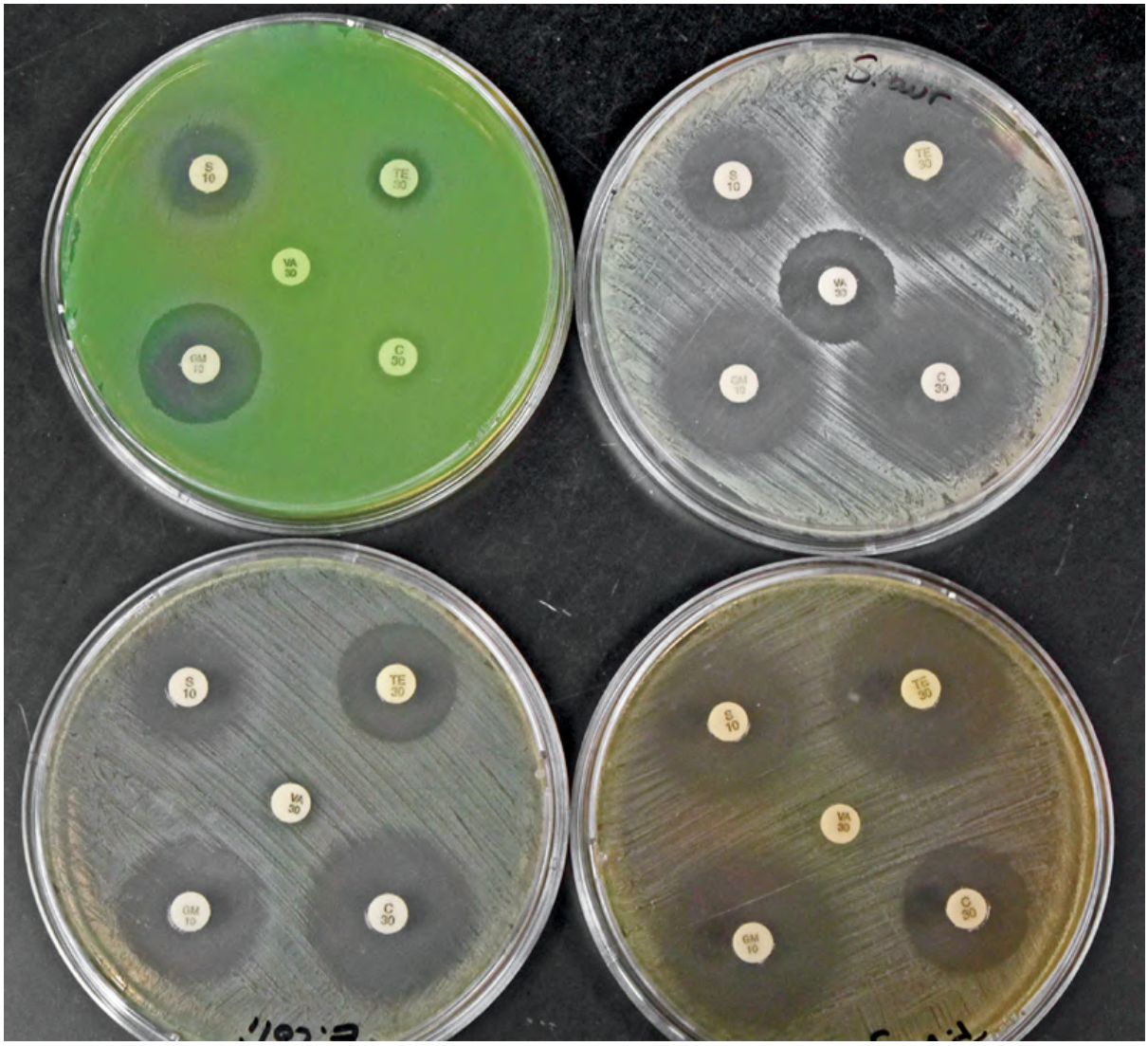


connect Resources are available through Connect, including interactive learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

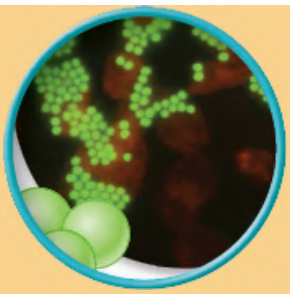
15

Diagnosing Infections



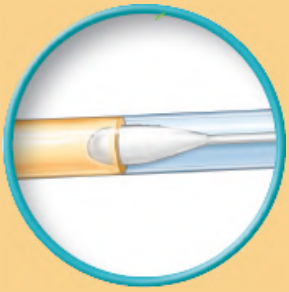
©McGraw-Hill Education/Lisa Burgess, photographer (test plates); Image provided by AdvanDx, Inc. (microbial identification); ©Steven Puetzer/Getty Images (genotypic methods); ©McGraw-Hill Education/Trish Ofenlock, photographer (health care worker)

IN THIS CHAPTER…



15.1 Preparation for the Survey of Microbial Diseases

1. List the three major categories of microbial identification techniques.
2. Provide a one-sentence description for each of these three categories.

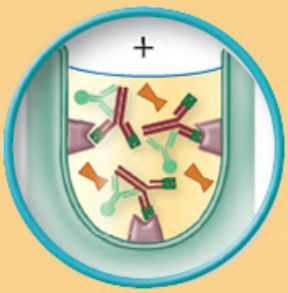


15.2 First Steps: Specimen Collection

3. Identify factors that may affect the identification of an infectious agent from a patient sample.
4. Compare the types of tests performed on microbial isolates versus those performed on patients themselves.

15.3 Phenotypic Methods

5. List at least three different tests that fall in the direct identification category.
6. Explain the main principle behind biochemical testing, and identify an example of such tests.
7. Discuss two major drawbacks of phenotypic testing methods that require culturing the pathogen.

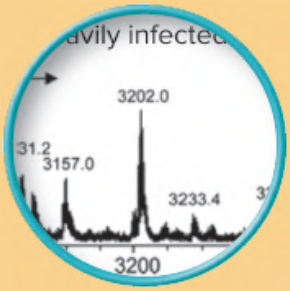


15.4 Immunologic Methods

8. Define the term *serology*, and explain the immunologic principle behind serological tests.
9. Identify two immunological diagnostic techniques that rely on a secondary antibody, and explain how they work.

15.5 Genotypic Methods

10. Explain why PCR is useful for infectious disease diagnosis.
11. Name two examples of techniques that employ hybridization.
12. Explain how whole-genome sequencing can be used for diagnosis.
13. Identify the situations in which pulsed-field gel electrophoresis is most useful.



15.6 Additional Diagnostic Technologies

14. Describe the benefits of "lab on a chip" technologies for global public health.
15. Identify an advantage presented by mass spectrometry and also by imaging techniques as diagnostic tools.

CASE FILE

Tracing the Cause

When I was a lab tech student, my preceptor and I were asked to go to the emergency room to draw blood from a patient. The lab requisition filled out by the nurse caring for the patient stated the patient had "fever for 1 week with a decreased level of consciousness."

On reporting to the ER, we were directed to the patient, who was lying on a gurney in an exam room. The patient was a 78-year-old diabetic gentleman who appeared pale and thin. We introduced ourselves and told the patient that we were there to draw blood. The patient barely acknowledged our presence and seemed to have difficulty staying awake. The ER nurses had already collected a urine specimen from the patient's Foley catheter for urinalysis and culture. They handed us the specimens they had collected, which had been labeled correctly with the patient's name and the collection date and time.

We proceeded to draw blood according to the physician's order. We obtained blood for a complete blood count, electrolytes, blood glucose, liver function, and cardiac markers. We also obtained blood from two different sites for blood cultures. Once we had collected all of the samples, we notified the nursing staff that we were finished. We labeled all the collection vials with the necessary information and returned to the lab.

After running all of the tests that could be performed in-house, we sent copies of all of the reports to the ER. The patient's hemoglobin and hematocrit were slightly low. The white blood cell count was very high. The patient's potassium was high, and the sodium level was low. Liver function was normal, as were cardiac markers. The urinalysis showed ketones and protein in the urine, evidence of dehydration but not of infection. The patient's serum glucose was high but not dangerously so.

On the basis of these lab studies, the patient was rehydrated with intravenous fluids and his potassium and sodium levels were soon corrected. He was also given insulin to lower his blood glucose. The patient was started on broad-spectrum IV antibiotics pending the results of the urine and blood cultures. He continued to run a high fever and was only semiconscious following admission to the ICU.

The preliminary urine cultures failed to identify any bacteria in the patient's urine. That left the blood cultures; when the preliminary results came back in 48 hours, the results revealed the presence of *Staphylococcus epidermidis* in the patient's blood. Sensitivity studies showed that the organism was sensitive to Penicillin G, rifampin, and

vancomycin. It also revealed that the microorganism was resistant to the ceftriaxone that the patient was currently receiving. The patient was switched to Penicillin G every 6 hours intravenously, and he made a relatively rapid recovery soon thereafter, with his fever disappearing within 36 hours.

- Why are blood cultures collected from two different sites?
- Why was it important for the laboratory staff to notify the nursing staff after the samples had been collected?

Case File Wrap-Up appears at the end of the chapter.

15.1 Preparation for the Survey of Microbial Diseases

Chapters 16 through 21 cover the most clinically significant bacterial, fungal, parasitic, and viral diseases. These chapters survey the most prevalent infectious conditions and the organisms that cause them. This chapter gets us started with an introduction to the how-to of diagnosing the infections.

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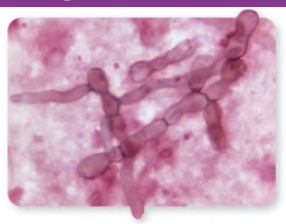
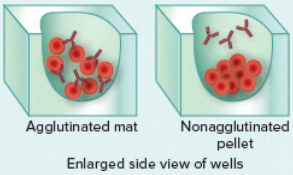
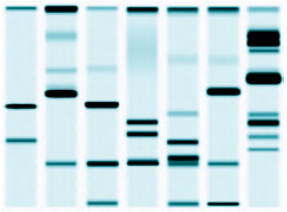
For many students (and professionals), the most pressing topic in microbiology is *how to identify unknown bacteria* in patient specimens or in samples from nature. As seen in **table**

15.1, methods microbiologists use to identify bacteria to the level of genus and species fall into three main categories: *phenotypic*, which includes a consideration of morphology (microscopic and macroscopic) as well as bacterial physiology or biochemistry; *immunologic*, which entails serological analysis; and *genotypic* (or genetic) techniques. Data from a cross section of such tests can produce a unique

Table 15.1 Methods of Microbial Identification

Category	Description	Example
Phenotypic	Observation of microbe's microscopic and macroscopic morphology, physiology, and biochemical properties	
Immunologic	Analysis of microbe using antibodies, or of patients' antibodies using prepackaged antigens	
Genotypic	Analysis of microbe's DNA or RNA	

Source: CDC/Arthur F. DiSalvo, M.D. (phenotypic); ©Steven Puetzer/Getty Images (genotypic)

profile for any bacterium. Increasingly, genetic means of identification are being used as a sole resource for identifying bacteria. We are in the middle of a revolution in infectious disease diagnosis.

Sophisticated genetic techniques have become commonplace and affordable for hospitals and diagnostic labs to adopt on a wide-scale basis. Many of these techniques take less time than needed to actually culture microorganisms and therefore result in quicker results and better patient outcomes. There has also been an increase in what is called point-of-care diagnosis. These are tests that can be performed at the bedside, meaning that samples do not have to be "sent out" and patient care can continue without delay.

It should be noted that some other techniques can assist in diagnosis. For example, computerized tomography (CT) scans are often used to diagnose peritonsillar abscesses, after which the causative agent can be identified using the techniques described in this chapter. Magnetic resonance imaging (MRI) and positron emission tomography (PET) scans are also used to find areas of deep tissue infection.

Phenotypic Methods

You will remember that phenotype refers to the traits that an organism is expressing in the present. So, put simply, phenotypic methods of identifying microbes involve examining their appearance and their behavior. In this context "behavior" is defined as what types of enzymatic activities it can carry out, what kind of physical conditions it thrives in, what antibiotics it is susceptible to, and the chemical composition of its walls and/or membranes.

Immunologic Methods

As you learned in chapter 13, one immune response to antigens is the production of antibodies, which are designed to bind tightly to specific antigens. The nature of the antibody response is exploited for diagnostic purposes when a patient sample is tested for the presence of specific antibodies to a suspected pathogen (antigen). Alternatively, microbial antigens in the patient's tissues can be tested with antibodies "off the shelf." These immunological methods can be easier than trying to isolate the microbe itself. Laboratory kits based on this technique are available for immediate identification of a number of pathogens.

©Nicolas/Getty Images

Genotypic Methods

Examining the genetic material itself (DNA and/or RNA) has revolutionized the identification and classification of bacteria. There are many advantages of genotypic methods over phenotypic methods, when they are available. The primary advantage is that culturing of the microorganisms is not always necessary. In recent decades, scientists have come to realize that there are many more microorganisms that we can't grow in the lab compared with those that we can. Numerous viable nonculturable (VNC) microbes are currently being identified in this manner, through studies such as the Human Microbiome Project. Another advantage is that genotypic methods are increasingly automated, producing rapid results that are often more precise than phenotypic methods.

15.1 LEARNING OUTCOMES—Assess Your Progress

1. List the three major categories of microbial identification techniques.
2. Provide a one-sentence description for each of these three categories.

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15.2 First Steps: Specimen Collection

Regardless of the method of diagnosis, specimen collection is the common point that guides the health care decisions of every member of a clinical team. Indeed, the success of identification and treatment depends on how specimens are collected, handled, stored, and cultured. Specimens can be taken by a clinical laboratory scientist or medical technologist, nurse, physician, or even by the patient. However, it is imperative that general aseptic procedures be used, including sterile sample containers and other tools to prevent contamination from the environment or the patient. **Figure 15.1** delineates the most common sampling sites and procedures.

In sites that normally contain resident microbiota, care should be taken to sample only the infected site and not the surrounding areas. Saliva is an especially undesirable contaminant because it contains millions of bacteria per milliliter, most of which are normal biota. Sputum, the mucus secretion that coats the lower respiratory surfaces, especially the lungs, is discharged by coughing or taken by a thin tube called a catheter to avoid contamination with saliva. In addition, throat and nasopharyngeal swabs should not touch the tongue, cheeks, or saliva. On occasion saliva samples are needed for dental diagnosis and are obtained by having the patient expectorate into a container.

Urine is taken aseptically from the bladder with a catheter designed for that site. Another method, called a "clean catch," is taken by washing the external urethra and collecting the urine midstream. The latter method inevitably incorporates a few normal biota into the sample, but these can usually be differentiated from pathogens in an actual infection. Sometimes diagnostic techniques require first-voided "dirty catch" urine. The mucous lining of the urethra, vagina, or cervix can be sampled with a swab or applicator stick.

Depending on the nature of a skin lesion, skin can be swabbed or scraped with a scalpel to expose deeper layers. Wounds are sampled either by swabbing or by using a punch biopsy tool. Sterile materials such as blood, cerebrospinal fluid, and tissue fluids must be taken by sterile needle aspiration. Antisepsis of the puncture site is extremely important in these cases. Additional sources of specimens are the eye, ear canal, synovial fluid, nasal cavity (all by swab), and diseased tissue that has been surgically removed (biopsied).

After proper collection, the specimen is promptly transported to a lab and stored appropriately (usually refrigerated) if it must be held for a time. Nonsterile samples in particular, such as urine, feces, and sputum, are especially prone to deterioration at room temperature. Special swab and transport systems are designed to collect the specimen and maintain it in stable condition for several hours. These devices provide nonnutritive maintenance media (so microbes survive but do not grow), a buffering system, and an anaerobic environment to prevent destruction of oxygen-sensitive bacteria.

Knowing how to properly collect, transport, and store specimens is an important aspect of many health professionals' jobs. In addition, labeling and identifying specimens, as well as providing an accurate patient history, are crucial to obtaining timely and accurate results.

Overview of Laboratory Techniques

Analyzing the patient for signs of microbial infection (i.e., fever, wound exudate, mucus production, abnormal lesion) comes first. After that, specimens are collected

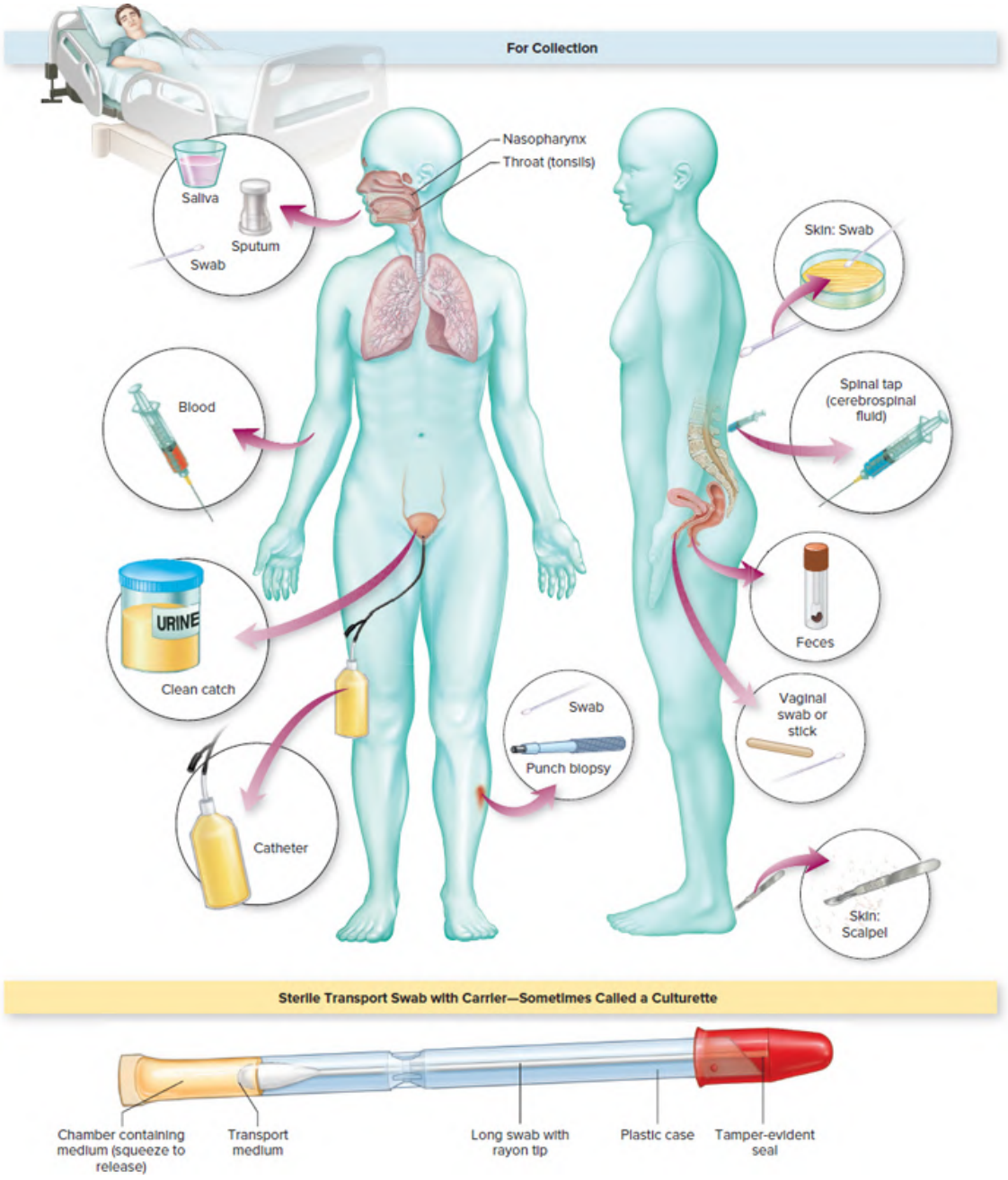

Figure 15.1 **Sampling sites and methods of collection for clinical laboratories**.

and analyzed. The time required for testing ranges from a few minutes in a rapid strep test to several weeks in a tuberculosis infection.

Results of specimen analysis are entered in a summary patient chart **(figure 15.2)** that can be used in assessment and treatment regimens. The form pictured here is an "old school" paper form. We're showing it because it gives a more comprehensive view of the various tests that can be requested than the more commonly used electronic record system, which shows different categories on separate screens. You may notice that it compiles information on tests

with which you are already familiar. As a health care provider, your understanding of and ability to communicate lab results are critical steps in the successful treatment of patients.

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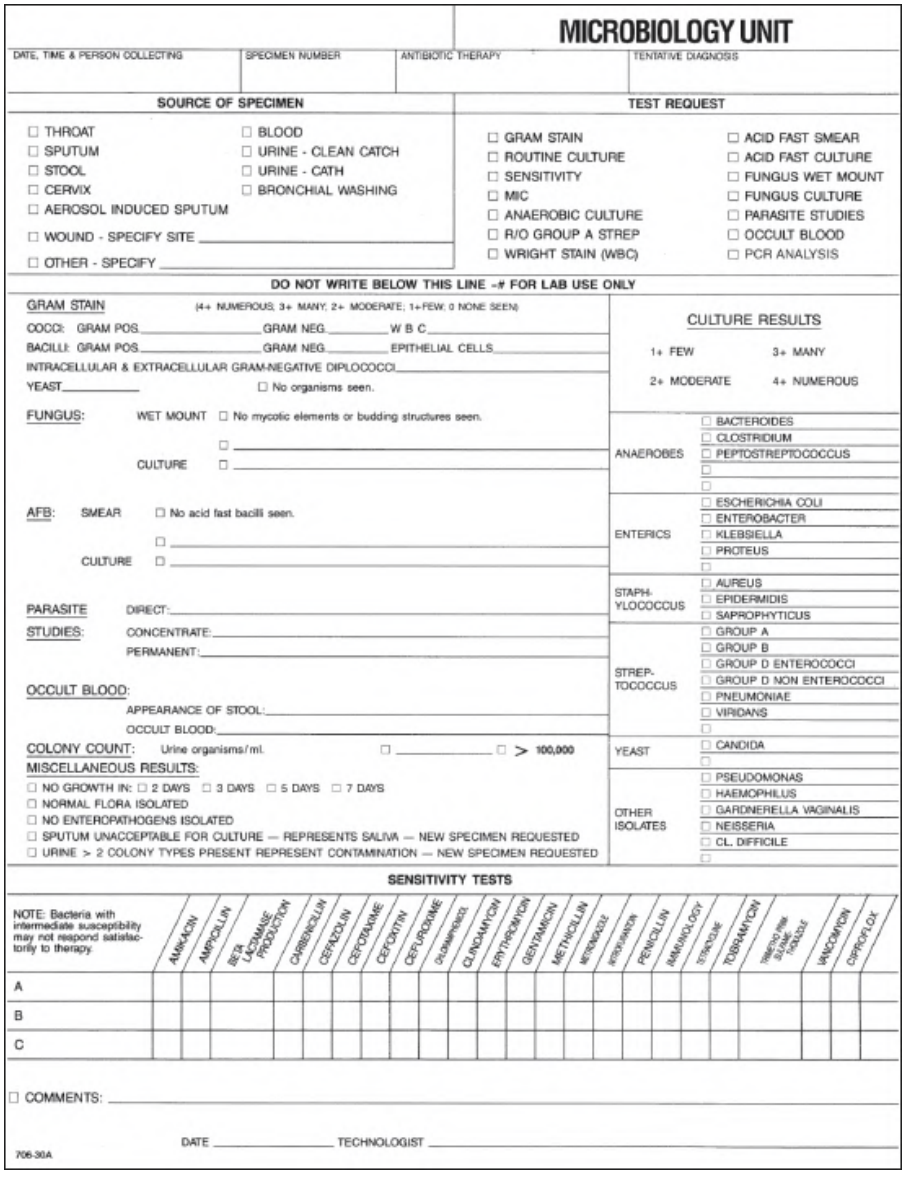
1. An RN is training a new staff nurse about proper specimen collection. Consequences of improper specimen collection may include all of the following, except
   - **a.** a delay in appropriate treatment.
   - **b.** exposing the patient to an unnecessary procedure.
   - **c.** having to repeat the test.
   - **d.** putting other patients at risk for incorrect treatment.
   - **e.** putting the patient at risk for incorrect treatment.



MICROBIOLOGY UNIT

DATE, TIME & PERSON COLLECTING | SPECIMEN NUMBER | ANTIBIOTIC THERAPY | TENTATIVE DIAGNOSIS

SOURCE OF SPECIMEN

☐ THROAT ☐ BLOOD
☐ SPUTUM ☐ URINE - CLEAN CATCH
☐ STOOL ☐ URINE - CATH
☐ CERVIX ☐ BRONCHIAL WASHING
☐ AEROSOL INDUCED SPUTUM
☐ WOUND - SPECIFY SITE _____
☐ OTHER - SPECIFY _____

TEST REQUEST

☐ GRAM STAIN ☐ ACID FAST SMEAR
☐ ROUTINE CULTURE ☐ ACID FAST CULTURE
☐ SENSITIVITY ☐ FUNGUS WET MOUNT
☐ MIC ☐ FUNGUS CULTURE
☐ ANAEROBIC CULTURE ☐ PARASITE STUDIES
☐ R/O GROUP A STREP ☐ OCCULT BLOOD
☐ WRIGHT STAIN (WBC) ☐ PCR ANALYSIS

DO NOT WRITE BELOW THIS LINE –# FOR LAB USE ONLY

GRAM STAIN (4+ NUMEROUS; 3+ MANY; 2+ MODERATE; 1+ FEW; 0 NONE SEEN)
COCCI: GRAM POS._____ GRAM NEG._____ W B C_____
BACILLI: GRAM POS._____ GRAM NEG._____ EPITHELIAL CELLS_____
INTRACELLULAR & EXTRACELLULAR GRAM-NEGATIVE DIPLOCOCCI_____
YEAST_____ ☐ No organisms seen.

FUNGUS: WET MOUNT ☐ No mycotic elements or budding structures seen.
☐ _____
CULTURE ☐ _____

AFB: SMEAR ☐ No acid fast bacilli seen.
☐ _____
CULTURE ☐ _____

PARASITE STUDIES: DIRECT:_____ CONCENTRATE:_____ PERMANENT:_____

OCCULT BLOOD: APPEARANCE OF STOOL:_____ OCCULT BLOOD:_____

COLONY COUNT: Urine organisms/ml. ☐ _____ ☐ > 100,000

MISCELLANEOUS RESULTS:
☐ NO GROWTH IN: ☐ 2 DAYS ☐ 3 DAYS ☐ 5 DAYS ☐ 7 DAYS
☐ NORMAL FLORA ISOLATED
☐ NO ENTEROPATHOGENS ISOLATED
☐ SPUTUM UNACCEPTABLE FOR CULTURE — REPRESENTS SALIVA — NEW SPECIMEN REQUESTED
☐ URINE > 2 COLONY TYPES PRESENT REPRESENT CONTAMINATION — NEW SPECIMEN REQUESTED

CULTURE RESULTS

1+ FEW 3+ MANY
2+ MODERATE 4+ NUMEROUS

ANAEROBES	☐ BACTEROIDES ☐ CLOSTRIDIUM ☐ PEPTOSTREPTOCOCCUS ☐ ☐
ENTERICS	☐ ESCHERICHIA COLI ☐ ENTEROBACTER ☐ KLEBSIELLA ☐ PROTEUS ☐
STAPHYLOCOCCUS	☐ AUREUS ☐ EPIDERMIDIS ☐ SAPROPHYTICUS
STREPTOCOCCUS	☐ GROUP A ☐ GROUP B ☐ GROUP D ENTEROCOCCI ☐ GROUP D NON ENTEROCOCCI ☐ PNEUMONIAE ☐ VIRIDANS ☐
YEAST	☐ CANDIDA ☐
OTHER ISOLATES	☐ PSEUDOMONAS ☐ HAEMOPHILUS ☐ GARDNERELLA VAGINALIS ☐ NEISSERIA ☐ CL. DIFFICILE ☐

SENSITIVITY TESTS

NOTE: Bacteria with intermediate susceptibility may not respond satisfactorily to therapy.

	AMIKACIN	AMPICILLIN	BETA LACTAMASE PRODUCTION	CARBENICILLIN	CEFAZOLIN	CEFOTAXIME	CEFOXITIN	CEFUROXIME	CHLORAMPHENICOL	CLINDAMYCIN	ERYTHROMYCIN	GENTAMICIN	METHICILLIN	MOXIFLOXACIN	NITROFURANTOIN	PENICILLIN	RIFAMPIN/RIFAMPICIN	STREPTOMYCIN	TOBRAMYCIN	TRIMETH-PRIM SULFAMETHOXAZOLE	VANCOMYCIN	CIPROFLOXACIN
A																						
B																						
C																						

☐ COMMENTS: _____

DATE _____ TECHNOLOGIST _____

706-30A

Figure 15.2 **Example of a clinical form used to report data on a patient's specimens.**

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15.2 LEARNING OUTCOMES—Assess Your Progress

3. Identify factors that may affect the identification of an infectious agent from a patient sample.
4. Compare the types of tests performed on microbial isolates versus those performed on patients themselves.

NCLEX® PREP

2. A clinical form used to report data on a patient's specimens may include
- **a.** *antibiotic history*.
- **b.** *patient symptoms*.
- **c.** *marital status*.
- **d.** *date and time of specimen collection*.
- **e.** *a, b, and d*.
- **f.** *b, c, and d*.

15.3 Phenotypic Methods

Immediate Direct Examination of Specimen

Direct microscopic observation of a fresh or stained specimen is one of the most rapid methods of determining presumptive and sometimes confirmatory microbial characteristics. The Gram stain and the acid-fast stain (see figure 2.18) are most often used for bacterial identification. As useful as these stains are, they can identify only a few organisms on their own. For that reason, we have a variety of other techniques.

Methods Requiring Growth

Selective and Differential Growth

Such a wide variety of media exist for microbial isolation that a certain amount of preselection must occur, based on the nature of the specimen. In cases in which the suspected pathogen is present in small numbers or is easily overgrown by normal biota, the specimen can be initially enriched with specialized media. Nonsterile specimens containing a diversity of bacterial species, such as urine and feces, are cultured on selective media to encourage the growth of only the suspected pathogen. For example, a large percentage of urinary tract infections are known to be caused by *Escherichia coli*, so selective media that will be sure to allow the growth of this common pathogen are chosen. Specimens are often inoculated into differential media to identify definitive characteristics, such as reactions in blood (blood agar) and fermentation patterns (mannitol salt and MacConkey agar).

A straightforward way to phenotypically identify specimens is to combine the results of tests such as Gram staining, growth on different media, and simple enzymatic tests. Usually a

Gram stain is the first test. In most cases, the specimen will be either gram-negative or gram-positive. This can form the starting point for a **dichotomous key**, a graphic method that is essentially a flowchart leading to the identification of specimens **(figure 15.3)**. The name "dichotomous" refers to the fact that at each branch of the flowchart there are two (di-) possible outcomes.

Biochemical Testing

The physiological reactions of bacteria to nutrients and other substrates provide excellent evidence of the types of enzyme systems present in a particular species. Knowing which enzymes an isolate has can often lead to its identity. Many of these tests are based on enzyme-mediated metabolic reactions that are visualized by a color change.

The microbe is cultured in a medium with a special substrate and then tested for a particular end product. Microbial expression of the enzyme is made visible by a colored dye; no coloration means it lacks the enzyme for utilizing the substrate in that particular way. The biochemical testing process has been fully automated by diagnostic companies. Even the growth and incubation step can be performed by the machines, and they can identify more than 2,500 different bacteria. With these machines, the sample is inoculated in some micro format, such as on a small card, inserted in a machine, and the whole process is performed by the machine. However,

Medical Moment

Qualitative Versus Quantitative Diagnosis

Laboratory tests may be *qualitative* or *quantitative*. Qualitative tests are used to determine whether a substance is present in a sample, while quantitative tests measure the amounts of the substance that are present.

A pregnancy test is a perfect example of a test that may be qualitative or quantitative. Most home pregnancy tests are qualitative in that they test for the presence or absence of the hormone known as human chorionic gonadotropin (hCG), which will appear in the urine of pregnant women several days after conception.

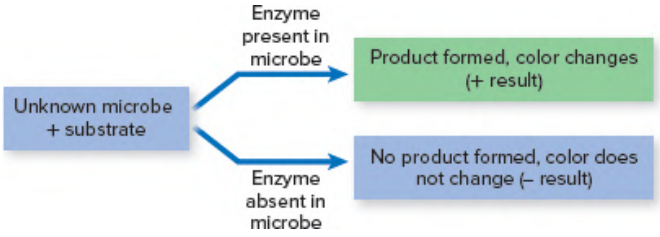
A quantitative pregnancy test is determined from a blood sample. The hCG levels should double every 2 days in the first few weeks of pregnancy. When hCG levels do not rise as expected, there may be a problem with the pregnancy (such as miscarriage); extremely high levels may be associated with twins. Therefore, measuring the exact amount of hCG present in the bloodstream provides the ordering physician with much more information than a qualitative pregnancy test can, which only indicates whether or not a woman is pregnant.

Q. What technique in this chapter do most home pregnancy test kits utilize?

Answer in Appendix B.

Antibodies are used to “diagnose” noninfectious conditions as well, such as pregnancy.
©laflor/E+/Getty Images

all over the world, and probably in your own microbiology lab course, the process is performed manually. Seeing it done manually makes it easier to visualize how it actually works **(figure 15.4)**. Additionally, direct biochemical testing of patient samples now can be performed without incubation, which produces results within hours instead of days.



Another type of phenotypic test is the MGIT system used to detect the growth of the slow-growing *Mycobacterium tuberculosis*. (MGIT stands for “mycobacterial growth indicator tube”.) This system monitors oxygen levels in a tube that has been inoculated with a patient specimen (sputum, blood). The tube contains a medium that encourages the growth of the tuberculosis bacterium (which is excruciatingly slow-growing). There is a silicon chip at the bottom of the tube that is impregnated with a fluorescent substance that is sensitive to oxygen levels. When first inoculated, there will be a lot of free oxygen in the medium, since even if the bacteria are present, there will not be many of them. But if the bacteria grow, they begin using the oxygen, and the decreased oxygen levels allow the silicon compound to fluoresce. In automated machines when a threshold level of fluorescence occurs, a visual and audible signal is given so a positive culture can be identified in the shortest possible time.

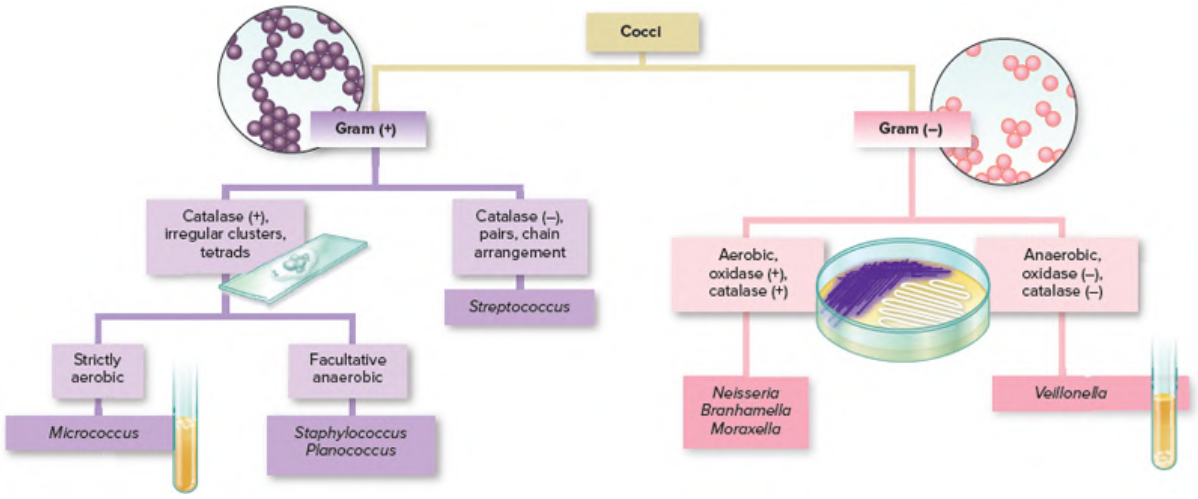


Figure 15.3 **Flowchart to separate primary genera of various cocci**. Identification scheme for cocci commonly involved in human diseases.

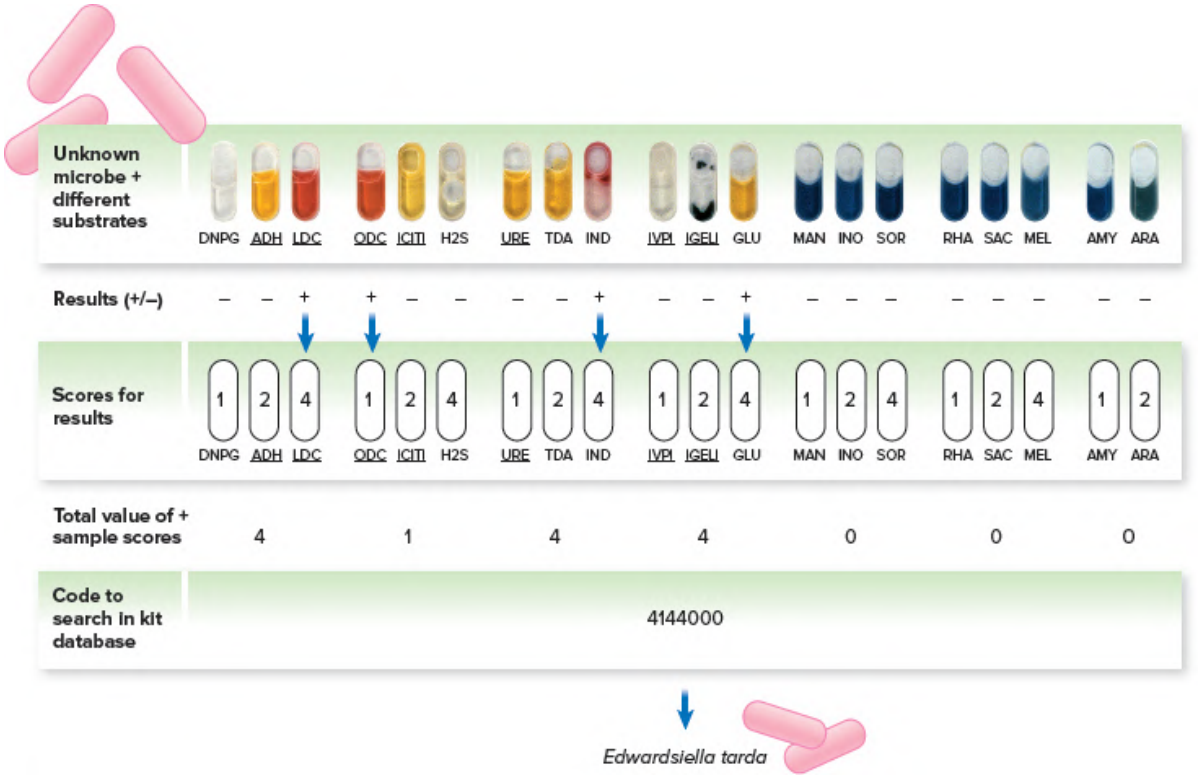


Figure 15.4 **Rapid biochemical test**. **(a)** Before inoculation. Each mini-tube in the strip contains a substrate such as a carbohydrate or the substrate for a particular enzyme that the unknown microbe might possess. **(b)** After inoculation with an unknown bacterium in a liquid medium. The colors of the wells indicate either a positive or negative reaction. The combination of colors are converted to a numerical code which indicates the identity of the bacterium. This is an API 20E® test strip manufactured by the company bioMérieux.

Source: CDC/Janice Haney Carr

Antimicrobial Susceptibility Testing

As important as identifying the microbial pathogen is identifying what antimicrobial drugs it can be treated with. With the crisis-level rise in antimicrobial resistance, it is important not to waste valuable treatment time by simply guessing what drugs to use to treat an infection. Most of the automated phenotypic systems incorporate a panel of commonly used antimicrobials for the particular infection site, and simultaneously test susceptibility while identifying the pathogen. This is commonly accomplished using an adaptation of the tube-

dilution method covered in chapter 12. Also, antimicrobial susceptibility tests can themselves provide the identity of some species, such as *Streptococcus, Clostridium*, and *Pseudomonas*.

Miscellaneous Tests

Phage typing relies on bacteriophages, viruses that attack bacteria in a very species-specific and strain-specific way. Such selection is useful in identifying some bacteria, primarily *Salmonella*, and is often used for tracing bacterial strains in epidemics. The technique of phage typing involves inoculating a lawn of bacterial cells onto agar, mapping it off into blocks, and applying a different phage to each sectioned area of growth **(figure 15.5)**. Cleared areas corresponding to lysed cells indicate sensitivity to that phage, and a bacterial identification may be determined from this pattern.

Susceptible animals are needed to cultivate bacteria such as *Mycobacterium leprae* and significant quantities of *Treponema pallidum*, whereas avian embryos and cell cultures are used to grow host cell-dependent rickettsias, chlamydias, and viruses.

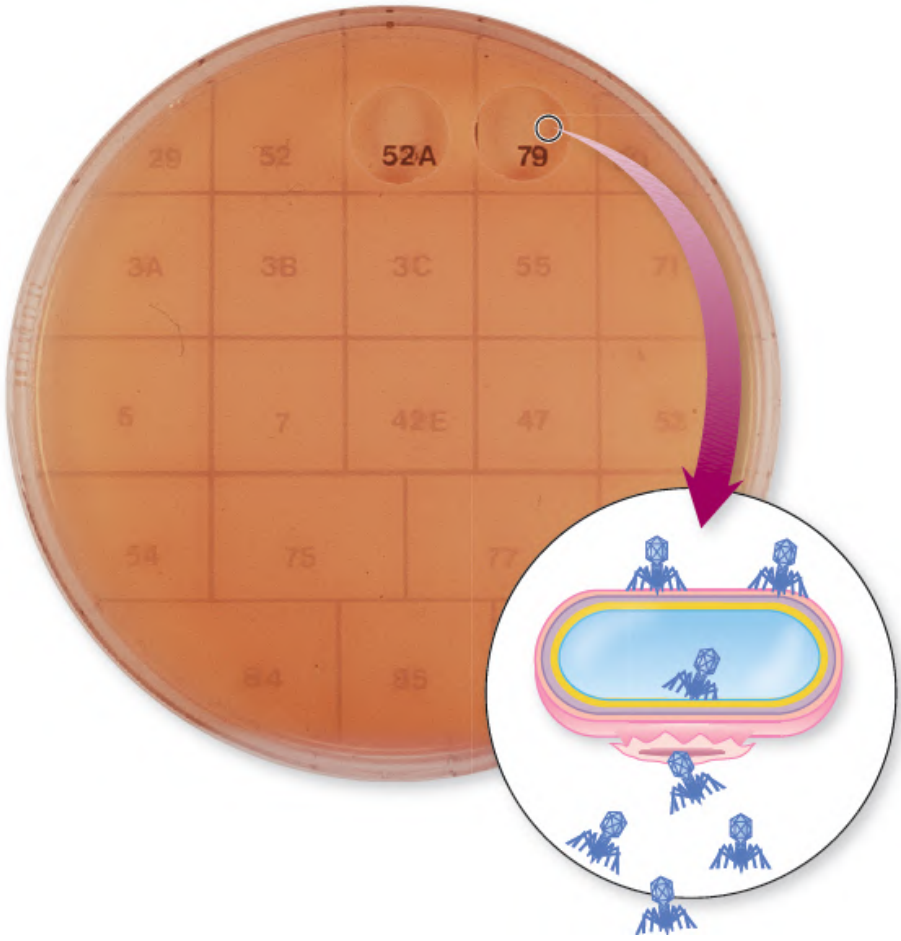


Figure 15.5 **Phage typing of an unknown bacterium**. A cleared area within a square of the bacterial lawn forms due to phage-induced lysis of cells, indicating sensitivity of the bacterium to the corresponding phage.
Courtesy The University of Leeds

NCLEX® PREP

3. When determining the clinical significance of cultures,

- **a.** *the number of microbes is significant.*
- **b.** *the presence of a single colony of a true pathogen may indicate the presence of the disease if the culture comes from a site known to be sterile (i.e., cerebrospinal fluid).*
- **c.** *the repeated isolation of a relatively pure culture of any microorganism can mean it is an agent of disease, although this is not always the case.*
- **d.** *a range of tests may be needed to identify a pathogen.*
- **e.** *all of the above are true.*

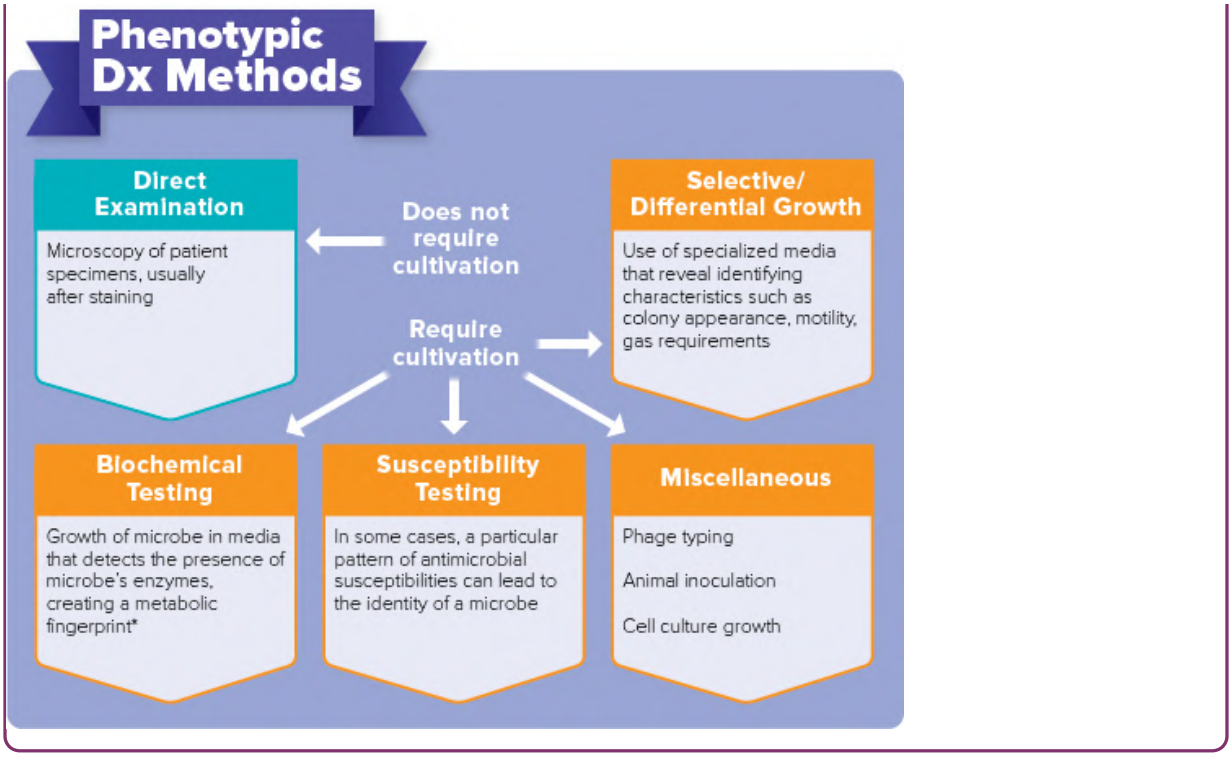
Determining Clinical Significance of Cultures

It is important to rapidly determine if an isolate from a specimen is clinically important or if it is merely a contaminant or normal biota. Although answering these questions may prove difficult, one can first focus on the number of microbes in a specimen. For example, a few colonies of *Escherichia coli* in a urine sample can simply indicate normal biota, whereas several hundred can mean active infection. In contrast, the presence of a single colony of a true pathogen, such as *Mycobacterium tuberculosis* in a sputum culture or an opportunist in sterile sites such as cerebrospinal fluid or blood, is highly suggestive of its role in disease. Furthermore, the repeated isolation of a relatively pure culture of any microorganism can mean it is an agent of disease.

As reliable as phenotypic methods can be, they have two major drawbacks: (1) When the microbe has to be cultured, it takes a minimum of 18 to 24 hours, and often longer; and (2) we are learning that many infectious conditions may be caused by nonculturable organisms, leaving open the possibility that the organism that we do culture is simply a bystander.

Table 15.2 summarizes phenotypic methods of diagnosis.

Table 15.2



*Newer methods may not require cultivation.

15.3 Phenotypic Methods

5. List at least three different tests that fall in the direct identification category.
6. Explain the main principle behind biochemical testing, and identify an example of such tests.
7. Discuss two major drawbacks of phenotypic testing methods that require culturing the pathogen.

15.4 Immunologic Methods

The antibodies formed during an immune reaction are important in combating infection, but they also form the basis of a powerful diagnostic strategy. Characteristics of antibodies (such as their quantity or specificity) can reveal the history of a patient's contact with microorganisms or other antigens. This is the underlying basis of serological testing. **Serology** is the branch of immunology that traditionally deals with *in vitro* diagnostic testing of serum.

Serological testing is based on the principle that antibodies have extreme specificity for antigens, so when a particular antigen is exposed to its specific antibody, it will fit like a hand in a glove. The ability to visualize this interaction macroscopically or microscopically provides a powerful tool for detecting, identifying, and quantifying antibodies—or conversely, antigens. One can detect or identify an unknown antibody using a known antigen, or one can use an antibody of known specificity to help detect or identify an unknown antigen **(figure 15.6)**. Even though the strategy

Syphilis is caused by *Treponema pallidum*.
Source: CDC

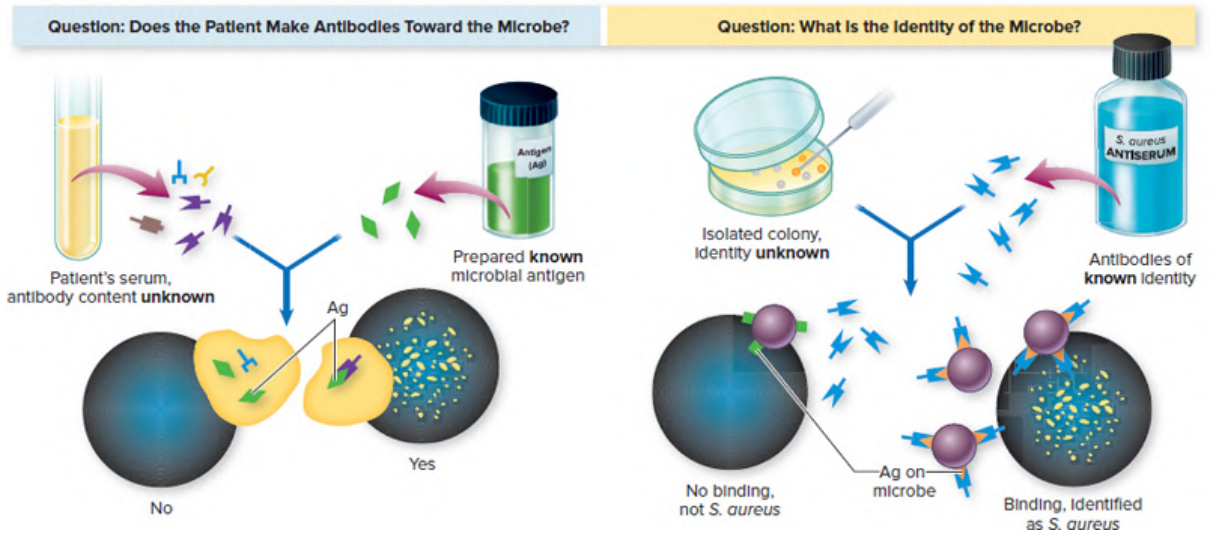


Figure 15.6 **Basic principles of serological testing using antibodies and antigens**.

is called "serology" we now test all kinds of body samples, not just sera. These include urine, cerebrospinal fluid, whole tissues, and saliva. These and other immune tests help to determine the immunologic status of patients, confirm a suspected diagnosis, or screen individuals for disease. The tests are summarized in **table 15.3**.

Different Kinds of Antigen-Antibody Interactions

The molecular basis of immunologic testing is the binding of an antibody (Ab) to a specific site (epitope) on an antigen (Ag). The binding reaction is not visible to the naked eye nor in light microscopy. But if the antigens and antibody are suspended in a solution, under the right circumstances the presence of binding can be seen as clumps in the solution.

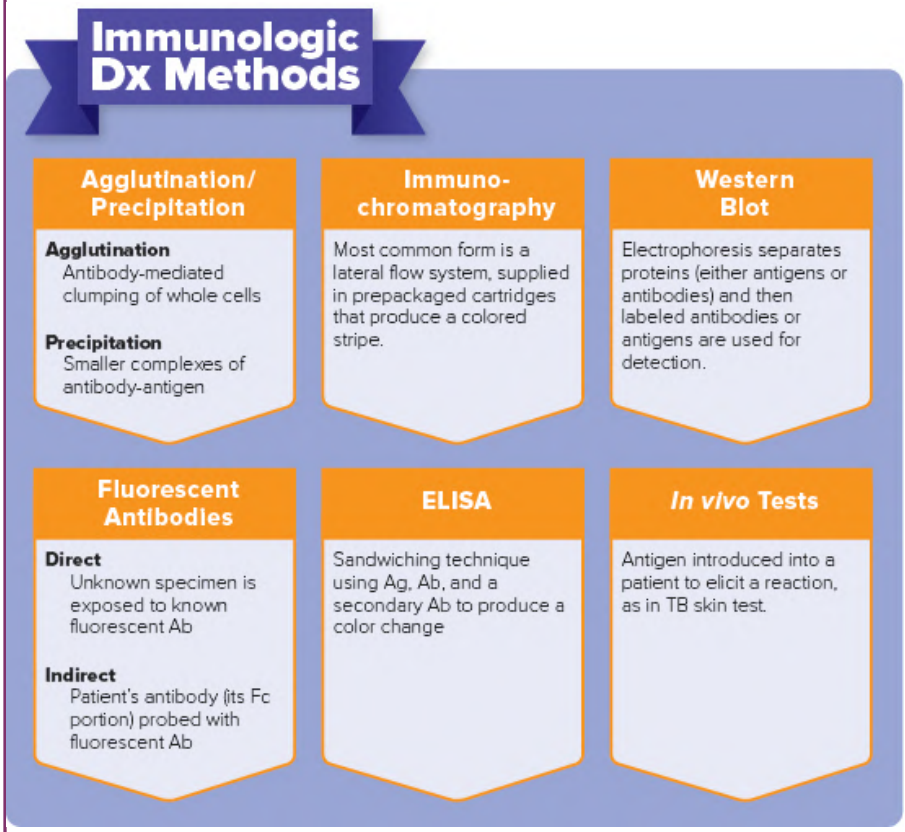
©Adam Gault/Getty Images

Agglutination and Precipitation Reactions

The essential differences between agglutination and precipitation as seen in table 15.3 are in size, solubility, and location of the antigen. In agglutination, the antigens are whole cells or organisms such as red blood cells, bacteria, or viruses displaying surface antigens **(figure 15.7);** in precipitation, the antigen examined is a soluble molecule. In both reactions, when antigen and antibody concentrations are optimal, one antigen is interlinked by several antibodies to form insoluble aggregates that settle out in solution. Agglutination is easily seen because it forms visible clumps of cells, such as in the *Weil-Felix reaction* used in diagnosing rickettsial infections. Agglutination is also used to determine blood compatibility.

These antigen-antibody binding reactions have been modified in many ways to make the reactions visible to the naked eye. One commonly used method is termed **immunochromatography**, often called the "lateral flow test." It can be found in drugstore pregnancy tests and rapid strep tests in the doctor's office. It usually consists of a plastic cartridge that contains some kind of porous material or polymer that directs fluid to flow in a particular direction. The fluid (the patient sample) will encounter antibodies along its route of flow. If the sample contains the correct antigen

Table 15.3



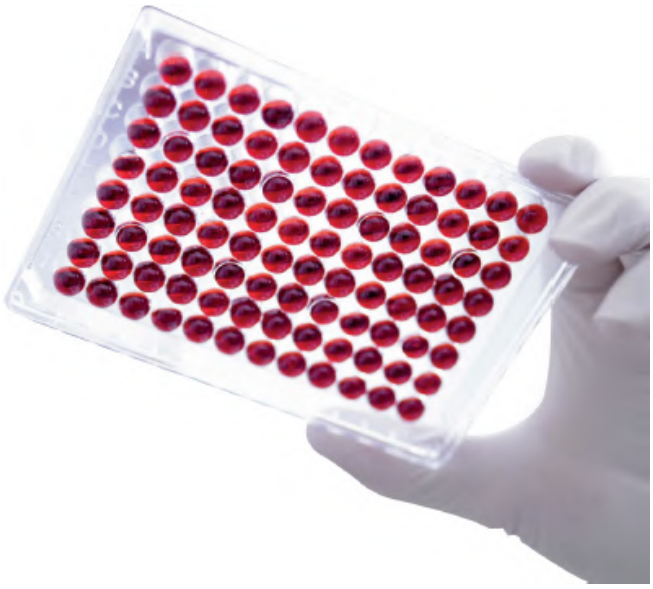
it will bind the antibodies and continue on to the next "station" in the cartridge. The next stage contains a third molecule that is impregnated on the paper in a stripe pattern. The third molecule binds the complexes and eventually this causes the stripe to change color. Thus, a microscopic antigen-antibody reaction is made visible to the naked eye **(figure 15.8)**.

Antibody Titers

An antigen-antibody reaction in liquid is read as a titer, or the concentration of antibodies in a sample. Titer is determined by serially diluting patient serum into test tubes or wells of a microtiter plate, all containing equal amounts of bacterial cells (antigen). Titer is defined as the highest dilution of serum that still produces agglutination. In general, the more a serum sample can be diluted and still react with antigen, the greater the concentration of antibodies and thus its titer. Antibody titers are often used to diagnose autoimmune disorders such as rheumatoid arthritis and lupus, and also to determine past exposure to certain diseases such as rubella.

Serotyping

Serotyping is an antigen-antibody technique for identifying, classifying, and subgrouping certain bacteria into categories called serotypes. This method employs antisera against cell antigens such as the capsule, flagellum, and cell wall. Serotyping



©Adam Gault/age fotostock

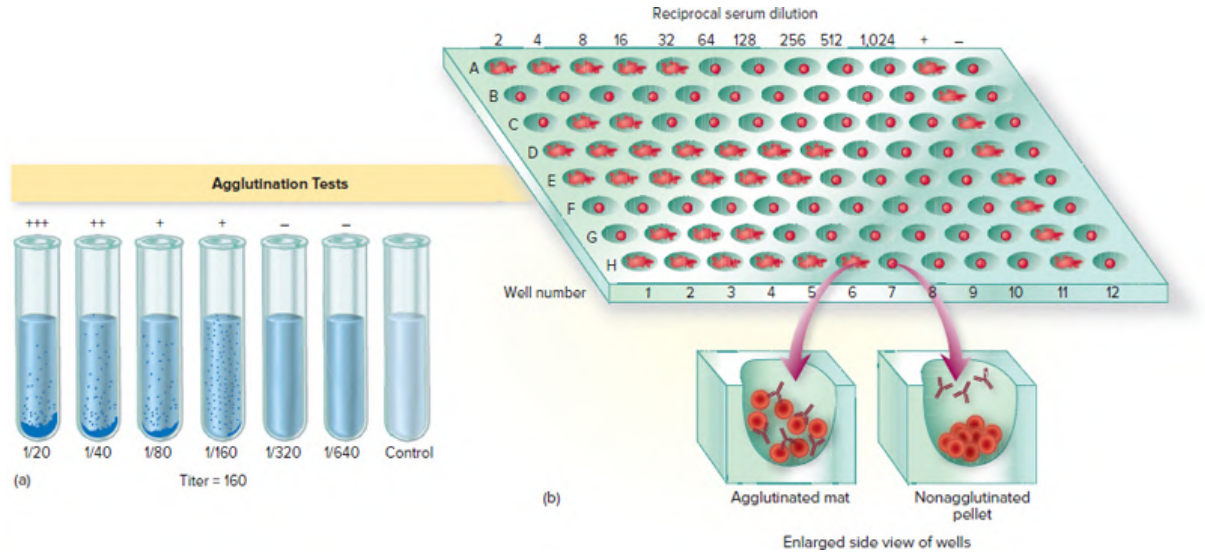


Figure 15.7 **Agglutination tests**. **(a)** Tube agglutination test for determining antibody titer. The same number of cells (antigen) is added to each tube, and the patient's serum is diluted in series. The titer in this example is 160 because there is no agglutination in the next tube in the dilution series (1/320). **(b)** A microtiter plate illustrating hemagglutination. The antibody is placed in wells 1–10. Positive controls (row 11) and negative controls (row 12) are included. Red blood cells are added to each well. If sufficient antibody is present to agglutinate the cells, they sink as a diffuse mat to the bottom of the well. If insufficient antibody is present, they form a tight pellet at the bottom.

is widely used in identifying *Salmonella* species and strains, and is the basis for differentiating the numerous pneumococcal and streptococcal serotypes.

The Western Blot Procedure

The **Western blot test** involves the separating of proteins by electrophoresis, and then using antibodies to detect the proteins **(figure 15.9)**. First, a sample of proteins obtained from cells after lysing them is separated via electrical charge within a gel. The proteins embedded in the gel are then transferred and immobilized to a special filter. The filter is next incubated with antibody solutions, some of which have been labeled with radioactive, fluorescent, or luminescent molecules. After incubation, sites of specific antigen-antibody binding will appear as a pattern of bands that can be compared with known positive and negative controls. It is a highly specific and sensitive way to identify or verify the presence of microbial-specific antigens or antibodies in a patient sample. Western blotting is the second (verification) test for preliminary antibody-positive HIV screening tests.

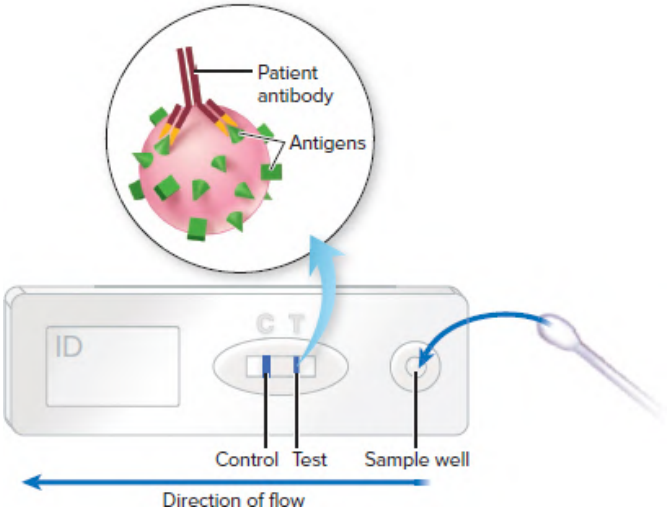


Figure 15.8 A lateral flow quick test relying on antigen-antibody binding. This is a positive test for *Neisseria gonorrhoeae*.

Immunofluorescence Testing

The fundamental tool in immunofluorescence testing is a fluorescent antibody—a monoclonal antibody labeled by a fluorescent dye. Fluorescent antibodies (FAbs) can be used for diagnosis in two ways. In *direct testing*, an unknown test specimen or antigen is fixed to a slide and exposed to a FAb solution of known composition. If antibody-antigen complexes form, they will remain bound to the sample and will be visualized by fluorescence microscopy, thus indicating a positive result **(figure 15.10)**. These tests are valuable for

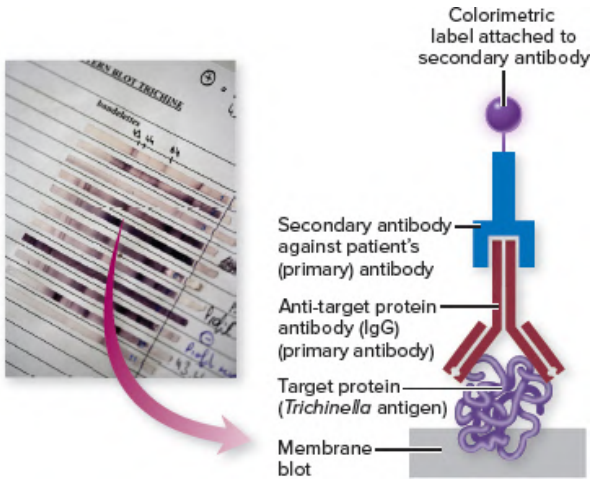


Figure 15.9 **The Western blot procedure**. Major antigens from the nematode *Trichinella* were separated via electrophoresis and transferred to a filter. The filter was incubated with the sera of 10 separate patients (in the 10 rows). Sera contain primary antibodies. Secondary antibodies (blue in the drawing) and a colorimetric label were added to visualize the patient's bound antibody (maroon in the drawing). Complexes of *Trichinella* antigen and patient antibody are visualized as bands.

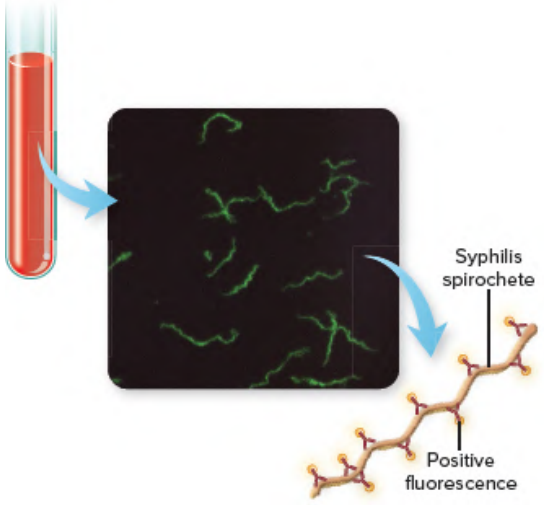


Figure 15.10 **Direct fluorescence antigen test**. Photomicrograph of a direct fluorescence test for *Treponema pallidum*, the syphilis spirochete.
Source: CDC/Russell (micrograph)

identifying and locating microbial antigens on cell surfaces or in tissues and in identifying the causative agents of syphilis, gonorrhea, and meningitis, among others.

In contrast, FAbs used in *indirect testing* recognize the Fc region of antibodies in patient sera (remember that antibodies are antigenic themselves!). Known antigen (i.e., bacterial cells) is added to the test serum, and binding of the fluorescent antibody is visualized through fluorescence microscopy. Fluorescing aggregates or cells indicate that the FAbs have complexed with the microbe-specific antibodies in the test serum. This technique is frequently used to diagnose syphilis and various viral infections.

Enzyme-Linked Immunosorbent Assay (ELISA)

The **ELISA** test, also known as enzyme immunoassay (EIA), uses an enzyme-linked indicator antibody to visualize antigen-antibody reactions. This technique also relies on a solid support such as a plastic microtiter plate that can *adsorb* (attract on its surface) the reactants **(figure 15.11)**.

The *indirect ELISA* test detects microbe-specific antibodies in patient sera. A known antigen is adsorbed to the surface of a well and mixed with unknown antibody. If an antibody-antigen complex forms, an added indicator antibody will bind, and subsequent development will produce a color change indicating a positive result. This is the common test used for antibody screening for HIV, various rickettsial species, hepatitis A and C, and *Helicobacter*. Because false positives can occur, a verification test (Western blot) may be necessary.

There are multiple ways to perform a direct ELISA. One of them is known as the sandwich test. In this setup, a known antibody is adsorbed to the bottom of a well and incubated with an unknown antigen. If an antibody-antigen complex forms, it will attract the indicator antibody, and color will develop in these wells, indicating a positive result. New cutting-edge detection systems utilize computer chips that sense minute changes in electrical current that occur when antibody-antigen complexes are formed.

Medical Moment

Understanding Lab Results

If you are planning to work in health care, it will be important for you to be able to interpret lab results and apply your knowledge to your patient's clinical situation. For example, your patient was admitted with a fever and a wound on the lower leg, and he is being treated with an antibiotic for his bacterial soft-tissue infection. Cultures were obtained from the infected area on admission and the patient was started empirically on broad-spectrum antibiotics. Over the next several hours, the infected area on the patient's lower leg increases in size and redness despite antibiotic therapy, and the patient's temperature remains elevated. You receive the patient's culture and antimicrobial susceptibilities report during the evening shift and realize that the antibiotic is not effective for the microorganism that has been identified. You notify the physician immediately, and the physician orders a different antibiotic based on the sensitivity report. The patient begins to respond to the new therapy within 24 hours—his temperature decreases and the redness begins to recede. Your decision to notify the physician when you did may have prevented the patient from getting sicker and may have shortened his hospital stay.

Q. What was more important in this case, your knowledge of which antibiotics are effective against which microbes, or your careful reading of the report?

Answer in Appendix B.

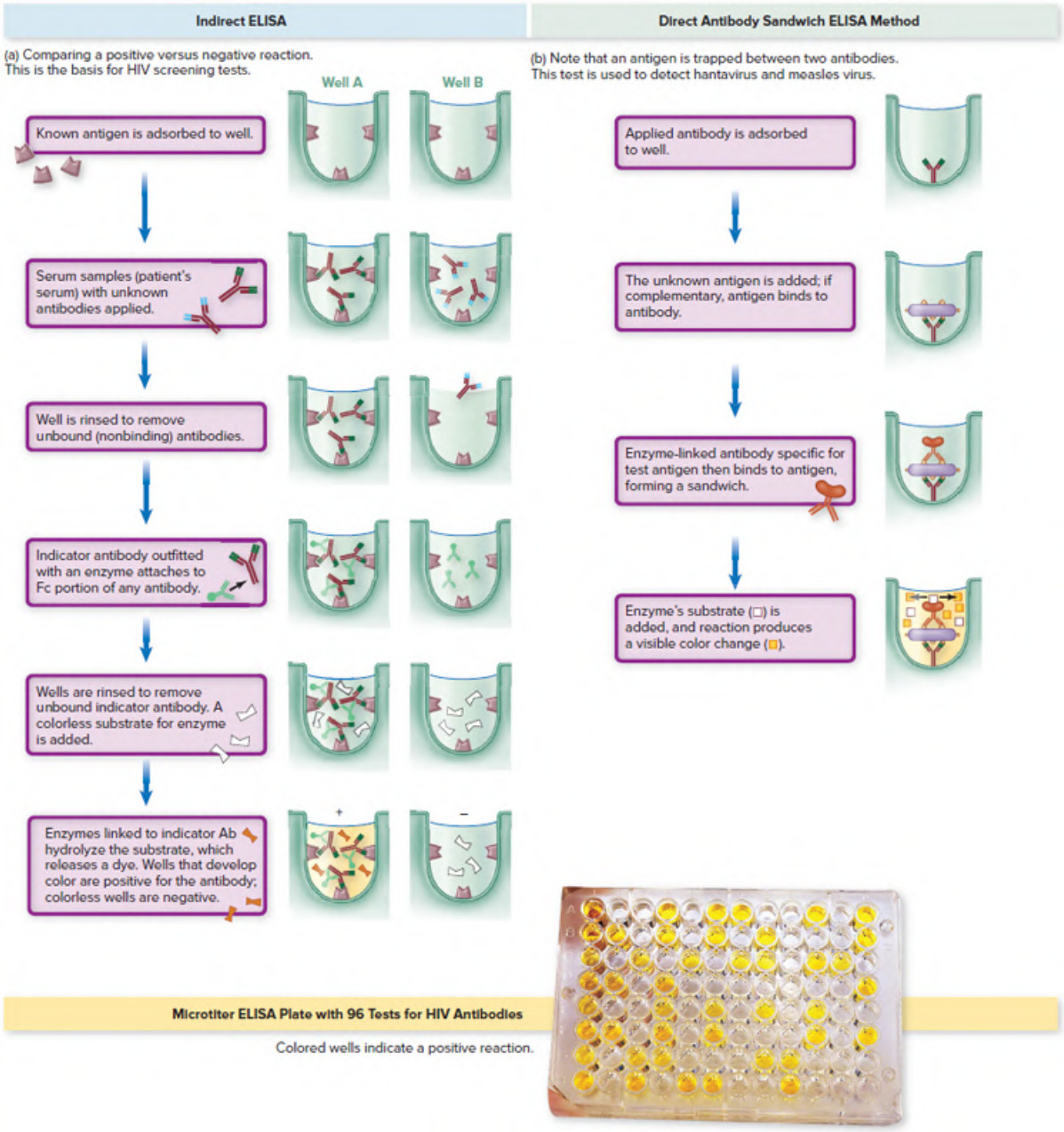


Figure 15.11 **Methods of ELISA testing**. **(a)** Indirect ELISAs detect patient antibody. Here, both positive and negative tests are illustrated. **(b)** Direct sandwich ELISAs detect patient antigen (microbes). Here, only the positive reaction is illustrated.

©Hank Morgan/Science Source

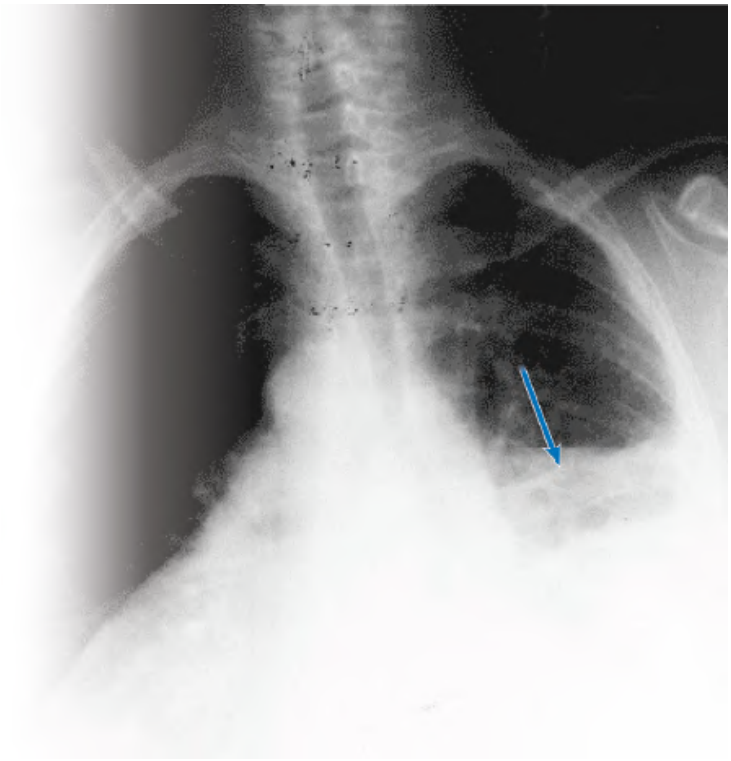
In Vivo Testing

In practice, *in vivo* tests employ principles similar to serological tests, except in this case an antigen is introduced into a patient to elicit some sort of visible reaction. The **tuberculin reaction**, where a small amount of purified protein derivative (PPD) from *Mycobacterium*

tuberculosis is injected into the skin, is a classic example. The appearance of a red, raised, thickened lesion in 48 to 72 hours can indicate previous exposure to tuberculosis (shown in figure 14.10).

General Features of Immune Testing

The most effective serological tests have a high degree of two characteristics: specificity and sensitivity. *Specificity* is the property of a test to focus on only a certain antibody or antigen and not to react with unrelated or distantly related ones. Put another way, a test with high specificity will have a low false positive rate. *Sensitivity* refers to the detection of even minute quantities of antibodies or antigens in a specimen. A test with high sensitivity will have a low false negative rate. Usually, a given test will be "better" at one or the other, and the tradeoff must be considered when choosing a test. For example in an epidemic of a deadly disease, such as Ebola, you might reason that you want to have a very high sensitivity to catch every case. People who test positive can then be screened using a different technique to determine if they actually have the disease. In other situations (for example, for that secondary screening test) you might want a higher specificity so that you don't have very many false positives.



A chest radiograph of a patient with Q fever pneumonia. The arrow shows areas of consolidation (infection). Courtesy EM Scrimgeour et el. First report of Q fever in Oman. Emerging Infectious Diseases. Jan-Feb 2000. vol. 6, no 1

15.4 LEARNING OUTCOMES—Assess Your Progress

8. Define the term *serology*, and explain the immunologic principle behind serological tests.

9. Identify two immunological diagnostic techniques that rely on a secondary antibody, and explain how they work.

15.5 Genotypic Methods

The sequence of nitrogenous bases within DNA or RNA is unique to every microorganism. Because of this and the many recent advances in genomic technology, nucleic-acid-based tests have become a mainstay of microbial identification. Genotypic methods are summarized in **table 15.4** and discussed below.

Polymerase Chain Reaction (PCR): The Backbone of New Diagnostics

Many nucleic acid tests use the polymerase chain reaction (PCR). PCR results in the production of numerous identical copies of DNA or RNA molecules within hours (see table 8.9). This method can amplify even minute quantities of nucleic acids present in a sample, which greatly improves the sensitivity of these tests. PCR amplification can be performed on genetic material from a wide variety of bacteria, viruses, protozoa, and fungi.

Adaptations and improvements of the PCR method are constantly being made. Some of the most important ones being used in diagnosis of infections are described next.

Medical Moment

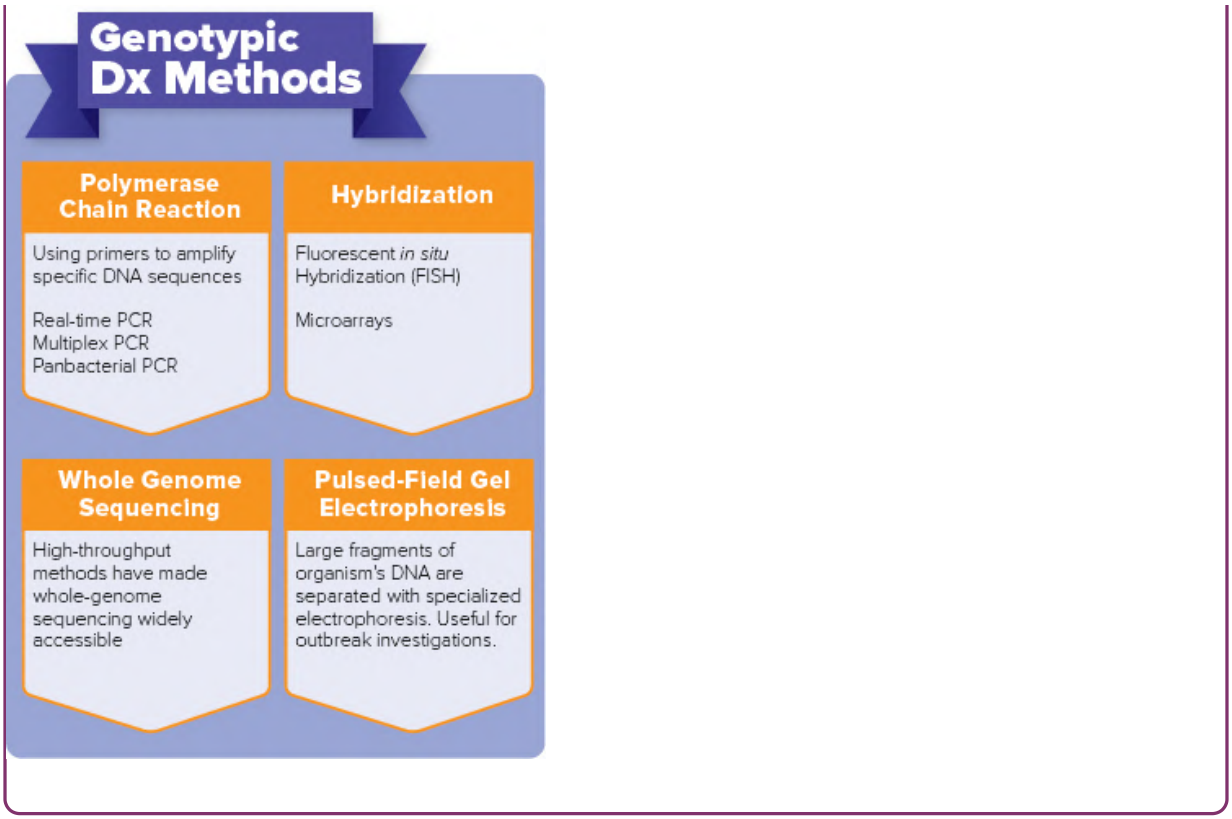
Detecting and Treating TB

In the phenotypic section earlier in this chapter you saw an example of a test used to detect the slow-growing bacterium that causes tuberculosis, Mycobacterium tuberculosis. One excellent example of how genotypic methods have been used to improve on previous methodologies is a relatively new assay called the GeneXpert MTB/RIF test. It is an automated test using nucleic acid amplification (basically the PCR method). It accomplishes two things that are very difficult and very slow to do with
M. tuberculosis: Identify the bacterium in a specimen and simultaneously assess its susceptibility to the front-line antibiotic, rifampin. This test is in a cartridge system and delivers answers to both those questions in 90 minutes, as opposed to weeks as was previously required. It is user-friendly and requires no special technical expertise, which makes it extremely useful in areas of the world where TB is endemic. It is a breakthrough that scientists have been working toward for decades.

Q. What are the consequences of taking several weeks to confirm a TB diagnosis, as opposed to 90 minutes?

Answer in Appendix B.

Table 15.4



Real-Time PCR

This technique is also known as "qPCR" because there is a quantitative aspect to it. It uses fluorescent labeling during the amplification procedure and the level of fluorescence is measured in real time as the reaction is running. It is fully automated and is faster than traditional PCR, because analysis of the DNA after the reaction is finished is not necessary. Another advance is that qPCR assays often assess the antimicrobial susceptibilities at the same time they are identifying the organism. Note: Real-time PCR is often confused with reverse-transcriptase PCR (you can see why). Reverse-transcriptase PCR is rightly referred to as RT-PCR, and involves the creation of DNA out of RNA ("reverse" transcription).

Multiplex PCR

This is a type of diagnostic PCR that contains primers for multiple organisms instead of just a single primer. As with biochemical panels, a multiplex PCR will contain primers for multiple organisms in the differential diagnosis for the patient's symptoms. Most often, multiplex PCR is also real-time PCR.

Panbacterial qPCR

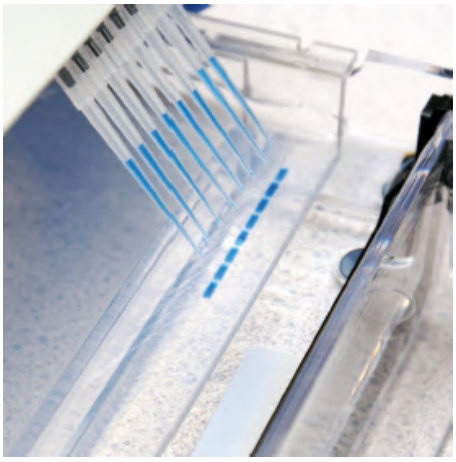
The prefix *pan*-means "universal," and this real-time PCR technique uses a primer (16srRNA) that is common to all bacteria. Thus, every bacterium in a sample will be amplified. It is particularly important when assessing a wound or infection that is

polymicrobial. This technique has the advantage that it can detect bacteria even after antibiotic treatment.

Hybridization: Probing for Identity

Hybridization is a technique that makes it possible to identify a microbe by analyzing segments of its genetic material. This requires small fragments of single-stranded DNA or RNA called **probes** that are known to be complementary to the specific sequences of nucleic acid isolated from a particular microbe. Base-pairing of the known probe to the nucleic acid can be observed providing evidence of the microbe's identity. These tests have become more convenient and portable over the years. Probes are typically fluorescently labeled or attached to an enzyme that triggers a colorimetric change when hybridization occurs. This is based on the property of dyes such as fluorescein and rhodamine, which emit visible light in response to ultraviolet. This property of fluorescence has found numerous applications in diagnostic testing.

Fluorescent *in situ* hybridization (FISH) techniques involve the application of fluorescently labeled probes to intact cells within a patient specimen or an environmental sample **(figure 15.12)**. Microscopic analysis is used to locate "glowing" cells and conclude the identity of a specific microbe. FISH is often used to confirm a diagnosis or to identify the microbial components within a biofilm. Due to the convenience and accuracy of new PCR methods, FISH methods are decreasing in use for diagnosis of infections, but continue to be used in cancer diagnoses, especially in personalized medicine, where a patient's DNA is examined for particular characteristics that can make a certain drug a better choice for them.



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Microarrays

Microarrays designed for infectious disease diagnosis are "chips" (absorbent plates) that contain gene sequences from potentially thousands of different possible infectious agents, selected based on the syndrome being investigated (such as respiratory infection, or meningitis symptoms). The arrays are selected based on a very large differential diagnosis; in other words, what possible microbes could cause disease in this syndrome? Arrays can be

made to contain bacterial, viral, and fungal genes in a single test. In this scenario, patient samples (sputum, cerebrospinal fluid) or the nucleic acids isolated from them are incubated with labeled gene sequences on the microarray. Matching sequences hybridize to the chip, and the label (in most cases it

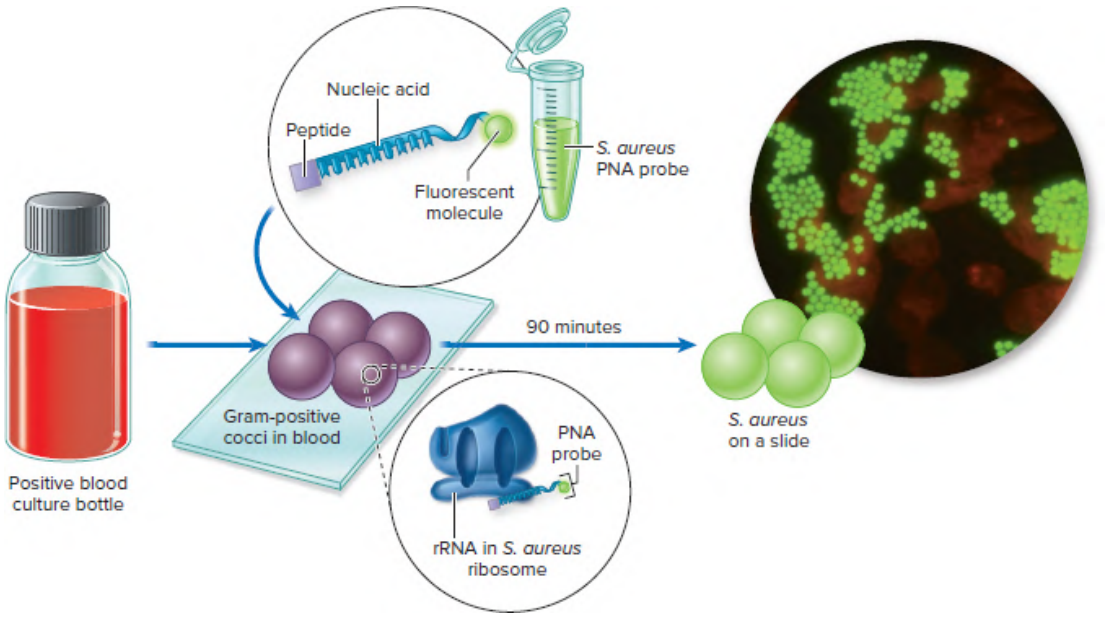


Figure 15.12 Peptide nucleic acid (PNA) FISH testing for *S. aureus*. Testing can identify blood-borne pathogens more quickly than other methods.
Image provided by AdvanDx, Inc. (*S. aureus*)

is fluorescence) is detected by a computer program, which provides the identity of the isolate or isolates **(figure 15.13)**.

Whole-Genome Sequencing

The cost of whole-genome sequencing, in terms of time and money, is becoming so low that this technique is becoming commonplace in clinical and epidemiological laboratories around the world. It is particularly useful for rapid analysis of outbreaks and drug-resistant organisms. More importantly, a single genome can be scanned and analyzed multiple times in a process called “deep sequencing,” which minimizes errors. Many scientists suspect that these types of sequencing will become so cheap and so routine that we will soon just “sequence everything” from a patient sample to find the one or more microbes causing symptoms.

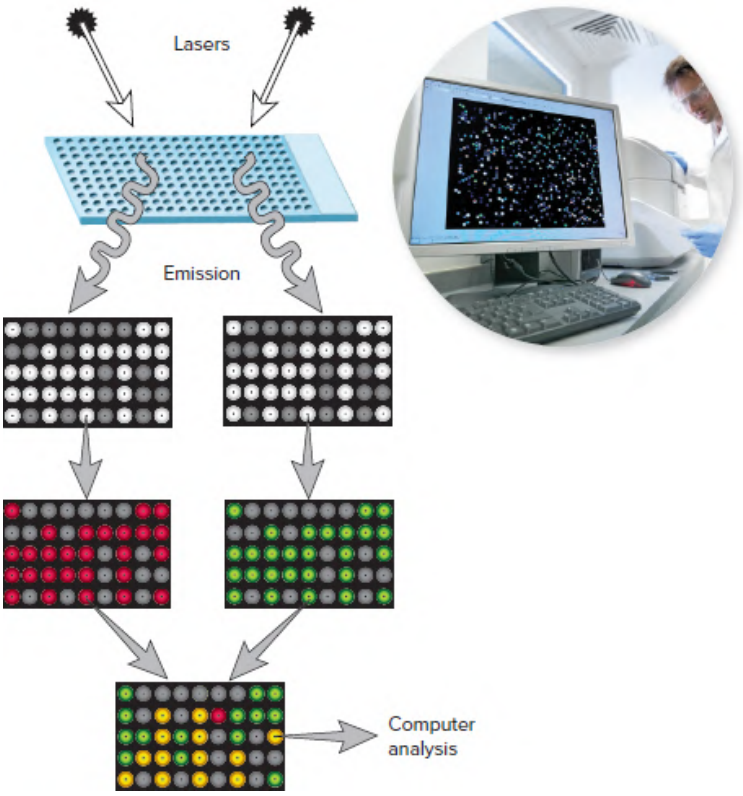


Figure 15.13 **Microarrays**. Digital view of a microarray.
©Cultura/Getty Images (photo)

Pulsed-Field Gel Electrophoresis: Microbial Fingerprints

In chapter 8, genetic fingerprinting was described as a method for analyzing short segments of DNA within a sample (see figure 8.17). Pulsed-field gel electrophoresis (PFGE) is a similar technique but it involves the separation of DNA fragments that are too large for conventional gel electrophoresis methods **(figure 15.14)**. This is accomplished by slowly applying alternating voltage levels to the gel from three different directions, allowing even similarly sized DNA fragments to fully separate. Because the DNA is subjected to restriction enzymes, single changes in the DNA sequence (from mutations) will result in fragments of different sizes. PFGE is often used in acute outbreaks of foodborne and other infections. PulseNet is a program established by the CDC that uses PFGE to assist in the investigation of possible infectious disease outbreaks, such as those caused by foodborne pathogens or contact with reptiles or backyard chickens. Scientists from public health facilities across the country

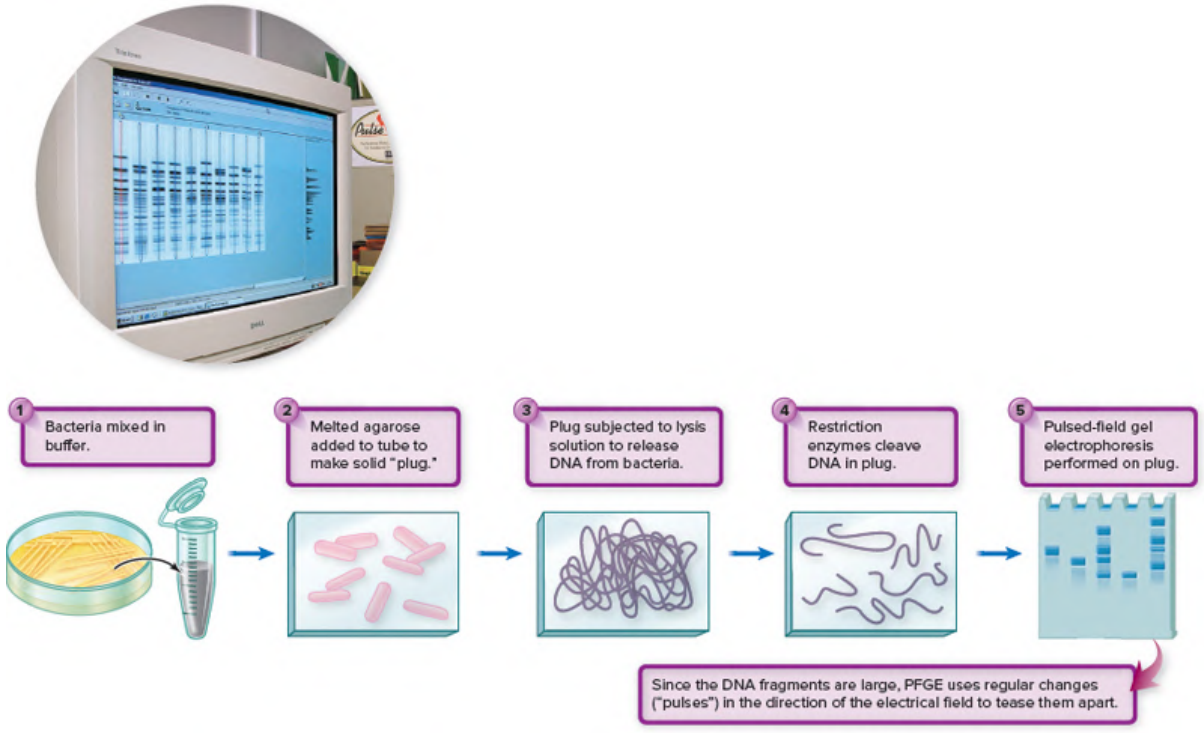


Figure 15.14 **Pulsed-field gel electrophoresis**.
Source: CDC

are able to rapidly communicate and compare PFGE data from patient specimens and other samples, allowing identification of outbreaks to occur within hours versus days or weeks. The CDC reports that they are in the process of transitioning their PulseNet system to using whole-genome sequencing, but PFGE is still useful in some applications.

Nucleic acid sequencing.
©Andrew Brookes/Getty Images

15.5 LEARNING OUTCOMES—Assess Your Progress

10. Explain why PCR is useful for infectious disease diagnosis.
11. Name two examples of techniques that employ hybridization.

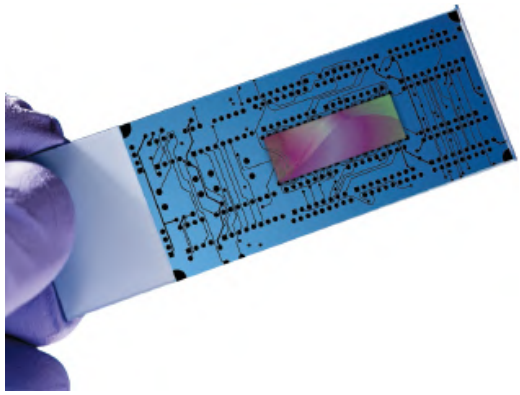
12. Explain how whole-genome sequencing can be used for diagnosis.
13. Identify the situations in which pulsed-field gel electrophoresis is most useful.

15.6 Additional Diagnostic Technologies

In addition to the traditional phenotypic and immunologic methods, you have seen in this chapter that highly automated processes for determining the genetic makeup of unknowns are being adopted at a rapid pace. In recent years breakthroughs in other scientific disciplines have also been brought into infectious disease diagnosis. We'll look at three of them: lab on a chip, mass spectrometry, and imaging technologies.

Lab on a Chip

Microarrays are contained on chips, as noted. Since the development of microarrays, many genetic tests have been miniaturized and placed on chips that are easy to use, requiring few supplies and little technical training. These have been facilitated by the use of microfluidics, using minuscule amounts of reagents and fluids to perform reactions that were previously done in large test tubes. This streamlines the testing and makes it easy to perform. DNA and RNA sequencing as well as PCR methods have been made available on "chips." While it is useful in clinical laboratories in developed countries, the biggest impact of labs on chips is predicted to be in developing countries, where diagnosis is often not possible due to lack of supplies, expertise, or even the refrigeration required to store an array of diagnostic reagents.



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Mass Spectrometry

Mass spectrometry has been utilized for years to determine the structure and composition of various chemical compounds and biological molecules. It is now being used, alone and in combination with other technologies such as PCR, to provide rapid and highly accurate microbial identification within just minutes. This technique, which is also often called MALDI-TOF, can be used to analyze a protein fingerprint from pure culture isolates or directly from samples isolated from patient specimens. It works by adding the patient sample to a metal plate and then striking it with a laser. This causes the sample to become ionized.

The ions from the sample are guided into a machine that separates them and identifies them according to their mass-to-charge ratio. This technology has been applied to the identification of bacteria, viruses, and fungi, so far, and is becoming commonplace in many clinical and research laboratories due to its ability to produce rapid, precise, and cost-effective results compared to conventional phenotypic, genotypic, and immunologic methods. One distinct advantage of this technique is that clinical laboratories can construct databases of local strains of microorganisms by running profiles of known microbes. This provides for an infinitely customizable and expandable database.

Mass spectrometry technologies can also be used to simultaneously detect antibiotic susceptibilities—obviously, an important advantage **(figure 15.15)**.

Imaging

For centuries imaging, in the form of X rays, has been used to assist in the diagnosis of tuberculosis. Now in the 21st century a variety of new imaging techniques

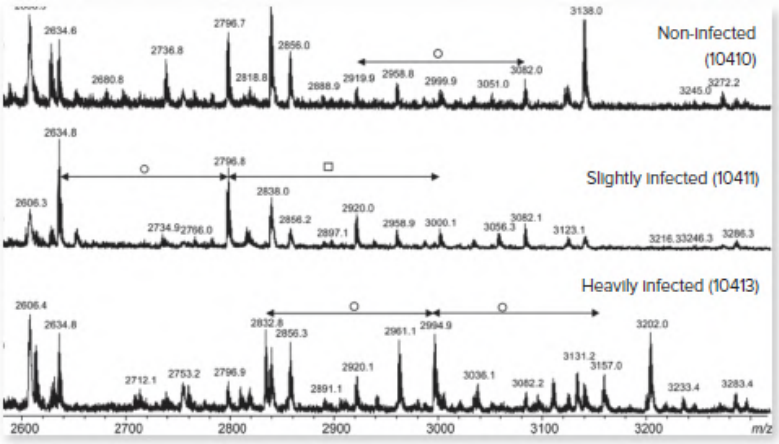

Figure 15.15 **Mass spectrometry of patient samples**.

Figure 15.16 **A patient undergoing MRI (magnetic resonance imaging) testing**.

©Pixtal/AGE Fotostock

have been deployed to help localize infections. They offer a pair of eyes to inaccessible places, and can save the patient an invasive biopsy. Infections associated with hip implants, for example, may be difficult to access through blood samples. The bacteria may be growing in biofilms on the implanted materials, or they may be growing in an abscess deep in the hip joint. Magnetic resonance imaging, computerized tomography (CT) scan, and positron emission tomography (PET) scans have been increasingly employed to find areas of localized infection in deep tissue, which can later be biopsied to aspirate samples for culture. In the event no infection is found on the image, the patient has been spared an invasive procedure **(figure 15.16)**.

Entirely New Diagnostic Strategies

Throughout the modern medical era, diagnosis of suspected microbial infections has focused on identifying the offending organism. But there is new thinking informing diagnosis and treatment. One strategy uses blood from a patient to check for the presence of seven genes that the host cells express in response to bacterial—but not viral—infection. If the seven genes are active, then the prescriber knows to prescribe an antibiotic. If they are not active, the symptoms are caused by virus(es) and therefore not going to be helped by antibiotics. This is an incredibly useful tool in a world where it is estimated that *the majority* of antibiotic prescriptions are incorrectly prescribed for conditions that are not caused by bacteria. Remember that many times in the United States (and most of the time in poorer countries) prescriptions are given without the benefit of diagnostic testing. Researchers at Stanford University, who developed this blood test, are working to make it more like *lab on a chip* technology, so that it can be quick and easy to use in all kinds of conditions.

Another "diagnostic" technique is under development by researchers in Boston. It is a blood test that scans for antibodies to more than 1,000 strains of viruses representing 206 species. The drop of blood contains the entire history of a person's exposure to viruses. There are wide-ranging implications in knowing this, from diagnosis of a particular set of symptoms to looking at the viruses that correlate with particular human cancers. In case you're wondering, of the 569 people who were initially tested from several continents, the average person has been exposed to about 10 viruses, while "overachievers" have been exposed to as many as 25. This technique certainly represents a new way of thinking about diagnosis—of past as well as present infections.

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15.6 LEARNING OUTCOMES—Assess Your Progress

14. Describe the benefits of "lab on a chip" technologies for global public health.
15. Identify an advantage presented by mass spectrometry and also by imaging techniques as diagnostic tools.

CASE FILE WRAP-UP

©McGraw-Hill Education/Trish Ofenlock, photographer (health care worker); ©McGraw-Hill Education/Lisa Burgess, photographer (test plate)

Increasing the number of blood cultures obtained increases the likelihood of isolating the offending organism and can be lifesaving. When two sets of blood cultures are obtained

and a pathogen is identified from both cultures, it is highly unlikely that the organism cultured is a contaminant. Both aerobic and anaerobic blood cultures are obtained to provide the best chance of isolating the organism.

Blood cultures are indicated before beginning parenteral antibiotic therapy in patients with fever and leukocytosis (elevated white blood cell count). It is important to obtain blood cultures before IV antibiotics are started because the antibiotics may inhibit the growth of the microorganism before it can be isolated.

The patient in the opening Case File had a fever with a decreased level of consciousness, both signs of sepsis. Blood cultures are used to identify microorganisms that have invaded the bloodstream, causing life-threatening septicemia. It was important for the lab workers to notify the nursing staff as soon as the cultures were collected so that the nursing staff could administer the initial dose of IV antibiotics.

©Adam Gault/Getty Images

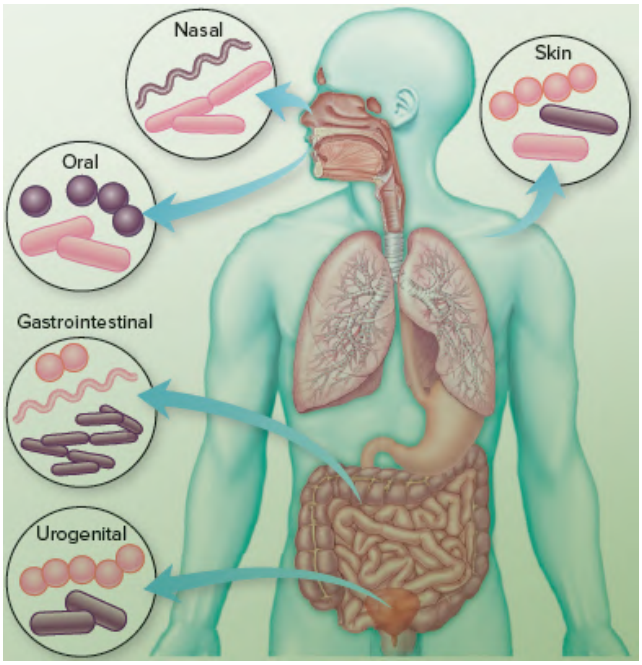
The Human Microbiome Project and Diagnosis of Noninfectious Disease

For decades, scientists have realized that culturing microbes for identification is not only time-consuming and inefficient but also incomplete. Many microbes are fastidious, requiring highly specialized growth conditions, and many others are considered "viable but nonculturable" (VNC). Some estimate that 99.9% of sequences found in the human body fall under the heading of VNC, and little is understood about the role that these elusive microbes play in the human microbiome and in disease.

Using techniques developed by J. Craig Venter in mapping the genome of microbes in the oceans, scientists have been able to map the genomes of VNC microorganisms in the Human Microbiome Project (HMP). Scientists at the Venter Institute developed multiple displacement amplification (MDA) technology. MDA is a non-PCR technique for copying fragments of DNA from a single cell until they reach the equivalent of the billions required for analysis. MDA is able to capture 90% of the genes from a single cell.

Using MDA, scientists are able to obtain information about susceptibility to antibiotics, signaling proteins used, and even how a VNC microbe lives and moves. All of this information is vital to the HMP. As you read in chapter 13, in the HMP, researchers sampled microbes from the mouth, nose, skin, intestine, and genitals of 242 volunteers (129 male and 113 female), resulting in over 5,000 samples from body sites. Using MDA, they collected about 3.5 terabases* of DNA sequences that have been entered into a gigantic comparative analysis system called the Integrated Microbial Genomes and Metagenomes for the Human Microbiome Project (IMG/M HMP).

Scientists point out that although there is only about a 0.01% difference between human genomes from one person to another, our microbiome genomes differ by as much as 50%. Researchers are beginning to use microbiome genome characteristics to predict who is at risk for type II diabetes, for example, and for leanness or obesity. While we should apply caution to "overinterpreting the microbiome," as some scientists say, there will clearly be important diagnostic tools coming from our knowledge of its genetics.



*A *terabase* is a genetic sequence of 10^{12} nucleic acid bases.

Chapter Summary

15.1 Preparation for the Survey of Microbial Diseases

- Microbiologists use three categories of techniques to diagnose infections: *phenotypic, immunologic*, and *genotypic*. In addition, some specialty technologies are being used.

15.2 On the Track of the Infectious Agent: Specimen Collection

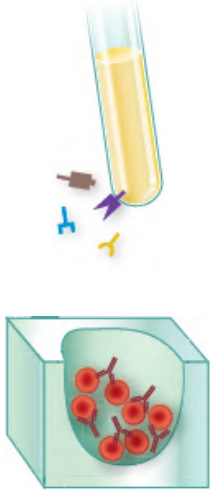
- The first step in clinical diagnosis (after patient observation) is obtaining a specimen. If this step is not performed correctly, specimen analysis will not be accurate no matter how good the test is.

15.3 Phenotypic Methods

- The main phenotypic methods include the direct examination of specimens, observing the growth of specimen cultures on special media, and biochemical testing of pure cultures.
- Most phenotypic methods require the culturing of the microbes. Furthermore, it does not account for infections that may be polymicrobial.

15.4 Immunologic Methods

- Serological testing can be performed on a variety of body fluids or tissues and is based on the principle that antibodies have extreme specificity for antigens.
- Agglutination reactions occur between antibody and antigens bound to cells, resulting in visible clumping. It is the basis of determining titer, or antibody concentration, in patient sera.
- Immunochromatographic methods use cartridges and lateral flow technology to make antibody-antigen interactions easily visible.
- Serotyping employs antisera against cellular antigens such as the capsule, flagellum, and cell wall to identify bacterial species and strains.
- In the Western blot procedure, proteins that have been separated by electrical current are identified by labeled antibodies.



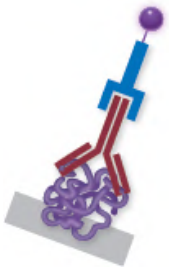
- Direct fluorescence antibody tests indicate the presence of microbial antigens and are useful in identifying infectious agents.
- The ELISA test is widely used to detect antigens (direct sandwich method) or antibodies (indirect method) in patient samples.
- *In vivo* serological testing, such as the tuberculin reaction, involves injection of antigen to elicit a visible immune response in the host.

15.5 Genotypic Methods

- The use of genotypic methods in microbial identification has grown exponentially.
- Polymerase chain reaction is used on its own for diagnosis, and also as a precursor to other genetic methods.
- Hybridization techniques exploit the base-pairing characteristics of nucleic acids. FISH methods and microarrays are two examples of hybridization techniques.
- Whole-genome sequencing is becoming widely available for diagnosis and outbreak analysis.
- Pulsed-field gel electrophoresis is particularly suited to outbreak investigations.
- High-throughput whole-genome sequencing is quickly becoming commonplace for diagnosing infectious diseases.

15.6 Additional Diagnostic Technologies

- *Lab on a chip* takes advantage of microfluidics to automate and miniaturize complex diagnostic procedures and can make them available to under-equipped locales.
- Mass spectrometry detects microbes via their protein fingerprints.
- Imaging techniques such as magnetic resonance imaging (MRI) and computerized tomography (CT) scans are increasingly used to visualize deep tissue infections.



SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	**A. Bloom's Level 1, 2–Remember and Understand (Choose one.)**	**B. Bloom's Level 3, 4–Apply and Analyze**	**C. Bloom's Level 5, 6–Evaluate and Create**
Evolution	**1**. When using pulsed-field gel electrophoresis, mutations in a microbe's genome will show up as **a**. a different pattern of bands. **b**. nonfluorescent bands. **c**. missing bands. **d**. all of these.	**2**. Explain why it is possible to identify some bacteria simply by knowing what antibiotic resistances they possess.	**3**. Serotyping identifies distinct members of the same species that are stably different from one another. Speculate about why these distinct serotypes are not different species instead.

ASM Concept/Competency	A. Bloom's Level 1, 2–Remember and Understand (Choose one.)	B. Bloom's Level 3, 4–Apply and Analyze	C. Bloom's Level 5, 6–Evaluate and Create
Cell Structure and Function	**4**. Mass spectrometry identifies microbes via **a**. fluorescent antibodies. **b**. cell surface carbohydrates. **c**. protein fingerprints. **d**. DNA profiling.	**5**. Name some bacterial structures that might be useful to target when using fluorescent antibodies designed for diagnosis, and justify your choices.	**6**. You perform a lumbar puncture on a patient with meningitis symptoms and see that the spinal fluid is cloudy. However, panbacterial PCR comes up with nothing. What is a likely explanation?
Metabolic Pathways	**7**. Which category of diagnosis is represented by studying a microbe's utilization of nutrients? **a**. phenotypic **b**. genotypic **c**. immunologic **d**. none of these	**8**. Write a paragraph that explains the mycobacterial growth indicator tube test to a middle school science class.	**9**. You inoculated a biochemical test strip with a patient isolate in a rural clinic you work in, and left it in the incubator overnight. When you came back in you saw that there had been a power outage (with

ASM Concept/Competency	A. Bloom's Level 1, 2– Remember and Understand (Choose one.)	B. Bloom's Level 3, 4–Apply and Analyze	C. Bloom's Level 5, 6–Evaluate and Create
			no backup power generator) and the temperature in the incubator was 25°C instead of 37°C. You examine the test strip and you see that the wells display growth. Do you trust the results or not? Why or why not?

ASM Concept/Competency	A. Bloom's Level 1, 2–Remember and Understand (Choose one.)	B. Bloom's Level 3, 4–Apply and Analyze	C. Bloom's Level 5, 6–Evaluate and Create
Information Flow and Genetics	**10**. Which of the following diagnostic techniques is most likely to be affected by changes in growth conditions of the specimen? **a**. phenotypic **b**. immunologic **c**. genotypic	**11**. You perform a Kirby-Bauer disk diffusion test to determine antibiotic susceptibilities of a clinical isolate. There is a clear zone of several millimeters around the penicillin disk, but there are two to three distinct colonies in the middle of the clear halo. What is the probable explanation for those colonies?	**12**. Why might culture conditions affect the results of a mass spectrometry method of diagnosis?

Microbial Systems	**13**. Which of the following techniques is most likely to reveal that an infection is in biofilm form? **a**. ELISA **b**. whole-genome sequencing **c**. PFGE	**14**. Why is it more important to use selective media when diagnosing a GI tract infection than when diagnosing a bloodstream infection?	**15**. What type of diagnostic method do you think would be most suited to determining if a bacterium causing a human infection was lysogenized by a bacteriophage that contains a virulence gene?

	d. imaging		
Impact of Microorganisms	**16**. T or F: Bacterial infection causes the expression of different human genes than does viral infection. **a**. T **b**. F	**17**. Most HIV tests look for patient antibody to the virus rather than the virus itself. Identify some advantages to this approach.	**18**. Nonhealing wounds on the surface of the body are often extremely difficult to manage, in part because the microbial cause of the lack of healing is often extremely difficult to identify. Create a list of reasons this might be the case.
Scientific Thinking	**19**. A test that results in a very large number of false positives probably has an unacceptable level of **a**. sensitivity. **b**. specificity.	**20**. When PCR is performed "by hand" (not with a prepackaged test kit), the lab environment has to be scrupulously clean. Explain why that might be.	**21**. What kind of a control would be important to run when performing a metabolic diagnostic test such as an API® strip?

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. From chapter 14, figure 14.10b. Imagine that this patient is being seen by his or her physician for this unknown rash. What rapid phenotypic test could suggest that this condition is caused by a bacterium? Could a rapid immunoassay or fluorescent procedure be

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connect Resources are available through Connect, including interactive learning tools, animations, and more.

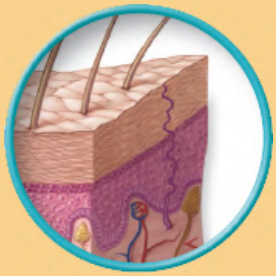
Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

16

Infectious Diseases Affecting the Skin and Eyes

©Syda Productions/Shutterstock (health care worker); ©Dennis MacDonald/Alamy Stock Photo (hospital); Source: CDC (biota of skin); Source: CDC/Courtesy World Health Organization (smallpox); Source: CDC/Janice Haney Carr/Jeff Hageman, M.H.S. (biota of eye); ©ISM/Medical Images (eye disease)

IN THIS CHAPTER…



16.1 The Skin and Its Defenses

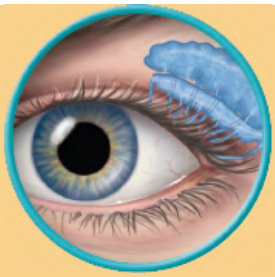
1. Describe the important anatomical features of the skin.
2. List the natural defenses present in the skin.

16.2 Normal Biota of the Skin

3. List characteristics of the skin's normal microbiota.

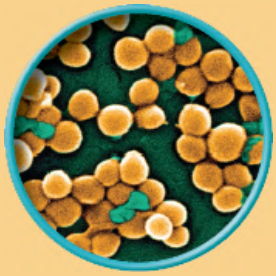
16.3 Skin Diseases Caused by Microorganisms

4. Explain the important features of the "Highlight Disease," MRSA skin and soft-tissue infection.
5. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Disease," maculopapular rash diseases.
6. Discuss important features of the other infectious skin diseases. These are impetigo, cellulitis, staphylococcal scalded skin syndrome, vesicular/pustular rash diseases, large pustular skin lesions, and cutaneous and superficial mycoses.
7. Discuss the relative dangers of rubella and rubeola viruses in different populations.



16.4 The Surface of the Eye and Its Defenses

8. Describe the important anatomical features of the eye.
9. List the natural defenses present in the eye.



16.5 Normal Biota of the Eye

10. List the types of normal biota presently known to occupy the eye.

16.6 Eye Diseases Caused by Microorganisms

11. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Disease," conjunctivitis.
12. Discuss the most important causes of keratitis.

CASE FILE

A Rash of Symptoms

I was a newly graduated nurse working in the emergency room of a large urban children's hospital when Wyatt was brought in by his mother. Wyatt was a 6-year-old who, until a few days prior, had been very healthy. Wyatt's mother told the triage nurse that Wyatt was very sick and had a rash "all over." As was policy, Wyatt was given a mask to wear because he was coughing and was sent back out to the waiting room after having a brief history taken and a set of vital signs.

Wyatt had been triaged as urgent due to his cough, high fever, and elevated heart rate, so it wasn't long before I called Wyatt to an exam room to perform a more in-depth history and head-to-toe assessment. According to Wyatt's mother, Wyatt had initially complained of a sore throat. Within a day, he also complained of a headache and developed a high fever, which his mother treated appropriately with acetaminophen. He then developed a cough, which his mother assumed was part of a "bad cold." She was not overly concerned until Wyatt developed a rash, which started on his face and head and rapidly spread to his chest and back, and then to his arms and legs.

I had Wyatt's mother undress him and put him in an examination gown, and it was only then that I realized the extent of Wyatt's rash. It was winter and Wyatt had been covered in clothing from head to toe, having only his jacket removed when seen by the triage nurse. Dressed in a gown, I saw that Wyatt's mother was not exaggerating—Wyatt's rash covered almost all of his body. It was a maculopapular rash and did not look like any childhood rash that I had ever seen, before or since.

The physician on duty came in to see Wyatt and immediately asked Wyatt to open his mouth. She asked the mother about Wyatt's immunizations, and his mother admitted that she did not believe in immunizations and Wyatt had never been immunized. The physician then turned immediately to me and asked me to put Wyatt in an isolation room. Upon my return, she spoke to Wyatt's mother, and I will never forget what she said: "Well, it was a very bad decision to not have Wyatt immunized, because now he has the measles. Not only does your son have the measles, but by bringing him here you exposed everyone in the waiting room to what can be a life-threatening illness." She then ordered a chest X ray and blood work to confirm the diagnosis and said that she would immediately notify the state health department.

- Why was a chest X ray performed?

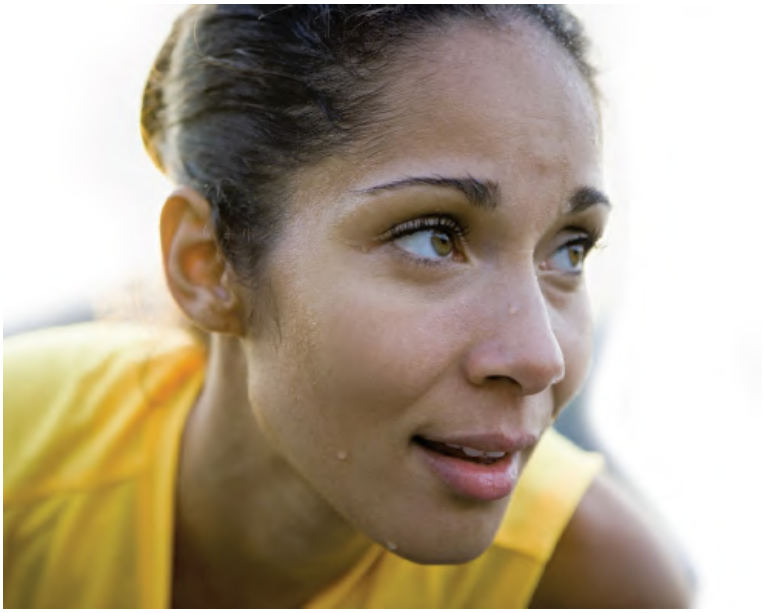
- Why was the state health department contacted?

Case File Wrap-Up appears at the end of the chapter.

16.1 The Skin and Its Defenses

The organs under consideration in this chapter—the skin and eyes—form the boundary between the human and the environment. The skin, together with the hair, nails, and sweat and oil glands, forms the **integument**. The skin has a total surface area of 1.5 to 2 square meters. Its thickness varies from 1.5 millimeters at places such as the eyelids to 4 millimeters on the soles of the feet. Several distinct layers can be found in this thickness, and we summarize them here. Follow **figure 16.1** as you read.

The outermost portion of the skin is the epidermis, which is further subdivided into four or five distinct layers. On top is a thick layer of epithelial cells called the stratum corneum, about 25 cells thick. The cells in this layer are dead and have migrated from the deeper layers during the normal course of cell division. They are packed with a protein called **keratin**, which the cells produce in very large quantities. Cells emerge from the deepest levels of the epidermis. Because this process is continuous, the entire epidermis is replaced every 25 to 45 days. Keratin gives the cells their ability to withstand damage, abrasion, and water penetration. The surface of the skin is termed *keratinized* for this reason. Below the stratum corneum are three or four more layers of epithelial cells. The lowest layer, the stratum basale, or basal



Sweat is cooling and it has several antimicrobial properties.
©Rubberball/Getty Images

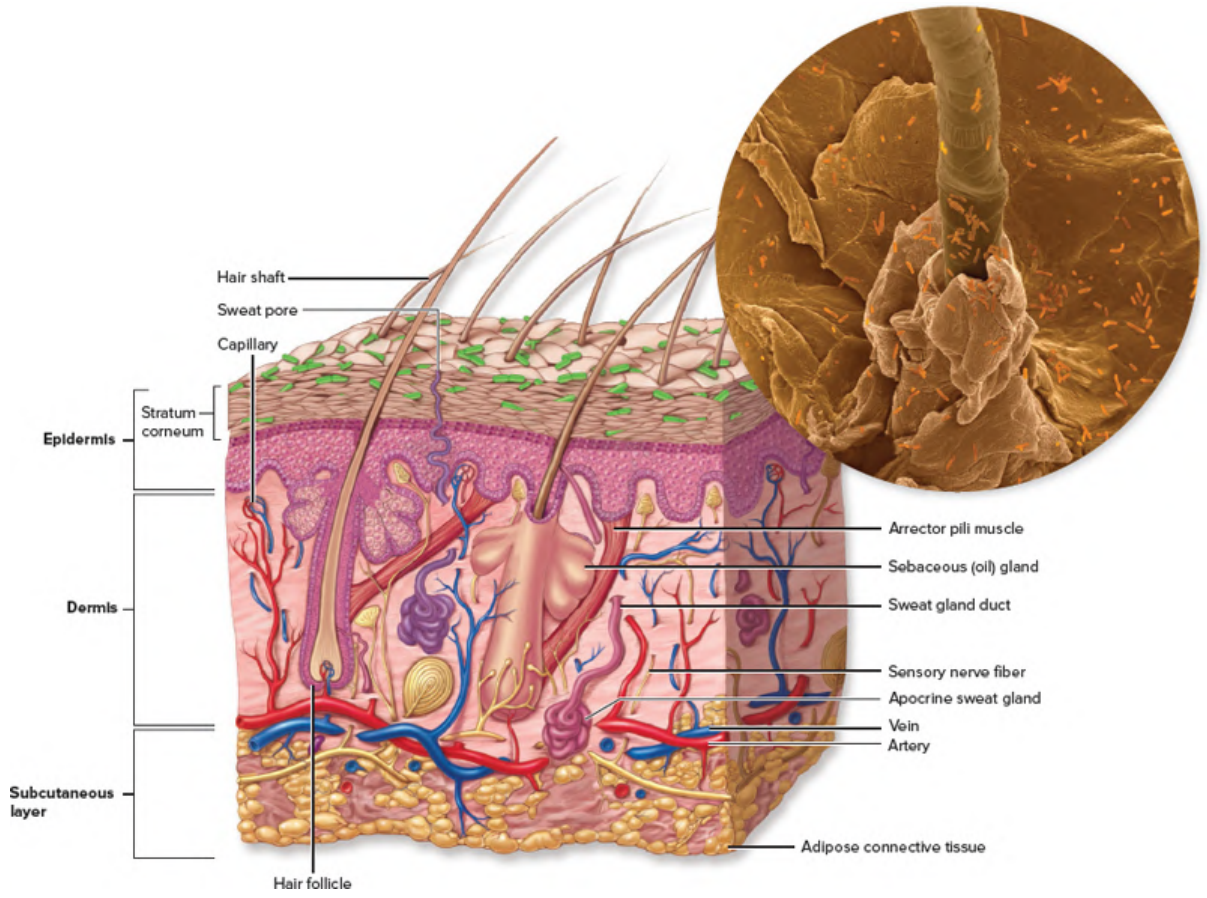


Figure 16.1 **A cross section of skin**.
©Dennis Kunkel Microscopy/Science Source

layer, is attached to the underlying dermis and is the source for all of the cells that make up the epidermis.

The dermis, underneath the epidermis, is composed of connective tissue instead of epithelium. This means that it is a rich matrix of fibroblast cells and fibers such as collagen, and it contains macrophages and mast cells. The dermis also harbors a dense network of nerves, blood vessels, and lymphatic vessels. Damage to the epidermis generally does not result in bleeding, whereas damage deep enough to penetrate the dermis results in broken blood vessels. Blister formation, the result of friction trauma or burns, represents a separation between the dermis and epidermis.

The "roots" of hairs, called follicles, are in the dermis. **Sebaceous** (oil) **glands** and scent glands are associated with the hair follicle. Separate sweat glands are also found in this tissue. All of these glands have openings on the surface of the skin, so they pass through the epidermis as well.

Millions of cells from the stratum corneum slough off every day, and attached microorganisms slough off with them. The skin is also brimming with antimicrobial substances. One of the most effective defenses of the skin is a class of molecules called antimicrobial peptides. These are positively charged chemicals that act by disrupting the negatively charged membranes of bacteria. There are many different types of these peptides, and they seem to be chiefly responsible for keeping the microbial count on skin relatively low.

The sebaceous glands' secretion, called **sebum**, has a low pH, which makes the skin inhospitable to many microorganisms. Sebum is oily due to its high concentration of lipids. The lipids can serve as nutrients for normal microbiota, but breakdown of the fatty acids contained in lipids leads to toxic by-products that inhibit the growth of microorganisms not adapted to the skin environment. This mechanism helps control the growth of potentially pathogenic bacteria. Sweat is also inhibitory to microorganisms, because of both its low pH and its high salt concentration. Lysozyme is an enzyme found in sweat (and tears and saliva) that specifically breaks down peptidoglycan, which you learned in chapter 3 is a unique component of bacterial cell walls.

16.1 LEARNING OUTCOMES—Assess Your Progress

1. Describe the important anatomical features of the skin.
2. List the natural defenses present in the skin.

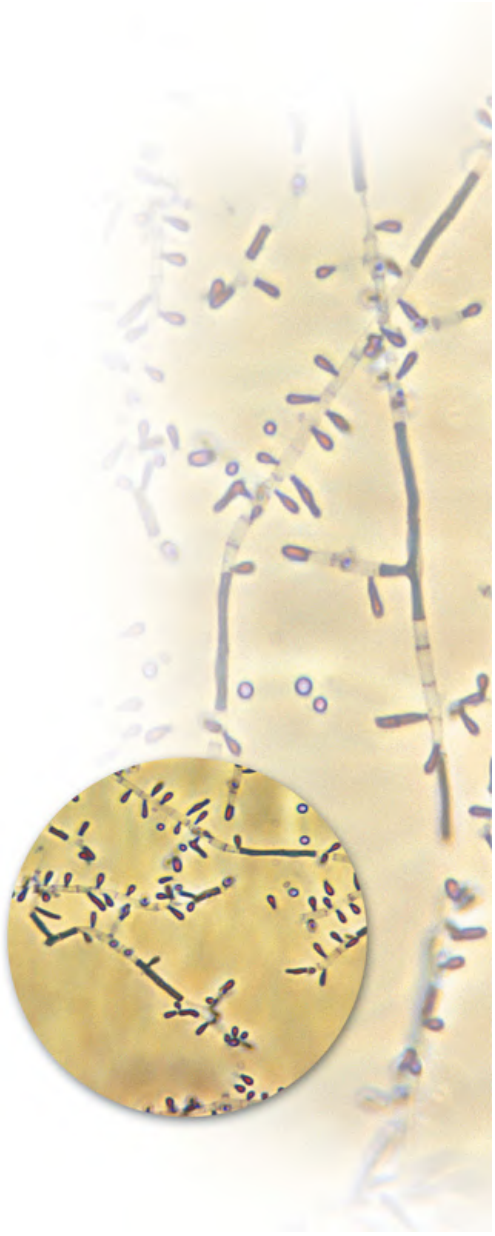
NCLEX® PREP

1. A nurse specializing in burn care is educating a nursing student about skin variations over the body. She states that the thickest skin is found on the _____ and the thinnest is on the _____.
   a. *chest; cheeks*
   b. *buttocks; nose*
   c. *plantar of the foot; eyelids*
   d. *abdomen; dorsum of the hands*

16.2 Normal Biota of the Skin

Microbes that do live on the skin surface as normal biota must be capable of living in the dry, salty conditions they find there. Microbes are relatively sparsely distributed over dry, flat areas of the body, such as on the back, but they can grow into dense populations in moist areas and skin folds, such as the underarm and groin areas. The normal microbiota also live in the protected environment of the hair follicles and glandular ducts.

Relying on cultivation-independent techniques such as 16s rRNA sequencing, the Human Microbiome Project (HMP) has begun to deliver fascinating information about our skin's biota. We are learning that hundreds of species of microbes, including some well-known pathogens, inhabit our epidermis, dermis, and subcutaneous skin layers. It is also common for different species to favor different areas of our bodies, and for different people to have different species. Last, in spite of this variation, it seems common for an individual's microbiota to remain relatively constant over time. In brief, the HMP is showing us that the skin microbiota is far more diverse than we imagined.



Dermatophytes are common fungal colonizers of skin.
Source: CDC

A Note About the Chapter Organization

In a clinical setting, patients present themselves to health care practitioners with a set of symptoms, and the health care team makes an “anatomical” diagnosis—such as a *generalized vesicular rash*. The anatomical diagnosis allows practitioners to narrow down the list of possible causes to microorganisms that are known to be capable of creating such a condition. Then the proper tests can be performed to arrive at an etiologic diagnosis (i.e., determining the exact microbial cause). So the order of events is as follows:

1. anatomical diagnosis,
2. differential diagnosis, and

3. etiologic diagnosis.

In this book, we organize diseases according to the anatomical diagnosis (which appears as a gold-colored heading). Then the agents in the differential diagnosis are each addressed, each of them appearing as turquoise headings. When we finish addressing each agent that could cause the condition, we sum them up in a Disease Table, whether there is only one possible cause or whether there are nine or ten.

In the Disease Tables you will also find a row featuring recommended treatment. Here we will identify the microbes that are on the CDC "Threat" list for their antibiotic resistance.

Defenses and Normal Biota of the Skin

	Defenses	Normal Biota
Skin	Keratinized surface, sloughing, low pH, high salt, lysozyme, antimicrobial peptides	*Streptococcus, Staphylococcus, Corynebacterium, Propionibacterium, Pseudomonas, Lactobacillus;* yeasts such as *Candida*

16.2 LEARNING OUTCOMES—Assess Your Progress

3. List characteristics of the skin's normal microbiota.

Figure 16.2 **A typical MRSA skin lesion**.
Source: CDC/Gregory Moran, M.D.

16.3 Skin Diseases Caused by Microorganisms

HIGHLIGHT DISEASE

MRSA Skin and Soft-Tissue Infection

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common cause of skin lesions in non-hospitalized people. (The hospitalized population is more likely to acquire systemic—bloodstream—infections from MRSA, addressed in chapter 18.) Even though the name mentions "methicillin," these strains are usually resistant to multiple antibiotics.

Staphylococcus aureus is a gram-positive coccus that grows in clusters, like a bunch of grapes. It is nonmotile. Much of its destructiveness is due to its array of superantigens (see chapter 12). It can be highly virulent, but it also appears as "normal" biota on the skin of one-third of the population. Strains that are methicillin-resistant are also found on healthy people.

This species is considered the sturdiest of all non-endospore-forming pathogens, with well-developed capacities to withstand high salt (7.5% to 10%), extremes in pH, and high temperatures (up to 60°C for 60 minutes). *S. aureus* also remains viable after months of air drying and resists the effect of many disinfectants and antibiotics.

Signs and Symptoms

MRSA infections of the skin tend to be raised, red, tender, localized lesions, often featuring pus and feeling hot to the touch **(figure 16.2)**. They occur easily in breaks in the skin caused by injury, shaving, or even just abrasion. They may localize around a hair follicle. Fever is a common feature.

Transmission and Epidemiology

MRSA is a common contaminant of all kinds of surfaces you touch daily, especially if the surfaces are not routinely sanitized. Gym equipment, airplane tray tables, electronic devices, razors, and so on, are all sources of indirect contact infection. Persons with active MRSA skin infections should keep them covered in order to avoid direct contact transmission to others.

Pathogenesis and Virulence Factors

All pathogenic *S. aureus* strains typically produce coagulase, an enzyme that coagulates plasma. Because 97% of all human isolates of *S. aureus* produce this enzyme, its presence is considered a highly diagnostic species characteristic.

Other enzymes expressed by *S. aureus* include hyaluronidase, which digests the intercellular "glue" (hyaluronic acid) that binds connective tissue in host tissues; staphylokinase, which digests blood clots; a nuclease that digests DNA (DNase); and lipases that help the bacteria colonize oily skin surfaces.

Culture and/or Diagnosis

Polymerase chain reaction (PCR) is routinely used to diagnose MRSA. Alternatively, cultivation on blood agar is a useful diagnostic technique **(figure 16.3)**. For heavily contaminated specimens, selective media such as mannitol salt agar are used. The production of catalase, an enzyme that breaks down hydrogen peroxide accumulated during oxidative metabolism, can be used to differentiate the staphylococci, which produce it, from the streptococci, which do not.

One key technique for separating *S. aureus* from other species of *Staphylococcus* is the coagulase test **(figure 16.4)**. By definition, any staph isolate that coagulates plasma is *S. aureus;* all others are coagulase-negative.

Prevention and Treatment

Prevention is only possible with good hygiene. Treatment of these infections often starts with incision of the lesion and drainage of the pus. Antimicrobial treatment should include more than one antibiotic. Current recommendations in the United States are for the use of vancomycin. These recommendations, of course, will change based on antibiotic-resistance patterns.

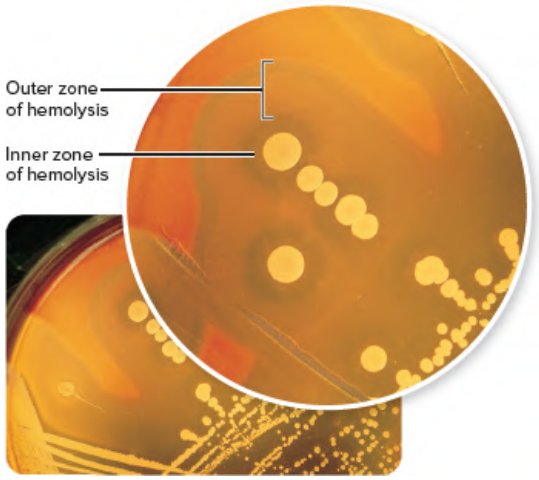


Figure 16.3 ***Staphylococcus aureus.*** Blood agar plate growing *S. aureus*. Some strains show two zones of hemolysis, caused by two different hemolysins. The inner zone is clear, whereas the outer zone is fuzzy and appears only if the plate has been refrigerated after growth.
©Kathleen Talaro

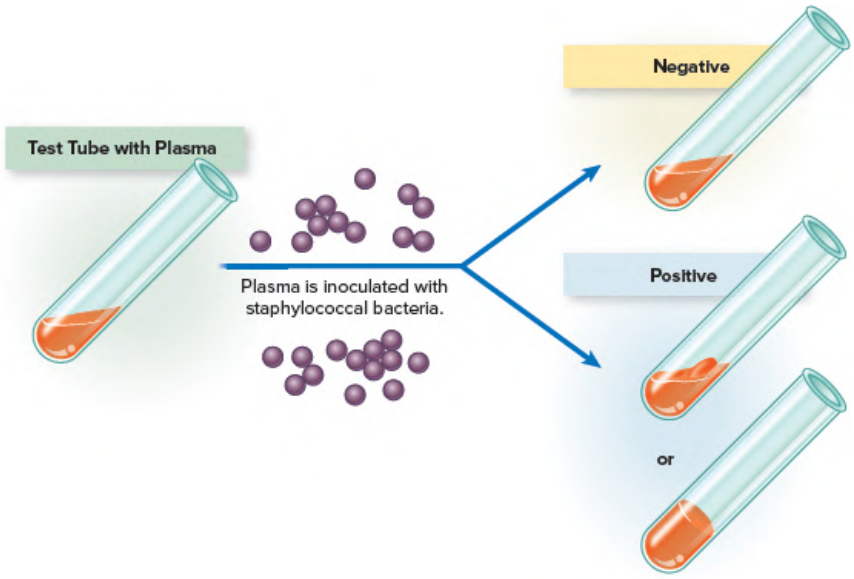


Figure 16.4 **The coagulase test**. Staphylococcal coagulase is an enzyme that reacts with factors in plasma to initiate clot formation. In the coagulase test, a tube of plasma is inoculated with the bacterium. If it remains liquid, the test is negative. If the plasma develops a lump or becomes completely clotted, the test is positive.

Disease Table 16.1 MRSA Skin and Soft-Tissue Infection

Causative Organism(s)	Methicillin-resistant *Staphylococcus aureus* (B) G+
Most Common Modes of Transmission	Direct contact, indirect contact
Virulence Factors	Coagulase, other enzymes, superantigens
Culture/Diagnosis	PCR, culture and Gram stain, coagulase and catalase tests, multitest systems
Prevention	Hygiene practices
Treatment	Vancomycin; in **Serious Threat** category in CDC Antibiotic Resistance Report
Epidemiology	Community-associated MRSA infections most common in children and young to middle-aged adults Incidence increasing in communities (decreasing in hospitals)

Highlight Disease

Maculopapular Rash Diseases

There are a variety of microbes that can cause the type of skin eruptions classified as *maculopapular*, a term denoting flat to slightly raised colored bumps.

Measles

Every year hundreds of thousands of children in the developing world die from this disease (about 367 a day), even though an extremely effective vaccine has been available since 1963. Health campaigns all over the world seek to make measles vaccine available to all and have been very effective. Since 2002, worldwide deaths from measles have dropped 74%. Ironically, it seems that more work and education need to be done in *developed* countries now. Many parents are opting to not have their children vaccinated, due to unfounded fears about the link between the vaccine and autism. We would do well to remember that before the vaccine was introduced, measles killed 6 million people worldwide each year.

Measles is also known as **rubeola**. Be very careful not to confuse it with the next maculopapular rash disease, rubella.

▶ Signs and Symptoms

The initial symptoms of measles are sore throat, dry cough, headache, conjunctivitis, lymphadenitis, and fever. In a short time, unusual oral lesions called *Koplik's spots* appear as a prelude to the characteristic red maculopapular **exanthem** (eg-zan′-thum) that erupts on the head and then progresses to the trunk and extremities until most of the body is covered **(figure 16.5)**. The rash gradually coalesces into red patches that fade to brown.

In a small number of cases, children develop laryngitis, bronchopneumonia, and bacterial secondary infections such as ear and sinus infections. Occasionally (1 in 100 cases), measles progresses to pneumonia or encephalitis, resulting in various central nervous system (CNS) changes ranging from disorientation to coma. Permanent brain damage or epilepsy can result.

The most serious complication is **subacute sclerosing panencephalitis (SSPE)**, a progressive neurological degeneration of the cerebral cortex, white matter, and brain stem. Once thought to be exceedingly rare (occurring in about 1 per million measles cases), a 2016

study found that the incidence is actually 1 in less than a thousand in children who get measles before being vaccinated. The pathogenesis of SSPE appears to involve a defective virus, one that has lost its ability to form a capsid and be released from an infected cell. Instead, it spreads unchecked through the brain by cell fusion, gradually destroying neurons and accessory cells and breaking down myelin. The disease causes profound intellectual and neurological impairment. The disease can occur years after the initial measles infection, and invariably leads to coma and death in a matter of months or years.

Pathogenesis and Virulence Factors

The virus implants in the respiratory mucosa and infects the tracheal and bronchial cells. From there, it travels to the lymphatic system, where it multiplies and then enters the bloodstream, a condition known as *viremia*. The bloodstream carries the virus to the skin and to various organs.

The measles virus induces the cell membranes of adjacent host cells to fuse into large syncytia (sin-sish′-uh), giant cells with many nuclei. These cells no longer perform their proper function. The virus seems proficient at disabling many aspects of the host immune response, especially cell-mediated immunity and delayed-type hypersensitivity.

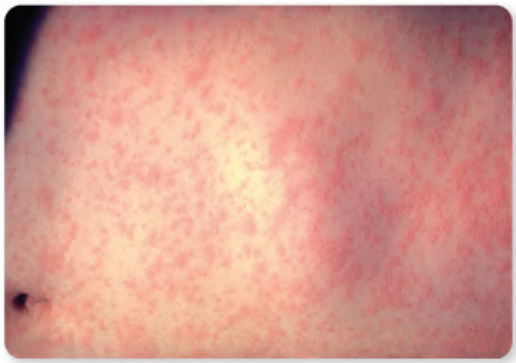


Figure 16.5 **The rash of measles**.
Source: CDC

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2. A pediatric client presents to the office with ruddy checks and a low-grade fever of 101°F. The client's mother states that the child has been acting normal, but now she notices the child has a slight rash, mainly on the torso. Which disease do you suspect based on this clinical presentation?

a. *measles*
b. *fifth disease*
c. *rubella*
d. *roseola*
e. *scarlet fever*

Transmission and Epidemiology

Measles is one of the most contagious infectious diseases, transmitted principally by respiratory droplets. There is no reservoir other than humans, and a person is infectious during the periods of incubation, prodrome phase, and the skin rash but usually not during convalescence. In December 2014, a measles outbreak began in the Disney theme parks in California. Eventually 111 measles cases would be associated with that outbreak. Many of the people diagnosed with measles were unvaccinated: Some of them were babies too young to be vaccinated, but many were children and adults who chose not to be vaccinated. In July 2015, a woman in Washington State died of measles, the first measles death in the United States in 12 years.

Culture and Diagnosis

The disease can be diagnosed on clinical presentation alone; but if further identification is required, an ELISA test is available that tests for patient IgM to measles antigen, indicating a current infection. A PCR test is also available.

Prevention

The MMR vaccine (for measles, mumps, and rubella) contains live attenuated measles virus, which confers protection for up to 20 years. A vaccine called ProQuad protects against measles, mumps, rubella, and varicella. Measles immunization is recommended for all healthy children at the age of 12 to 15 months, with a booster before the child enters school.

Treatment

Treatment relies on reducing fever, suppressing cough, and replacing lost fluid. Complications require additional remedies to relieve neurological and respiratory symptoms and to sustain nutrient, electrolyte, and fluid levels. Vitamin A supplements are recommended by some physicians. They have been found effective in reducing the symptoms and decreasing the rate of complications.

Rubella

This disease is also known as German measles. Rubella is derived from the Latin for "little red," and that is a good way to remember it because it causes a relatively minor rash disease with few complications. Sometimes it is called the 3-day measles. The only exception to this mild course of events is when a fetus is exposed to the virus while in its mother's womb (*in utero*). Serious damage can occur, and for that reason women of childbearing years must be sure to be vaccinated before they plan to conceive.

Signs and Symptoms

The two clinical forms of rubella are referred to as postnatal infection, which develops in children or adults, and **congenital** (prenatal) infection of the fetus, expressed in the newborn as various types of birth defects.

Postnatal Rubella A rash of pink macules and papules first appears on the face and progresses down the trunk and toward the extremities, advancing and resolving in about 3 days. The rash is milder looking than the measles rash **(Disease Table 16.2)**. Adult rubella is often characterized by joint inflammation and pain rather than a rash.

Congenital Rubella Rubella is a strongly **teratogenic** virus. Transmission of the rubella virus to a fetus *in utero* can result in a serious complication called **congenital rubella** (**figure 16.6**). The mother is able to transmit the virus even if she is asymptomatic. Infection in the first trimester is most likely to induce miscarriage or multiple permanent defects in the newborn. The most common of these is deafness and may be the only defect seen in some babies. Other babies may experience cardiac abnormalities, ocular lesions, deafness, and mental and physical retardation in varying combinations. Less drastic sequelae that usually resolve in time are anemia, hepatitis, pneumonia, carditis, and bone infection.

Figure 16.6 **An infant born with congenital rubella can display a papular pink or purple rash.**
©James Stevenson/Science Source

A Note About Statistics in the Disease Tables

Each condition we study is summarized in a disease table. The last row of each table contains information about the epidemiology of the disease. The type of epidemiological information that is most relevant to a particular disease can vary. For example, it is vitally important to know the numbers of new cases (incidence) of some diseases. This is the case for measles in the United States (see Disease Table 16.2), as we track the resurgence of a disease once controlled by vaccination. For other conditions, such as fifth disease in the same table, it is more useful to know how many people have been affected by the time they reach a certain age. Prevalence, or the current number of people affected by the condition, is another common measure. And for some diseases the most informative statistic is how deadly they are (mortality rate). These tables contain the most useful information about each condition.

Causative Agent

The rubella virus is a *Rubivirus*, in the family *Togaviridae*. The virus has the ability to stop mitosis, which is an important process in a rapidly developing embryo and fetus. It also induces apoptosis (programmed cell death) of normal tissue cells. This inappropriate cell death can do irreversible harm to organs it affects. And last, the virus damages vascular endothelium, leading to poor development of many organs.

Transmission and Epidemiology

Rubella is a disease with worldwide distribution. Infection is initiated through contact with respiratory secretions and occasionally urine. The virus is shed during the prodromal phase and

up to a week after the rash appears. Congenitally infected infants are contagious for a much longer period of time. Because the virus is only moderately communicable, close living conditions are required for its spread. Rubella and congenital rubella syndrome were declared eliminated from the United States in 2004, and from the Americas in 2015.

Culture and Diagnosis

Because it mimics other diseases, rubella should not be diagnosed on clinical grounds alone. IgM antibody to rubella virus can be detected early using an ELISA technique or a latex-agglutination card. Women in developed countries routinely undergo antibody testing at the beginning of pregnancy to determine their immune status.

Prevention and Treatment

The attenuated rubella virus vaccine is usually given to children in the combined form (MMR or MMRV vaccination) at 12 to 15 months and a booster at 4 or 6 years of age. The vaccine for rubella can be administered on its own, without the measles and mumps components. Postnatal rubella is generally benign and requires only symptomatic treatment. No specific treatment is available for the congenital manifestations.

Fifth Disease

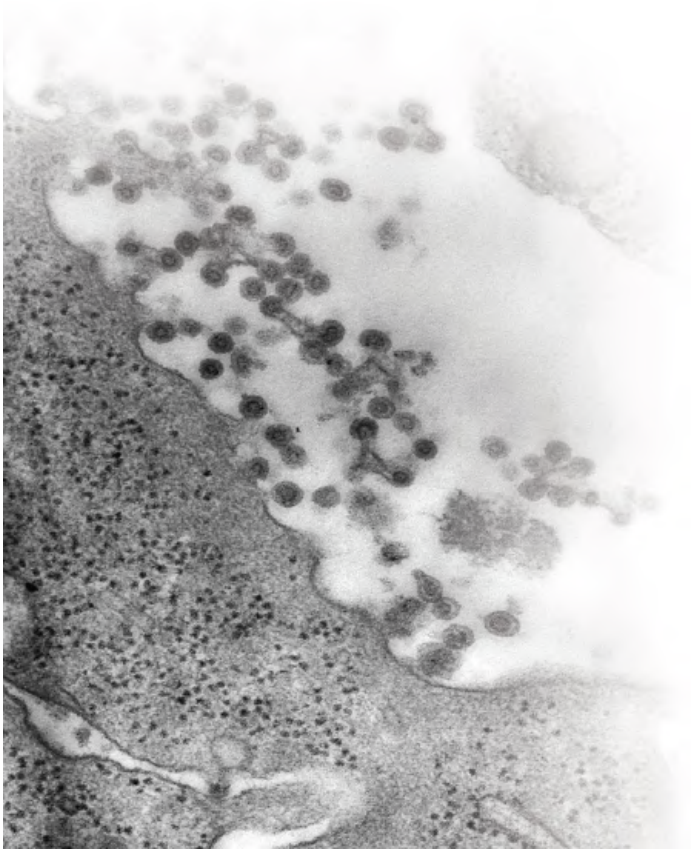
This disease, more precisely called *erythema infectiosum*, is so named because about 100 years ago it was the fifth of the diseases recognized by doctors to cause rashes in children. The first four were scarlet fever, measles, rubella, and another rash that was thought to be distinct but was probably not. Fifth disease is a very mild disease that often results in a characteristic "slapped-cheek" appearance because of a confluent reddish rash that begins on the face. Within 2 days, the rash spreads on the body but is most prominent on the arms, legs, and trunk. The rash of fifth disease may reoccur for several weeks and may be brought on by any activity that increases body heat (i.e., exercise, fever, sunlight, warm baths, and even high emotion).

The causative agent is parvovirus B19. You may have heard of "parvo" as a disease of dogs, but strains of this virus group infect humans. Fifth disease is usually diagnosed by the clinical presentation, but sometimes it is helpful to rule out rubella by testing for IgM against rubella.

This infection is very contagious. There is no vaccine and no treatment for this usually mild disease.

Roseola

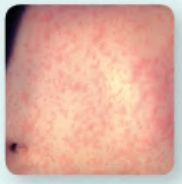
This disease is common in young children and babies. It is sometimes known as "sixth disease." It can result in a maculopapular rash, but a high percentage (up to 70%) of cases proceed without the rash stage. Children sick with this disease exhibit a high fever (up to 41°C, or 105°F) that comes on quickly and lasts for up to 3 days. Seizures may occur during this period, but other than that patients remain alert and do not



Source: CDC/Fred Murphy and Sylvia Whitfield

Disease Table 16.2 Maculopapular Rash Diseases

Disease	Measles (Rubeola)	Rubella	Fifth Disease	Roseola	Other Conditions to Consider
Causative Organism(s)	Measles virus V	Rubella virus V	Parvovirus B19 V	Human herpesvirus 6 V	Other conditions resulting in a rash that may look similar to these maculopapular conditions should be considered: Zika virus disease (covered in chapter 17) Scarlet fever (chapter 19) Secondary syphilis (chapter 21) Rocky Mountain
Most Common Modes of Transmission	Droplet contact	Droplet contact	Droplet contact, direct contact	Unknown	
Virulence Factors	Syncytium formation, ability to suppress CMI	In fetuses: inhibition of mitosis, induction of apoptosis, and damage to vascular endothelium	—	Ability to remain latent	
Culture/Diagnosis	Clinical diagnosis; ELISA or PCR	Acute IgM, acute/convalescent IgG	Usually diagnosed clinically	Usually diagnosed clinically	

Disease	Measles (Rubeola)	Rubella	Fifth Disease	Roseola	Other Conditions to Consider
Prevention	Live attenuated vaccine (MMR or MMRV)	Live attenuated vaccine (MMR or MMRV)	–	–	spotted fever (chapter 18)
Treatment	No antivirals; vitamin A, antibiotics for secondary bacterial infections	–	–	–	
Distinguishing Features of the Rashes	Starts on head, spreads to whole body, lasts over a week	Milder red rash, lasts approximately 3 days	"Slapped-face" rash first, spreads to limbs and trunk, tends to be confluent rather than distinct bumps	High fever precedes rash stage; rash not always present	
Epidemiological Features	Incidence increasing in North America; in developing countries incidence is 30 million cases/yr and 1 million deaths	Eliminated from the Americas; worldwide: 100,000 infants/yr born with congenital rubella syndrome	60% of population seropositive by age 20	> 90% seropositive; 90% of disease cases occur before age of 2	
Appearance of Lesions	 Source: CDC	Source: CDC	©Dr. P. Marazzi/Science Source	©Scott Camazine/Science Source	

act terribly ill. On the fourth day, the fever disappears, and it is at this point that a rash can appear, first on the chest and trunk and less prominently on the face and limbs. By the time the rash appears, the disease is almost over.

Roseola is caused by a human herpesvirus called HHV-6. Like all herpesviruses, it can remain latent in its host indefinitely after the disease has cleared. Very occasionally, the virus reactivates in childhood or adulthood, leading to mononucleosis-like or hepatitis-like symptoms. It is thought that 100% of the U.S. population is infected with this virus by adulthood. Some people experienced the disease roseola when they became infected, and some of them did not. The suggestion has been made that this virus causes other disease conditions later in life, such as multiple sclerosis. No vaccine and no treatment exist for roseola. **Disease Table 16.2**.

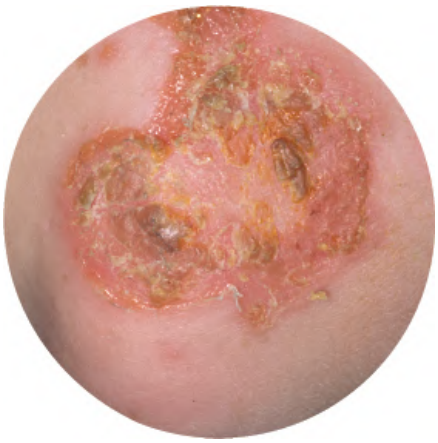


Figure 16.7 Impetigo lesion on the face.
©Hercules Robinson/Alamy Stock Photo

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3. A patient has been diagnosed with impetigo but has been noncompliant with medical treatment. What complication is associated with untreated impetigo?

a. *future antibiotic allergies*
b. *measles*
c. *increased likelihood of roseola*
d. *glomerulonephritis*
e. *septicemia*

Impetigo

Impetigo is a superficial bacterial infection that causes the skin to flake or peel off **(figure 16.7)**. It is not a serious disease but is highly contagious, and children are the primary victims. Impetigo can be caused by either *Staphylococcus aureus* or *Streptococcus pyogenes*, and some cases are probably caused by a mixture of the two. It has been suggested that *S. pyogenes* begins all cases of the disease, and in some cases *S. aureus* later takes over and becomes the predominant bacterium cultured from lesions. Because *S. aureus* produces a bacteriocin (toxin) that can destroy *S. pyogenes*, it is possible that *S. pyogenes* is often missed in culture-based diagnosis.

Impetigo, whether it is caused by *S. pyogenes, S. aureus*, or both, is highly contagious and transmitted through direct contact but also via fomites and mechanical vector transmission. The peak incidence is in the summer and fall.

The only current prevention for impetigo is good hygiene. Vaccines are in development for both of the etiologic agents, but none are currently available.

Signs and Symptoms

The "lesion" of impetigo looks variously like peeling skin, crusty and flaky scabs, or honey-colored crusts. Lesions are most often found around the mouth, face, and extremities, though they can occur anywhere on the skin. It is very superficial and it itches. The symptomatology does not indicate whether the infection is caused by *Staphylococcus* or *Streptococcus*.

Impetigo Caused by *Staphylococcus aureus*

The most important virulence factors relevant to *S. aureus* impetigo are exotoxins called exfoliative toxins A and B, which are coded for by a phage that infects some *S. aureus* strains. At least one of the toxins attacks a protein that is very important for epithelial cell-to-cell binding in the outermost layer of the skin. Breaking up this protein leads to the characteristic blistering seen in the condition. The breakdown of skin architecture also facilitates the spread of the bacterium.

Impetigo Caused by *Streptococcus pyogenes*

Streptococcus pyogenes is thoroughly described in chapter 19. The important features are briefly summarized here, and the features pertinent to impetigo are listed in **Disease Table 16.3**.

S. pyogenes is a gram-positive coccus and is beta-hemolytic on blood agar. In addition to impetigo, it causes streptococcal pharyngitis (strep throat), scarlet fever, pneumonia, puerperal fever, necrotizing fasciitis, serious bloodstream infections, and poststreptococcal conditions such as rheumatic fever.

If the precise etiologic agent must be identified, there are well-established methods for identifying group A streptococci. Refer to chapter 19.

Pathogenesis and Virulence Factors

Like *S. aureus*, this bacterium possesses a huge arsenal of enzymes and toxins. Some of these are listed in Disease Table 16.3.

Rarely, impetigo caused by *S. pyogenes* can be followed by acute poststreptococcal glomerulonephritis (see chapter 19). The strains that cause impetigo never cause rheumatic fever, however.

S. pyogenes is more often the cause of impetigo in newborns, and *S. aureus* is more often the cause of impetigo in older children, but both can cause infection in either age group.

Medical Moment

Scabies

Scabies is a parasitic disease caused by infestation of the human itch mite (*Sarcoptes* sp.). It is typical for only 10 to 15 mites to invade initially. The mites burrow into the epidermis, where the female lives and lays eggs. The eggs hatch and larva mature in pouches underneath the skin's surface. The site of mite infestation develops a papular rash and can be extremely itchy. The burrowing sites may be visible as thin gray lines under the skin. Symptoms develop 2 to 6 weeks after infestation for those who have not been exposed to scabies previously.

Scabies infection is transmitted by prolonged skin contact or occasionally by fomites. It occurs most commonly in crowded areas, such as child care facilities, prisons, and nursing homes. Medication is available to kill the mites and their eggs. It is recommended that linens and clothing of infected persons and their household contacts be washed and dried in high heat to prevent transmission or reinfection.

Q. What does the fact that scabies can be transmitted by fomites suggest about the mite's need for a living host?

Answer in Appendix B.

Source: National Institute of Allergy and Infectious Diseases (NIAID)/NIH/USHHS

Disease Table 16.3 Impetigo

Causative Organism(s)	*Staphylococcus aureus* B **G+**	*Streptococcus pyogenes* **G+** B
Most Common Modes of Transmission	Direct contact, indirect contact	Direct contact, indirect contact
Virulence Factors	Exfoliative toxin A, coagulase, other enzymes	Streptokinase, plasminogen-binding ability, hyaluronidase, M protein
Culture/Diagnosis	Routinely based on clinical signs, when necessary, culture and Gram stain, coagulase and catalase tests, multitest systems, PCR	Routinely based on clinical signs, when necessary, culture and Gram stain, coagulase and catalase tests, multitest systems, PCR
Prevention	Hygiene practices	Hygiene practices
Treatment	In uncomplicated cases, no treatment; topical mupirocin or retapamulin, oral dicloxacillin, cephalexin, or TMP-SMZ; (MRSA is in **Serious Threat** category in CDC Antibiotic Resistance Report)	In uncomplicated cases, no treatment; topical mupirocin or retapamulin; (erythromycin-resistant *Streptococcus pyogenes* is in **Concerning Threat** category in CDC Antibiotic Resistance Report)
Distinguishing Features	Seen more often in older children, adults	Seen more often in newborns
Epidemiological Features	Incidence approximately 2—5% of children in temperate climates; higher in tropical areas	

Disease Table 16.4 Cellulitis

Causative Organism(s)	Methicillin-resistant *Staphylococcus aureus* B **G+**	*Streptococcus pyogenes* B **G+**	Other bacteria or fungi
Most Common Modes of Transmission	Parenteral implantation	Parenteral implantation	Parenteral implantation
Virulence Factors	Exfoliative toxin A, coagulase, other enzymes	Streptokinase, plasminogen-binding ability, hyaluronidase, M protein	—
Culture/Diagnosis	Based on clinical signs	Based on clinical signs	Based on clinical signs
Prevention	—	—	—
Treatment	Oral or IV antibiotic (dicloxacillin if sensitive, vancomycin if MRSA); surgery sometimes necessary; (MRSA is in **Serious Threat** category in CDC Antibiotic Resistance Report)	Oral or IV antibiotic (penicillin); surgery sometimes necessary; (erythromycin-resistant *Streptococcus pyogenes* is in **Concerning Threat** category in CDC Antibiotic Resistance Report)	Aggressive treatment with oral or IV antibiotic; surgery sometimes necessary
Distinguishing Features	—	—	More common in immunocompromised
Epidemiological Features	Incidence highest among males 45–64		

Cellulitis

Cellulitis is a condition caused by a fast-spreading infection in the dermis and in the subcutaneous tissues below. It causes pain, tenderness, swelling, and warmth. Fever and swelling of the lymph nodes draining the area may also occur. Frequently, red lines leading away from the area are visible (a phenomenon called *lymphangitis*). This symptom is the result of microbes and inflammatory products being carried by the lymphatic system. Bacteremia could develop with this disease, but uncomplicated cellulitis has a good prognosis.

Cellulitis generally follows introduction of bacteria or fungi into the dermis, either through trauma or by subtle means (with no obvious break in the skin). The most common causes of the condition in healthy people are *Staphylococcus aureus* and *Streptococcus pyogenes*, although almost any bacterium and some fungi can cause this condition in an immunocompromised patient. In infants, group B streptococci are a frequent cause (see chapter 21).

Medical Moment

Scrum Pox: Herpes Gladiatorum

Herpes gladiatorum is caused by herpes simplex virus type 1 (HSV-1). It often affects athletes such as wrestlers who come into close contact with each other (it is sometimes called “scrum pox” or “mat herpes” by rugby players or wrestlers). Outbreaks of the infection may occur in gyms or fitness centers.

The lesions occur in clusters of blisters that may or may not be painful. The lesions generally heal within a week to 10 days. Once a person has been infected with HSV-1, the person is infected for life and can pass the infection on to others. Periodic outbreaks can occur during times of stress, trauma, or sun exposure. During the first episode, the infected person may experience fever, sore throat, headache, and swollen glands. Some individuals can be infected with the virus but will fail to develop any skin lesions. These individuals carry the virus and can still pass the virus to others through skin-to-skin contact.

Q. What are the official names for the modes of transmission for herpes gladiatorum?

Answer in Appendix B.

Mild cellulitis responds well to oral antibiotics chosen to be effective against both *S. aureus* and *S. pyogenes*. Keep in mind that if it is caused by *S. aureus*, the bacterium is usually of the methicillin-resistant variety (MRSA). More involved infections and infections in immunocompromised people require intravenous antibiotics. If there are extensive areas of tissue damage, surgical debridement (duh-breed’-munt) is warranted. **Disease Table 16.4**.

Staphylococcal Scalded Skin Syndrome (SSSS)

This syndrome is another **dermolytic** condition caused by *Staphylococcus aureus*. It affects mostly newborns and babies, although children and adults can experience the infection. Transmission may occur when caregivers carry the bacterium from one baby to another. Adults in the nursery can also directly transfer *S. aureus* because approximately 30% of adults are asymptomatic carriers. Carriers can harbor the bacteria in the nasopharynx, axilla, perineum, and even the vagina. (Fortunately, only about 5% of *S. aureus* strains are lysogenized by the type of phage that codes for the toxins responsible for this disease.)

Like impetigo, this is an exotoxin-mediated disease. The phage-encoded exfoliative toxins A and B are responsible for the damage. Unlike impetigo, the toxins enter the bloodstream from some

focus of infection (the throat, the eye, or sometimes an impetigo infection) and then travel to the skin throughout the body. These toxins cause **bullous lesions**, which often appear first around the umbilical cord (in neonates) or in the diaper or axilla area. A split occurs in the epidermal tissue layers just above the stratum basale (see figure 16.1). Widespread desquamation of the skin follows, leading to the burned appearance referred to in the name **(figure 16.8)**.

Once a tentative diagnosis of SSSS is made, immediate antibiotic therapy should be instituted. **Disease Table 16.5**.

Vesicular or Pustular Rash Diseases

There are two diseases that present as generalized "rashes" over the body in which the individual lesions contain fluid. The lesions are often called *pox*, and the two diseases are chickenpox and smallpox **(Disease Table 16.6)**. Chickenpox is very common and mostly benign, but even a single case of smallpox constitutes a public health emergency. Both are viral diseases.

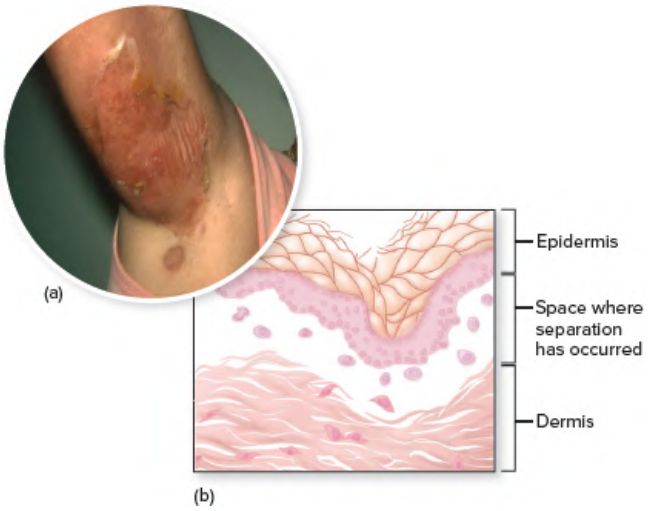

Figure 16.8 Staphylococcal scalded skin syndrome (SSSS) in the underarm area. **(a)** Exfoliative toxin produced in local infections causes blistering and peeling away of the outer layer of skin. **(b)** Drawing of a segment of skin affected with SSSS. The point of epidermal shedding, or desquamation, is in the epidermis. The lesions will heal well because the level of separation is so superficial.

©DermPics/Science Source (a)

Disease Table 16.5 Scalded Skin Syndrome

Causative Organism(s)	*Staphylococcus aureus* B G+
Most Common Modes of Transmission	Direct contact, droplet contact
Virulence Factors	Exfoliative toxins A and B
Culture/Diagnosis	Histological sections; culture performed but false negatives common because toxins alone are sufficient for disease
Prevention	Eliminate carriers in contact with neonates
Treatment	Immediate systemic antibiotics (current recommendation is cloxacillin)
Distinguishing Features	Split in skin occurs *within* epidermis
Epidemiological Features	Mortality 1%–5% in children, 50%–60% in adults

Chickenpox

After an incubation period of 10 to 20 days, the first symptoms to appear are fever and an abundant rash that begins on the scalp, face, and trunk and radiates in sparse crops to the extremities. Skin lesions progress quickly from macules and papules to itchy vesicles filled with a clear fluid. In several days, they encrust and drop off, usually healing completely but sometimes leaving a tiny pit or scar. Lesions number from a few to hundreds and are more abundant in adolescents and adults than in young children. **Figure 16.9** contains images of the chickenpox lesions in a child and in an adult. The lesion distribution is *centripetal*, meaning that there are more in the center of the body and fewer on the extremities. The distribution seen with smallpox is just the opposite. The illness usually lasts 4 to 7 days; new lesions stop appearing after about 5 days. Patients are considered contagious until all of the lesions have crusted over.

Approximately 0.1% of chickenpox cases are followed by encephalopathy, or inflammation of the brain caused by the virus. It can be fatal, but in most cases recovery is complete.

Figure 16.9 **Images of chickenpox and smallpox.**

©David White/Alamy Stock Photo (chickenpox on face); ©Picture Partners/Alamy Stock Photo (chickenpox on back); Source: CDC (chickenpox on shoulder); Source: CDC/Dr. Robinson (smallpox on arm); Source: CDC/Dr. John Noble, Jr. (smallpox on face); ©Everett Collection Historical/Alamy Stock Photo (smallpox on back)

▶ Shingles

After recuperation from chickenpox, the virus enters into the sensory nerve endings of cutaneous spinal nerve branches, especially those that serve the skin of the chest and head. From there, it becomes latent in the ganglia and may reemerge as **shingles** (also known as **herpes zoster**) with its characteristic asymmetrical distribution on the skin of the trunk or head **(figure 16.10)**.

Shingles develops abruptly after reactivation by such stimuli as psychological stress, X-ray treatments, immunosuppressive and other drug therapy, surgery, or a

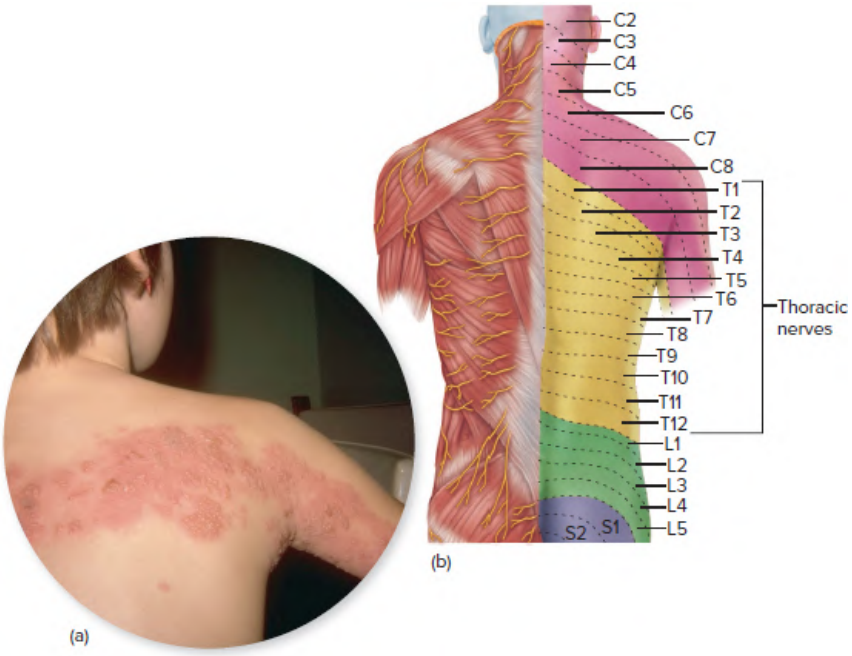

Figure 16.10 **Varicella-zoster virus reemergence as shingles**. **(a)** Clinical appearance of shingles lesions. **(b)** Dermatomes are areas of the skin served by a single ganglion. Shingles generally affects a single dermatome for this reason.

©Logical Images, Inc. All rights reserved (a)

developing malignancy. The virus is believed to migrate down the ganglion to the skin, where multiplication resumes and produces crops of tender, persistent vesicles. Inflammation of the ganglia and the pathways of nerves can cause pain and tenderness, known as postherpetic neuralgia, that can last for several months. Involvement of cranial nerves can lead to eye inflammation and ocular and facial paralysis.

Note On Bioterror Agents

The Centers for Disease Control and Prevention maintain a list of the most dangerous infectious agents that would be most logically used for a bioterror attack. There are three categories: A, B, and C. Category A agents (the most dangerous) are those that are (1) easily disseminated, or transmissible person-to-person; (2) result in high mortality rates; (3) could incite panic and social disruption; and (4) require special or unique actions for preparedness. Category A agents will be pointed out in each of the disease tables in the next six chapters.

Causative Agent

Human herpesvirus 3 (HHV-3, also called **varicella** [var″-ih′sel′-ah]) causes chickenpox, as well as the condition called herpes zoster or shingles. The virus is sometimes referred to as the varicella-zoster virus (VZV). Like other herpesviruses, it is an enveloped DNA virus.

Pathogenesis and Virulence Factors

HHV-3 enters the respiratory tract, attaches to respiratory mucosa, and then invades and enters the bloodstream. The viremia disseminates the virus to the skin, where the virus causes adjacent cells to fuse and eventually lyse, resulting in the characteristic lesions. The virus enters the sensory nerves at this site, traveling to the dorsal root ganglia.

The ability of HHV-3 to remain latent in ganglia is an important virulence factor, because resting in this site protects it from attack by the immune system and provides a reservoir of virus for the reactivation condition of shingles.

Transmission and Epidemiology

Humans are the only natural hosts for HHV-3. The virus is harbored in the respiratory tract but is communicable from both respiratory droplets and the fluid of active skin lesions. People can acquire a chickenpox infection by being exposed to the fluid of shingles lesions.

Infected persons are most infectious a day or two prior to the development of the rash. Chickenpox is so contagious that if you are exposed to it you almost certainly will get it.

Prevention

Live attenuated vaccine was licensed in 1995. In 2006, the FDA approved a unique vaccine called Zostavax. It is intended for adults age 60 and over and is for the prevention of shingles.

Treatment

Uncomplicated varicella is self-limiting and requires no therapy aside from alleviation of discomfort. Secondary bacterial infection is treated with topical or systemic antibiotics. Oral acyclovir or related antivirals should be administered within 24 hours of onset of the rash to people considered to be at risk for serious complications. Under no circumstances should aspirin be administered, as this may lead to Reye's syndrome.

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4. A nurse in an urgent care office is caring for a patient with shingles infection. Following education for the patient and family, the nurse evaluates the efficacy of her teaching. All of the following statements by the patient are true regarding shingles, except:

- **a.** *Shingles is caused by a reactivation of the varicella virus that may remain latent in the ganglia of nerve fibers for years.*
- **b.** *Shingles may cause eye inflammation and facial paralysis.*
- **c.** *Psychological stress can reactivate the virus that causes shingles.*
- **d.** *You can come down with shingles by being exposed to the fluid in shingles lesions.*

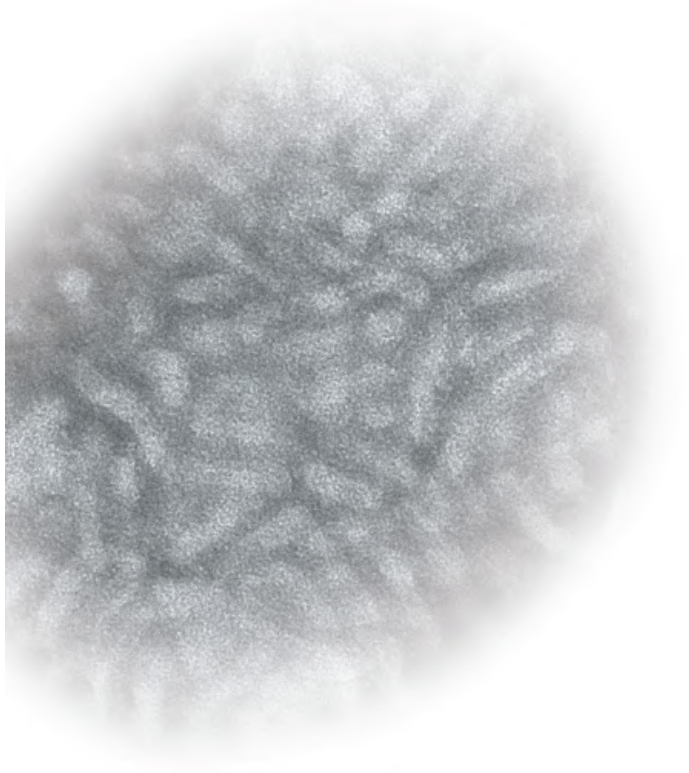
Smallpox

Largely through the World Health Organization's comprehensive global efforts, naturally occurring smallpox is now a disease of the past. However, after the terrorist attacks on the United States on

September 11, 2001, and the anthrax bioterrorism shortly thereafter, the U.S. government began taking the threat of smallpox bioterrorism very seriously. Vaccination, which had been discontinued, was once again offered to certain U.S. populations.

Signs and Symptoms

Infection begins with fever and malaise, and later a rash begins in the pharynx, spreads to the face, and progresses to the extremities. Initially, the rash is *macular*, evolving in turn to *papular, vesicular*, and *pustular* before eventually crusting over,



Vaccinia virus, the cause of cowpox, is used as a vaccine against smallpox, caused by the variola virus.
Source: CDC

leaving nonpigmented sites pitted with scar tissue. There are two principal forms of smallpox: variola minor and variola major. Variola major is a highly virulent form that causes toxemia, shock, and intravascular coagulation. People who have survived any form of smallpox nearly always develop lifelong immunity.

It is vitally important for health care workers to be able to recognize the early signs of smallpox. The diagnosis of even a single suspected case must be treated as a health and law enforcement emergency. The symptoms of variola major progress as follows: After the prodrome period of high fever and malaise, a rash emerges, first in the mouth. Severe abdominal and back pain sometimes accompany this phase of the disease. A rash appears on the skin and spreads throughout the body within 24 hours. The rash typically occurs more on the extremities (a centrifugal distribution; see figure 16.9).

By the third or fourth day of the rash, the bumps become larger and fill with a thick opaque fluid. A major distinguishing feature of this disease is that the pustules are indented in the middle. Also, patients report that the lesions feel as if they contain a BB pellet. Within a few days, these pustules begin to scab over. After 2 weeks, most of the lesions will have crusted over. The patient remains contagious until the last scabs fall off because the crusts contain the virus. During the entire rash phase, the patient is very ill. The lesions occur at the dermal level, which is the reason that scars remain after the lesions are healed.

A patient with variola minor has a rash that is less dense and is generally less ill than someone with variola major.

Causative Agent

The causative agent of smallpox, the variola virus, is an orthopoxvirus, an enveloped DNA virus. Other members of this group are the monkeypox virus and the vaccinia virus from which smallpox vaccine is made. Variola is a hardy virus, surviving outside the host longer than most viruses.

Transmission and Epidemiology

Smallpox is spread primarily through droplets, although fomites such as contaminated bedding and clothing can also spread it.

In the early 1970s, smallpox was endemic in 31 countries. Every year, 10 to 15 million people contracted the disease, and approximately 2 million people died from it. By 1977, after 11 years of intensive effort by the world health community, the last natural case occurred in Somalia. Currently, samples of stored smallpox virus exist in only two verified locations, at the CDC in Atlanta, Georgia, and at a state research center in Koltsovo, Russia.

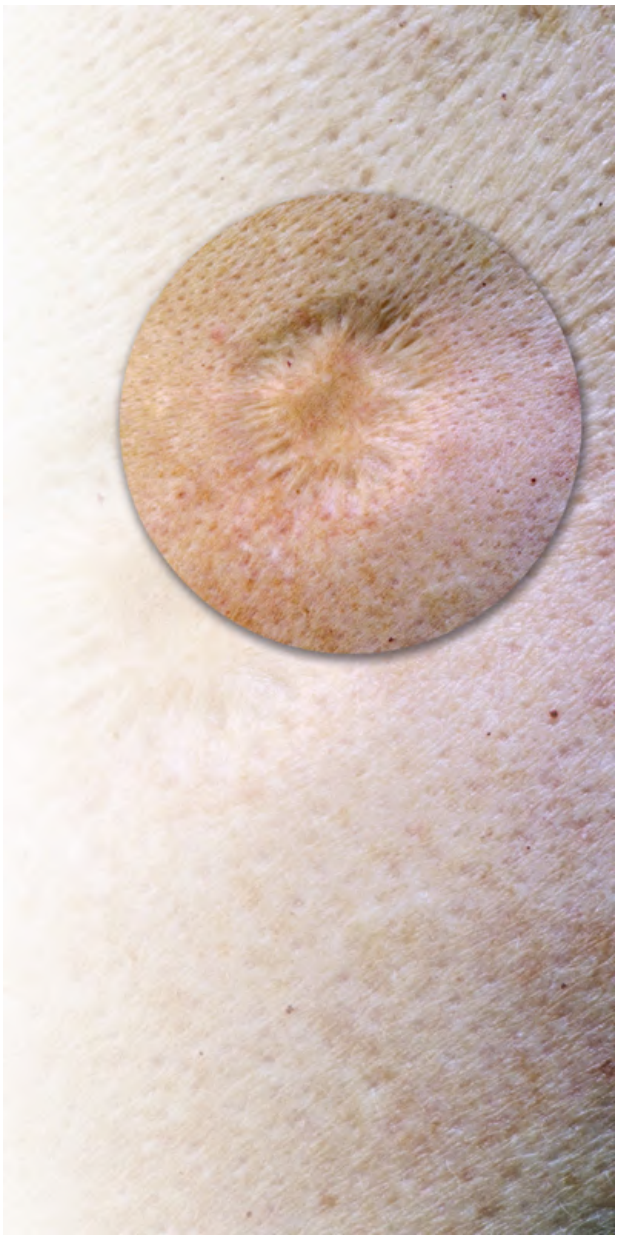
Prevention

In chapter 13, you read about Edward Jenner and his development of vaccinia virus to inoculate against smallpox. To this day, the vaccination for smallpox is based on the vaccinia virus. Immunizations were stopped in the United States in 1972. Since the terrorist events of 2001, some military branches are requiring that their personnel take the vaccination before deploying to certain parts of the world. Even though officially smallpox does not exist anymore except in labs in Russia and the United States, there are fears that other entities may have stockpiled the virus for use in biological warfare or terror attacks. That is why the United States maintains a stockpile of smallpox vaccine to use in the case of a smallpox attack.

Vaccination is also useful for postexposure prophylaxis, meaning that it can prevent or lessen the effects of the disease after you have already been infected with it.

Treatment

Two drugs have been proven effective in laboratory tests, but not in humans. They are tecovirimat and cidofovir. The U.S. government also maintains a supply of these drugs to use in case of a public health emergency. **Disease Table 16.6**.



Typical scar from a smallpox lesion
©SPL/Science Source

Disease Table 16.6 Vesicular/Pustular Rash Diseases

Disease	Chickenpox	Smallpox
Causative Organism(s)	Human herpesvirus 3 (varicella-zoster virus) V	Variola virus V
Most Common Modes of Transmission	Droplet contact, inhalation of aerosolized lesion fluid	Droplet contact, indirect contact
Virulence Factors	Ability to fuse cells, ability to remain latent in ganglia	Ability to dampen, avoid immune response
Culture/Diagnosis	Based largely on clinical appearance; PCR is available	Based largely on clinical appearance; if suspected refer to CDC

Disease	Chickenpox	Smallpox
Prevention	Live attenuated vaccine; there is also vaccine to prevent reactivation of latent virus (shingles)	Live virus vaccine (vaccinia virus)
Treatment	None in uncomplicated cases; acyclovir for high risk	Tecovirimat, cidofovir
Distinguishing Features	No fever prodrome; lesions are superficial and in centripetal distribution (more in center of body)	Fever precedes rash, lesions are deep and in centrifugal distribution (more on extremities)
Epidemiological Features	Chickenpox: vaccine decreased hospital visits by 88%, ambulatory visits by 59%	Last natural case worldwide was in 1977
Appearance of Lesions	©Gabriel Blaj/Alamy Stock Photo	Source: CDC/Dr. Charles Farmer, Jr.

Large Pustular Skin Lesions

Leishmaniasis

Two infections that result in large lesions (greater than a few millimeters across) deserve mention in this chapter on skin infections. The first is leishmaniasis, a zoonosis transmitted among various mammalian hosts by female sand flies. This infection can express itself in several different forms, depending on which species of the protozoan *Leishmania* is involved. Cutaneous leishmaniasis is a localized infection of the capillaries of the skin caused by *L. tropica*, found in Mediterranean, African, and Southeast Asian regions. A form of mucocutaneous leishmaniasis called espundia is caused by *L. brasiliensis*, endemic to parts of Central and South America. It affects both the skin and mucous membranes. Another form of this infection is systemic leishmaniasis.

Leishmania is transmitted to the mammalian host by the sand fly when it ingests the host's blood. The disease is endemic to equatorial regions that provide favorable conditions for the sand fly. At particular risk are travelers or immigrants who have never had contact with the protozoan and lack specific immunity.

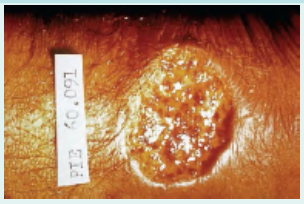
There is no vaccine; avoiding the sand fly is the only prevention.

Cutaneous Anthrax

This form of anthrax is the most common and least dangerous version of infection with *Bacillus anthracis*. (The spectrum of anthrax disease is discussed fully in chapter 18.) It is caused by endospores entering the skin through small cuts or

Disease Table 16.7 Large Pustular Skin Lesions

Disease	Leishmaniasis	Cutaneous Anthrax
Causative Organism(s)	*Leishmania* spp. (P)	*Bacillus anthracis* (B) G+
Most Common Modes of Transmission	Biological vector (sand fly)	Direct contact with endospores

Disease	Leishmaniasis	Cutaneous Anthrax
Virulence Factors	Multiplication within macrophages	Endospore formation; capsule, lethal factor, edema factor
Culture/Diagnosis	Usually microscopic visualization	Culture on blood agar; serology, PCR performed by CDC
Prevention	Avoiding sand fly	Avoid contact; vaccine available but not widely used
Treatment	Sodium stibogluconate, pentamidine	Ciprofloxacin, plus two additional antibiotics
Distinguishing Features	Mucocutaneous and systemic forms	Can be fatal
Epidemiological Features	Untreated visceral leishmaniasis mortality rate is 100%; 10% for cutaneous leishmaniasis	Untreated cutaneous anthrax mortality rate: 20%; treated mortality rate less than 1%
Appearance of Lesions	 Source: CDC	©Medical-on-Line/Alamy Stock Photo

abrasions. Germination and growth of the pathogen in the skin are marked by the production of a papule that becomes increasingly necrotic and later ruptures to form a painless, black **eschar** (ess′-kar) **(Disease Table 16.7)**. In the fall of 2001, 11 cases of cutaneous anthrax occurred in the United States as a result of bioterrorism (along with 11 cases of inhalational anthrax). Mail workers and others contracted the infection when endospores were sent through the mail. The infection can be naturally transmitted by contact with hides of infected animals (especially goats).

Left untreated, even the cutaneous form of anthrax is fatal approximately 20% of the time. A vaccine exists but is recommended only for high-risk persons and the military. **Disease Table 16.7**.

Cutaneous and Superficial Mycoses

Ringworm

A group of fungi that is collectively termed **dermatophytes** causes a variety of body surface conditions. These mycoses are strictly confined to the nonliving epidermal tissues (stratum corneum) and their derivatives (hair and nails). All these conditions have different names that begin with the word **tinea** (tin′-ee-ah), which derives from the erroneous belief that they were caused by worms. That misconception is also the reason these diseases are often called *ringworm*—ringworm of the scalp (tinea capitis), beard (tinea barbae), body (tinea corporis), groin (tinea cruris), foot (tinea pedis), and hand (tinea manuum). (Don't confuse these "tinea" terms with genus and species names. It is simply an old practice for naming the conditions.) Most of these conditions are caused by one of three different dermatophytes, which are discussed here.

The signs and symptoms of ringworm conditions are summarized in **table 16.1**.

An anthrax lesion.
Source: CDC

Bacillus anthracis is responsible for anthrax.
Source: CDC/Dr. William A. Clark (Bacillus anthracis)

Table 16.1 Signs and Symptoms of Cutaneous Mycoses

Ringworm of the Scalp (Tinea Capitis)	This mycosis results from the fungal invasion of the scalp and the hair of the head, eyebrows, and eyelashes.	
Ringworm of the Beard (Tinea Barbae)	This tinea, also called *barber's itch*, affects the chin and beard of adult males. Although once a common after effect of unhygienic barbering, it is now contracted mainly from animals.	
Ringworm of the Body (Tinea Corporis)	This extremely prevalent infection of humans can appear nearly anywhere on the body's glabrous (smooth and bare) skin.	
Ringworm of	Sometimes known as *jock itch*, crural ringworm occurs mainly in males on the groin, perianal skin,	

the Groin (Tinea Cruris)	scrotum, and, occasionally, the penis. The fungus thrives under conditions of moisture and humidity created by sweating.	
Ringworm of the Foot (Tinea Pedis)	Tinea pedis has more colorful names as well, including athlete's foot and jungle rot. Infections often begin with blisters between the toes that burst, crust over, and can spread to the rest of the foot and nails.	
Ringworm of the Nail (Tinea Unguium)	Fingernails and toenails, being masses of keratin, are often sites for persistent fungus colonization. The first symptoms are usually superficial white patches in the nail bed. A more invasive form causes thickening, distortion, and darkening of the nail.	

Source: CDC (Tinea Capitis); Source: CDC (Tinea Barbae); ©Biophoto Associates/Science Source (Tinea Corporis); ©Dr. Harout Tanielian/Science Source (Tinea Cruris); ©Dr. P. Marazzi/Science Source (Tinea Pedis); Source: CDC/Dr Edwin P Ewing, Jr. (Tinea Unguium)

Causative Agents

There are about 39 species in the genera *Trichophyton, Microsporum*, and *Epidermophyton* that can cause the preceding conditions **(figure 16.11)**. The causative agent of a given type of ringworm varies from one geographic location to another and is not restricted to a particular genus and species.

Diagnosis of tinea of the scalp caused by some species is aided by use of a long-wave ultraviolet lamp that causes infected hairs to fluoresce. Samples of hair, skin scrapings, and nail debris treated with heated potassium hydroxide (KOH) show a thin, branching fungal mycelium if infection is present.

Pathogenesis and Virulence Factors

The dermatophytes have the ability to invade and digest keratin, which is naturally abundant in the cells of the stratum corneum. They have also been found to suppress the ability of the immune system to respond to them, ensuring their long-term persistence. The fungi do not invade deeper epidermal layers.

Transmission and Epidemiology

Transmission of the fungi that cause these diseases is via direct and indirect contact with other humans or with infected animals. Some of these fungi can be acquired from the soil. (Interestingly, cultures that walk barefoot most of the time have very low levels of athlete's foot, suggesting that it is the confined atmosphere of a shoe that encourages fungal growth.)

Therapy is usually a topical antifungal agent. Ointments containing tolnaftate, miconazole, itraconazole, terbinafine, or thiabendazole are applied regularly for several weeks. Some drugs work by speeding up loss of the outer skin layer.

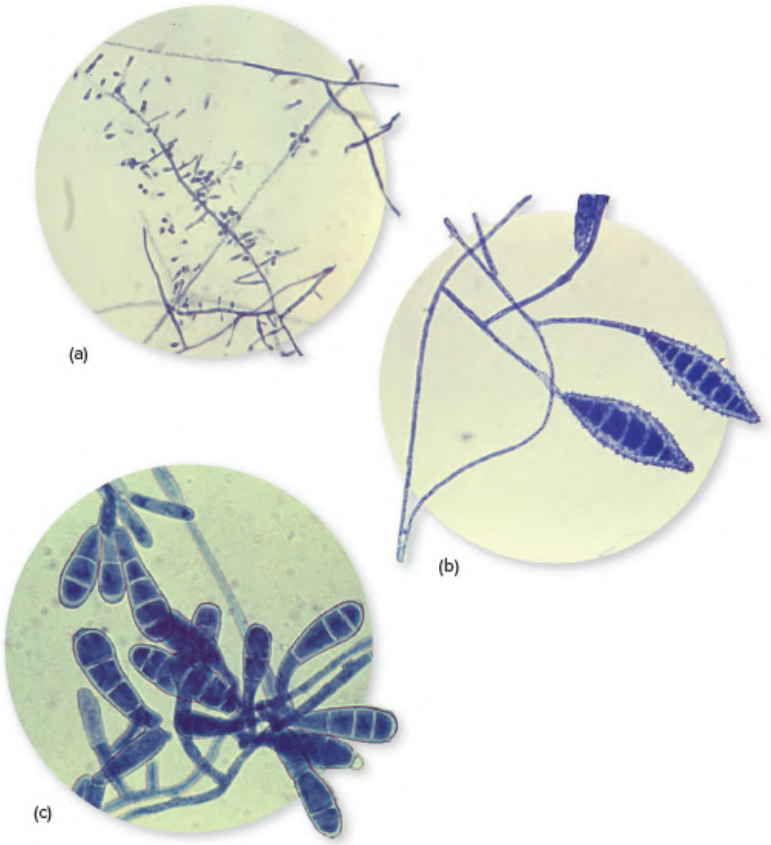


Figure 16.11 **Examples of dermatophyte spores**. **(a)** Regular, numerous microconidia of *Trichophyton*. **(b)** Macroconidia of *Microsporum canis*, a cause of ringworm in cats, dogs, and humans. **(c)** Smooth-surfaced macroconidia in clusters characteristic of *Epidermophyton*.
Source: CDC/Dr. Lucille K. George

Superficial Mycoses

Agents of **superficial mycoses** involve the outer epidermal surface and are ordinarily innocuous infections with cosmetic rather than outright disease-causing effects. Tinea versicolor is caused by the yeast genus *Malassezia*, a genus that has at least 10 species living on human skin. The yeast feeds on the high oil content of the skin glands. Even though these yeasts are very common (carried by nearly 100%

Disease Table 16.8 Cutaneous and Superficial Mycoses

Disease	Cutaneous Infections	Superficial Infections (Tinea Versicolor)
Causative Organism(s)	*Trichophyton, Microsporum, Epidermophyton* F	*Malassezia* species F
Most Common Modes of Transmission	Direct and indirect contact, vehicle (soil)	Endogenous "normal biota"
Virulence Factors	Ability to degrade keratin, invoke hypersensitivity, avoidance of immune response	–
Culture/Diagnosis	Microscopic examination, KOH staining, culture	Usually clinical, KOH can be used
Prevention	Avoid contact	None
Treatment	Topical tolnaftate, itraconazole, terbinafine, miconazole, thiabendazole, oral terbinafine	Topical antifungals

Disease	Cutaneous Infections	Superficial Infections (Tinea Versicolor)
Epidemiological Features	Among schoolchildren, 0%–19% prevalence, in humid climates up to 30%	Highest incidence among adolescents

of humans tested), in some people its growth elicits mild, chronic scaling and interferes with production of pigment by melanocytes. The trunk, face, and limbs may take on a mottled appearance **(figure 16.12)**. Other superficial skin conditions in which *Malassezia* is implicated are folliculitis, psoriasis, and seborrheic dermatitis (dandruff). **Disease Table 16.8**.

16.3 LEARNING OUTCOMES—Assess Your Progress

4. Explain the important features of the "Highlight Disease," MRSA skin and soft-tissue infection.
5. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Disease," maculopapular rash diseases.
6. Discuss important features of the other infectious skin diseases. These are impetigo, cellulitis, staphylococcal scalded skin syndrome, vesicular/pustular rash diseases, large pustular skin lesions, and cutaneous and superficial mycoses.
7. Discuss the relative dangers of rubella and rubeola viruses in different populations.

16.4 The Surface of the Eye and Its Defenses

The eye is a complex organ with many different tissue types, but for the purposes of this chapter we consider only its exposed surfaces, the *conjunctiva* and the *cornea* **(figure 16.13)**. The **conjunctiva** is a very thin membranelike tissue that covers the eye (except for the cornea) and lines the eyelids. It secretes an oil- and mucus-containing fluid that lubricates and protects the eye surface. The **cornea** is the dome-shaped central portion of the eye lying over the iris (the colored part of the eye). It has five to six layers of epithelial cells that can regenerate quickly if they are superficially damaged. It has been called "the windshield of the eye."

Figure 16.12 **Tinea versicolor**. Mottled, discolored skin pigmentation shown on two different skin tones. This is characteristic of superficial skin infection by *Malassezia furfur*.
©Biophoto Associates/Science Source (light skin); ©4FR/iStock/Getty Images (medium skin)

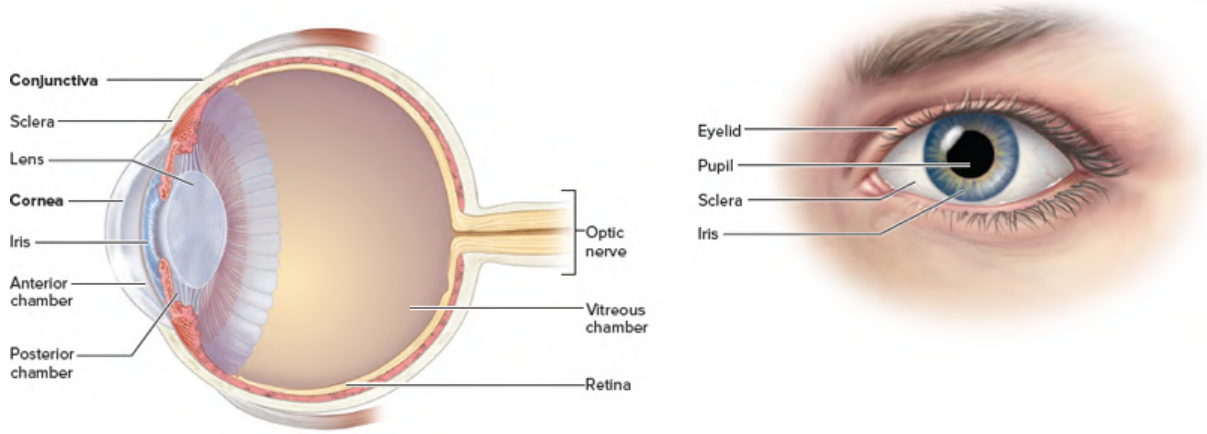


Figure 16.13 **The anatomy of the eye**.

The eye's best defense is the film of tears, which consists of an aqueous fluid, oil, and mucus. The tears are formed in the lacrimal gland at the outer and upper corner of each eye **(figure 16.14)**, and they drain into the lacrimal duct at the inner corner. Tears contain sugars, lysozyme, and lactoferrin. These last two substances have antimicrobial properties. The mucous layer contains

proteins and sugars and plays a protective role. And, of course, the flow of the tear film prevents the attachment of microorganisms to the eye surface.

Because the eye's primary function is vision, anything that hinders vision would be counterproductive. For that reason, inflammation does not occur in the eye as readily as it does elsewhere in the body. Flooding the eye with fluid containing a large number of light-diffracting objects, such as lymphocytes and phagocytes, in response to every irritant would mean almost constantly blurred vision. So even though the eyes are relatively vulnerable to infection (not being covered by keratinized epithelium), the evolution of the vertebrate eye has been toward reduced innate immunity and a corresponding reduction in inflammatory response. This characteristic is sometimes known as **immune privilege**.

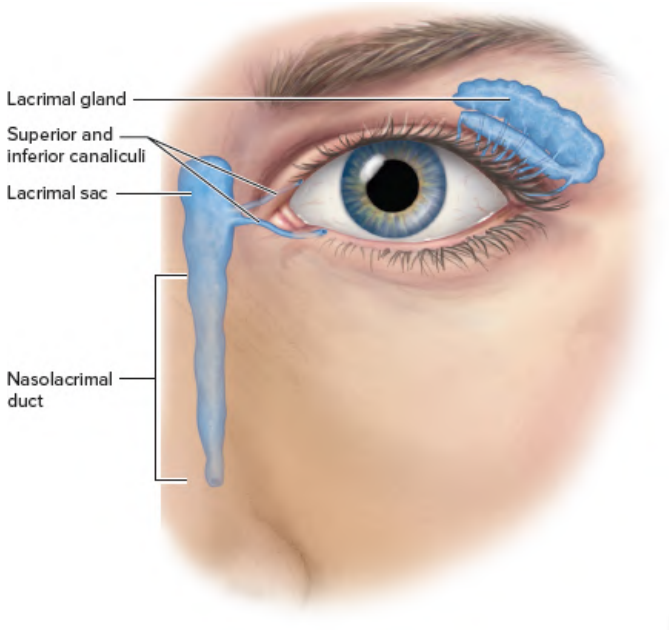


Figure 16.14 **The lacrimal apparatus of the eye**.

16.4 LEARNING OUTCOMES—Assess Your Progress

8. Describe the important anatomical features of the eye.
9. List the natural defenses present in the eye.

16.5 Normal Biota of the Eye

The eye was previously thought to be only sparsely populated by microbiota. 16s rRNA analysis of the healthy eye microbiome has revealed a more robust population, showing a lot of diversity in the bacteria found. In many cases *Corynebacterium* is the dominant genus. To a large extent the eye microbiome resembles that of the skin.

Defenses and Normal Biota of the Eyes

	Defenses	Normal Biota
Eyes	Mucus in conjunctiva and in tears, lysozyme and lactoferrin in tears	*Corynebacterium* species and other skin colonizers

16.5 LEARNING OUTCOMES—Assess Your Progress

10. List the types of normal biota presently known to occupy the eye.

©Comstock Images/PictureQuest

16.6 Eye Diseases Caused by Microorganisms

In this section, we cover the infectious agents that cause diseases of the surface structures of the eye—namely, the cornea and conjunctiva.

Figure 16.15 **Neonatal conjunctivitis**.
©Medical-on-Line/Alamy Stock Photo

Highlight Disease

Conjunctivitis

Infection of the conjunctiva is relatively common. It can be caused by specific microorganisms that have a predilection for eye tissues, by contaminants that proliferate due to the presence of a contact lens or an eye injury, or by accidental inoculation of the eye by a traumatic event.

Signs and Symptoms

Just as there are many different causes of conjunctivitis, there are many different clinical presentations. Most bacterial infections produce a milky discharge, whereas viral infections tend to produce a clear watery exudate. It is typical for a patient to wake up in the morning with an eye "glued" shut by secretions that have accumulated and solidified through the night. Some conjunctivitis cases are caused by an allergic response, and these often produce copious amounts of clear fluid as well. The informal name for common conjunctivitis is pinkeye.

Causative Agents and Their Transmission

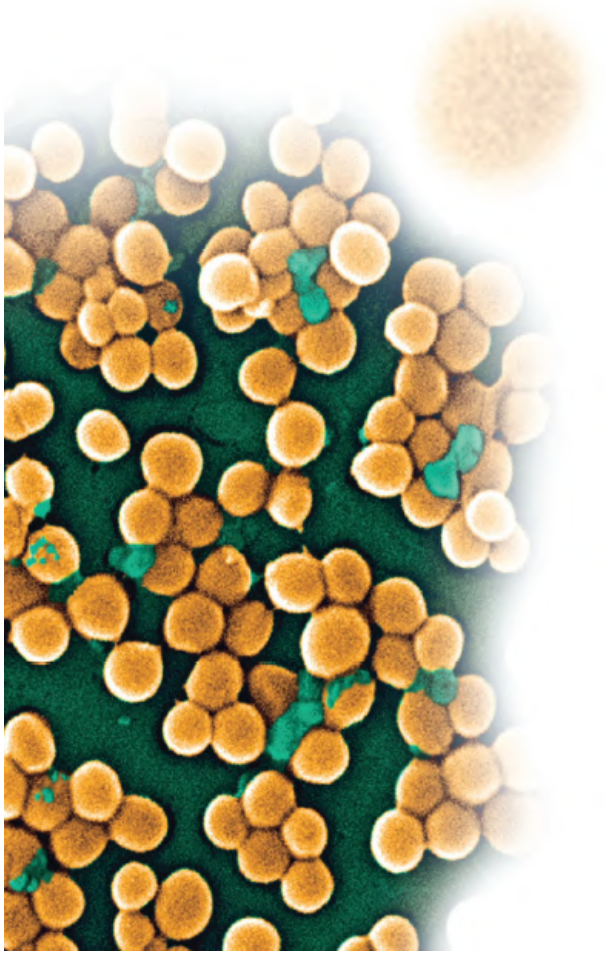
Cases of neonatal eye infection with *Neisseria gonorrhoeae* or *Chlamydia trachomatis* are usually transmitted vertically from a genital tract infection in the mother (discussed in chapter 21). Either one of these eye infections can lead to serious eye damage if not treated promptly **(figure 16.15)**. Note that herpes simplex can also cause neonatal conjunctivitis, but it is often accompanied by generalized herpes infection (covered in chapter 21).

Bacterial conjunctivitis in other age groups is most commonly caused by *Staphylococcus epidermidis, Streptococcus pyogenes*, or *Streptococcus pneumoniae*, although *Haemophilus influenzae* and *Moraxella* species are also frequent causes. *N. gonorrhoeae* and *C. trachomatis* can also cause conjunctivitis in adults. These infections may result from autoinoculation from a genital infection or from sexual activity, although *N. gonorrhoeae* can be part of the normal biota in the respiratory tract. A wide variety of bacteria, fungi, and protozoa can contaminate contact lenses and lens cases and then be transferred to the eye, resulting in disease that may be very serious. Viral conjunctivitis is commonly caused by adenoviruses, although other viruses

may be responsible. Both bacterial and viral conjunctivitis are transmissible by direct and even indirect contact and are usually highly contagious.

Prevention and Treatment

Newborn children in the United States are administered antimicrobials in their eyes after delivery to prevent neonatal conjunctivitis from either *N. gonorrhoeae* or *C. trachomatis*. Treatment of those infections, if they are suspected, is started before lab results are available and usually is accomplished with erythromycin, both topical and oral. If *N. gonorrhoeae* is confirmed, oral therapy is usually switched to ceftriaxone. If antibacterial therapy is prescribed for other conjunctivitis cases, it should cover all possible bacterial pathogens. Ciprofloxacin eyedrops are a common choice. Erythromycin or gentamicin are also often used. Because conjunctivitis is usually diagnosed based on clinical signs, a physician may prescribe prophylactic antibiotics even if a viral cause is suspected. If symptoms don't begin improving within 48 hours, more extensive diagnosis may be performed. **Disease Table 16.9** lists the most common causes of conjunctivitis. Keep in mind that other microorganisms can also cause conjunctival infections.



Source: CDC/Janice Haney Carr/Jeff Hageman, M.H.S.

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5. Upon inspection of a client's face, the nurse notes a milky discharge coming from the right eye. The client relates that upon arising this morning, he felt as if his eyes were "tightly shut." The nurse is getting ready to physically examine the client. Based on this information, the priority action taken by the nurse is to

a. *ask the client if he used any different products on his face.*
b. *flush out the eye with sterile water.*
c. *obtain gloves to perform an examination.*
d. *note whether the client has allergies to food or medication.*

Disease Table 16.9 Conjunctivitis

Disease	Neonatal Conjunctivitis	Bacterial Conjunctivitis	Viral Conjunctivitis
Causative Organism(s)	*Chlamydia trachomatis* or *Neisseria gonorrhoeae* B **G−**	*Streptococcus pneumoniae, Staphylococcus epidermidis, Staphylococcus aureus, Haemophilus influenzae, Moraxella*, and also *Neisseria gonorrhoeae, Chlamydia trachomatis* B	Adenoviruses and others V
Most Common Modes of Transmission	Vertical	Direct, indirect contact	Direct, indirect contact
Virulence Factors	—	—	—
Culture/Diagnosis	Gram stain and culture	Clinical diagnosis	Clinical diagnosis
Prevention	Screen mothers, apply antibiotic ophthalmic solution to newborn eyes	Hygiene	Hygiene
Treatment	Topical and oral antibiotics; (antibiotic-resistant *N. gonorrhoeae* is in **Urgent Threat** category in CDC Antibiotic Resistance Report)	Trimethoprim/polymyxin B for routine cases; azithromycin or levofloxacin for more serious cases (several of these bacteria appear in the CDC Antibiotic Resistance Report)	None, although antibiotics often given because type of infection not distinguished
Distinguishing Features	In babies <28 days old	Mucopurulent discharge	Serous (clear) discharge
Epidemiological Features	Less than 0.5% in developed world; higher incidence in developing world	More common in children	More common in adults

Keratitis

Keratitis is a more serious eye condition than conjunctivitis. Damage of deeper eye tissues occurs and can lead to complete corneal destruction. Any microorganism can cause this condition, especially after trauma to the eye, but this section focuses on two of the most common causes: miscellaneous bacteria (which cause 80% of the infectious keratitis cases), and herpes simplex virus. This virus can cause keratitis in the absence of predisposing trauma.

The usual cause of herpetic keratitis is a "misdirected" reactivation of (oral) herpes simplex virus type 1 (HSV-1). The virus, upon reactivation, travels into the ophthalmic rather than the mandibular branch of the trigeminal nerve. Infections with HSV-2 can also occur as a result of a sexual encounter with the virus or transfer of the virus from the genital to eye area. Blindness due

to herpes is the leading infectious cause of blindness in the United States. Bacterial and fungal causes of keratitis are more common in developing countries.

The viral condition is treated with trifluridine or acyclovir or both.

Disease Table 16.10 Keratitis

Causative Organism(s)	Herpes simplex virus (V)	Miscellaneous bacteria, or *Acanthamoeba* (B) (P)
Most Common Modes of Transmission	Reactivation of latent virus, although primary infections can occur in the eye	Often traumatic introduction (parenteral)
Virulence Factors	Latency	Various
Culture/Diagnosis	Usually clinical diagnosis; viral culture or PCR if needed	Various
Prevention	—	—
Treatment	Topical trifluridine +/– oral acyclovir	Specific antimicrobials
Epidemiological Features	One-third worldwide population infected; in United States, annual incidence of 500,000	

In the last few years, another form of keratitis has been increasing in incidence. An amoeba called *Acanthamoeba* has been causing serious keratitis cases, especially in people who wear contact lenses. This free-living amoeba is everywhere—it lives in tap water, freshwater lakes, and the like. The infections are usually associated with less-than-rigorous contact lens hygiene, or previous trauma to the eye **(figure 16.16). Disease Table 16.10**.

Figure 16.16 ***Acanthamoeba*** **infection of the eye**.
©ISM/Medical Images

16.6 LEARNING OUTCOMES—Assess Your Progress

11. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Disease," conjunctivitis.

12. Discuss the most important causes of keratitis.

CASE FILE WRAP-UP

©Syda Productions/Shutterstock (health care worker); ©Dennis MacDonald/Alamy Stock Photo (hospital)

Wyatt, the patient in the chapter opening case file, was admitted to the hospital after his X ray confirmed a mild case of pneumonia, a complication of his measles. He was given IV antibiotics to treat his pneumonia and fluids to counter dehydration, as well as antipyretics to treat his fever and make him more comfortable. He was discharged home after 4 days and recovered completely.

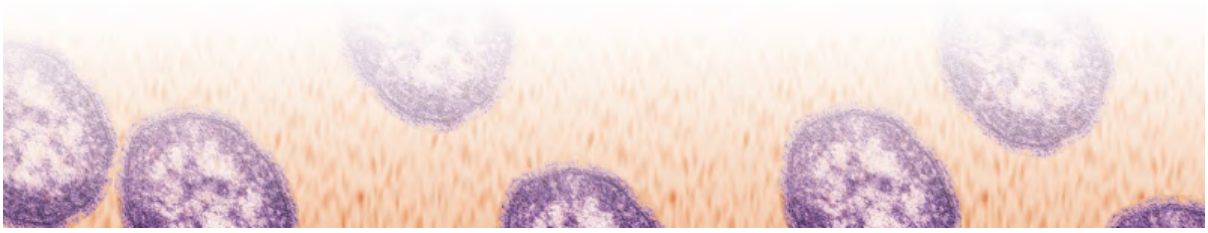
The physician contacted the state health department, as must be done legally. State health department investigators contacted all of Wyatt's contacts to ensure they had not fallen ill. A few contacts were found who had never been vaccinated because their parents shared Wyatt's mother's erroneous view of vaccines. These children were quarantined at home to prevent the potential spread of the disease. Thankfully, all of the patients in the hospital waiting room were found to be immune to the disease and no one else became ill. Wyatt was assumed to have contracted measles while on a family vacation abroad.

Summing Up

Taxonomic Organization Microorganisms Causing Diseases of the Skin and Eyes

Microorganism	Pronunciation	Disease Table
Gram-positive bacteria		
Staphylococcus aureus	staf″-uh-lo-kok′-us are′-ee-us	MRSA skin and soft tissue infection, 16.1
		Impetigo, 16.3
		Cellulitis, 16.4
		Scalded skin syndrome, 16.5
Streptococcus pyogenes	strep″-tuh-kok′-us pie′-ah″-gen-eez	Impetigo, 16.3
		Cellulitis, 16.4
		Scarlet fever, 16.2
Bacillus anthracis	buh-sill′-us an′-thray″-sus	Cutaneous anthrax, 16.7
Gram-negative bacteria		
Neisseria gonorrhoeae	nye-seer″-ee-uh′ gon′-uh-ree″-uh	Neonatal conjunctivitis, 16.9
Chlamydia trachomatis	kluh-mi″-dee-uh′ truh-koh′-muh-tis	Neonatal conjunctivitis, 16.9
DNA viruses		
Human herpesvirus 3	hew′-mun hur″-peez-vie′-russ	Chickenpox, shingles, 16.6
Variola virus	vayr′-ee-oh″-luh vie′-russ	Smallpox, 16.6
Parvovirus B19	par″-voh-vie′-russ	Fifth disease, 16.2
Human herpesvirus 6	hew′-mun hur″-peez-vie′-russ	Roseola, 16.2
Herpes simplex virus	hur″-peez sim′-plex vie′-russ	Keratitis, 16.10
Adenovirus	a″-duh-no-vie′-russ	Conjuctivitis, 16.9

Microorganism	Pronunciation	Disease Table
RNA viruses		
Measles virus	mee′-zulls vie′-russ	Measles, 16.2
Rubella virus	roo′-bell″-uh vie′-russ	Rubella, 16.2
Fungi		
Trichophyton	try″-ko-fie′-tahn	Ringworm, 16.8
Microsporum	my″-krow′-spoor′-um	Ringworm, 16.8
Epidermophyton	ep′-uh-dur″-moh-fie′-tahn	Ringworm, 16.8
Malassezia spp.	mal′-uh-see″-zee-uh	Superficial mycoses, 16.8
Protozoa		
Leishmania spp.	leesh-mayn″-ee-uh	Leishmaniasis, 16.7
Acanthamoeba	ay-kanth″-uh-mee′-buh	Keratitis, 16.10



Source: CDC/Cynthia Goldsmith

The Microbiome

C-Section Babies Have a Different Microbiome than Those Delivered Vaginally

©Kevin Landwer-Johan/Getty Images

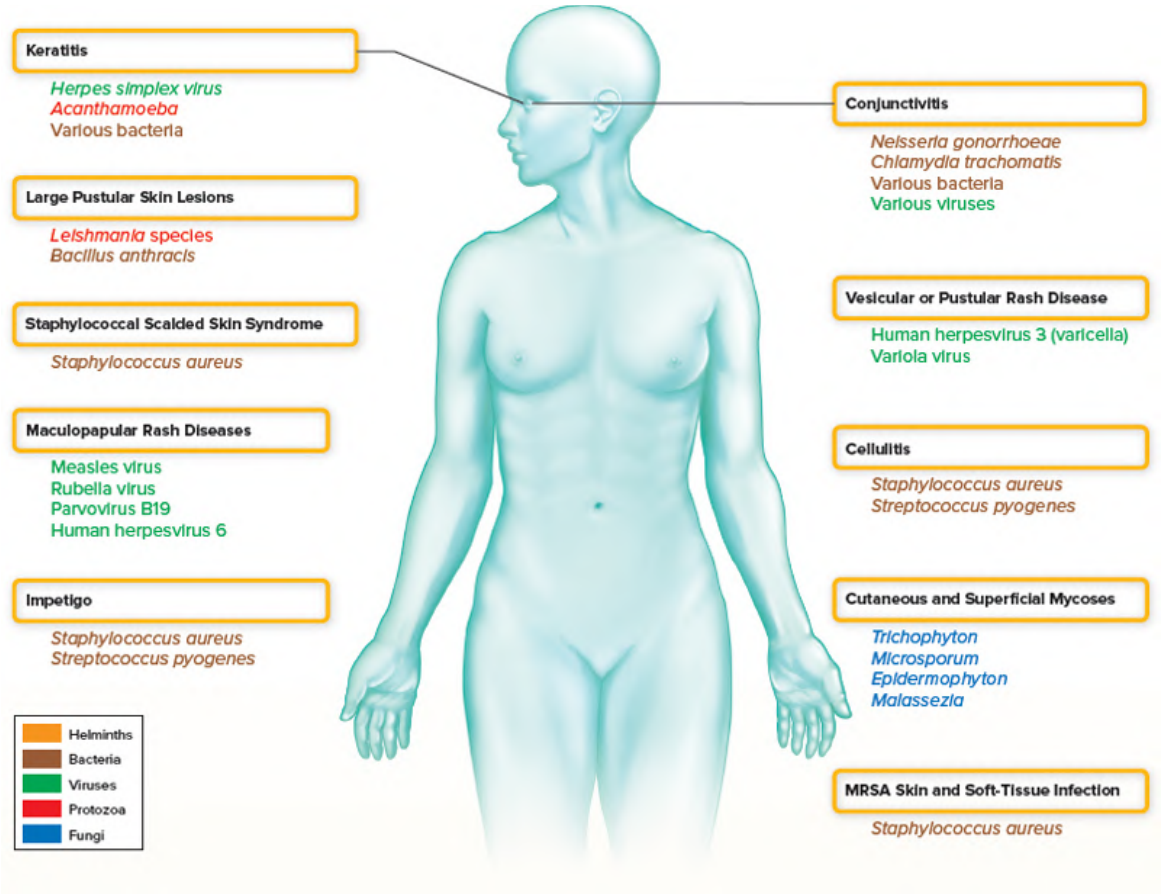
Think about your circle of female family and friends: How many of them have delivered babies via C-section? In some regions of the United States, the rates of C-section approach 50%. In some other countries (such as Brazil), it can be as high as 90%. But microbiome science suggests that it might not be the best start for babies—at least with respect to their gut

microbiome. And we know how important the gut microbiome is. We have talked about how it can influence many aspects of our health, even outside our guts.

Scientists studied the gut microbiome of babies who were delivered vaginally versus that of those delivered via C-section. The vaginally born babies were colonized with vaginal and fecal bacteria from the mother. That might sound bad, but the bacteria help babies digest milk and are generally protective to gut health. On the other hand, the guts of babies delivered through C-section are generally colonized by the skin bacteria of their mother and of their caregiver. Skin bacteria are not meant to become the microbiome in the gut. Several studies have shown that C-section babies are more prone to infections, and even more prone to allergies and obesity later in life.

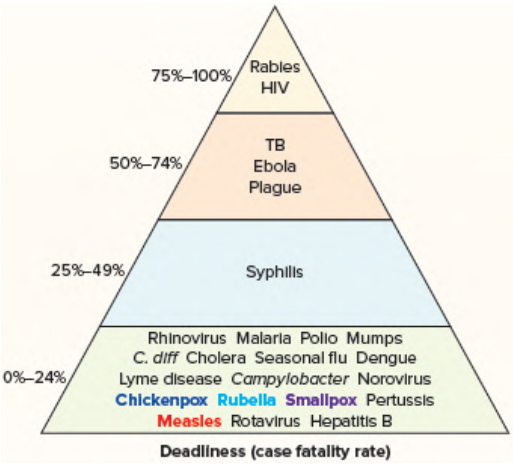
Scientists are researching a simple technique to change the initial colonization of C-section babies. An hour before the C-section surgery, a gauze packet is placed into the mother's vagina; immediately after the baby is delivered, the gauze is wiped over the baby's mouth and skin in an attempt to preempt the establishment of skin microbes as the baby's microbiome. It is not yet a medically accepted procedure, but research is continuing.

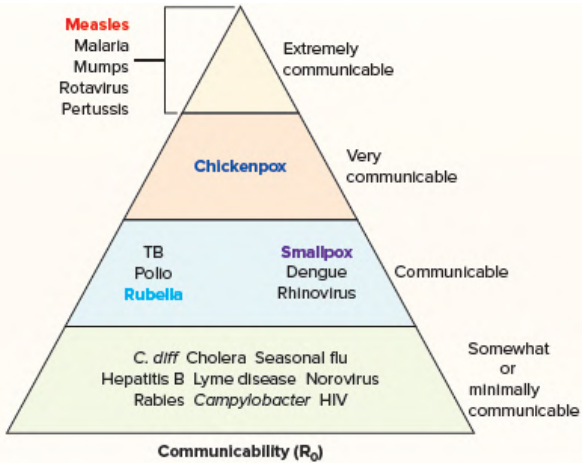
Infectious Diseases Affecting
The Skin and Eyes



System Summary Figure 16.17

Deadliness and Communicability of Selected Diseases of the Skin and Eyes





Chapter Summary

16.1 The Skin and Its Defenses

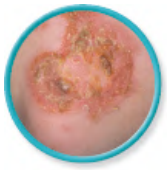
- The epidermal cells contain the protein keratin, which "waterproofs" the skin and protects it from microbial invasion.
- Other defenses include antimicrobial peptides, low pH sebum, antimicrobial peptides, and high salt and lysozyme in sweat.

16.2 Normal Biota of the Skin

- The Human Microbiome Project and related efforts are revealing that the normal skin microbiota are far more diverse than we had previously understood from cultivation methods.
- The normal biota includes hundreds of species of microorganisms, differs from region to region of the body, differs between people, and yet can be constant over time on the same person.

16.3 Skin Diseases Caused by Microorganisms

- **MRSA Skin and Soft-Tissue Infection**
  - Serious skin lesions caused by methicillin-resistant *S. aureus* are on the rise in the community environment.
  - MRSA isolates are commonly found on healthy people and on community surfaces.
- **Maculopapular Rash Diseases**
  - **Measles:** Measles or *rubeola* results in characteristic red maculopapular rash that erupts on the head and then progresses to the trunk and extremities until most of the body is covered. The MMR vaccine (measles, mumps, and rubella) and MMRV (measles, mumps, rubella, and varicella) contain attenuated measles virus.
  - **Rubella:** Also known as German measles, can appear in two forms: postnatal and congenital (prenatal) infection of the fetus. The MMR and MMRV vaccines contain protection from rubella.
  - **Fifth Disease:** Also called *erythema infectiosum*, fifth disease is a very mild but highly contagious disease that often results in characteristic "slapped-cheek" appearance. Causative agent is parvovirus B19.
  - **Roseola:** Can result in a maculopapular rash; is caused by a human herpesvirus called HHV-6.
  - **Impetigo:** A highly contagious superficial infection that can cause skin to peel or flake off. Causative organisms can be either *Staphylococcus aureus* or *Streptococcus pyogenes* or both.
  - **Cellulitis:** Results from a fast-spreading infection of the dermis and subcutaneous tissue below. Most commonly caused by *S. aureus* or *S. pyogenes*.
- **Staphylococcal Scalded Skin Syndrome (SSSS):** Caused by *S. aureus*. Affects mostly newborns and babies and is similar to a systemic form of impetigo.
- **Vesicular or Pustular Rash Diseases**
  - **Chickenpox:** Skin lesions progress quickly from macules and papules to itchy vesicles filled with clear fluid.
    - **Shingles:** Chickenpox virus becomes latent in the ganglia and may reemerge to cause shingles. Human herpesvirus 3 (varicella) causes chickenpox, as well as herpes zoster or shingles.
  - **Smallpox:** Naturally occurring smallpox has been eradicated from the world. Causative agent is the variola virus, an enveloped DNA virus.
- **Large Pustular Skin Lesions**
  - **Leishmaniasis:** A zoonosis transmitted by the female sand fly when it ingests the host's blood.
  - **Cutaneous Anthrax:** Most common and least dangerous version of infections with *Bacillus anthracis*.
- **Cutaneous and Superficial Mycoses**
  - **Ringworm:** A group of fungi collectively termed dermatophytes cause mycoses in the nonliving epidermal tissues, hair, and nails. Often called "ringworm." Species in the genera *Trichophyton, Microsporum*, and *Epidermophyton* are the cause.
  - **Superficial Mycoses:** Agents of superficial mycoses, such as *Malassezia* species, involve only the outer epidermis.



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16.4 The Surface of the Eye and Its Defenses

- The flushing action of the tears, which contain lysozyme and lactoferrin, is the major protective feature of the eye.

16.5 Normal Biota of the Eye

- The eye has similar microbes as the skin but in lower numbers.
- As with the skin, cultivation-independent methods are showing that the eye is home to many more species than we previous thought.

16.6 Eye Diseases Caused by Microorganisms

- **Conjunctivitis:** Infection of the conjunctiva (commonly called pinkeye) can be caused by either bacteria or viruses. Bacteria are the most frequent cause. Both bacterial and viral conjunctivitis are highly contagious.
- **Keratitis:** A more serious eye infection than conjunctivitis. Herpes simplex viruses (HSV-1 and HSV-2) and *Acanthamoeba* cause additional forms of the disease.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. Which of the following infectious agents has evolved to maintain a persistent state in its host? **a**. variola virus **b**. herpes virus **c**. vaccinia virus **d**. Staphylococcus aureus	**2**. Can you think of a plausible argument for why hospital cases of MRSA are declining and community cases of MRSA are increasing?	**3**. Smallpox has ravaged human populations for thousands of years. When it first came to the Americas in the 1500s it had a high case-fatality rate. By the end of the 1800s it manifested as a much milder disease. Speculate on why that might be.
Cell Structure and Function	**4**. What is an antimicrobial enzyme found in sweat, tears, and saliva that can break down bacterial cell walls? **a**. lysozyme **b**. beta-lactamase **c**. catalase **d**. hyaluronidase	**5**. What cell structure of *Bacillus anthracis* makes it capable of surviving in powdered form, and naturally in soil, for indefinite periods of time?	**6**. Both *Staphylococcus aureus* and *Streptococcus pyogenes* are "pyogenic." What does that mean and what attributes of the two bacteria are likely to contribute to that phenomenon?

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Metabolic Pathways	**7**. Which of the following organisms produces an enzyme that breaks down hydrogen peroxide? Streptococcus pyogenes Staphylococcus aureus MRSA **d**: two of these	**8**. *Streptococcus pneumoniae*, which is a common inhabitant of healthy upper respiratory tracts, produces hydrogen peroxide to fight off microbial competitors. How is it possible then that *Staphylococcus aureus* is frequently a co-colonizer?	**9**. Some antifungal drugs inhibit an enzyme called squalene epoxidase. What does this enzyme do and why is it an effective target for selective toxicity to the fungus and not the host?
Information Flow and Genetics	**10**. Which of these techniques has detected the larger number of normal microbiota on skin surfaces? **a**. culturing **b**. 16s rRNA sequencing antibody probing gel electrophoresis **d**.	**11**. The first person to die of measles in the United States in 12 years was a woman in Washington State in July 2015. What kind of laboratory tests would you perform to determine whether the virus was the same that circulated at Disney in 2014?	**12**. Herpesviruses cause several of the diseases in this chapter. Considering some features all of these conditions have in common, hypothesize what the viral DNA does after initial invasion of a host cell.

Microbial Systems	**13**. Which of the following conditions is most likely to be a polymicrobial infection? **a**. measles **b**. rubella **c**. leishmaniasis **d**. impetigo	**14**. Describe how a virus infection of human cells can lead to syncytia.	**15**. Research the bacterium *Micavibrio aeruginosavorus*, which is often found as normal biota of the eye. Formulate a theory on how it keeps pathogens from harming the eye.
Impact of Microorganisms	**16**. *Staphylococcus aureus* is part of the differential diagnosis of which of the following diseases? **a**. impetigo **b**. maculopapular rash **c**. both of these **d**. neither of these	**17**. Compare and contrast the effects of the rubeola virus and the rubella virus on humans.	**18**. In a single (unvaccinated) family one winter, the 12-year-old and 4-year-old came down with chickenpox. The mother and father had experienced the disease as children and were unaffected this time. The 3-month-old baby did not become ill, either. However, 4 months later the baby exhibited a shingles outbreak. Using immunological phenomena, explain what was likely going on with the baby.
Scientific Thinking	**19**. Which steps of the diagnostic process are in order? **a**. Differential diagnosis, anatomic diagnosis, etiologic diagnosis **b**. Anatomic diagnosis, etiologic diagnosis,	**20**. Explain why detecting a single case of smallpox would be considered a public health—and national security—emergency.	**21**. Construct a testable hypothesis for examining whether receiving the chickenpox vaccine results in fewer cases of shingles in the population.

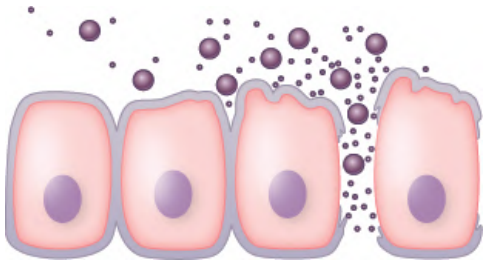
	differential diagnosis **c**. Anatomic diagnosis, differential diagnosis, etiologic diagnosis **d**. none of these		

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From chapter 11, figure 11.2**. How does this figure help explain impetigo caused by *Staphylococcus aureus* or *Streptococcus pyogenes?*



connect Resources are available through Connect, including interactive learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

17

Infectious Diseases Affecting the Nervous System

©Science Photo Library/Alamy Stock Photo (blood cells); Source: CDC/DR. Al Jenny (diseased tissue); ©Exactostock/SuperStock (health care worker)

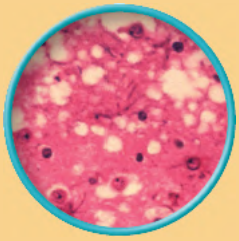
IN THIS CHAPTER…

17.1 The Nervous System and Its Defenses

1. Describe the important anatomical features of the nervous system.
2. List the natural defenses present in the nervous system.

17.2 Normal Biota of the Nervous System

3. Discuss the current state of knowledge of the normal microbiota of the nervous system.



17.3 Nervous System Diseases Caused by Microorganisms

4. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Diseases," meningitis and Zika virus disease.
5. Identify the most common and also the most deadly of the multiple possible causes of meningitis.
6. Explain the difference between the oral polio vaccine and the inactivated polio vaccine, and under which circumstances each is appropriate.
7. Discuss important features of the diseases most directly involving the brain. These are meningoencephalitis, encephalitis, and subacute encephalitis.
8. Identify which encephalitis-causing viruses you should be aware of in your geographic area.
9. Name three causes of subacute encephalitis and discuss the epidemiology of each.
10. Discuss important features of the disease rabies.
11. Compare and contrast the diseases tetanus and botulism.

CASE FILE

Time Is of the Essence

I was working in an assisted living facility. As an RN, I supervised the care of all the patients in the facility. My responsibilities included preparing and administering medications, performing daily patient assessments, and responding to changes in patient condition. I worked with a team of nursing assistants who helped residents with activities of daily living. Supervising a large number of patients was a challenge but I could depend on my team to keep me alerted if any of our residents needed immediate attention.

One morning, a nursing assistant alerted me that Betty, an 86-year-old female, was lethargic and difficult to arouse. Betty was an active and cognitively appropriate resident. She had been diagnosed with a lower respiratory tract infection a few days prior. I was concerned when I entered her room and found her in bed and abnormally drowsy. She had a high temperature and seemed sensitive to the lights in the room. We called for emergency services to transport her to the hospital.

Once at the hospital, the medical team in the emergency department performed a thorough neurological assessment. Betty was confused and lethargic. She continued to exhibit photophobia and reported a headache. The provider decided to institute intravenous antibiotic therapy, as he was concerned for meningitis. Cefotaxime and vancomycin were ordered STAT. Labs were drawn, including a complete blood count (CBC) with differential, metabolic panel, and blood cultures. The provider performed a lumbar puncture.

Betty was admitted to an inpatient medical unit with presumed meningitis. The team planned to continue her antibiotic therapy and monitor her neurological status closely.

- What findings on a lumbar puncture are diagnostic of meningitis?
- How is medication dosing adjusted to cross the blood-brain barrier?

Case File Wrap-Up appears at the end of the chapter.

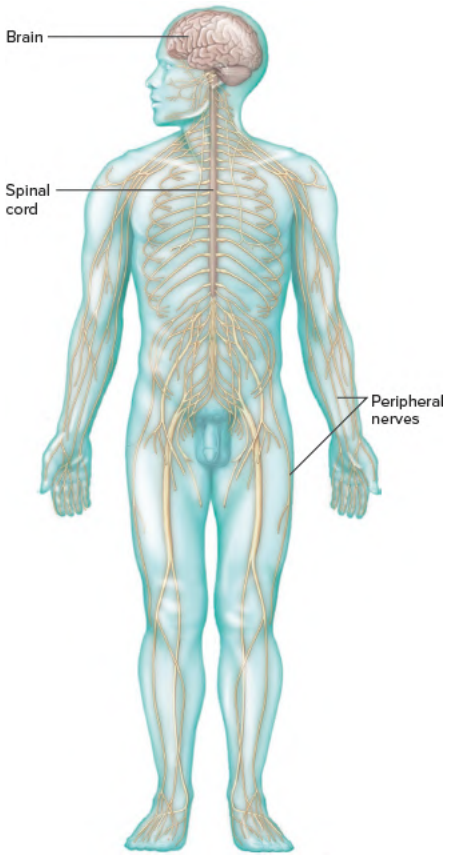


Figure 17.1 **The two component parts of the nervous system**. The central nervous system and the peripheral nerves.

17.1 The Nervous System and Its Defenses

The nervous system has two component parts: the central nervous system (CNS), consisting of the brain and spinal cord, and the peripheral nervous system (PNS), which contains the nerves that emanate from the brain and spinal cord to sense organs and to the periphery of the body **(figure 17.1)**. The nervous system performs three important functions—sensory, integrative, and motor. The sensory function is fulfilled by sensory receptors at the ends of peripheral nerves. They generate nerve impulses that are transmitted to the central nervous system. There, the impulses are translated, or integrated, into sensation or thought, which in turn drives the motor function. The motor function necessarily involves structures outside of the nervous system, such as muscles and glands.

The brain and the spinal cord are dense structures made up of cells called **neurons**. They are both surrounded by bone. The brain is situated inside the skull, and the spinal cord lies within the spinal column **(figure 17.2)**, which is composed of a stack of interconnected bones called vertebrae. The soft tissue of the brain and spinal cord is encased within a tough casing of three membranes called the **meninges**. The layers of these three membranes, from outermost to innermost position, are the dura mater, the arachnoid mater, and the pia mater. Between the arachnoid mater and pia mater is the subarachnoid space (i.e., the space under the arachnoid mater). The subarachnoid space is filled with a clear serumlike fluid called cerebrospinal fluid (CSF). The CSF provides nutrition to the CNS, while also providing a liquid cushion for the sensitive brain and spinal cord. The meninges are a common site of infection, and microorganisms can often be found in the CSF when meningeal infection **(meningitis)** occurs.

The peripheral nervous system consists of nerves and ganglia. A ganglion is a swelling in the nerve where the cell bodies of the neurons aggregate. Nerves are bundles of neuronal axons that receive and transmit nerve signals. The axons and dendrites of adjacent neurons communicate with each other over a very small space, called a synapse. Chemicals called neurotransmitters are released from one cell and act on the next cell in the synapse.

The defenses of the nervous system are mainly structural. The bony casings of the brain and spinal cord protect them from traumatic injury. The cushion of surrounding CSF also serves a protective function. The entire nervous system is served by the vascular system, but the interface between the blood vessels serving the brain and the brain itself is different from that of other areas of the body and provides a third structural protection. The cells that make up the walls of the blood vessels allow very few molecules to pass through. In other parts of the body, there is freer passage of ions, sugars, and other metabolites through the walls of blood vessels. The restricted permeability of blood vessels in the brain is called the **blood-brain barrier**, and it prohibits most microorganisms from passing into the central nervous system. The drawback of this phenomenon is that drugs and antibiotics are difficult to introduce into the CNS also.

The CNS is considered an "immunologically privileged" site. These sites are able to mount only a partial, or at least a different, immune response when exposed to immunologic challenge. The functions of the CNS are so vital for the life of an organism that even temporary damage that could potentially result from "normal" immune responses would be very detrimental. The uterus and parts of the eye are other immunologically privileged sites. Specialized cells in the central nervous system perform defensive functions. Microglia are a type of cell having phagocytic capabilities, and brain macrophages also exist in the CNS, although the activity of both of these types of cells is thought to be less than that of phagocytic cells elsewhere in the body.

NCLEX® PREP

1. The term *immunologically privileged* means that
- **a.** *the CNS can only mount a partial immune response when faced with an immunologic challenge.*
- **b.** *the CNS is impervious to microorganisms.*
- **c.** *the CNS is protected against all microorganisms by a barrier (the blood-brain barrier).*
- **d.** *the immune response of the CNS is superior to that of other body sites.*

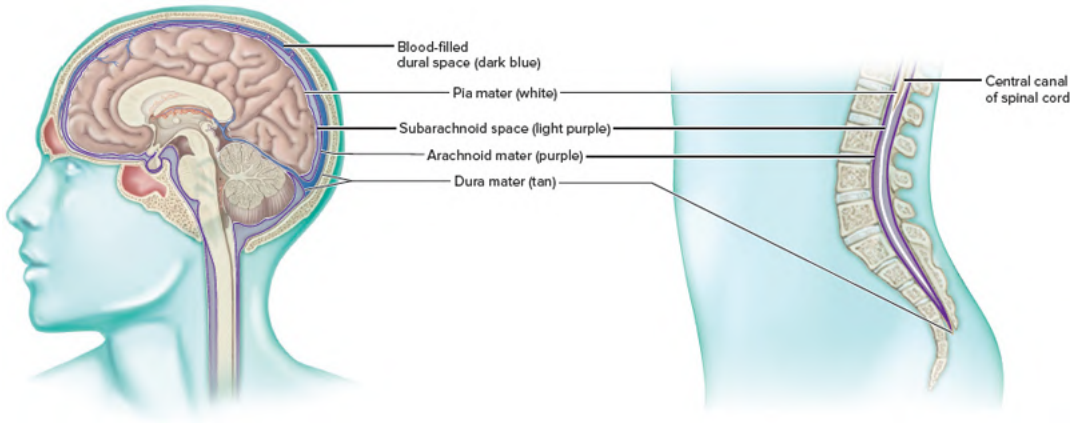


Figure 17.2 **Detailed anatomy of the brain and spinal cord**.

17.1 LEARNING OUTCOMES—Assess Your Progress

1. Describe the important anatomical features of the nervous system.
2. List the natural defenses present in the nervous system.

17.2 Normal Biota of the Nervous System

It is still believed that there is no normal biota in either the CNS or PNS, and that finding microorganisms of any type in these tissues represents a deviation from the healthy state. Viruses such as herpes simplex live in a dormant state in the nervous system between episodes of acute disease, but they are not considered normal biota. Having said that, there is a lot of research suggesting that the gut microbiome influences the nervous system in many ways. In fact the very development of the brain, the blood-brain barrier, and proper construction of peripheral nerves are influenced by the microbiome in the developing gut.

Nervous System Defenses and Normal Biota

	Defenses	Normal Biota
Nervous System	Bony structures, blood-brain barrier, microglial cells, and macrophages	None

17.2 LEARNING OUTCOMES—Assess Your Progress

3. Discuss the current state of knowledge of the normal microbiota of the nervous system.

NCLEX® PREP

2. What is the significance of the blood-brain barrier with respect to medication administration?
   a. *It allows for the absorption of all medications in the CNS.*
   b. *Medications must be able to penetrate the blood-brain barrier to be effective in reaching the CNS.*
   c. *The blood-brain barrier is a nonpermeable membrane and as such prevents absorption of all medications.*
   d. *Selective permeability is a characteristic of the blood-brain barrier, allowing medications with low lipid solubility to reach the CNS.*

©Science Photo Library RF/Getty Images

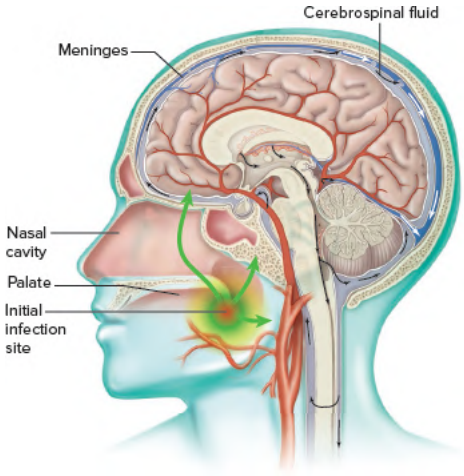


Figure 17.3 **Dissemination of the meningococcus from a nasopharyngeal infection**. Bacteria spread to the roof of the nasal cavity, which borders a highly vascular area at the base of the brain. From this location, they can enter the blood, causing meningococcemia, and escape into the cerebrospinal fluid, leading to infection of the meninges.

17.3 Nervous System Diseases Caused by Microorganisms

HIGHLIGHT DISEASE

Meningitis

Meningitis, an inflammation of the meninges, is an excellent example of an anatomical syndrome. Many different microorganisms can cause an infection of the meninges, and they produce a similar set of symptoms. Noninfectious causes of meningitis exist as well, but they are much less common than the infections listed here.

The more serious forms of acute meningitis are caused by bacteria, but it is thought that their entrance to the CNS is often facilitated by coinfection or previous infection with respiratory viruses. Meningitis in neonates is most often caused by different microorganisms than those causing the disease in children and adults, and therefore it is described separately in the following section.

Whenever meningitis is suspected, lumbar puncture (spinal tap) is performed to obtain CSF, which is then examined by Gram stain and/or culture. Most physicians will begin treatment with a broad-spectrum antibiotic immediately and shift treatment if necessary after a diagnosis has been confirmed.

▶ Signs and Symptoms

No matter the cause, meningitis results in these typical symptoms: severe headache, painful or stiff neck, fever, and nausea and vomiting. Early symptoms may be mistaken for flu symptoms. Photophobia (sensitivity to light) may also be noted. Skin rashes may be present in specific types of meningitis. There is usually an increased number of white blood cells in the CSF. Specific microorganisms may cause additional, and sometimes characteristic, symptoms, which are described in the individual sections that follow.

Like many other infectious diseases, meningitis can manifest as acute or chronic disease. Some microorganisms are more likely to cause acute meningitis, and others are more likely to cause chronic disease.

In a normal healthy person, it is very difficult for microorganisms to gain access to the nervous system. Those that are successful usually have specific virulence factors.

Neisseria meningitidis

Neisseria meningitidis appears as gram-negative diplococci (round cells occurring in joined pairs) and is commonly known as the meningococcus. It is often associated with epidemic forms of meningitis. This organism causes the most serious form of acute meningitis and accounts for 15% to 20% of all meningitis cases. Most cases occur in young children, because vaccination of otherwise healthy children against this disease is not recommended until age 11. Although 12 different strains with different capsular antigens exist, serotypes B, C, and Y responsible for most cases of infection in the United States. In Africa other serotypes are prominent, and two serotypes (A and W) are associated with the Hajj, an annual pilgrimage made by Muslims to the city of Mecca in Saudi Arabia.

▶ Pathogenesis and Virulence Factors

The bacterium enters the body via the upper respiratory tract, moves into the blood, rapidly penetrates the meninges, and produces symptoms of meningitis. The most serious complications of meningococcal infection are due to meningococcemia **(figure 17.3)**, which can accompany meningitis but can also occur on its own. The pathogen releases endotoxin into the generalized circulation, which is a potent stimulus for certain white blood cells. Damage to the blood vessels caused by cytokines released by the white blood cells leads to vascular collapse, hemorrhage, and crops of lesions called **petechiae** (pee-tee′-kee-eye) on the trunk and appendages. A *petechia* (singular) is a small, 1 to 2 mm red or purple spot that may occur anywhere on the body **(figure 17.4)**.

In a small number of cases, meningococcemia becomes an overwhelming disease with a high mortality rate.

The disease has a sudden onset, marked by fever higher than 40°C or 104°F, sore throat, chills, delirium, severe widespread areas of bleeding under the skin, shock, and coma. Generalized intravascular clotting, cardiac failure, damage to the adrenal glands, and death can occur within a few hours. The bacterium has an IgA protease and a capsule, both of which counter the body's defenses.

Transmission and Epidemiology

Because meningococci do not survive long in the environment, these bacteria are usually acquired through close contact with secretions or droplets.

Meningococcal meningitis has a sporadic or epidemic incidence in late winter or early spring. The continuing reservoir of infection is humans who harbor the pathogen in the nasopharynx. The scene is set for transmission when carriers live in close quarters with nonimmune individuals, as might be expected in families, day care facilities, college dormitories, and military barracks. The highest risk groups are young children (6 to 36 months old) and older children and young adults (10 to 20 years old). Cases peak in January and February in the United States.

Figure 17.4 **Vascular damage associated with meningococcal meningitis**.
Source: CDC/Mr. Gust

Culture and Diagnosis

Suspicion of bacterial meningitis constitutes a medical emergency, and differential diagnosis must be done with great haste and accuracy. It is most important to confirm (or rule out) meningococcal meningitis, because it can be rapidly fatal. Treatment is usually begun with this bacterium in mind until it can be ruled out. Cerebrospinal fluid, blood, or nasopharyngeal samples are stained and observed directly for the typical gram-negative diplococci. Cultivation is the preferred method of diagnosis because it also enables a quick assessment of antimicrobial susceptibilities. Specific rapid tests are also available for detecting the capsular polysaccharide or the cells directly from specimens without culturing.

It is usually necessary to differentiate this species from normal *Neisseria* that also live in the human body and can be present in infectious fluids. Immediately after collection, specimens are streaked on Modified Thayer-Martin medium (MTM) or chocolate agar and incubated in a high CO_2 atmosphere. Presumptive identification of the genus is obtained by a Gram stain and oxidase testing on isolated colonies **(figure 17.5)**.

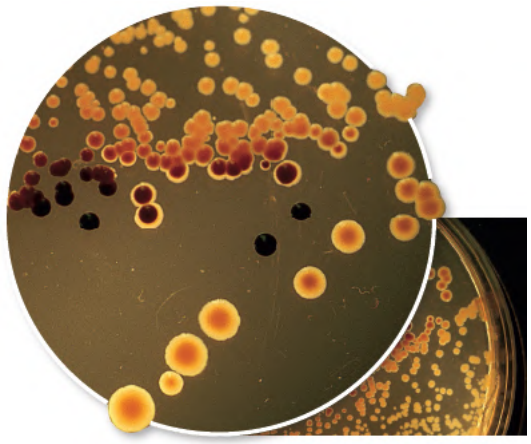


Figure 17.5 The oxidase test. A drop of oxidase reagent is placed on a suspected *Neisseria* or *Branhamella* colony. If the colony reacts with the chemical to produce a purple to black color, it is oxidase-positive; those that remain white to tan are oxidase-negative. Because several species of gram-negative rods are also oxidase-positive, this test is presumptive for these two genera only if a Gram stain has verified the presence of gram-negative cocci.

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Prevention and Treatment

The background infection rate in most populations is about 1%, so well-developed natural immunity to the meningococcus appears to be the rule. A sort of natural immunization occurs during the early years of life as one is exposed to the meningococcus and its close relatives. Because even treated meningococcemial disease has a mortality rate of up to 15%, it is vital that antibiotic therapy begin as soon as possible with one or more drugs; it is generally given in high doses intravenously. Patients may also require treatment for shock and intravascular clotting.

When family members, medical personnel, or children in day care or school have come in close contact with infected people, they should receive a vaccination. Preventive therapy with rifampin or tetracycline may also be warranted. In the United States, immunization begins at the age of 11 followed by a booster dose. Vaccines are also available for younger children and for adults over the age of 55 who are at high risk for infection. Routine immunization is with one of two meningococcal vaccines that protect against serotypes A, C, W, and Y. At about the time a booster is needed (16 years of age), the CDC recommends additionally the first dose of the new vaccine effective against serotype B, the serotype that has caused several outbreaks on college campuses in recent years.

Disease Table 17.1 Meningitis

Causative Organism(s)	*Neisseria meningitidis* (B) **G−**	*Streptococcus pneumoniae* (B) **G+**	*Haemophilus influenzae* (B) **G−**	*Listeria monocytogenes* (B) **G+**	*Cryptococcus neoformans* (F)	*Coccidioides immitis* (F)	Viruses (V)
Most Common Modes of Transmission	Droplet contact	Droplet contact	Droplet contact	Vehicle (food)	Vehicle (air, dust)	Vehicle (air, dust, soil)	Droplet contact
Virulence Factors	Capsule, endotoxin, IgA protease	Capsule, induction of apoptosis, hemolysin and hydrogen peroxide production	Capsule	Intracellular growth	Capsule, melanin production	Granuloma (spherule) formation	Lytic infection of cells
Culture/Diagnosis	Gram stain/culture of CSF, blood, rapid antigenic tests, oxidase test	Gram stain/culture of CSF	Culture on chocolate agar	Cold enrichment, rapid methods	Negative staining, biochemical tests, DNA probes, cryptococcal antigen test	Identification of spherules, cultivation on Sabouraud's agar	Initially, absence bacteria/fungi/pro followed by viral culture or antigen

Prevention	Conjugated vaccine; ciprofloxacin, rifampin, or ceftriaxone used to protect contacts	Two vaccines: PCV13 and PPSV23	Hib (*H. influenzae* serotype b) vaccine, ciprofloxacin, rifampin, or ceftriaxone	Cooking food, avoiding unpasteurized dairy products	–	Avoiding airborne endospores	–
Treatment	Penicillin, ceftriaxone, chloramphenicol	Vancomycin + ceftriaxone; in **Serious Threat** category in CDC Antibiotic Resistance Report	Ceftriaxone	Ampicillin, trimethoprim-sulfamethoxazole	Amphotericin B and fluconazole	Fluconazole or Amphotericin B	Usually none (unl specific virus iden and specific antivi exists)
Distinctive Features	Petechiae, meningococcemia, rapid decline	Serious, acute, most common meningitis in adults	Serious, acute, less common in United States since vaccine became available	Asymptomatic in healthy adults; meningitis in neonates, elderly, and immunocompromised	Acute or chronic, most common in AIDS patients	Almost exclusively in endemic regions	Generally milder t bacterial or fungal
Epidemiological Features	United States: 550 cases in 2013; meningitis "belt" in Africa: 1,000 cases per 100,000 annually	U.S. incidence before vaccine for children: 7.7 hospitalizations per 100,000. After vaccine for children: 2.6 per 100,000	Before Hib vaccine, 300,000–400,000 deaths worldwide per year	Mortality can be as high as 33%	In United States, mainly a concern for HIV+ patients; 90% drop in incidence in the 1990s due to better management of AIDS; worldwide: 1 million new cases per year	Incidence in endemic areas: 200–300 annually	In United States, 4 meningitis cases c by viruses: 26,000 42,000 hospitalizations/y

Streptococcus pneumoniae

You will see in chapter 19 that *Streptococcus pneumoniae*, also referred to as the **pneumococcus**, causes the majority of bacterial pneumonias. Meningitis is also caused by this bacterium. Indeed, it is the most frequent cause of community-acquired meningitis and is also very severe. It does not cause the petechiae associated with meningococcal meningitis, and that difference is useful diagnostically. As many as 25% of pneumococcal meningitis patients will also have pneumococcal pneumonia. Pneumococcal meningitis is most likely to occur in patients with underlying susceptibility, such as alcoholic patients and patients with sickle-cell disease or those with absent or defective spleen function.

This bacterium is covered thoroughly in chapter 19, because it is a common cause of ear infections and pneumonia. It obviously has the potential to be highly pathogenic, while appearing as normal biota in many people. It can penetrate the respiratory mucosa; gain access to the bloodstream; and then, under certain conditions, enter the meninges.

Like the meningococcus, this bacterium has a polysaccharide capsule that protects it against phagocytosis. It also produces an alpha-hemolysin and hydrogen peroxide, both of which have been shown to induce damage in the CNS. It also appears capable of inducing brain cell apoptosis.

The bacterium is a small gram-positive flattened coccus that appears in end-to-end pairs. It has a distinctive appearance in a Gram stain of cerebrospinal fluid. Staining or culturing the nasopharynx is not useful because it is often normal biota there. Many strains of the bacterium are resistant to the first-line antibiotic, penicillin. In pneumococcal meningitis, initial treatment with vancomycin + ceftriaxone is recommended. If the isolate comes back as penicillin-sensitive (the cerebrospinal fluid must be cultured before beginning antibiotic treatment), then treatment can be switched.

Two vaccines are available for *S. pneumoniae:* a thirteen-valent conjugated vaccine (Prevnar), which is recommended as part of the childhood immunization schedule, and a 23-valent polysaccharide vaccine (Pneumovax 23), which is available for adults. Current recommendations for unvaccinated adults call for initial vaccination with Prevnar, followed by Pneumovax 6 to 12 months later.

Diagram of the meninges of the brain.
©McGraw-Hill Education

©Dr. Gary Gaugler/Science Source

Haemophilus influenzae

The meningitis caused by this bacterium is severe. Before the vaccine was introduced in 1988, it was a very common cause of severe meningitis and death. In the course of the last 13 years, meningitis caused by this bacterium is much less common in the United States, a situation that can always change if a lower percentage of people get the vaccine and herd immunity is compromised. Cases that do occur in the United States are now mostly caused by nonserotype B strains. Globally, it is still common, and is an important cause of the disease in children under the age of 5.

Listeria monocytogenes

Listeria monocytogenes is a gram-positive bacterium that ranges in morphology from coccobacilli to long filaments in palisades formation **(figure 17.6)**. Cells do not produce capsules or endospores and have from one to four flagella. *Listeria* is not fastidious and is resistant to cold, heat, salt, pH extremes, and bile. It grows inside host cells and can move directly from an infected host cell to an adjacent healthy cell.

Listeriosis in healthy adults is often a mild or subclinical infection with nonspecific symptoms of fever, diarrhea, and sore throat. However, listeriosis in elderly or immunocompromised patients, fetuses, and neonates (described later) usually affects the brain and meninges and results in septicemia. (*Septicemia* is a term that means the multiplication of bacteria in the bloodstream.) Some strains target the heart. The death rate is around 20%. Pregnant women are especially susceptible to infection, which can be transmitted to the fetus prenatally when the microbe crosses the placenta or postnatally through the birth canal. Intrauterine infections usually result in premature abortion and fetal death.

Apparently, the primary reservoir is soil and water, and animals, plants, and food are secondary sources of infection. Most cases of listeriosis are associated with ingesting contaminated dairy products, poultry, and meat. Recent epidemics have spurred an in-depth investigation into the prevalence of *L. monocytogenes* in

these sources. The pathogen has been isolated in 10% to 15% of ground beef and in 25% to 30% of chicken and turkey carcasses and is also present in 5% to 10% of luncheon meats, hot dogs, and cheeses.

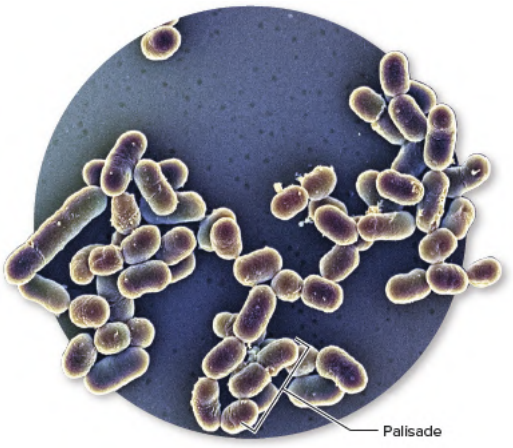

Figure 17.6 ***Listeria monocytogenes***. The bacterium is generally rod shaped. In Gram stains, individual cells tend to stack up in structures called palisades. That arrangement, pointed out here, is more obvious on a Gram stain where many more bacteria are seen.
©Dr. Gary Gaugler/Science Source

Diagnosing listeriosis is hampered by the difficulty in isolating it. The chances of isolation, however, can be improved by using a procedure called *cold enrichment*, in which the specimen is held at 4°C and periodically plated onto media, but this procedure can take 4 weeks. Rapid diagnostic kits using ELISA, immunofluorescence, and gene probe technology are now available for direct testing of dairy products and cultures. Antibiotic therapy should be started as soon as listeriosis is suspected. Ampicillin and trimethoprim-sulfamethoxazole are the first choices, followed by erythromycin. Prevention can be improved by adequate pasteurization temperatures and by proper washing, refrigeration, and cooking of foods that are suspected of being contaminated with animal manure or sewage. Pregnant women are cautioned by the U.S. Food and Drug Administration not to eat soft, unpasteurized cheeses.

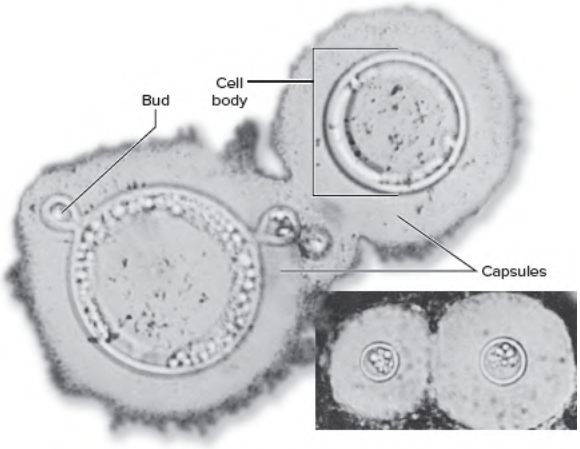

Figure 17.7 ***Cryptococcus neoformans*** **from infected spinal fluid stained negatively with India ink**. Halos around the large spherical yeast cells are thick capsules. Also note the buds forming on one cell. Encapsulation is a useful diagnostic sign for cryptococcosis, although the capsule is fragile and may not show up in some preparations (150×).
©Gordon Love, M.D. VA, North CA Healthcare System, Martinez, CA (both)

Cryptococcus neoformans

The fungus *Cryptococcus neoformans* causes a more chronic form of meningitis with a more gradual onset of symptoms, although in AIDS patients the onset may be fast and the course of the disease more acute. It is sometimes classified as a meningoencephalitis (inflammation of both brain and meninges). Headache is the most common symptom, but nausea and neck stiffness are very common. This fungus is a widespread resident of human habitats. It has a spherical to ovoid shape, with small, constricted buds and a large capsule that is important in its pathogenesis **(figure 17.7)**.

The primary ecological niche of *C. neoformans* is the bird population. It is prevalent in urban areas where pigeons congregate, and it proliferates in the high-nitrogen environment of droppings that accumulate on pigeon roosts. Masses of dried yeast cells are readily scattered into the air and dust. Its role as an opportunist is supported by evidence that healthy humans have strong resistance to it and that frank (obvious) infection occurs primarily in debilitated patients.

By far the highest rates of cryptococcal meningitis occur among patients with AIDS. This meningitis is frequently fatal. Other conditions that predispose individuals to infection are steroid treatment, diabetes, and cancer. It is not considered communicable among humans.

Prevention and Treatment

Systemic cryptococcosis requires immediate treatment with amphotericin B and fluconazole over a period of weeks or months. There is no prevention.

Coccidioides species

This fungus causes a condition that is often called "Valley Fever" in the U.S. Southwest. The morphology of *Coccidioides* is very distinctive. At 25°C, it forms a moist white to brown colony with abundant, branching, septate hyphae. These hyphae fragment into thick-walled, blocklike **arthroconidia** (arthrospores) at maturity **(figure 17.8*a*)**. On special media incubated at 37°C to 40°C, an arthrospore germinates into the parasitic phase, a small, spherical cell called a spherule **(figure 17.8*b*)** that can be found in infected tissues as well.

There are two species that cause this disease, found in different geographical places. *C. immitis* causes disease in California, and *C. posadasii* in northern Mexico, Central and South America, and the American Southwest, especially in Arizona. Sixty percent of all infections occur in Arizona. **Figure 17.9** shows the incidence of this disease from 1998 to 2015 in the United States.

Pathogenesis and Virulence Factors

This is a true systemic fungal infection of high virulence, as opposed to an opportunistic infection. It usually begins with pulmonary infection but can disseminate quickly throughout the body. Coccidioidomycosis of the meninges is the most serious manifestation. All persons inhaling the arthrospores probably develop some degree of infection, but

Meningitis-causing agents have their own research branch at the CDC.
Source: CDC/Maryam I. Daneshvar, Ph.D.

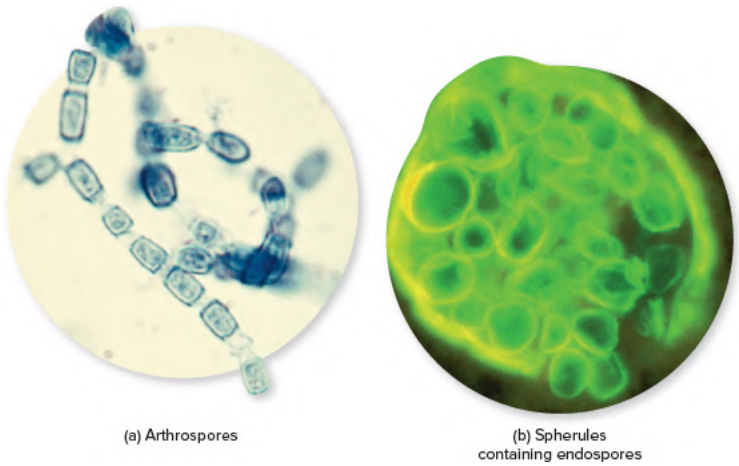

Figure 17.8 **Two phases of *Coccidioides* infection**. **(a)** Arthrospores are present in the environment and are inhaled. **(b)** In the lungs, the brain, or other tissues, arthrospores develop into spherules that are filled with endospores. Endospores are released and induce damage.
Source: CDC/Dr. Hardin (a); ©Science Source (b)

certain groups have a genetic susceptibility that gives rise to more serious disease. After the arthrospores are inhaled, they develop into spherules in the lungs. These spherules release scores of endospores into the lungs. (Unfortunately, *endospores* is the term used for this phase of the infection even though we have learned that endospores are *bacterial* structures.) At this point, the patient either experiences mild respiratory symptoms, which resolve themselves, or the endospores cause disseminated disease. Disseminated disease can include meningitis, osteomyelitis, and skin granulomas.

The highest incidence of coccidioidomycosis, estimated at 100,000 cases per year, occurs in the southwestern United States, although it also occurs in Mexico and parts of Central and South America. Especially concentrated reservoirs exist in the San Joaquin Valley of California and in southern Arizona. Outbreaks are usually associated with farming activity, archeological digs, construction, and mining. Climate change, which is drying out much of the southwestern and western United States, is predicted to increase the incidence and range of this disease in the western United States.

Viruses

It is estimated that four of five meningitis cases are caused by one of a wide variety of viruses. Because no bacteria or fungi are found in the CSF in viral meningitis, the condition is often called *aseptic meningitis*. Aseptic meningitis may occasionally have noninfectious causes.

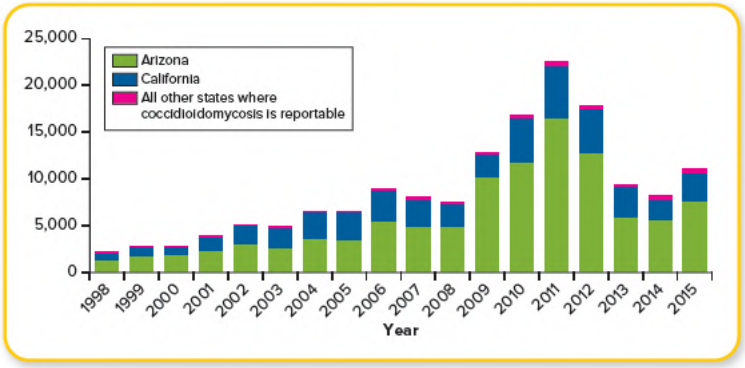

Figure 17.9 **Number of reported coccidioidomycosis cases in the United States, 1998–2015**.
Source: CDC

Note About Differential Diagnosis

Meningitis is a disease that illustrates how important it is for a clinician to think epidemiologically. When encountering a set of symptoms such as those of meningitis, a health care worker should be able to access a differential diagnosis in his or her mind. If the patient lives in Arizona, you definitely want to include *Coccidioides* in your differential diagnosis. Knowing the patient's vaccination history can help rule out some causative agents. And finally, if you look at our table of possible causative agents, you see that 80% of meningitis cases are caused by unidentified viruses, and are mild. Here's where you see that simply knowing a list of possible causative agents is not enough. A little more information will tell you how likely each of them is. **Each Disease Table in this book serves to summarize the differential diagnosis**.

There's a saying among physicians: "When you hear hoofbeats, think horses, not zebras." This refers to the fact that you should consider the simplest possibility, which in the case of meningitis would be a virus, resulting in uncomplicated disease. But if the patient is very sick upon presentation, or has signs of petechiae on the skin, you err on the side of safety and treat for *Neisseria* until it is ruled out.

Disease Table 17.2 Neonatal and Infant Meningitis

Causative Organism(s)	*Streptococcus agalactiae* B **G+**	*Escherichia coli*, strain K1 B **G−**	*Listeria monocytogenes* B **G+**	*Cronobacter sakazakii* B **G−**
Most Common Modes of Transmission	Vertical (during birth)	Vertical (during birth)	Vertical	Vehicle (baby formula)
Virulence Factors	Capsule	–	Intracellular growth	Ability to survive dry conditions
Culture/Diagnosis	Culture mother's genital tract on blood agar; CSF culture of neonate	CSF Gram stain/culture	Cold enrichment, rapid methods	Chromogenic differential agar, or rapid detection kits
Prevention	Culture and treatment of mother	–	Cooking food, avoiding unpasteurized dairy products	Safe preparation and use of, or avoidance of, powdered formula
Treatment	Penicillin G plus aminoglycosides	Ceftazidime or cefepime +/− gentamicin	Ampicillin, trimethoprim-sulfamethoxazole	Begin with broad-spectrum drugs until susceptibilities determined
Distinctive Features	Most common; positive culture of mother confirms diagnosis	Suspected if infant is premature		–
Epidemiological Features	Before intrapartum antibiotics introduced in 1996: 1.8 cases per	Estimated at 0.2–5 per 1,000 live births; 20% of pregnant	Mortality can be as high as 33%	Rare (a handful of documented cases in United States

	1,000 live births After intrapartum antibiotics: 0.32 cases per 1,000 live births	women colonized		annually), but deadly

The majority of cases of viral meningitis occur in children, and 90% are caused by enteroviruses. A common cause of viral meningitis is initial infection with HSV-2, concurrent with a genital infection. But many other viruses also gain access to the central nervous system on occasion.

Viral meningitis is generally milder than bacterial or fungal meningitis, and it is usually resolved within 2 weeks. The mortality rate is less than 1%. Diagnosis begins with the failure to find bacteria, fungi, or protozoa in CSF and can be confirmed, depending on the virus, by viral culture or specific antigen tests. In most cases, no treatment is indicated.

The organisms in the differential diagnosis for acute meningitis are summarized in **Disease Table 17.1**.

Neonatal Meningitis

Meningitis in newborns is almost always a result of infection transmitted by the mother, either *in utero* or during passage through the birth canal. As more premature babies survive, the rates of neonatal meningitis increase, because the condition is favored in patients with immature immune systems. In the United States, the two most common causes are *Streptococcus agalactiae* (known as group B streptococcus) and *Escherichia coli. Listeria monocytogenes* is also found frequently in neonates. It has already been covered here but is included in **Disease Table 17.2** as a reminder that it can cause neonatal cases as well. In the developing world, neonatal meningitis is more commonly caused by other organisms.

Streptococcus agalactiae

This species of *Streptococcus* belongs to group B of the streptococci. It colonizes 10% to 30% of female genital tracts and is the most frequent cause of neonatal meningitis

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(for details about this condition in women, see chapter 21). The treatment for neonatal disease is Penicillin G, sometimes supplemented with an aminoglycoside. Women who are considered high risk (previous baby with group B streptococcal disease, early rupture of membranes, premature labor) are typically screened for the presence of these bacteria by means of a cervical and rectal swab at between 35 and 37 weeks of gestation. Women who are found to harbor the bacteria are offered intravenous antibiotics at the beginning of active labor and throughout labor until delivery is accomplished to avoid passing the bacteria to their infant during the birthing process. Penicillin is the drug of choice. If the mother is allergic to penicillin, IV erythromycin or cefuroxime may be administered instead.

Escherichia coli

The K1 strain of *E. coli* is the second most common cause of neonatal meningitis. Most babies who suffer from this infection are premature, and their prognosis is poor. About 20% to 30% of them die, even with aggressive antibiotic treatment, and those who survive often have brain damage.

The bacterium is usually transmitted from the mother's birth canal. It causes no disease in the mother but can infect the vulnerable tissues of a neonate. It seems to have a predilection for the tissues of the central nervous system. Ceftazidime or cefepime +/− gentamicin is usually administered intravenously.

Cronobacter sakazakii

Cronobacter, formerly known as *Enterobacter sakazakii*, is found mainly in the environment but has been implicated in outbreaks of neonatal and infant meningitis transmitted through contaminated powdered infant

formula. Although cases of *Cronobacter* meningitis are rare, mortality rates can reach 40%. The FDA and the CDC advise hospitals to use ready-to-feed or concentrated liquid formulas. They also recommend that home caregivers wash their hands and use clean feeding equipment when preparing formula, use fresh formula for each feeding, and discard any leftover formula. **Disease Table 17.2**.

HIGHLIGHT DISEASE

Zika Virus Disease

Starting in 2015 and continuing into 2016, an epidemic of babies born with abnormally small heads—a condition called microcephaly—became obvious in Brazil. The cause was quickly determined to be the Zika virus. Zika has been known to scientists since the mid-1900s, but the manifestation of hundreds of brain-damaged babies was new. Zika was first discovered in Uganda in Africa, where it seems to have circulated among human populations for some time. In the first half of the 20th century the virus migrated to western Africa and then to Southeast Asia. In 2015 it was found to be circulating in Brazil, the first evidence of the virus in the Americas. In February 2016, the World Health Organization declared Zika an international health emergency. That emergency was ended in November of the same year. However, the virus continues to be a threat, and pregnant women in particular are cautioned not to travel to Zika-endemic areas.

▶ Signs and Symptoms

When adults are infected with Zika, they can experience a range of symptoms, from none at all, to a skin rash, conjunctivitis, and muscle and joint pain. The virus also seems to trigger Guillain-Barre syndrome (GBS) in some adults. GBS is a neurological condition that can occur after infections with certain bacteria and viruses, and sometimes after exposure to vaccines. Infection with the bacterium *Campylobacter jejuni*, a common foodborne bacterium, is one of the more common

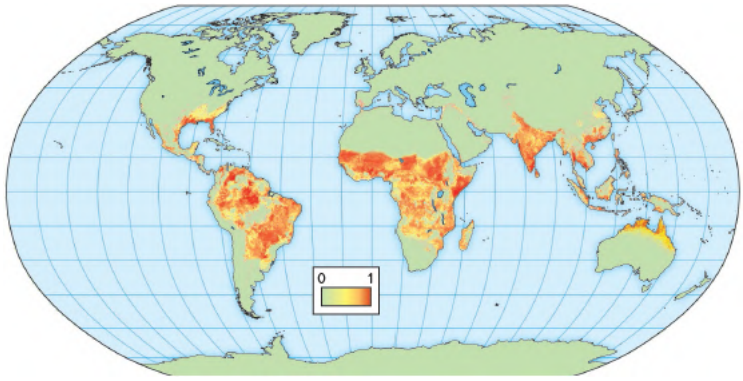


Figure 17.10 Map of distribution of *Aedes aegypti* in 2015. The mosquito transmits Zika virus, yellow fever virus, dengue fever virus, and others. Green indicates a probability of zero that the mosquito is present; red indicates 100% probability. The mosquito's habitat continues to expand.
Source: CDC

triggers of GBS. Zika infection has been found during this recent epidemic to lead to GBS. The syndrome is characterized by the immune system attacking peripheral nerves. The effects can be long-lasting, though usually they resolve completely. In a certain percentage of cases respiratory muscles can be affected; and death may follow.

Far more serious consequences affect babies who acquire Zika during gestation. In these cases it can cause congenital *Zika virus syndrome*. The most obvious manifestation is the characteristically small head, although it is not present in every case. Additional symptoms of the syndrome include vision problems, involuntary movements, seizures, and irritability. Symptoms of brain stem dysfunction such as swallowing problems are common as well.

Source: CDC

Causative Agent

Zika virus is an RNA virus in the Flaviviridae family. It is closely related to the viruses causing dengue fever, West Nile fever, and yellow fever.

Transmission and Epidemiology

Scientists are still unraveling the pattern of Zika transmission and spread around the world. We do know that it is transmitted by the bite of the *Aedes* mosquito. The virus has also been transmitted via sexual intercourse with infected individuals. And of course, it can be transmitted vertically *in utero*.

During 2016, the virus spread rapidly through the Americas, and continues to do so. Its mosquito host, the genus *Aedes*, is spread throughout the world. **Figure 17.10** depicts the likely habitat of one common *Aedes* species, *Aedes aegypti*. As of mid-2017, 175 symptomatic cases were reported in the United States, 174 of them in travelers returning from affected areas, and one of them acquired through sexual transmission.

Prevention and Treatment

Currently there is no vaccine for Zika, but the recent epidemics have spurred intense research in this area. Early stage clinical trials of an investigational vaccine, called ZPIV for Zika Purified Inactivated Virus, began in late 2016. At this point the only treatment is to provide supportive measures, both for adults and infants. Patients experiencing GBS should receive intensive physical therapy and may require mechanical ventilation.

In late 2016, the state of Florida started releasing genetically modified male mosquitoes near Key West, an area experiencing Zika transmission. When females mate

Disease Table 17.3 Zika Virus Infection

Causative Organism(s)	Zika virus V
Most Common Modes of Transmission	Vertical, vector-borne, sexual contact likely through blood transfusions (not yet confirmed)
Virulence Factors	–
Culture/Diagnosis	PCR testing
Prevention	Avoiding mosquitoes; vaccine in trials
Treatment	Supportive
Epidemiological Features	Originated in Africa but spreading throughout world, current outbreak started in 2015

with these males, their offspring die. Officials are testing strategies like this to combat the worst mosquito-borne diseases.

Medical Moment

Acute Flaccid Myelitis

Acute flaccid myelitis (AFM) is a broad term for conditions affecting the spinal cord causing a loss of muscle function, tone, and reflexes. It is often accompanied by cranial nerve deficits. Organisms known to cause these symptoms include West Nile virus, poliovirus, and adenoviruses. AFM is diagnosed by clinical assessment, along with the presence of spinal cord lesion(s) on MRI imaging.

In 2014, case reports began to surface of AFM with no identifiable pathogen. Occurring more commonly in children, patients present with a sudden loss of muscle tone and mobility. Muscle weakness may progress rapidly to respiratory failure, requiring mechanical ventilation. Recovery of function is variable with some patients having near full recovery after months of rehabilitation, whereas others remain completely paralyzed.

While these AFM cases are presumed to have a viral cause, the CDC reported in January 2017 that they have been unable to isolate a consistent pathogen in samples of cerebrospinal fluid from affected patients. Occurrence of this variation of AFM is rare but increasing in frequency, causing much effort to be invested in researching the causative organism.

Q. What other disease of the nervous system is characterized by a flaccid form of paralysis?

Answer in Appendix B.

Source: CDC

Poliomyelitis

Poliomyelitis (poh″-lee-oh′my″-eh′ly′tis) (polio) is an acute enteroviral infection of the spinal cord that can cause neuromuscular paralysis. Because it often affects small children, in the past it was called *infantile paralysis*. No civilization or culture has escaped the devastation of polio. The efforts of a WHO campaign have significantly reduced the global incidence of polio. It was the campaign's goal to eradicate all of the remaining wild polioviruses by 2000, and then by 2005. It didn't happen. Eventually, billionaire Bill Gates got involved and contributed $700 million to help eradicate the disease. In 2014, India was declared polio-free. However, also during that year, the World Health Organization declared a public health emergency with respect to polio because it had spread to eight countries in Africa and the Middle East, threatening to undo progress toward its eradication.

Signs and Symptoms

Most infections are contained as short-term, mild viremia. Some people develop mild nonspecific symptoms of fever, headache, nausea, sore throat, and myalgia. If the viremia persists, viruses can be carried to the central nervous system through its blood supply. The virus then spreads along specific pathways in the spinal cord and

brain. The virus is **neurotropic**, that is, it attacks the nervous system. It infiltrates the motor neurons of the anterior horn of the spinal cord, although it can also attack spinal ganglia, cranial nerves, and motor nuclei.

In paralytic disease, invasion of motor neurons causes various degrees of flaccid (floppy) paralysis over a period of a few hours to several days. Depending on the level of damage to motor neurons, paralysis of the muscles of the legs, abdomen, back, intercostals, diaphragm, pectoral girdle, and bladder can result. In rare cases of **bulbar poliomyelitis**, the brain stem, medulla, or even cranial nerves are affected. This situation leads to loss of control of cardiorespiratory regulatory centers, requiring mechanical respirators. In time, the unused muscles begin to atrophy, growth is slowed, and severe deformities of the trunk and limbs develop. Common sites of deformities are the spine, shoulder, hips, knees, and feet. Because motor function but not sensation is compromised, the crippled limbs are often very painful.

In recent times, a condition called post-polio syndrome (PPS) has been diagnosed in long-term survivors of childhood infection. PPS manifests as a progressive muscle deterioration that develops in about 25% to 50% of patients several decades after their original polio attack.

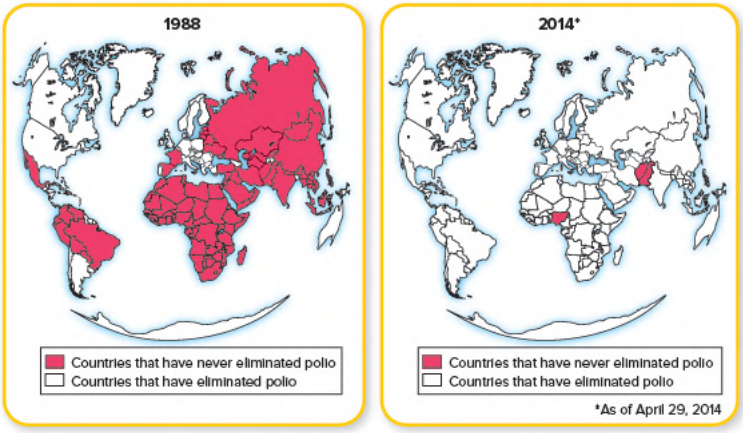


Figure 17.11 **Progress in eliminating polio**.
Source: CDC

Causative Agent

The poliovirus is in the family *Picornaviridae*, genus *Enterovirus*—named for its small (*pico*) size and its RNA genome. It is nonenveloped and nonsegmented. The naked capsid of the virus confers chemical stability and resistance to acid, bile, and detergents. By this means, the virus survives the gastric environment and other harsh conditions, which means it is transmitted easily.

Pathogenesis and Virulence Factors

After being ingested, polioviruses adsorb to receptors of mucosal cells in the oropharynx and intestine. Here, they multiply in the mucosal epithelia and lymphoid tissue. Multiplication results in large numbers of viruses being shed into the throat and feces, and some of them leak into the blood. Depending on the number of viruses in the blood and their duration of stay there, an individual may exhibit no symptoms, mild nonspecific symptoms such as fever or short-term muscle pain, or devastating paralysis.

Transmission and Epidemiology

Humans are the only known reservoir. The virus is passed within the population through food, water, hands, objects contaminated with feces, and mechanical vectors. Although the 20th century saw a very large rise in paralytic polio cases, it was also the century during which effective vaccines were developed. The infection was eliminated from the Western Hemisphere in the late 20th century. Since the virus cannot exist outside of a human, it is possible to totally eradicate the disease if we eliminate (i.e., vaccinate) all susceptible hosts. Decades of work with international governments, Rotary International, the CDC, the WHO, Unicef, and the Bill and Melinda Gates

Foundation have led us to the point where only three countries still see polio transmission: Afghanistan, Nigeria, and Pakistan **(figure 17.11)**. A massive effort involving 20 million volunteers going door-to-door vaccinating over 3 billion children in the last 20 years has led us to this point.

Prevention and Treatment

Treatment of polio rests largely on alleviating pain and suffering. During the acute phase, muscle spasm, headache, and associated discomfort can be alleviated by pain-relieving drugs. Respiratory failure may require artificial ventilation maintenance. Prompt physical therapy to diminish crippling deformities and to retrain muscles is recommended after the acute febrile phase subsides.

The mainstay of polio prevention is vaccination as early in life as possible, usually in four doses starting at about 2 months of age. Adult candidates for immunization are travelers and members of the armed forces. The two forms of vaccine currently in use are inactivated poliovirus vaccine (IPV), developed by Jonas Salk in 1954, and oral poliovirus vaccine (OPV), developed by Albert Sabin in the 1960s.

For many years, the oral vaccine was used in the United States because it is easily administered by mouth, but it is not free of medical complications. It contains an attenuated virus that can multiply in vaccinated people and be spread to others. In very rare instances, the attenuated virus reverts to a neurovirulent strain that causes disease rather than protects against it. For this reason, IPV, using killed virus, is the only vaccine used in the United States. **Disease Table 17.4**.

Disease Table 17.4 Poliomyelitis

Causative Organism(s)	Poliovirus (V)
Most Common Modes of Transmission	Fecal-oral, vehicle
Virulence Factors	Attachment mechanisms
Culture/Diagnosis	Viral culture, serology
Prevention	Live attenuated (OPV) (developing world) or inactivated vaccine (IPV) (developed world)
Treatment	None, palliative, supportive
Epidemiological Features	Polio has been 99% eradicated; still endemic in Pakistan, Afghanistan, and Nigeria as of 2017

Meningoencephalitis

Two microorganisms cause a distinct disease called *meningoencephalitis* (disease in both the meninges and brain), and they are both amoebas. *Naegleria fowleri* and *Acanthamoeba* are accidental parasites that invade the body only under unusual circumstances.

Naegleria fowleri

Most cases of *Naegleria* infection reported worldwide occur in people who have been swimming in warm, natural bodies of freshwater. Infection can begin when amoebas are forced into human nasal passages as a result of swimming, diving, or other aquatic activities. Once the amoeba is inoculated into the favorable habitat of the nasal mucosa, it burrows in, multiplies, and uses the olfactory nerve to migrate into the brain and surrounding structures. The result is primary amoebic meningoencephalitis (PAM), a rapid, massive destruction of brain and spinal tissue that causes hemorrhage and coma and invariably ends in death within a week or so **(figure 17.12)**. Note that this organism is very common—children often carry the amoeba as harmless biota, especially during the summer months, and the series of events leading to disease is exceedingly rare.

Unfortunately, *Naegleria* meningoencephalitis advances so rapidly that treatment usually proves futile. Studies have indicated that early therapy with amphotericin B, sulfadiazine, or tetracycline in some combination can be of some benefit. Because of the wide distribution of the amoeba and its hardiness, no general method of control exists. Public swimming pools and baths must be adequately chlorinated and checked periodically for the amoeba.

Acanthamoeba

This protozoan differs from *Naegleria* in its portal of entry. It invades broken skin, the conjunctiva, and occasionally the lungs and urogenital epithelia. It causes a meningoencephalitis somewhat similar to that of *Naegleria*. The course of infection is lengthier but nearly as deadly, with only a 2% to 3% survival rate. The

disease is called granulomatous amoebic meningoencephalitis (GAM). At special risk for infection are people with traumatic eye injuries, contact lens wearers, and AIDS patients exposed to contaminated water. We discussed ocular infections in chapter 16. Cutaneous and CNS infections with this organism are occasional complications in AIDS.

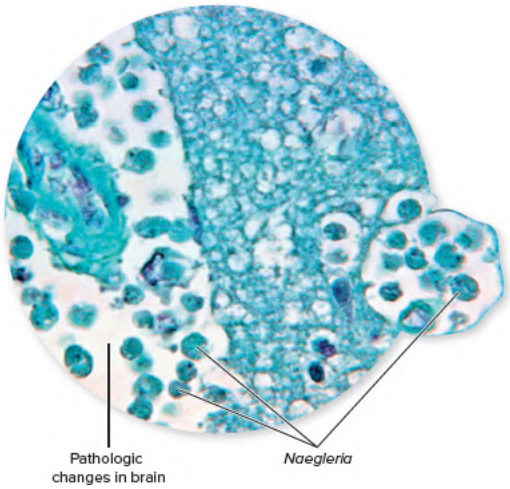


Figure 17.12 ***Naegleria fowleri*** **in the brain**. The trophozoite form invades brain tissue, destroying it.
Source: CDC

Disease Table 17.5 Meningoencephalitis

Disease	Primary Amoebic Meningoencephalitis	Granulomatous Amoebic Meningoencephalitis
Causative Organism(s)	*Naegleria fowleri* (P)	*Acanthamoeba* (P)
Most Common Modes of Transmission	Vehicle (exposure while swimming in water)	Direct contact
Virulence Factors	Invasiveness	Invasiveness
Culture/Diagnosis	Examination of CSF; brain imaging, biopsy	Examination of CSF; brain imaging, biopsy
Prevention	Limit warm freshwater or untreated tap water entering nasal passages	–
Treatment	Amphotericin B; mostly ineffective	Surgical excision of granulomas; ketoconazole may help
Epidemiological Features	United States: 0–7 cases a year; 97% case-fatality rate. Spreading to northern states as climate warms.	Predominantly occurs in immunocompromised patients

Pools are chlorinated to prevent *Naeglaria* and other outbreaks.
©Lena Mirisola/Cultura Exclusive/Getty Images

Acute Encephalitis

Encephalitis (inflammation of the brain) can present as acute or **subacute**. It is always a serious condition, as the tissues of the brain are extremely sensitive to damage by inflammatory processes. Acute encephalitis is almost always caused by viral infection. One category of viral encephalitis is caused by viruses borne by insects (called *arboviruses*, which is short for *arthropod-borne viruses*), including West Nile virus. Alternatively, other viruses, such as the JC virus and members of the herpes family, are causative agents. Bacteria such as those covered under meningitis can also cause encephalitis, but the symptoms are usually more pronounced in the meninges than in the brain.

The signs and symptoms of encephalitis vary, but they may include behavior changes or confusion because of inflammation. Decreased consciousness and seizures frequently occur. Symptoms of meningitis are often also present. Few of these agents have specific treatments, but because swift initiation of acyclovir therapy can save the life of a patient suffering from herpesvirus encephalitis, most physicians will begin empiric therapy with acyclovir in all seriously ill neonates and most other patients showing evidence of encephalitis. Treatment will, in any case, do no harm in patients who are infected with other agents.

Arthropod-Borne Viruses (Arboviruses)

Most arthropods that serve as infectious disease vectors feed on the blood of hosts. Infections show a peak incidence when the arthropod is actively feeding and reproducing, usually from late spring through early fall. Warm-blooded vertebrates also maintain the virus during the cold and dry seasons. Humans can serve as dead-end, accidental hosts, as in equine encephalitis, or they can be a maintenance reservoir, as in yellow fever (discussed in chapter 18).

Arboviral diseases have a great impact on humans. Although exact statistics are unavailable, it is believed that millions of people acquire infections each year and thousands of them die. One common outcome of arboviral infection is an acute fever, often accompanied by rash. Viruses that primarily cause these symptoms are covered in chapter 18.

The arboviruses discussed in this chapter can cause encephalitis, and we consider them as a group because the symptoms and management are similar. The transmission and epidemiology of individual viruses are different, however, and are discussed for each virus in **Table 17.1**.

▶ Pathogenesis and Virulence Factors

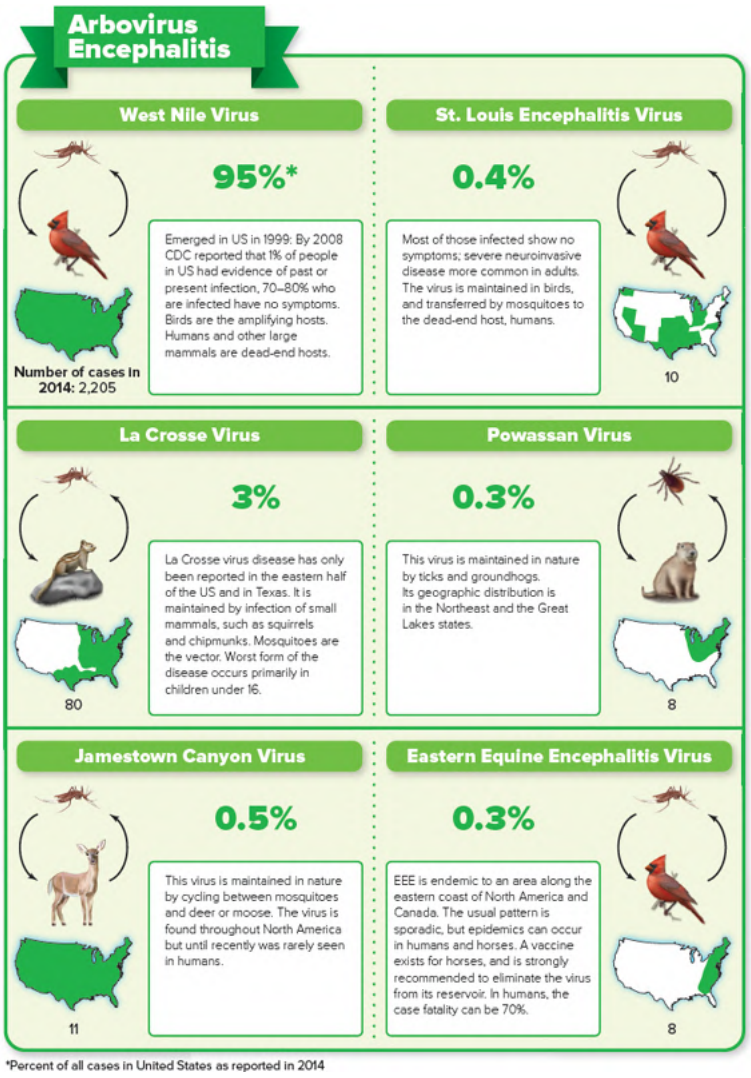
Arboviral encephalitis begins with an arthropod bite, the release of the virus into tissues, and its replication in nearby lymphatic tissues. Prolonged viremia establishes the virus in the brain, where inflammation can cause swelling and damage to the brain, nerves, and meninges. Symptoms are extremely variable and can include coma, convulsions, paralysis, tremor, loss of coordination, memory deficits, changes in speech and personality, and heart disorders. In some cases, survivors experience some degree of permanent brain damage. Young children and the elderly are most sensitive to injury by arboviral encephalitis. Having said that, most people who are infected will actually show no symptoms.

Public health officials regularly take samples of standing water in rain gutters, swimming pools, and elsewhere, looking for larval mosquitoes like those seen here.
Source: CDC

Culture and Diagnosis

Except during epidemics, detecting arboviral infections can be difficult. The patient's history of travel to endemic areas or contact with vectors, along with serum analysis,

Table 17.1



Source: ArboNET, Arboviral Diseases Branch, Centers for Disease Control and Prevention.

Disease Table 17.6 Acute Encephalitis

Causative Organism(s)	Arboviruses (West Nile virus, La Crosse virus, Jamestown Canyon virus, St. Louis encephalitis virus, Powassan virus, eastern equine encephalitis virus) V	Herpes simplex 1 or 2 V	JC virus V
Most Common Modes of Transmission	Vector (arthropod bites)	Vertical or reactivation of latent infection	? Ubiquitous
Virulence Factors	Attachment, fusion, invasion capabilities	–	–
Culture/Diagnosis	History, rapid serological tests, nucleic acid amplification tests	Clinical presentation, PCR, Ab tests, growth of virus in cell culture	PCR of cerebrospinal fluid
Prevention	Insect control	Maternal screening for HSV	None
Treatment	None	Acyclovir	Zidovudine or other antivirals
Distinctive Features	History of exposure to insect important	In infants, disseminated disease present; rare between 30 and 50 years	In severely immunocompromised, especially AIDS
Epidemiological Features	See incidence rates in table 17.1	HSV-1 most common cause of encephalitis; 2 cases per million	Affects 5% of adults with untreated AIDS

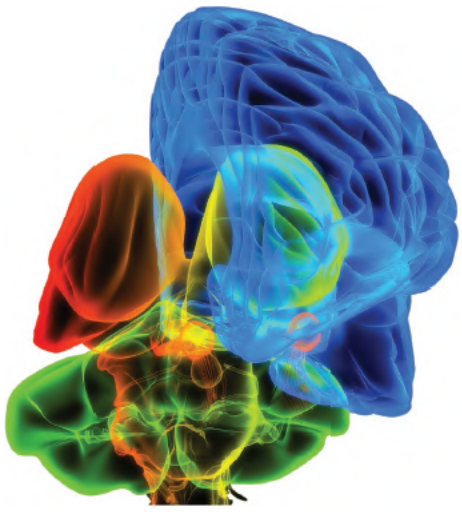
		per year	

is highly supportive of a diagnosis. Rapid serological and nucleic acid amplification tests are available for some of the viruses.

Treatment

No satisfactory treatment exists for any of the arboviral encephalitides (plural of *encephalitis*). As mentioned earlier, empiric acyclovir treatment may be begun in case the infection is actually caused by either herpes simplex virus or varicella zoster. Treatment of the other infections relies entirely on support measures to control fever, convulsions, dehydration, shock, and edema.

Most of the control safeguards for arbovirus disease are aimed at the arthropod vectors. Mosquito abatement by eliminating breeding sites and by broadcast-spreading insecticides has been highly effective in restricted urban settings. Birds play a role as reservoirs of the virus, but direct transmission between birds and humans does not occur.



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Herpes Simplex Virus (HSV)

Herpes simplex type 1 and 2 viruses can cause encephalitis in newborns born to HSV-positive mothers. In this case, the virus is disseminated and the prognosis is poor. Older children and young adults (ages 5 to 30), as well as older adults (over 50 years old), are also susceptible to herpes simplex encephalitis caused most commonly by HSV-1. In these cases, the HSV encephalitis usually represents a reactivation of dormant HSV from the trigeminal ganglion.

It should be noted the varicella-zoster virus (see chapter 16) can also reactivate from the dormant state, and it is responsible for rare cases of encephalitis.

JC Virus

The **JC virus (JCV)** gets its name from the initials of the patient in whom it was first diagnosed as the cause of illness. Serological studies indicate that asymptomatic infection

with this polyoma virus is commonplace. In patients with immune dysfunction, especially in those with AIDS, it can cause a condition called **progressive multifocal leukoencephalopathy** (loo″-koh-en-sef″uh-lop′-uh-thee) **(PML)**. This uncommon but generally fatal infection is a result of JC virus attack of accessory brain cells. The infection demyelinizes certain parts of the cerebrum. This virus should be considered when encephalitis symptoms are observed in AIDS patients. Recently a few deaths from this condition have been prevented with high doses of zidovudine. **Disease Table 17.6**.

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3. An infectious disease team is analyzing the epidemiology of vector-borne diseases, including those transmitted by arthropods. All of the following are arboviral diseases, except:

a. *polio*
b. *West Nile encephalitis*.
c. *yellow fever*.
d. *St. Louis encephalitis*.

Subacute Encephalitis

When encephalitis symptoms take longer to show up, and when the symptoms are less striking, the condition is termed *subacute encephalitis*. The most common cause of subacute encephalitis is the protozoan *Toxoplasma*. Another form of subacute encephalitis can be caused by persistent measles virus as many as 7 to 15 years after the initial infection. A class of infectious agents known as prions can cause a condition called spongiform encephalopathy. Finally, the differential diagnosis of subacute encephalitis should consider a variety of infections with primary symptoms elsewhere in the body (see the last column of Disease Table 17.6).

Toxoplasma gondii

This protozoal infection in the fetus and in immunodeficient people, especially those with AIDS, is severe and often fatal. Although infection in otherwise healthy people is generally unnoticed, recent data suggest that it may have subtle but profound effects on their brain and the responses it controls. People with a history of *Toxoplasma* infection are often more likely to display thrill-seeking behaviors and seem to have slower reaction times.

Signs and Symptoms

Most cases of toxoplasmosis are asymptomatic or marked by mild symptoms such as sore throat, lymph node enlargement, and low-grade fever. In patients whose immunity is suppressed by infection, cancer, or drugs, the outlook may be grim. The infection causes a more chronic or subacute form of encephalitis than do most viruses, often producing extensive brain lesions and fatal disruptions of the heart and lungs. A pregnant woman with toxoplasmosis has a 33% chance of transmitting the infection to her fetus. Congenital infection occurring in the first or second trimester is associated with stillbirth and severe abnormalities such as liver and spleen enlargement, liver failure, hydrocephalus, convulsions, and damage to the retina that can result in blindness.

Note About Neglected Parasitic Infections

You may have the impression that the United States has a low number of infections with protozoa and helminths, and these are primarily "developing country" diseases. Unfortunately, that is not the case. The CDC has recently designated five diseases as "neglected parasitic infections (NPIs)" in the United States:

- Chagas disease (in chapter 18)—caused by *Trypanosome cruzi*, a protozoan
- Neurocysticercosis (chapter 20)—caused by the tapeworm *Taenia sodium*
- Toxocariasis (chapter 20)—caused by the roundworm *Toxocara*
- Toxoplasmosis (this chapter)—caused by the protozoan *Toxoplasma gondii*
- Trichomoniasis (chapter 21)—caused by the protozoan *Trichomonas vaginalis*

The numbers of people experiencing disease from these five organisms is in the millions—and that's just in the United States. We will point out the NPIs as we encounter them in the rest of this book.

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4. For which disease processes are immunizations available as a method of prevention? Select all that apply.

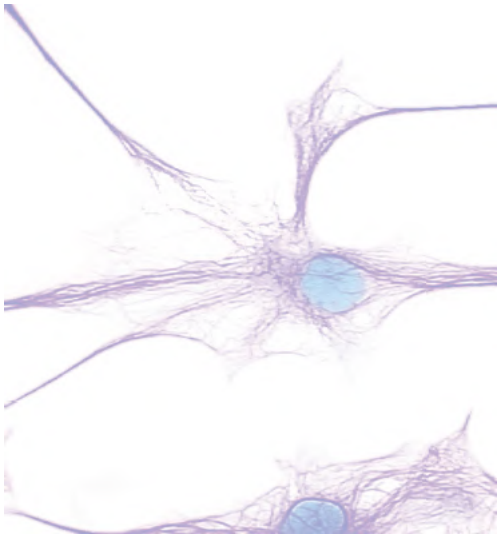
a. *Cryptococcus neoformans*
b. *Listeria monocytogenes*
c. *Haemophilus influenzae*
d. *Streptococcus pneumoniae*
e. *Neisseria meningitidis*

Pathogenesis and Virulence Factors

Toxoplasma is an obligate intracellular parasite, making its ability to invade host cells an important factor for virulence.

Transmission and Epidemiology

T. gondii is a very successful parasite with so little host specificity that it can attack at least 200 species of birds and mammals. However, the parasite undergoes a sexual phase in the intestine of cats and is then released in feces, where it becomes an infective *oocyst* that survives in moist soil for several months. These forms eventually enter an asexual cyst state in tissues, called a *pseudocyst*. Most



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of the time, the parasite does not cycle in cats alone and is spread by oocysts to intermediate hosts, including rodents and birds. The cycle returns to cats when they eat these infected prey animals. Cattle and sheep can also be infected.

Scientists have discovered that the protozoan crowds into a part of the rat brain that usually directs the rat to avoid the smell of cat urine (a natural defense against a domestic rat's major predator). When *Toxoplasma* infects rat brains, the rats lose their fear of cats. Infected rats are then easily eaten by cats, ensuring the continuing *Toxoplasma* life cycle. All other neurological functions in the rat are left intact.

Humans appear to be constantly exposed to the pathogen. The rate of prior infections, as detected through serological tests, can be as high as 90% in some populations. Many cases are caused by ingesting pseudocysts in undercooked contaminated meat, and other sources include contact with other mammals or even dirt and dust contaminated with oocysts. Fetuses may become infected when tachyzoites cross the placenta.

In view of the fact that the oocysts are so widespread and resistant, hygiene is of paramount importance in controlling toxoplasmosis. Adequate cooking or freezing below −20°C destroys both oocysts and tissue cysts. Oocysts can also be avoided by washing the hands after handling cats or soil possibly contaminated with cat feces, especially sandboxes and litter boxes. Pregnant women should be especially attentive to these rules and should never clean the cat's litter box. *Toxoplasma* infection of the fetus can result in miscarriage, premature birth, or babies born with brain and/or vision problems.

Measles Virus: Subacute Sclerosing Panencephalitis

Subacute sclerosing panencephalitis is sometimes called a "slow virus infection." The symptoms appear years after an initial measles episode and are different from those of immune-mediated postinfectious encephalitis, described earlier. SSPE seems to be caused by direct viral invasion of neural tissue. SSPE has no effective treatment and is

always fatal. It is not clear what factors lead to persistence of the virus in some people. SSPE's important features are listed in **Disease Table 17.7**.

Prions

As you read in chapter 5, prions are *proteinaceous infectious particles* containing, apparently, no genetic material. They are known to cause diseases called **transmissible spongiform encephalopathies (TSEs)**, neurodegenerative diseases with long incubation periods but rapid progressions once they begin. The human TSEs are **Creutzfeldt-Jakob disease (CJD)**, Gerstmann-Strussler-Scheinker disease, and fatal familial insomnia. TSEs are also found in animals and include a disease called scrapie in sheep and goats, transmissible mink encephalopathy, and bovine spongiform encephalopathy (BSE). This last disease is commonly known as mad cow disease and was in the headlines in the 1990s due to its apparent link to a variant form of Creutzfeldt-Jakob human disease in Great Britain. In 2016 a new prion was discovered, one that seems to cause a disease known as multiple system atrophy, a condition similar to Parkinson's disease. There is some hope that diseases such as Alzheimer's and Parkinson's will someday be linked to actual prion infection. We consider CJD in this section.

Signs and Symptoms of CJD

Symptoms of CJD include altered behavior, dementia, memory loss, impaired senses, delirium, and premature senility. Uncontrollable muscle contractions continue until death, which usually occurs within 1 year of diagnosis.

Prions were found in the meat of cattle fed with animal scraps. The highest number of cases occurred in the 1990s in the United Kingdom but isolated cases have been found in the United States as recently as 2014.

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Table 17.7 Subacute Encephalitis

Causative	*Toxoplasma gondii* (P)	Subacute sclerosing	Prions	Other conditions

Organism(s)		panencephalitis		to consider
Most Common Modes of Transmission	Vehicle (meat) or fecal-oral	Persistence of measles virus	CJD = direct/parenteral contact with infected tissue, or inherited vCJD = vehicle (meat, parenteral)	Other conditions may display subacute encephalitis symptoms:
Virulence Factors	Intracellular growth	Cell fusion, evasion of immune system	Avoidance of host immune response	Rickettsial diseases-Rocky Mountain spotted fever (chapter 18)
Culture/Diagnosis	Serological detection of IgM, culture, histology	EEGs, MRI, serology (Ab versus measles virus)	Biopsy, image of brain	Lyme disease (chapter 18)
Prevention	Personal hygiene, food hygiene	None	Avoiding infected meat or instruments; no prevention for inherited form	*Bartonella* or *Anaplasma* disease (chapter 18)
Treatment	Pyrimethamine and/or leucovorin and/or sulfadiazine	None	None	Tapeworm disease—*Taenia solium* (chapter 20)
Distinctive Features	Subacute, slower development of disease	History of measles	Long incubation period; fast progression once it begins	Syphilis—*Treponema pallidum* (chapter 21)
Epidemiological Features	15%–29% of U.S. population is seropositive; internationally, seroprevalence is up to 90%; disease occurs in 3%–15% of AIDS patients; designated a "neglected parasitic infection" in the United States by the CDC	Research in 2016 suggests it is 6–14 times more common than previously realized.	CJD: 1 case per year per million worldwide; seen in older adults vCJD: 98% cases originated in United Kingdom	

Causative Agent of CJD

The transmissible agent in CJD is a prion. In some forms of the disease, it involved the transformation of a normal host protein (called PrP), a protein that is supposed to function to help the brain develop normally. Once this happens, the abnormal PrP itself becomes catalytic and able to spontaneously convert other normal human PrP proteins into the abnormal form. This becomes a self-propagating chain reaction that creates a massive accumulation of altered PrP, leading to plaques, spongiform damage (i.e., holes in the brain) **(figure 17.13)**, and severe loss of brain function.

Using the term *transmissible agent* may be a bit misleading, however, as some cases of CJD arise through genetic mutation of the *PrP* gene, which can be a heritable trait. So it seems that although one can acquire a defective PrP protein via transmission, one can also have an altered *PrP* gene passed on through heredity.

Prions are incredibly hardy "pathogens." They are highly resistant to chemicals, radiation, and heat. They can withstand prolonged autoclaving.

Transmission and Epidemiology

In the late 1990s, it became apparent that humans were contracting a variant form of CJD (vCJD) after ingesting meat from cattle that had been afflicted by bovine spongiform encephalopathy. Presumably, meat products had been contaminated with fluid or tissues infected with the prion. Cases of this disease are centered around Great Britain, where many cows were found to have BSE. The median age at death of

patients with vCJD is 28 years. In contrast, the median age at death of patients with other forms of CJD is 68 years.

Health care professionals should be aware of the possibility of CJD in patients, especially when surgical procedures are performed, as cases have been reported of transmission of CJD via contaminated surgical instruments. Due to the heat and chemical resistance of prions, normal disinfection and sterilization procedures are usually not sufficient to eliminate them from instruments and surfaces. The latest CDC guidelines for handling of CJD patients in a health care environment should be consulted. There is no known treatment for CJD, and mortality appears to be 100%; but there is active research into treatments for prion diseases. **Disease Table 17.7**.

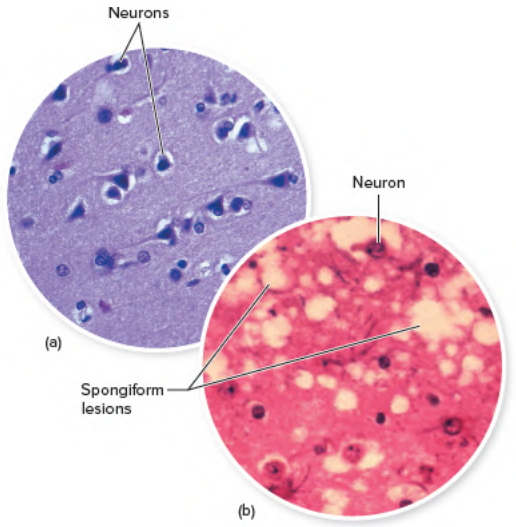

Figure 17.13 **The microscopic effects of spongiform encephalopathy.** **(a)** Normal cerebral cortex section, showing neurons and glial cells. **(b)** Sectioned cortex in CJD patient shows numerous round holes, producing a "spongy" appearance. This destroys brain architecture and causes massive loss of neurons and glial cells.
©M. Abbey/Science Source (a); Source: CDC/DR. Al Jenny (b)

Rabies

Rabies is a slow, progressive zoonotic disease characterized by a fatal encephalitis. It is so distinctive in its pathogenesis and its symptoms that we discuss it separately from the other encephalitides. It is distributed nearly worldwide, except for perhaps a few countries that have remained rabies-free by practicing rigorous animal control.

Signs and Symptoms

The average incubation period of rabies is 1 to 2 months or more, depending on the wound site, its severity, and the inoculation dose. The incubation period is shorter in facial, scalp, or neck wounds because of closer proximity to the brain. The prodromal phase begins with fever, nausea, vomiting, headache, fatigue, and other nonspecific symptoms.

Until recently, humans were never known to survive rabies. But a handful of patients have recovered in recent years after receiving intensive, long-term treatment.

Pathogenesis and Virulence Factors

Infection with rabies virus typically begins when an infected animal's saliva enters a puncture site. The virus occasionally is inhaled or inoculated through the membranes of the eye. The rabies virus remains up to a week at the trauma site, where it multiplies. The virus then gradually enters nerve endings and advances toward the ganglia, spinal cord, and brain. Viral multiplication throughout the brain is eventually followed by migration to such diverse sites as the eye, heart, skin, and oral cavity. The infection cycle is completed when the virus replicates in the salivary gland and is shed into the saliva. Untreated rabies proceeds through several distinct stages that almost inevitably end in death, unless post-exposure vaccination is performed before symptoms begin.

Scientists have discovered that virulence is associated with an envelope glycoprotein that seems to give the virus its ability to spread in the CNS and to invade certain types of neural cells.

Transmission and Epidemiology

The primary reservoirs of the virus are wild mammals such as canines, skunks, raccoons, badgers, cats, and bats that can spread the infection to domestic dogs and cats. Both wild and domestic mammals can spread the disease to humans through bites, scratches, and inhalation of droplets. The annual worldwide total for human rabies is estimated to be about 35,000 to 50,000 cases, but only a tiny number of these cases occur in the United States. The majority of these are transmitted to humans from bats. Most U.S. cases of rabies occur in wild animals (about

6,000 to 7,000 cases per year), while dog rabies has declined **(figure 17.14)**. Globally, over 90% of human rabies cases are still acquired from dogs.

Bats are one of the vectors for rabies.
©Hugh Lansdown/Shutterstock

The epidemiology of animal rabies in the United States varies. The most common wild animal reservoir hosts are raccoons, bats, and skunks. Regional differences in the dominant reservoir also occur. Skunks are the most common carriers of rabies in California, while raccoons are the predominant carriers in the East, and foxes dominate in Texas.

Diagnosis requires multiple tests. Reverse transcription PCR is used with saliva samples but must be accompanied by detection of antibodies to the virus in serum or spinal fluid. Skin biopsies are also used.

Prevention and Treatment

A bite from a wild or stray animal demands assessment of the animal, meticulous care of the wound, and a specific treatment regimen. A wild mammal, especially a skunk, raccoon, fox, or coyote that bites without provocation, is presumed to be rabid, and therapy for rabies is immediately begun. Rabies has been transmitted to humans in the absence of a bite; aerosols of bat saliva are thought to be capable of transmitting the virus. For that reason people who have found a bat in their house can be encouraged to undergo the postexposure prophylaxis regimen.

Rabies is one of the few infectious diseases for which a combination of passive and active postexposure immunization is indicated (and successful). Initially the wound is infused with human rabies immune globulin (HRIG) to impede the spread of the virus, and globulin is also injected intramuscularly to provide immediate systemic protection. A full course of vaccination is started simultaneously. The routine postexposure vaccination entails intramuscular or intradermal injection on days 0, 3, 7, and 14, sometimes with two additional boosters. High-risk groups such as veterinarians, animal handlers, laboratory personnel, and travelers

Disease Table 17.8 Rabies

Causative Organism(s)	Rabies virus V
Most Common Modes of Transmission	Parenteral (bite trauma), droplet contact
Virulence Factors	Envelope glycoprotein
Culture/Diagnosis	Direct fluorescent antigen testing
Prevention	Inactivated vaccine
Treatment	Postexposure passive and active immunization; induced coma and ventilator support if symptoms have begun
Epidemiological Features	United States: 1–5 cases per year Worldwide: 35,000–55,000 cases annually

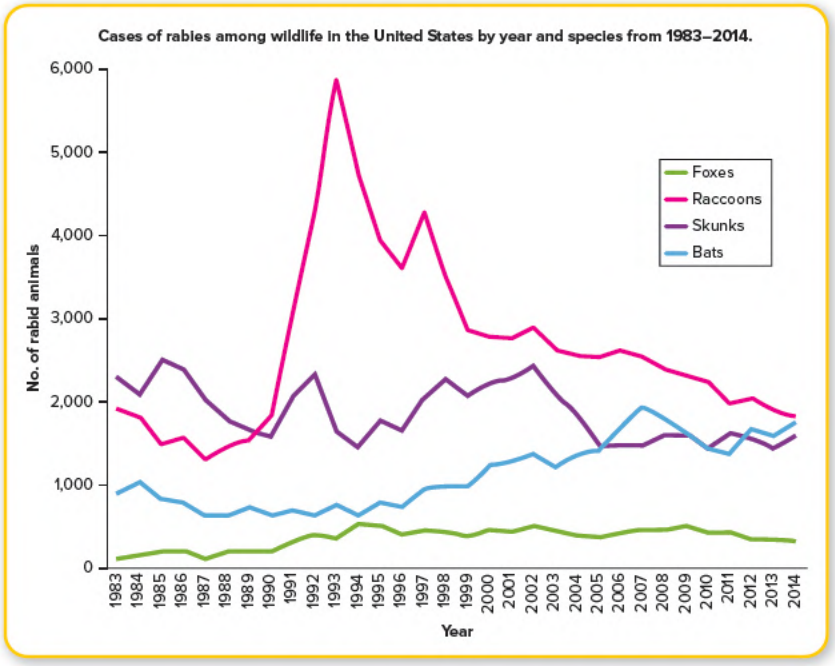

Figure 17.14 Most common carriers of rabies in the United States from 1983 to 2014. In the early 1990s raccoons were by far the most common, but in 2014 their infections had decreased and they were approximately equal to bats and skunks.
Source: CDC

should receive three doses to protect against possible exposure. A DNA vaccine for rabies is in development. **Disease Table 17.8**.

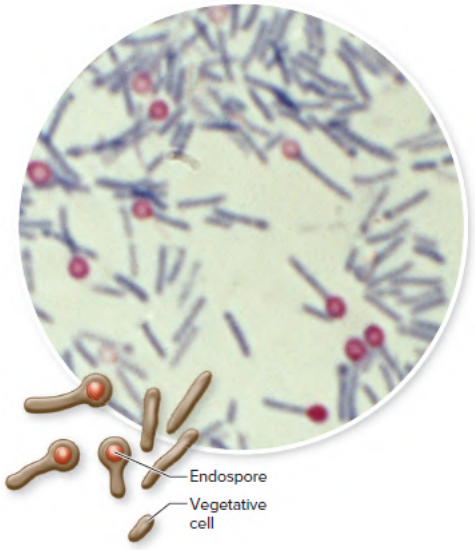

Figure 17.15 ***Clostridium tetani*.** Its typical tennis racket morphology is created by terminal endospores that swell the end of the cell (170×).
©Biophoto Associates/Science Source

Tetanus

Tetanus is a neuromuscular disease whose alternate name, lockjaw, refers to an early effect of the disease on the jaw muscle. The etiologic agent, *Clostridium tetani*, is a common resident of cultivated soil and the gastrointestinal tracts of animals. It is a gram-positive, spore-forming rod. The endospores it produces often cause the vegetative cell to swell **(figure 17.15)**. The endospores are produced only under anaerobic conditions.

Signs and Symptoms

C. tetani releases a powerful exotoxin that is a neurotoxin, **tetanospasmin**, that binds to target sites on peripheral motor neurons, spinal cord and brain, and in the sympathetic nervous system. The toxin acts by blocking the inhibition of muscle contraction. Without inhibition of contraction, the muscles contract uncontrollably, resulting in spastic paralysis. The first symptoms are clenching of the jaw, followed in succession by extreme arching of the back, flexion of the arms, and extension of the legs **(figure 17.16)**. Lockjaw confers the bizarre appearance of *risus sardonicus* (sardonic grin), which looks eerily as though the person is smiling **(figure 17.17)**. Death most often occurs due to paralysis of the respiratory muscles and respiratory arrest.

Pathogenesis and Virulence Factors

The mere presence of endospores in a wound is not sufficient to initiate infection because the bacterium is unable to invade damaged tissues readily. It is also a strict anaerobe, and the endospores cannot become established unless tissues at the site of the wound are necrotic and poorly supplied with blood, conditions that favor germination.

As the vegetative cells grow, the tetanospasmin toxin is released into the infection site. The toxin spreads to nearby motor nerve endings in the injured tissue, binds to them, and travels via axons to the ventral horns of the spinal cord (figure 17.16). The toxin blocks the release of neurotransmitters. Only a small amount of toxin is required to initiate the symptoms.

Transmission and Epidemiology

Endospores usually enter the body through accidental puncture wounds, burns, umbilical stumps, frostbite, and crushed body parts. The incidence of tetanus is low in North America. Most cases occur among geriatric patients and intravenous drug abusers. Historically, however, the worldwide incidence of maternal and neonatal tetanus has been high, causing 60,000 deaths each year. In response, the World Health Organization (WHO) has made dramatic global progress in reducing mortality through the promotion of more hygienic delivery practices and vaccination.

Prevention and Treatment

A patient with a clinical appearance suggestive of tetanus should immediately receive antitoxin therapy with human tetanus immune globulin (TIG) and Penicillin G.

The recommended vaccination series for 1- to 3-month-old babies consists of three injections of DTaP (diphtheria, tetanus, and acellular pertussis) given 2 months apart, followed by booster doses about 1 and 4 years later. Alternately, they may be vaccinated with a vaccine called "DT," which protects only against diphtheria and tetanus. Children thus immunized probably have protection for 10 years. At that point, and every 10 years thereafter, they should receive a dose of TD, tetanus-diphtheria vaccine. Additional protection against neonatal tetanus may be achieved by vaccinating pregnant women, whose antibodies will be passed to the fetus. Toxoid

Figure 17.16 **Late-stage tetanus**.
Source: CDC

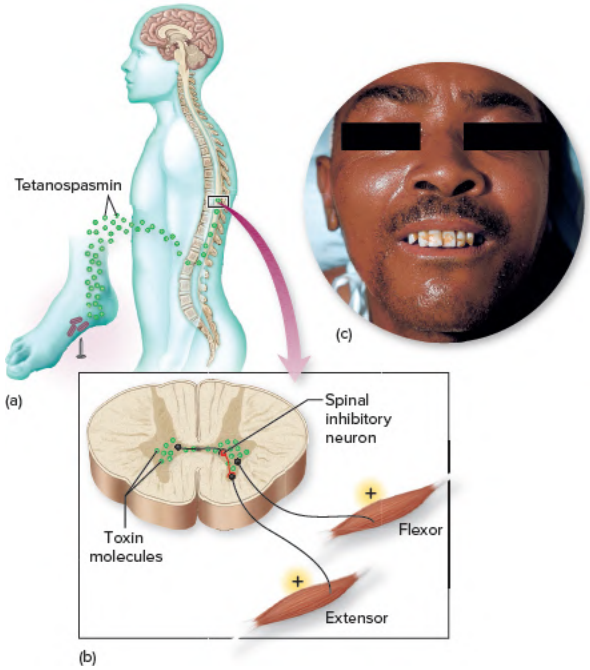

Figure 17.17 **The events in tetanus**. **(a)** After traumatic injury, bacteria infecting the local tissues secrete tetanospasmin, which is absorbed by the peripheral axons and is carried to the target neurons in the spinal column. **(b)** In the spinal cord, the toxin attaches to the junctions of regulatory neurons that inhibit inappropriate contraction. Released from inhibition, the muscles, even opposing members of a muscle group, receive constant stimuli and contract uncontrollably. **(c)** Muscles contract spasmodically, without regard to regulatory mechanisms or conscious control. Note the clenched jaw, called *risus sardonicus*.
Source: CDC/Dr. Thomas F. Sellers/Emory University (c)

Disease Table 17.9 **Tetanus**

Causative Organism(s)	*Clostridium tetani* B G+
Most Common Modes of Transmission	Parenteral, direct contact
Virulence Factors	Tetanospasm exotoxin
Culture/Diagnosis	Symptomatic
Prevention	Tetanus toxoid immunization
Treatment	Combination of passive antitoxin and tetanus toxoid active immunization, metronidazole; sedation
Epidemiological Features	United States: Approximately 30 cases/year; worldwide, +/− 60,000 newborn deaths annually in developing countries

should also be given to injured persons who have never been immunized, have not completed the series, or whose last booster was received more than 10 years previously. The vaccine can be given simultaneously with passive TIG immunization to achieve immediate as well as long-term protection. **Disease Table 17.9**.

Passive tetanus immunoglobulin is given immediately to halt the progress of the toxin molecules.
©Image Source/Getty Images

Botulism

Botulism is an **intoxication** (i.e., caused by an exotoxin) associated with eating poorly preserved foods, although it can also occur as a true infection. Until recent times, it was relatively common and frequently fatal, but modern techniques of food preservation and medical treatment have reduced both its incidence and its fatality rate.

▶ Signs and Symptoms

There are three major forms of botulism, distinguished by their means of transmission and the population they affect. **Table 17.2** summarizes these. The symptoms are largely the same in all three forms, however. From the circulatory system, an exotoxin called the **botulinum toxin** travels to its principal site of action, the neuromuscular junctions of skeletal muscles. The effect of botulinum is to prevent the release of the neurotransmitter, acetylcholine, that initiates the signal for muscle contraction. This results in the

Table 17.2 Three Types of Botulism

Transmission and Epidemiology	Culture and Diagnosis

		Transmission and Epidemiology	Culture and Diagnosis
Infant Botulism	**Infection followed by intoxication**	This is currently the most common type of botulism in the United States, with approximately 75 cases reported annually. The exact food source is not always known, although raw honey has been implicated in some cases, and the endospores are common in dust and soil. Apparently, the immature state of the neonatal intestine and microbial biota allows the endospores to gain a foothold, germinate, and give off neurotoxin. As in adults, babies exhibit flaccid paralysis, usually manifested as a weak sucking response, generalized loss of tone (the "floppy-baby syndrome"), and respiratory complications. Although adults can also ingest botulinum endospores in contaminated vegetables and other foods, the adult intestinal tract normally inhibits this sort of infection.	Finding the toxin or the organism in the feces confirms the diagnosis.
70%–75%*			
Foodborne Botulism	**Pure intoxication**	Many botulism outbreaks occur in home-processed foods, including canned vegetables, smoked meats, and cheese spreads. Several factors in food processing can lead to botulism. Endospores can be present on the vegetables or meat at the time of gathering and are difficult to remove completely. When contaminated food is put in jars and steamed in a pressure cooker that does not reach reliable pressure and temperature, some endospores survive (botulinum endospores are highly heat resistant). At the same time, the pressure is sufficient to evacuate the air and create anaerobic conditions. Storage of the jars at room temperature favors endospore germination and vegetative growth, and one of the products of the cell's metabolism is botulinum, the most potent microbial toxin known. Bacterial growth may not be evident in the appearance of the jar or can or in the food's taste or texture, and only minute amounts of toxin may be present. Botulism is never transmitted from person to person.	Some laboratories attempt to identify the toxin in the offending food. Alternatively, if multiple patients present with the same symptoms after ingesting the same food, a presumptive diagnosis can be made. The cultivation of *C. botulinum* in feces is considered confirmation of the diagnosis since the carrier rate is very low.
20%–30%			
Wound Botulism	**Infection followed by intoxication**	Perhaps three or four cases of wound botulism occur each year in the United States. In this form of the disease, endospores enter a wound or puncture, much as in tetanus, but the symptoms are similar to those of foodborne botulism. Increased cases of this form of botulism are being reported in intravenous drug users as a result of needle puncture.	The toxin should be demonstrated in the serum, or the organism should be grown from the wound.
<3%			

*Percentage of botulism cases in United States that are of this type

opposite effect of tetanus. The type of paralysis is called flaccid paralysis, where muscle contraction is inhibited **(figure 17.18)**. The usual time before onset of symptoms is 12 to 72 hours, depending on the size of the dose. Neuromuscular symptoms first affect the muscles of the head and include double vision, difficulty in swallowing, and dizziness, but there is no sensory or mental lapse. Later symptoms are descending muscular paralysis and respiratory compromise. In the past, death resulted from respiratory arrest, but mechanical respirators have reduced the fatality rate to about 10%.

You are probably familiar with the use of the botulinum toxin, or "Botox," to relieve facial wrinkles. This treatment exploits the action of the exotoxin to inhibit muscle contractions, leading to smoother surface skin. Botox treatment (in developed countries) is currently being used for several medical conditions that have nothing to do with vanity. People suffering from migraine, excessive sweating, urinary incontinence, spasmocity associated with multiple sclerosis, and even tennis elbow are experiencing relief from Botox injections.

Causative Agent

Clostridium botulinum, like *Clostridium tetani*, is an endospore-forming anaerobe that does its damage through the release of an exotoxin. *C. botulinum* commonly inhabits soil and water and occasionally the intestinal tract of animals. It is distributed worldwide but occurs most often in the Northern Hemisphere. The species has seven distinctly different types (designated A, B, C, D, E, F, and G) that vary in distribution among animals, regions of the world, and types of exotoxin. Human disease is usually associated with types A, B, E, and F, and animal disease with types A, B, C, D, and E.

Both *C. tetani* and *C. botulinum* produce neurotoxins; but tetanospasmin, the toxin made by *C. tetani*, results in spastic paralysis (uncontrolled muscle contraction).

Figure 17.18 **Infant botulism**. The child is 6 weeks old and displays the flaccid paralysis characteristic of botulism.
Source: CDC

In contrast, botulinum, the *C. botulinum* neurotoxin, results in flaccid paralysis, a loss of ability to contract the muscles.

Disease Table 17.10 Botulism

Causative Organism(s)	*Clostridium botulinum* (B) G+
Most Common Modes of Transmission	Vehicle (foodborne toxin, airborne organism); direct contact (wound); parenteral (injection)
Virulence Factors	Botulinum exotoxin
Culture/Diagnosis	Culture of organism; demonstration of toxin
Prevention	Food hygiene; toxoid immunization available for laboratory professionals
Treatment	Antitoxin, Penicillin G for wound botulism, supportive care
Epidemiological Features	United States: 75% of botulism is infant botulism; approximately 100–150 cases annually Category **A bioterror agent;** 1 gm of aerosolized botulinum toxin estimated to be able to kill 1.5 million people

Culture and Diagnosis

Diagnostic standards are slightly different for the three different presentations of botulism. A suspected case of botulism should trigger a phone call to the state health department or the CDC before proceeding with diagnosis or treatment.

Prevention and Treatment

The CDC maintains a supply of antitoxin, which, when administered soon after diagnosis, can prevent the worst outcomes of the disease. Patients are also managed with respiratory and cardiac support systems. In all cases, hospitalization is required and recovery takes weeks. There is an overall 5% mortality rate.

17.3 LEARNING OUTCOMES—Assess Your Progress

4. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Diseases," meningitis and Zika virus disease.
5. Identify the most common and also the most deadly of the multiple possible causes of meningitis.
6. Explain the difference between the oral polio vaccine and the inactivated polio vaccine, and under which circumstances each is appropriate.
7. Discuss important features of the diseases most directly involving the brain. These are meningoencephalitis, encephalitis, and subacute encephalitis.
8. Identify which encephalitis-causing viruses you should be aware of in your geographic area.
9. Name three causes of subacute encephalitis and discuss the epidemiology of each.
10. Discuss important features of the disease rabies.

11. Compare and contrast the diseases tetanus and botulism.

CASE FILE WRAP-UP

©Exactostock/SuperStock (health care worker); ©Science Photo Library/Alamy Stock Photo (blood cells)

Meningitis is an infection of the meninges, which surround the brain and spinal cord. In Betty's case it is possible that the organism causing her respiratory illness migrated from the nasal cavity to the brain. The most common form of meningitis in adults is caused by *Streptococcus pneumoniae*, the same organism that causes most cases of bacterial pneumonia.

Lumbar puncture is an important part of the diagnostic workup for meningitis. A needle is inserted into the spinal canal to obtain a sample of cerebral spinal fluid (CSF). During the procedure, a manometer is used to measure the pressure in the CNS system. Elevated pressure is indicative of swelling in the brain. Lab results from a lumbar puncture can help clarify the pathogen—viral, fungal, or bacterial. For a patient with bacterial meningitis, one would expect low glucose, high protein, and high white blood cell count in the CSF.

Due to the high risk of complications and mortality from meningitis, antibiotics should be administered as soon as possible, regardless of whether the CSF sample has been collected. Prompt and well-monitored treatment is essential to prevent the neurological complications of meningitis, including hearing loss, intellectual deficits, partial paralysis, and seizures.

Monitoring serum drug levels is an essential part of care. The metabolic panel gives an overview of organ function, particularly the kidney and liver, both of which play an important role in clearing medications from the bloodstream. If organ dysfunction causes accumulation of an antibiotic, toxicity may occur. In the case of Betty's meningitis, monitoring medication levels was important for another reason. The blood-brain barrier is a protective barrier that prohibits the introduction of any substances to the central nervous system. In order for the antibiotics to be effective in treating the infection, this barrier must be overcome. In patients with meningitis, higher doses and/or more frequent administration of antibiotics is necessary to make sure they get to the CNS.

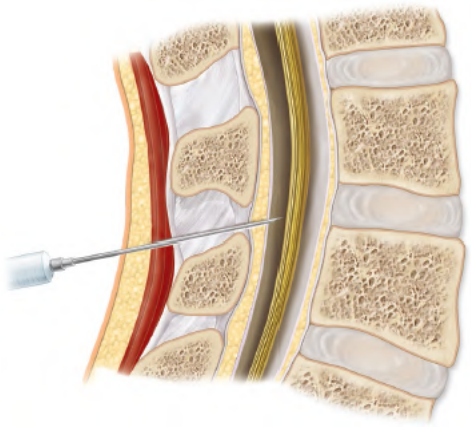

Diagram of the needle placement for lumbar puncture.
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Summing Up

Taxonomic Organization Microorganisms Causing Disease in the Nervous System

Microorganism	Pronunciation	Disease Table
Gram-positive endospore-forming bacteria		
Clostridium botulinum	klos-trid″-ee-um bot′-yew-lin″-um	Botulism, 17.10
Clostridium tetani	klos-trid″-ee-um tet′-a-nie	Tetanus, 17.9
Gram-positive bacteria		
Streptococcus agalactiae	strep″-tuh-kok′-us ay-ga-lact′-tee-ay	Neonatal meningitis, 17.2
Streptococcus pneumoniae	strep″-tuh-kok′-us nu-mo′-nee-ay	Meningitis, 17.1
Listeria monocytogenes	lis-teer′-ee-uh mon′-oh-sy-toj″-eh-nees	Meningitis, 17.1 Neonatal meningitis, 17.2
Gram-negative bacteria		
Cronobacter sakazakii	krow″-no-bak′-tur sock″-uh-zock′-ee	Neonatal meningitis, 17.2
Escherichia coli	esh′-shur-eesh″-ee-uh col′-eye	Neonatal meningitis, 17.2
Haemophilus influenzae	huh-mah′-fuh-luss in′-floo-en″-zay	Meningitis, 17.1
Neisseria meningitidis	nye-seer″-ee-uh′ men′-in-jit″-ih-dus	Meningitis, 17.1
DNA viruses		
Herpes simplex virus type 1 and 2	hur′-peez sim′-plex vie′-russ	Encephalitis, 17.6
JC virus	jay′-cee″ vie′-russ	Progressive multifocal leukoencephalopathy, 17.6
RNA viruses		
Arboviruses West Nile virus, La Crosse virus, Jamestown Canyon virus, St. Louis encephalitis virus, Powassan virus, eastern equine encephalitis virus	ar′-bow-vie′-russ-suz west ny′-l vie′russ, luh-kross vie′russ, jeymz′-toun kan′-yun vie′russ, saynt lew′iss en-cef ′-uh-ly″-tuss vie′russ, poe-was′-uhn vie′russ, ee′-stern ee′-cwine en-sef-uh-ly′-tuss vie'russ	Encephalitis, 17.6
Poliovirus	poh′-lee-oh vie′-russ	Poliomyelitis, 17.4
Rabies virus	ray′-bees vie′-russ	Rabies, 17.8
Zika virus	zee′kuh vie′-rus	Zika virus, 17.3
Fungi		
Cryptococcus neoformans	crip-tuh-kok′-us nee′-oh-for″-mans	Meningitis, 17.1
Coccidioides	cox-sid″-ee-oid′-ees	Meningitis, 17.1
Prions		

Microorganism	Pronunciation	Disease Table
Creutzfeldt-Jakob prion	croytz′-felt yaw′-cob pree′-on	Subacute encephalitis, 17.7
Protozoa		
Acanthamoeba	ay-kanth″-uh-mee′-buh	Meningoencephalitis, 17.5
Naegleria fowleri	nay-glar′-ee-uh fow-lahr′-ee	Meningoencephalitis, 17.5
Toxoplasma gondii	tox′-oh-plas″-mah gon′-dee-eye	Subacute encephalitis, 17.7

The Gut and the Brain

Have you ever heard the term "gut-brain axis"? For many years it has been recognized that there is an important and comprehensive connection between the gastrointestinal tract and the brain. They are connected through hormonal, endocrine, and neuronal mechanisms, so that one affects the other. This connection is so important that the gut is sometimes called "the second brain." We know this instinctively, since our gut reacts when we think certain thoughts, such as "I have to give a class presentation in 5 minutes." Situations and thoughts that make us extremely uneasy have a noticeable effect on our digestive system.

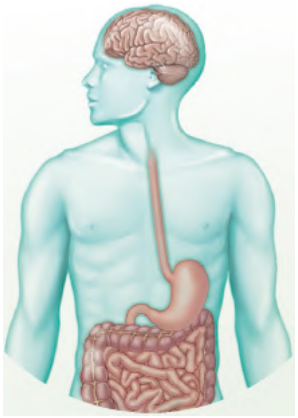
Since the early 2000s we have realized that there is another huge influence on our central nervous system that comes from the gut: our gut microbiota. It may seem incredible, but the composition of our gut microbiota has been shown to be closely correlated with the following characteristics of our brain biology:

- The way our brain develops *in utero*. The gut microbiome appears to influence the number of neurons created during embryonic development and the number of neurons that are disposed of as part of the normal process of brain development before birth.
- The relative activity of microglia—the resident phagocytic cells in the brain, which account for 10% to 15% of all brain cells. With a disrupted (or absent) microbiota, these cells have less immune responsiveness.
- The process of myelination of nerves. Myelination occurs during fetal development and afterwards. It lays down a fatty layer (myelin) around nerves to enhance signal conduction. The process is notably disrupted when the gut microbiota is not present or is significantly altered. This can have a significant effect on nerve function, both in the central and in the peripheral nervous system.
- The creation of a sound blood-brain barrier (BBB). With a disrupted or absent microbiota, the BBB is leaky and doesn't function the way it does in animals with healthy microbiota.

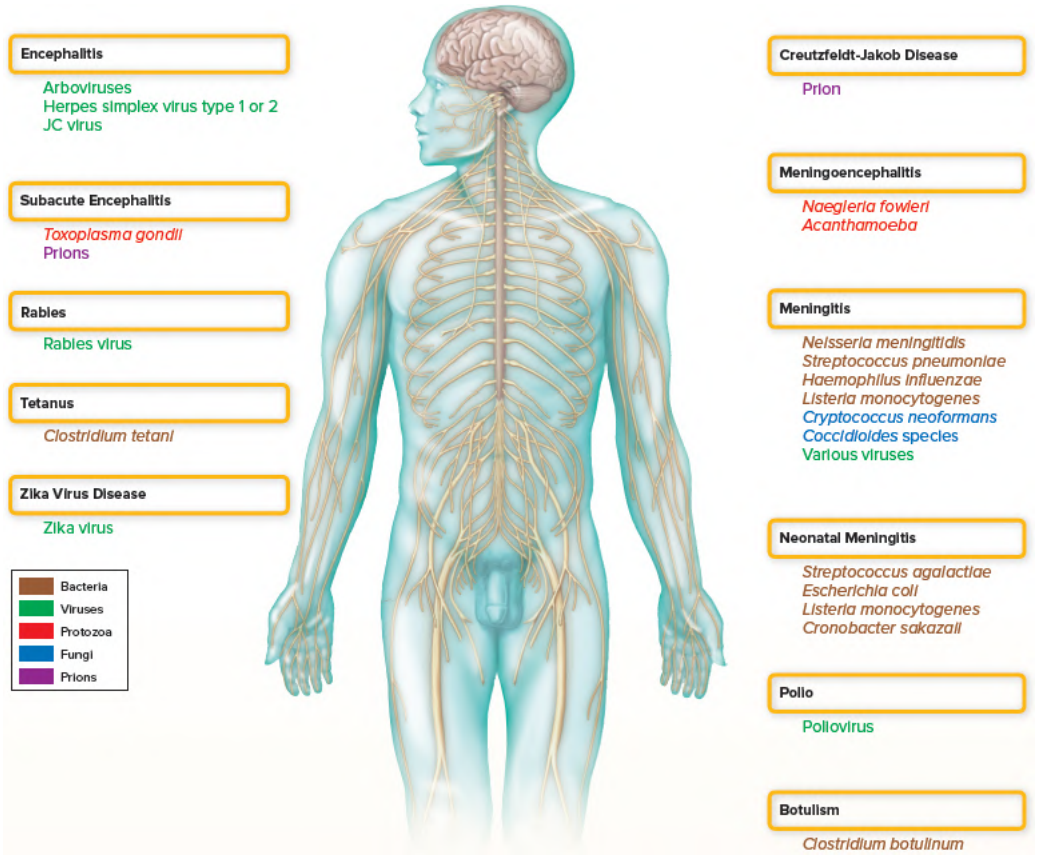
What is the upshot of these phenomena? Recent research is finding many connections between healthy neural functioning of organisms and their microbiome—and the findings described here suggest the underlying mechanistic reasons for this. We have mentioned elsewhere that there are correlative studies linking the gut microbiome and such disorders as depression, schizophrenia, and even autism spectrum disorder. Clearly, this is a rich area for exploration.

Note About Correlation vs. Causation This area of research is a good example to illustrate a very important distinction in science. When we say that it is a rich area of research, it is "rich" in two senses. There will certainly be groundbreaking findings, of the type where we can say definitively that "an *x* type of microbiome leads to (or causes) *y* neurological outcome." These will indeed lead to better management of neurological disorders via the microbiome. There will also certainly be "red herrings"—studies that find an association between the microbiome and certain brain conditions, that turn out to be unsubstantiated. This is the way scientific research proceeds, since early studies usually only establish *correlation* (that two phenomenon were seen together), and it takes more in-depth studies to establish *causation* (that one factor actually resulted in the other). Unfortunately, news outlets don't often appreciate the distinction in these two types of findings, so you

will frequently see media reports of new studies that merely found a correlation, breathlessly proclaiming that "Factor A causes Factor B."

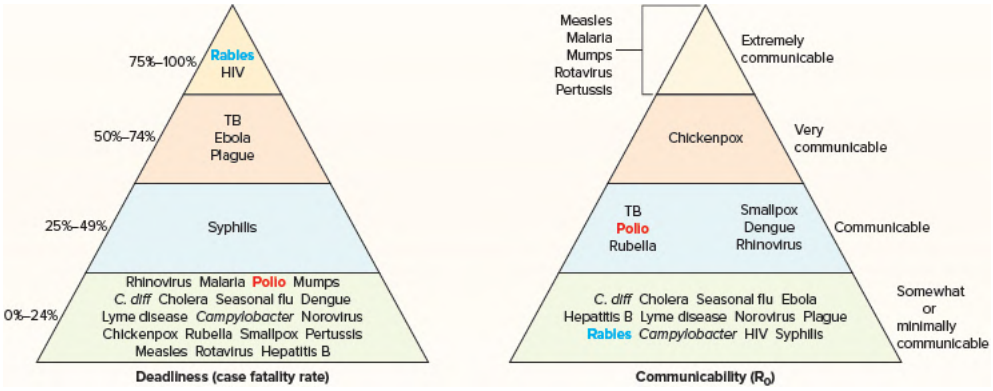


Infectious Diseases Affecting
The Nervous System



System Summary Figure 17.19

Deadliness and Communicability of Selected Diseases of the Nervous System



Chapter Summary

17.1 The Nervous System and Its Defenses

- The nervous system has two parts: the central nervous system (the brain and spinal cord), and the peripheral nervous system (nerves and ganglia).
- The soft tissue of the brain and spinal cord is encased within the tough casing of three membranes called the *meninges*. The subarachnoid space is filled with a clear serumlike fluid called cerebrospinal fluid (CSF).
- The nervous system is protected by the *blood-brain barrier*, which limits the passage of substances from the bloodstream to the brain and spinal cord.

17.2 Normal Biota of the Nervous System

- At the present time, we believe there is no normal biota in either the central nervous system (CNS) or the peripheral nervous system (PNS).

17.3 Nervous System Diseases Caused by Microorganisms

- **Meningitis:** Inflammation of the meninges. The more serious forms are caused by bacteria, often facilitated by coinfection or previous infection with respiratory viruses.
  - *Neisseria meningitidis:* Gram-negative diplococcus; causes most serious form of acute meningitis.
  - *Streptococcus pneumoniae:* Gram-positive coccus; most frequent cause of community-acquired pneumococcal meningitis.
  - *Haemophilus influenzae:* Declined sharply because of vaccination.
  - *Listeria monocytogenes:* Most cases are associated with ingesting contaminated dairy products, poultry, and meat.
  - *Cryptococcus neoformans:* Fungus; causes chronic form with more gradual onset of symptoms.
  - *Coccidioides* species: "Valley Fever"; begins in lungs but can disseminate quickly throughout body; highest incidence in southwestern United States, Mexico, and parts of Central and South America.

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- Viruses: Very common, particularly in children; 90% are caused by enteroviruses.

- **Neonatal Meningitis:** Usually transmitted vertically. Primary causes are *Streptococcus agalactiae, Escherichia coli, Cronobacter sakazakii*, and *Listeria monocytogenes*.
- **Zika virus disease:** Declared a global public health emergency in 2016 by the World Health Organization. In neonates can cause microcephaly; in adults symptoms may be unnoticed, mild, or trigger the onset of Guillain-Barre syndrome.
- **Poliomyelitis:** Acute enterovirus infection of spinal cord; can cause neuromuscular paralysis. After a massive effort, most of the world is polio-free, but until it is eradicated from Nigeria, Pakistan, and Afghanistan it threatens the world.

- **Meningoencephalitis:** Caused mainly by two amoebas, *Naegleria fowleri* and *Acanthamoeba*.
- **Acute Encephalitis:** Usually caused by viral infection. Arboviruses carried by arthropods often are responsible. Begins with arthropod bite, release of virus into tissues, and replication in nearby lymphatic tissues.
  - West Nile virus: Emerged in United States in 1999; now widespread. Most cases subclinical but dangerous for infants and the elderly. Maintained in birds and transmitted by mosquitoes.
  - LaCrosse virus: Restricted to the eastern half of the United States and Texas. Maintained in small mammals and transmitted by mosquitoes.
  - Jamestown Canyon virus: Human infection becoming more common. Maintained in deer and moose and transmitted by mosquitoes.
  - St. Louis Encephalitis virus: Adults more likely to show symptoms. Maintained in birds and transmitted by mosquitoes.
  - Powassan virus: In northeastern United States and Great Lakes states. Maintained in groundhogs and transmitted by ticks.
  - Eastern equine encephalitis virus: Endemic to east coast of United States. Epidemics can occur in horses and humans. High case-fatality rate.
  - Nonarboviral causes: Herpes simplex virus, JC virus.
- **Subacute Encephalitis:** Symptoms take longer to manifest.
  - *Toxoplasma gondii:* Protozoan, causes toxoplasmosis, most common form of subacute encephalitis. Relatively asymptomatic in the healthy; can be severe in immunodeficient people and fetuses.
  - Subacute sclerosing panencephalitis: a long-term fatal consequence of prior measles infection.
  - Prions: Proteinaceous infectious particles containing no genetic material. Cause transmissible spongiform encephalopathies (TSEs), neurodegenerative diseases with long incubation periods but rapid progressions once they begin.
- **Rabies:** Slow, progressive zoonotic disease characterized by fatal encephalitis. Rabies virus is in the family *Rhabdoviridae*.
- **Tetanus:** Neuromuscular disease, also called lockjaw; caused by *Clostridium tetani* neurotoxin, *tetanospasmin*, which binds target sites on spinal neurons, blocks inhibition of muscle contraction.
- **Botulism:** Caused by exotoxin of *C. botulinum;* associated with eating poorly preserved foods; can also occur as true infection. Three major forms: foodborne botulism, infant botulism, and wound botulism.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. Which pathogen has evolved to make its rodent host less avoidant of cats? **a**. *Cryptococcus neoformans* **b**. *Neisseria meningitidis* **c**. rabies virus **d**. *Toxoplasma gondii*	**2**. *Neisseria meningitidis* occurs as noninvasive normal biota in the nares (noses) of approximately 35% of the population. Those that have developed the ability to be invasive, entering the nervous system, are said to be at an "evolutionary dead end." Speculate on why the invasive strains are characterized with that label.	**3**. Speculate on why the poliovirus has changed over evolutionary time to cause severe disease less often in humans, while a disease such as rabies has not become less virulent for humans over time.
Cell Structure and Function	**4**. What cellular structure do several of the organisms that cause meningitis share? **a**. capsule **b**. pilus **c**. fimbria **d**. endospore	**5**. Why is it necessary only to include the exotoxin (in toxoid form) in vaccines for tetanus and botulism?	**6**. Justify the use of only a Gram stain in diagnosing some types of meningitis.
Metabolic Pathways	**7**. Which of the following organisms is anaerobic? **a**. *poliovirus* **b**. *Cryptococcus* **c**. *Clostridium* **d**. *Coccidioides*	**8**. When cultivating specimens for *N. meningitidis*, nonselective chocolate agar is often used. But when culturing for another *Neisseria* species, *N. gonorrhoeae*, a different medium, Modified Thayer-Martin (MTM) medium, which contains multiple antibiotics, is preferred. Speculate on why this is.	**9**. What do you expect to happen to the geographical distribution of *Coccidioides* cases over the next 10 years? Justify your answer.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Information Flow and Genetics	**10**. Which disease is caused by an infectious agent that carries no nucleic acid? **a**. CJD **b**. rabies **c**. polio **d**. meningitis	**11**. Why would PCR be the best method for identifying microbes in the cerebrospinal fluid if antibiotic treatment was begun before the fluid was sampled?	**12**. Scientists have analyzed the "transcriptome" (the RNA transcripts) of mouse brains that have been infected with *Toxoplasma*. In what ways might that be informative?

Microbial Systems	**13**. The normal gut microbiota in adults, but not infants, inhibit the growth of which pathogen? **a**. *Neisseria meningitidis* **b**. *Clostridium botulinum* **c**. *Clostridium tetani* **d**. *Naegleria fowleri*	**14**. Botulinum toxin has been proposed as a treatment for the symptoms of tetanus. Discuss why this might be useful.	**15**. Recent research shows that when scientists infect *Aedes aegypti* with a bacterium called *Wolbachia*, they become less likely to spread Zika and other viruses to humans. Speculate on what *Wolbachia* may be doing in the mosquito.
Impact of Microorganisms	**16**. Subacute encephalitis can be caused by **a**. *Toxoplasma gondii*. **b**. *Streptococcus agalactiae*. **c**. *Naegleria fowleri*. **d**. *Haemophilus influenzae*.	**17**. "Accidental herd immunity" conferred by the oral polio vaccine in the developing world has aided the elimination of polio from many countries. Explain what accidental herd immunity is and how it works.	**18**. Conduct research about the multistage meningitis outbreak linked to steroid injections in 2012. Speculate as to why this "nonpathogenic" fungal species caused such severe disease and death.
Scientific Thinking	**19**. Mosquito eradication could change the epidemiology of **a**. polio. **b**. Zika. **c**. West Nile. **d**. two of these.	**20**. The paralytic form of poliomyelitis began to occur in epidemics in North America and Europe in the early part of the 1900s. Many scientists believe this increase in incidence and in disease manifestations was due to significant	**21**. In The Microbiome feature, the link between the gut microbiome and brain health is discussed. The feature also mentions that many of the published studies show correlation, and

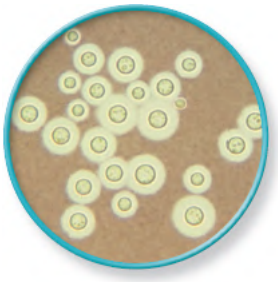
		improvements in sanitation that occurred around that time. Use what you know about how the virus is transmitted to justify this seemingly puzzling outcome. (Hint: Maternal antibodies might be involved.)	not causation. Using laboratory mice as your subjects, design an experiment that could show that prolonged antibiotic exposure disrupts the gut microbiome and *causes* altered brain development.

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From chapter 2, figure 2.19*a***. Without looking back to the figure in chapter 2, speculate on which meningitis-causing organism you are seeing here. How could your presumptive diagnosis be confirmed?



Source: CDC/Dr. Leanor Haley

connect Resources are available through Connect, including interactive learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

18

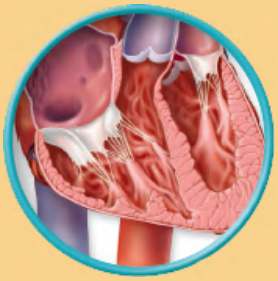
Infectious Diseases Affecting the Cardiovascular and Lymphatic Systems

Source: Library of Congress [LC-DIG-highsm-11900] (forest); ©ZouZou/Shutterstock (health care worker)

IN THIS CHAPTER…

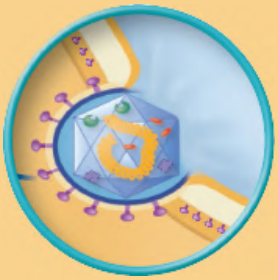
18.1 The Cardiovascular and Lymphatic Systems and Their Defenses

1. Describe the important anatomical features of the cardiovascular and lymphatic systems.
2. List the natural defenses present in the cardiovascular and lymphatic systems.



18.2 Normal Biota of the Cardiovascular and Lymphatic Systems

3. Explain the "what" and the "why" of the normal biota of the cardiovascular and lymphatic systems.



18.3 Cardiovascular and Lymphatic System Diseases Caused by Microorganisms

4. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Diseases" malaria and HIV.
5. Discuss the epidemiology of malaria.
6. Describe the epidemiology of HIV infection in the developing world.
7. Discuss the important features of infectious cardiovascular conditions that have more than one possible cause. These are the two forms of endocarditis, septicemia, hemorrhagic fever diseases, and nonhemorrhagic fever diseases.
8. Identify factors that distinguish hemorrhagic and nonhemorrhagic fever diseases.
9. Outline the series of events that may lead to septicemia and how it should be prevented and treated.
10. Discuss the important features of infectious cardiovascular diseases that have only one possible cause. These are plague, tularemia, Lyme disease, infectious mononucleosis, Chagas disease, and anthrax.
11. Describe what makes anthrax a good agent for bioterrorism, and list the important presenting signs to look for in patients.

CASE FILE

Bitten by a Tick

Miguel, a 28-year-old male, presented to the emergency department where I was working as a nurse practitioner. Miguel reported that for the past 5 days, he had been experiencing a persistent headache and fatigue. He had an intermittent fever with chills. Miguel sought medical attention with his primary care provider and was sent to the emergency department with concern for a serious or systemic illness.

I brought Miguel into a treatment room and checked his vital signs. He had a fever and reported a persistent headache. His heart rate was high and blood pressure was normal. He was able to answer my questions, though he appeared lethargic.

I gathered more information about Miguel's health history. He worked in construction and had been working on a new job site for the past month. He had no significant past medical history. He denied recent travel or exposure to infectious disease. With his unremarkable health history, I began to think broadly about what could have caused his symptoms. Living in rural Pennsylvania, exposure to ticks was common in forested areas. I asked Miguel if he had any recent insect bites. He recalled finding a tick on the back of his neck 1 week prior. Upon my exam, I found a red area where the tick bite had occurred. The central red area was surrounded by another red ring, resembling a bull's eye.

Based on his symptoms, I suspected that Miguel might have Lyme disease. I ordered a 3-week course of antibiotics and instructed him to begin treatment immediately. To confirm the diagnosis, I ordered a serum enzyme immunoassay test. I also counseled Miguel about the use

of appropriate protective clothing and DEET insect repellant to prevent tick bites at his job site.

- Why is it important to treat Lyme disease early?
- Will Miguel be cured of the disease after antibiotic therapy?

Case File Wrap-Up appears at the end of the chapter.

©Purestock/Getty Images

18.1 The Cardiovascular and Lymphatic Systems and Their Defenses

The Cardiovascular System

The *cardiovascular system* is the pipeline of the body. It is composed of the blood vessels, which carry blood to and from all regions of the body, and the heart, which pumps the blood. This system moves the blood in a closed circuit, and it is therefore known as the *circulatory system*. The cardiovascular system provides tissues with oxygen and nutrients and carries away carbon dioxide and waste products, delivering them to the appropriate organs for removal. A closely related but largely separate system, the *lymphatic system*, is a major source of immune cells and fluids, and it serves as a one-way passage, returning fluid from the tissues to the cardiovascular system. **Figure 18.1** shows you how the two systems work together. You first saw this figure in chapter 12.

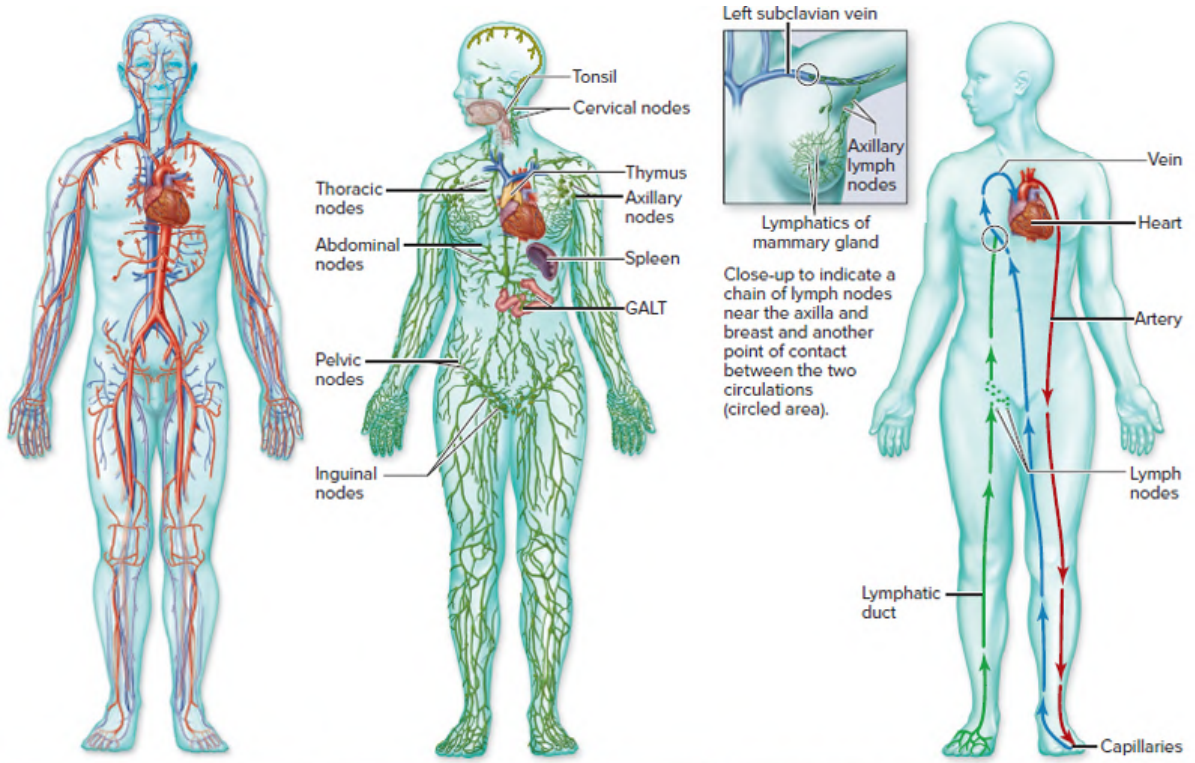


Figure 18.1 **The anatomy of the cardiovascular and lymphatic systems**.

The heart is a fist-size muscular organ that pumps blood through the body. It is divided into two halves, each of which is divided into an upper and lower chamber **(figure 18.2)**. The upper chambers are called atria (singular, *atrium*), and the lower are ventricles. The entire organ is encased in a fibrous covering, the pericardium, which is an occasional site of infection. The actual wall of the heart has three layers: from outer to inner, they are the epicardium, the myocardium, and the endocardium. The endocardium also covers the valves of the heart, and it is a relatively common target of microbial infection.

The blood vessels consist of *arteries, veins*, and *capillaries*. Arteries carry oxygenated blood away from the heart under relatively high pressure. They branch into smaller vessels called arterioles. Veins actually begin as smaller venules in the periphery of the body and coalesce into veins. The smallest blood vessels, the capillaries, connect arterioles to venules. Both arteries and veins have walls made of three layers of tissue. The innermost layer is composed of a smooth epithelium called endothelium. Its smooth surface encourages the smooth flow of cells and platelets through the system. The next layer is composed of connective tissue and muscle fibers. The outside layer is a thin layer of connective tissue. Capillaries, the smallest vessels, have walls made of only one layer of endothelium.

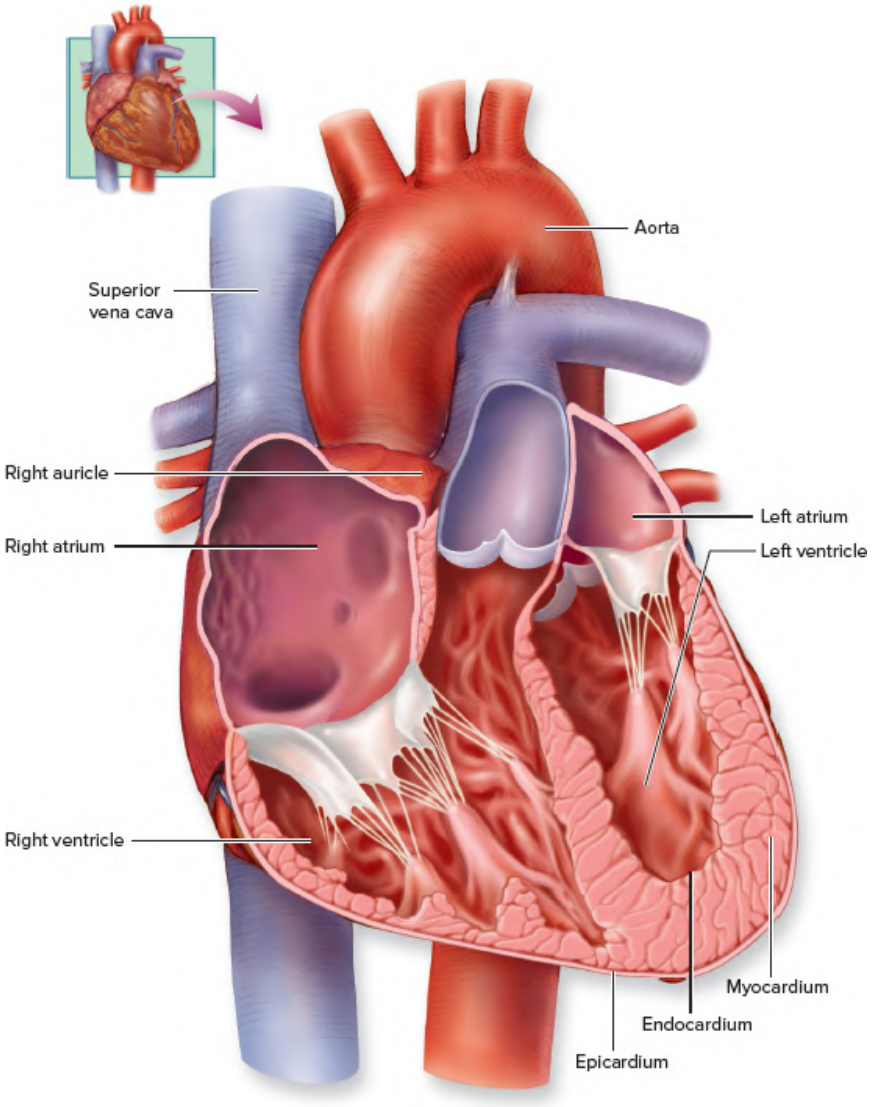
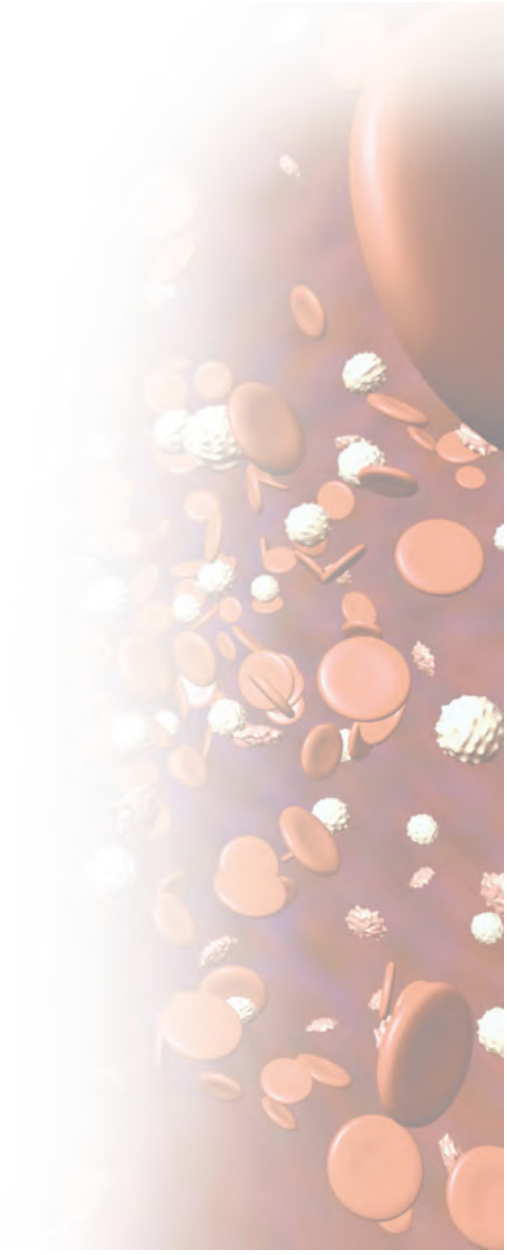


Figure 18.2 **The heart**.



Conceptual image of blood cells in an artery.

The Lymphatic System

The lymphatic system consists mainly of the lymph vessels, which roughly parallel the blood vessels; lymph nodes, which cluster at body sites such as the groin, neck, armpit, and intestines; and the spleen. It serves to collect fluid that has left the blood vessels and entered tissues, filter it of impurities and infectious agents, and return it to the blood.

Defenses of the Cardiovascular and Lymphatic Systems

The cardiovascular system is highly protected from microbial infection. Microbes that successfully invade the system, however, gain access to every part of the body, and every system may potentially be affected. For this reason, bloodstream infections are called **systemic infections**.

There are multiple modes of defense against infection in the bloodstream. The blood is full of leukocytes, with approximately 5,000 to 10,000 white blood cells per milliliter of blood. The various types of white blood cells include the lymphocytes, responsible for specific immunity, and the phagocytes, which are so critical to nonspecific as well as specific immune responses. Very few microbes can survive in the blood with so many defensive elements. That said, a handful of infectious agents have nonetheless evolved sophisticated mechanisms for avoiding blood-borne defenses.

Medical conditions involving the blood often have the suffix *-emia*. For instance, viruses that cause meningitis can travel to the nervous system via the bloodstream. Their presence in the blood is called **viremia**. When fungi are in the blood, the condition is termed **fungemia**, and bacterial presence is called **bacteremia**, a general term denoting only their *presence*. Bacteria frequently are introduced into the bloodstream during the course of daily living. Brushing your teeth or tearing a hangnail can introduce bacteria from the mouth or skin into the bloodstream; this situation is usually temporary. But when bacteria flourish and grow in the bloodstream, the condition is termed **septicemia**. Septicemia (also called sepsis) can very quickly lead to cascading immune responses, resulting in decreased systemic blood pressure, which can lead to **septic shock**, a life-threatening condition.

18.1 LEARNING OUTCOMES—Assess Your Progress

1. Describe the important anatomical features of the cardiovascular and lymphatic systems.
2. List the natural defenses present in the cardiovascular and lymphatic systems.

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1. A patient has been diagnosed with inflammation of the inner lining of the heart, secondary to a bacterial infection. What is the term for this condition?
   a. *bacterial carditis*
   b. *endocarditis*
   c. *epicarditis*
   d. *myocarditis*
   e. *pericarditis*

18.2 Normal Biota of the Cardiovascular and Lymphatic Systems

Like the nervous system, the cardiovascular and lymphatic systems are “closed” systems with no normal access to the external environment. Therefore, it has long been believed that they possess no normal biota. Thus, in the healthy state, no microorganisms *colonize* either the lymphatic or cardiovascular systems. Of course, this is biology, and it is never quite that simple. Recent studies from the Human Microbiome Project have suggested that the bloodstream is not completely sterile, even during periods of apparent health. There is evidence that the blood cells do contain bacteria of various types. It is tempting to speculate

that these low-level microbial "infections" may contribute to diseases for which no etiology has previously been found or for conditions currently thought to be noninfectious.

Cardiovascular and Lymphatic Systems Defenses and Normal Biota

	Defenses	Normal Biota
Cardiovascular System	Blood-borne components of nonspecific and specific immunity–including phagocytosis, specific immunity	Unclear
Lymphatic System	Numerous immune defenses reside here.	Unclear

18.2 LEARNING OUTCOMES—Assess Your Progress

3. Explain the "what" and the "why" of the normal biota of the cardiovascular and lymphatic systems.

18.3 Cardiovascular and Lymphatic System Diseases Caused by Microorganisms

Categorizing cardiovascular and lymphatic infections according to clinical presentation is somewhat difficult because most of these conditions are systemic, with effects on multiple organ systems. We start with two extremely important conditions, malaria and HIV.

Note on the Global Fund to Fight AIDS, Tuberculosis, and Malaria

In 2002 a unique partnership between governments, the private sector, civil society, and people affected by AIDS, tuberculosis, or malaria was formed. Its aim is to raise money and distribute it to programs that work in countries where the epidemics are the worst, and to save lives. The Bill and Melinda Gates Foundation is a major contributor to the Global Fund. And it has been very successful. There are one-third fewer deaths due to those "Big 3" diseases in countries where the Global Fund is active. They report that 17 million lives have been saved since 2002, at the rate of about 2 million a year. This chapter covers malaria and AIDS, and tuberculosis will be addressed in chapter 19.

HIGHLIGHT DISEASE

Malaria

From prehistoric time until the present, malaria has been one of the greatest afflictions, in the same rank as bubonic plague, influenza, and tuberculosis. Even now, as the dominant protozoal disease, it threatens 40% of the world's population every year. The origin of the name is from the Italian words *mal*, "bad," and *aria*, "air."

► Signs and Symptoms

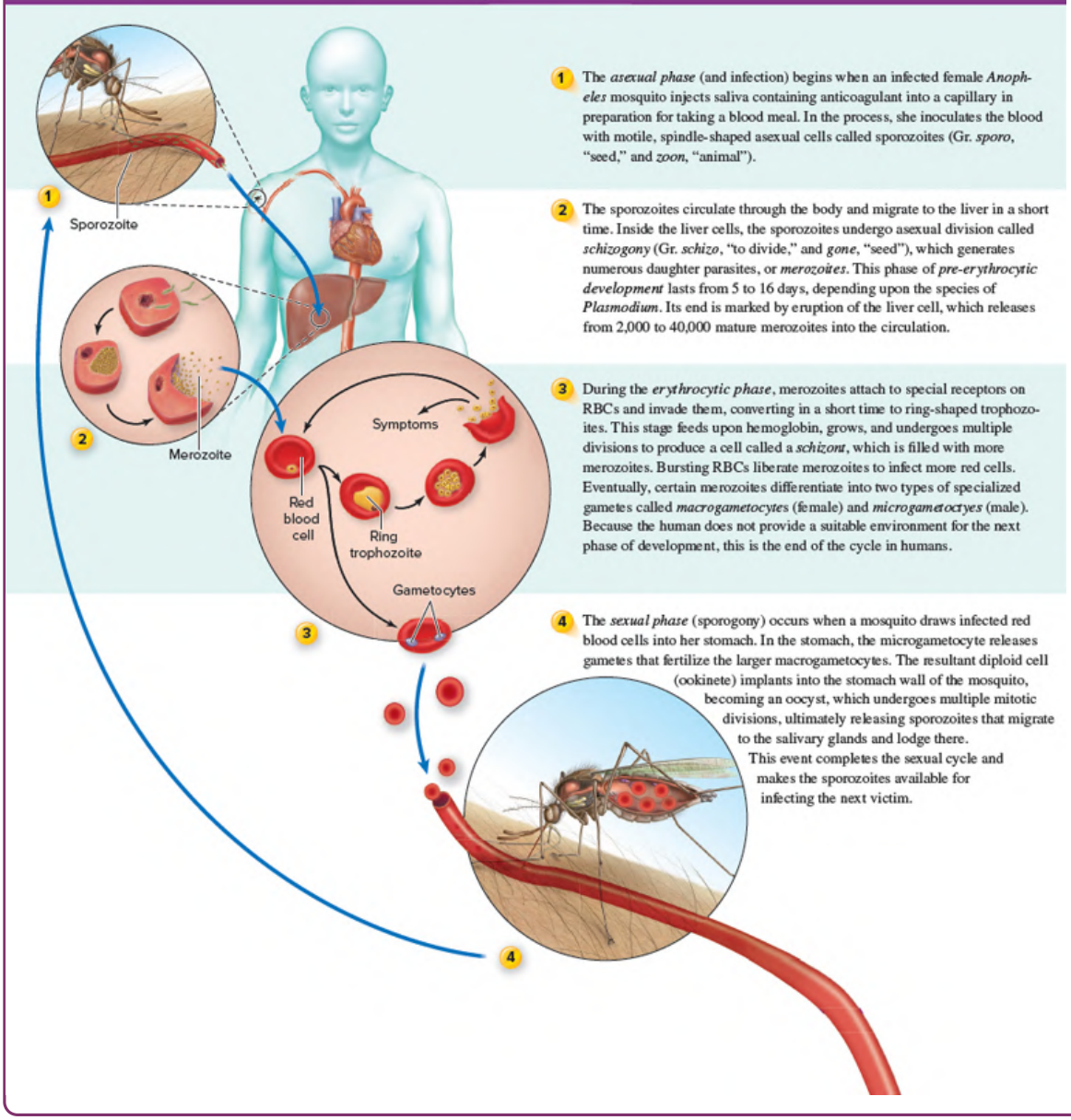
After a 10- to 16-day incubation period, the first symptoms are malaise, fatigue, vague aches, and nausea with or without diarrhea, followed by bouts of chills, fever, and sweating. These symptoms occur at 48- or 72-hour intervals, as a result of the synchronized rupturing of red blood cells. The interval, length, and regularity of symptoms reflect the type of malaria. Patients with falciparum malaria, the most virulent type, often display persistent fever, cough, and weakness for weeks without relief. Complications of malaria are hemolytic anemia from lysed blood cells and organ enlargement and rupture due to cellular debris that accumulates in the spleen, liver, and kidneys. One of the most serious complications of falciparum malaria is termed *cerebral malaria*. In this condition, small blood vessels in the brain become obstructed due to the increased ability of red blood cells (RBCs) to adhere to vessel walls (a condition called *cytoadherence* induced by the protozoan). The resulting decrease in oxygen in brain tissue can result in coma and death. In general, malaria has the highest death rate in the acute phase, especially in children. Certain kinds of malaria (those caused by *Plasmodium vivax* and *P. ovale*) are subject to relapses because some infected liver cells harbor dormant protozoans for up to 5 years.

Mosquitos transmit the malaria protozoan.
©Renaud Visage/Digital Vision/Getty Image

► Causative Agent

Plasmodium species are protozoa in the sporozoan group. The genus *Plasmodium* contains over 200 species, but only five are known to commonly infect humans: *P. malariae, P. vivax, P. knowlesi, P. ovale*, and *P. falciparum*. The five species show variations in the pattern and severity of disease. For instance, *P. falciparum* is responsible for the vast majority of deaths.

Table 18.1 Life Cycle of the Malarial Parasite



Development of the malarial parasite is divided into two distinct phases: the asexual phase, carried out in the human, and the sexual phase, carried out in the mosquito. **Table 18.1** lists the steps of the malarial life cycle.

Figure 18.3 illustrates the ring trophozoite stage in a malarial infection.

Figure 18.3 The ring trophozoite stage in a *Plasmodium falciparum* infection. A smear of peripheral blood shows ring forms in red blood cells. Some RBCs have multiple trophozoites.
Courtesy Stephen B. Aley, PhD., University of Texas at El Paso

Pathogenesis and Virulence Factors

The invasion of the merozoites into RBCs leads to the release of fever-inducing chemicals into the bloodstream. Chills and fevers often occur in a cyclic pattern. *Plasmodium* also metabolizes glucose at a very high rate, leading to hypoglycemia in the human host. The damage to RBCs results in anemia. The accumulation of malarial products in the liver and the immune stimulation in the spleen can lead to enlargement of these organs. Individual protozoa within a host can express distinctly different surface antigens, making it difficult for the host immune system to battle.

Transmission and Epidemiology

All forms of malaria are spread primarily by the female *Anopheles* mosquito. Although malaria was once distributed throughout most of the world, the control of mosquitoes in temperate areas has successfully restricted it mostly to a belt extending around the equator **(figure 18.4)**. Despite this achievement, approximately 200 million new cases are still reported each year, about 90% of them in Africa. The most frequent victims are children and young adults, of whom between 500,000 and 1 million die annually. A particular form of the malarial protozoan causes damage to the placenta in pregnant women, leading to excess mortality among fetuses and newborns. The total case rate in the United States is about 1,000 to 2,000 new cases a year. While most of these are found in people who acquired it in a known endemic area, locally transmitted infections are on the rise.

Culture and Diagnosis

Malaria can be diagnosed definitively by the discovery of a typical stage of *Plasmodium* in stained blood smears (see figure 18.3). Newer serological procedures have made diagnosis more accurate while requiring less skill to perform. Other indications are knowledge of the patient's residence or travel in endemic areas and symptoms such as recurring chills, fever, and sweating.

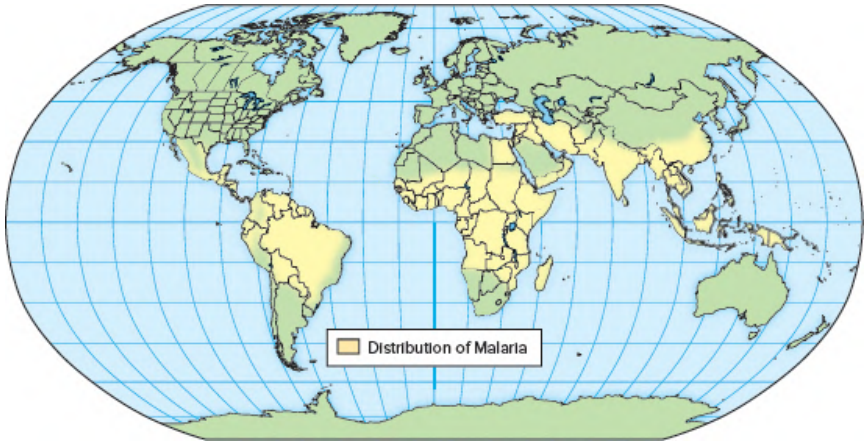


Figure 18.4 The malaria belt. Yellow zones outline the major regions that harbor malaria. The malaria belt corresponds to a band around the equator.

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2. After returning from international travel, a 4-year-old female is diagnosed with falciparum malaria. All of the following complications may be associated with this disease, except
- **a.** *splenic rupture*.
- **b.** *acute kidney injury*.
- **c.** *altered mental status*.
- **d.** *peptic ulcer*.
- **e.** *anemia*.

Figure 18.5 An African family sits under a treated mosquito net from the UNICEF mosquito nets program.
©Anthony Asael/Art in All of Us/Corbis News/Getty Images

Prevention

Malaria prevention is attempted through long-term mosquito abatement and human chemoprophylaxis. Abatement includes elimination of standing water that could serve as a breeding site and spraying of insecticides to reduce populations of adult mosquitoes, especially in and near human dwellings. Scientists have also tried introducing sterile male mosquitoes into endemic areas in an attempt to decrease mosquito populations. Humans can reduce their risk of infection considerably by using netting, screens, and repellants; by remaining indoors at night; and by taking weekly doses of prophylactic drugs. (Western travelers to endemic areas are usually prescribed antimalarials for the duration of their trips.) The WHO and other international organizations focus on efforts to distribute bed nets and to teach people how to dip the nets into an insecticide **(figure 18.5)**. The use of bed nets has been estimated to reduce childhood mortality from malaria by 20%. Here is an area where we can report some success: Bed-net use has tripled in 16 of 20 sub-Saharan African countries since 2000.

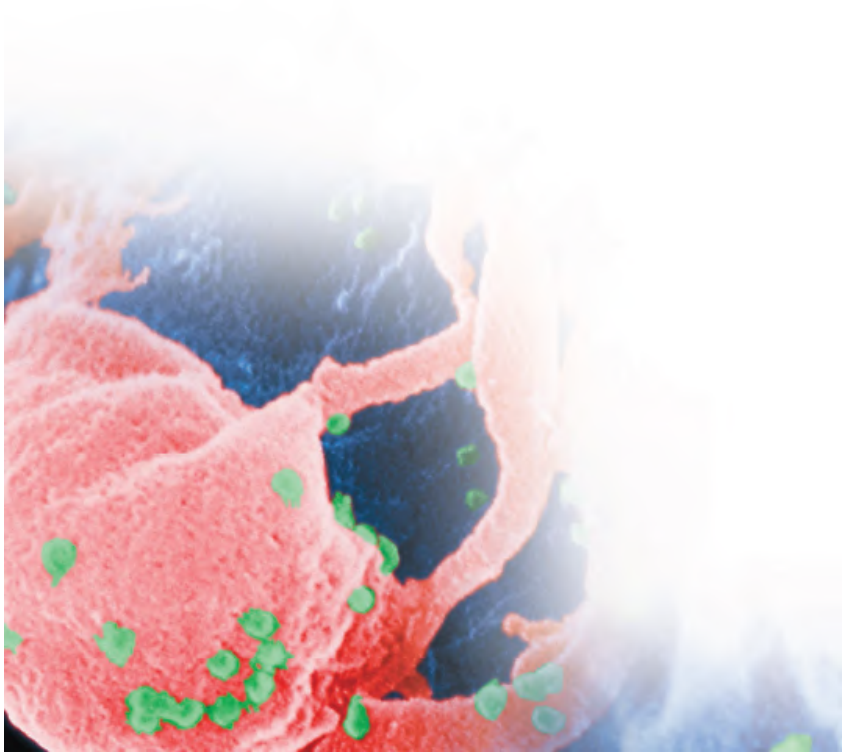
The best protection would come from a malaria vaccine, and scientists have struggled for decades to develop one. In 2016 a major breakthrough in vaccine research occurred. A vaccine called RTS,S—effective against *P. falciparum* (the most deadly and most common strain in Africa)—was found to be highly effective in children. Beginning in 2018 it will be used in pilot vaccination programs in sub-Saharan Africa.

Treatment

Quinine has long been a mainstay of malaria treatment. Chloroquine, the least toxic type, is used in nonresistant forms of the disease. The malarial protozoan has developed resistance to nearly every drug used for its treatment. Currently, a drug called artemisinin, derived from a plant, is recommended in many situations. It should be used in combination with other drugs to prevent resistance from developing. In areas where the protozoan is not resistant to chloroquine, that should be used. **Disease Table 18.1**.

Disease Table 18.1 Malaria

Causative Organism(s)	*Plasmodium falciparum*, *P. vivax, P. ovale, P. malariae*, *P. knowlesi* (P)
Most Common Modes of Transmission	Biological vector (mosquito), vertical
Virulence Factors	Multiple life stages; multiple antigenic types; ability to scavenge glucose, GPI, cytoadherence
Culture/Diagnosis	Blood smear; serological methods, PCR in cases where protozoa not detectable in blood
Prevention	Mosquito control; use of bed nets; vaccine for children in endemic areas now in use; prophylactic antiprotozoal agents
Treatment	Artemisinin, combination therapy, or chloroquine; consult WHO
Epidemiological Features	United States: cases are generally in travelers or immigrants; internationally, 200 million cases in "malaria belt"; 0.5 million deaths per year; more deadly in children



HIV budding out of an infected immune cell.
Source: CDC/Cynthia Goldsmith

Highlight Disease

HIV Infection and AIDS

Signs and Symptoms

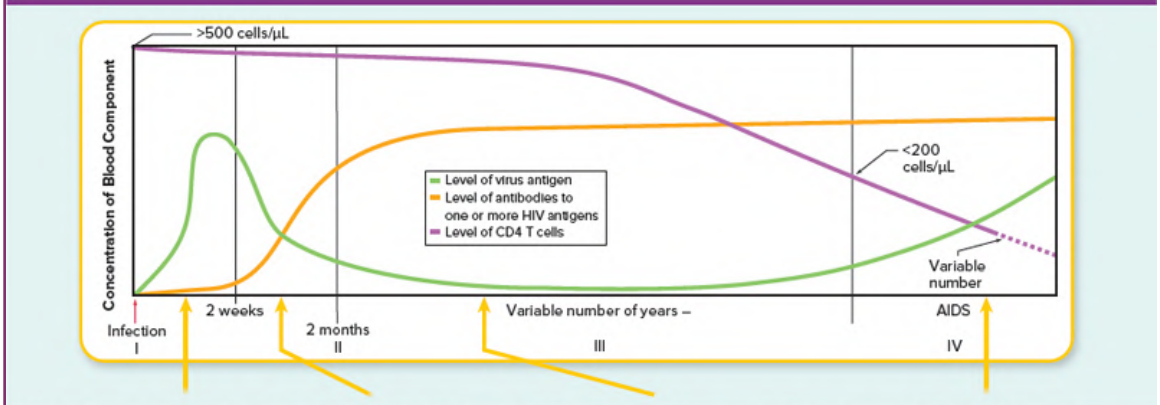
A spectrum of clinical signs and symptoms is associated with human immunodeficiency virus (HIV) infection. Symptoms in HIV infection are directly tied to two things: the level of virus

in the blood and the level of T cells in the blood. To understand the progression, follow **table 18.2** closely.

The table shows two different lines that correspond to virus and T cells in the blood. Another line depicts the amount of antibody against the virus. Note that the table depicts the course of HIV infection in the absence of medical intervention or chemotherapy.

Initial symptoms may be fatigue, diarrhea, weight loss, and neurological changes, but most patients first notice infection because of one or more opportunistic infections or neoplasms (cancers). These conditions are known as AIDS-defining illnesses (ADIs) and are detailed in **table 18.3**. Other disease-related symptoms appear to accompany severe immune deregulation, hormone imbalances, and metabolic disturbances. Pronounced wasting of body mass is a consequence of weight loss, diarrhea, and poor nutrient absorption.

Table 18.2 The Progression of HIV in the Absence of Treatment



Initial infection is often attended by vague, mononucleosis-like symptoms that soon disappear. This phase corresponds to the initial high levels of virus (the green line above). Antibodies are not yet abundant.	In the second phase, virus numbers in blood drop dramatically and antibody levels become detectable. CD4 T cells begin to decrease in number.	A long period of mostly asymptomatic infection ensues. During this time, which can last from 2 to 15 years, lymphadenopathy may be the prominent symptom. During the mid- to late-asymptomatic period, the number of T cells in the blood is steadily decreasing. Once the T-cell level reaches a (low) threshold, the symptoms of AIDS ensue.	Once T cells drop below 200 cells/μL, AIDS results. Note that even though antibody levels remain high, virus levels in the blood begin to rise.

Table 18.3 AIDS-Defining Illnesses

Skin and/or Mucous Membranes (Includes Eyes)	Nervous System	Cardiovascular and Lymphatic System or Multiple Organ Systems	Respiratory Tract	Gastrointestinal Tract	Genitourinary and/or Reproductive Tract

Skin and/or Mucous Membranes (Includes Eyes)	Nervous System	Cardiovascular and Lymphatic System or Multiple Organ Systems	Respiratory Tract	Gastrointestinal Tract	Genitourinary and/or Reproductive Tract
Cytomegalovirus retinitis (with loss of vision) Herpes simplex chronic ulcers (>1 month duration) Kaposi's sarcoma	Cryptococcosis, extrapulmonary HIV HIV encephalopathy Lymphoma; primarily in brain Progressive multifocal leukoencephalopathy Toxoplasmosis of the brain	Coccidioidomycosis, disseminated or extrapulmonary Cytomegalovirus (other than liver, spleen, nodes) Histoplasmosis Burkitt's lymphoma Immunoblastic lymphomas *Mycobacterium kansasii*, disseminated or extrapulmonary *Mycobacterium tuberculosis*, disseminated or extrapulmonary *Salmonella* septicemia, recurrent Wasting syndrome	Candidiasis of trachea, bronchi, or lungs Herpes simplex bronchitis or pneumonitis *Mycobacterium avium* complex Tuberculosis (*Mycobacterium tuberculosis*) *Pneumocystis jiroveci* pneumonia Pneumonia, recurrent Cryptococcosis	Candidiasis of esophagus, GI tract Herpes simplex chronic ulcers (>1 month duration) or esophagitis Isosporiasis, intestinal Cryptosporidiosis, chronic intestinal (>1 month duration)	Invasive cervical carcinoma (HPV) Herpes simplex chronic ulcers (>1 month duration)

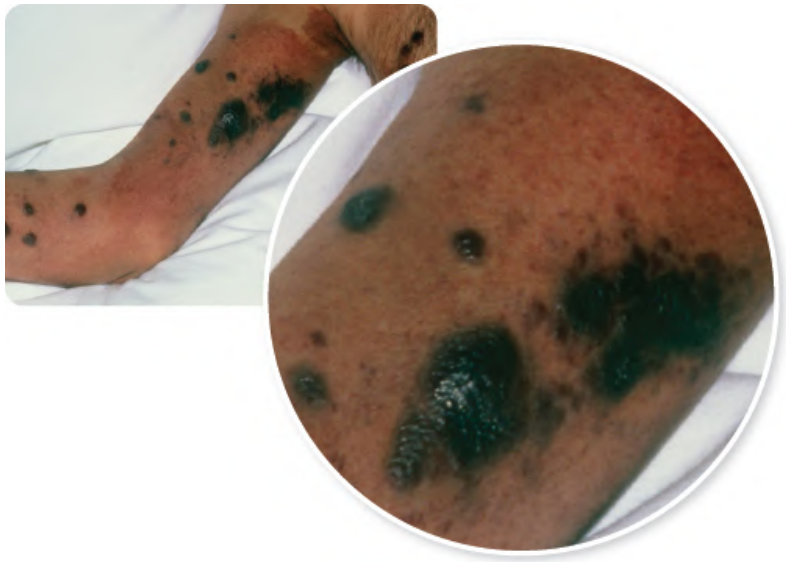


Figure 18.6 **Kaposi's sarcoma**.
©SPL/Science Source

Some of the most virulent complications are neurological. Lesions occur in the brain, meninges, spinal column, and peripheral nerves. Patients with nervous system involvement

show some degree of withdrawal, persistent memory loss, spasticity, sensory loss, and progressive AIDS dementia. **Figure 18.6** depicts a common ADI, Kaposi's sarcoma.

Causative Agent

HIV is a retrovirus in the genus *Lentivirus*. Many retroviruses have the potential to cause cancer and produce dire, often fatal diseases and are capable of altering the host's DNA in profound ways. They are named "retroviruses" because they reverse the usual order of transcription. They contain an unusual enzyme called reverse transcriptase (RT) that catalyzes the replication of double-stranded DNA from single-stranded RNA. Not only can this retroviral DNA be incorporated into the host genome as a provirus that can be passed on to progeny cells, but some retroviruses also transform cells (make them malignant) and regulate certain host genes.

HIV and other retroviruses display structural features typical of enveloped RNA viruses **(figure 18.7*a*)**. The outermost component is a lipid envelope with transmembrane glycoprotein spikes that mediate viral adsorption to the host cell. HIV can only infect host cells that present the required receptors, which is a combination receptor consisting of the CD4 marker plus a coreceptor called CCR-5. The virus uses these receptors to gain entrance to several types of leukocytes and tissue cells **(figure 18.7*b*)**.

Pathogenesis and Virulence Factors

HIV enters a mucous membrane or the skin and travels to dendritic cells beneath the epithelium. In the dendritic cell, the virus grows and is shed from the cell without killing it. The virus is amplified by macrophages in the skin, lymph organs, bone marrow, and blood. One of the great ironies of HIV is that it infects and destroys many of the very cells needed to combat it, including the helper (T4 or CD4) class of lymphocytes, monocytes, macrophages, and even B lymphocytes. The virus is adapted to docking onto its host cell's surface receptors. It then induces viral fusion with the cell membrane and creates syncytia.

Table 18.4 illustrates the life cycle of the virus.

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3. An RN works at a clinic for patients infected with human immunodeficiency virus (HIV). He provides education about AIDS-defining illnesses (ADIs). Which of the following are characterized as ADIs? Select all that apply.

a. *Kaposi's sarcoma*
b. *Tuberculosis, caused by Mycobacterium tuberculosis*
c. *Brucellosis*
d. *Acute lymphocytic leukemia*
e. *HIV encephalopathy*

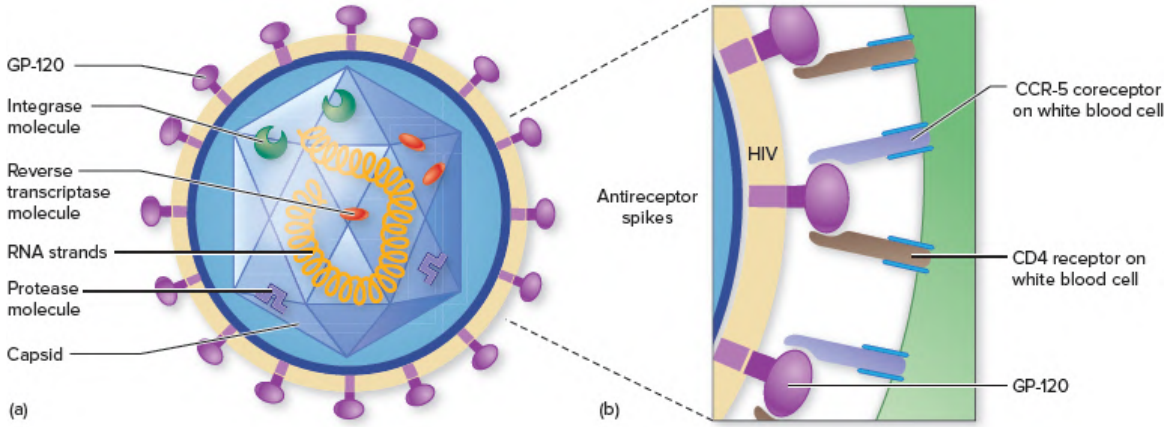
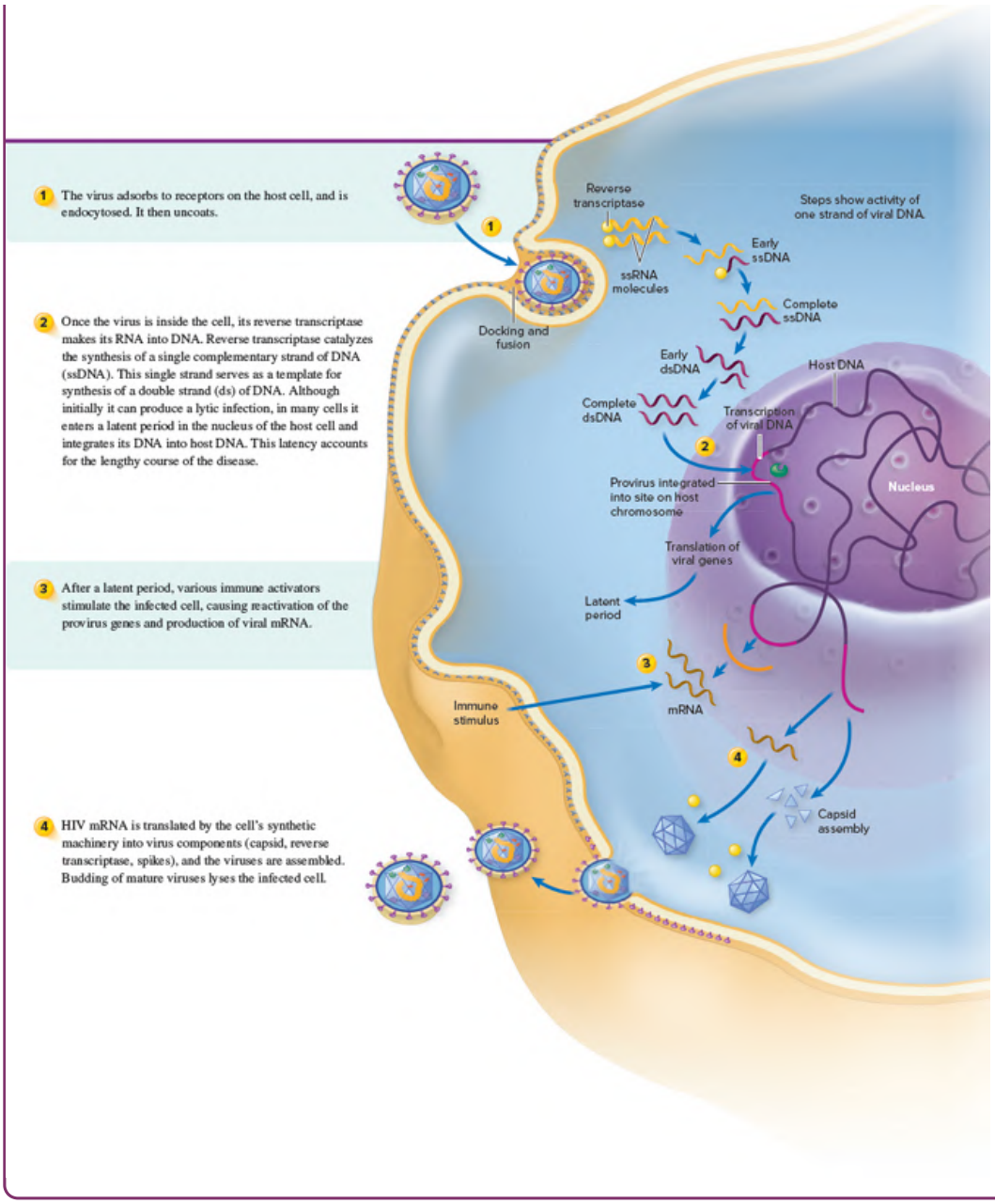


Figure 18.7 **The general structure of HIV**. **(a)** The virus consists of glycoprotein (GP) spikes in the envelope, two identical RNA strands, and several molecules of reverse transcriptase, protease, and integrase encased in a protein capsid. **(b)** The snug attachment of HIV glycoprotein molecules to their specific receptors on a human cell membrane. These receptors are CD4 and a coreceptor called CCR-5 (fusin) that permit docking with the host cell and fusion with the cell membrane.

Table 18.4 The Multiplication Cycle of HIV



Transmission

HIV transmission occurs mainly through two forms of contact: sexual intercourse and transfer of blood or blood products **(figure 18.8)**. Babies can also be infected before or during birth, as well as through breast feeding. The mode of transmission is similar to that of hepatitis B virus, except that the AIDS virus does not survive for as long outside the host and it is far more sensitive to heat and disinfectants. Additionally, HIV is not transmitted through saliva, as hepatitis B can be. Health care workers should be aware that fluids they may come in contact with during childbirth or invasive procedures can also transmit the virus. These are amniotic fluid, synovial fluid, and spinal fluid.

In 2016 scientists traced the origins of HIV in the United States. Although the epidemic did not begin until the 1980s, the virus was present in some people in the United States as early as the 1970s. The AIDS quilt was launched in the late 1980s. Each panel commemorates a single victim of the disease.
Source: Photographs in the Carol M. Highsmith Archive, Library of Congress, Prints and Photographs Division.

Semen and vaginal secretions also harbor free virus and infected white blood cells; thus, they are significant factors in sexual transmission. The virus can be isolated from urine, tears, sweat, and saliva in the laboratory—but in such small numbers that these fluids are not considered sources of infection. Because breast milk contains significant numbers of leukocytes, neonates who have escaped infection prior to and during birth can still become infected through nursing.

Epidemiology

Since the beginning of the AIDS epidemic in the early 1980s, more than 35 million people have died worldwide. The best global estimate of the number of individuals currently infected with HIV is 37 million (2015), with over 1.2 million in the United States. There is good news: Between 2005 and 2014, there has been a 19% decrease in new infections in the

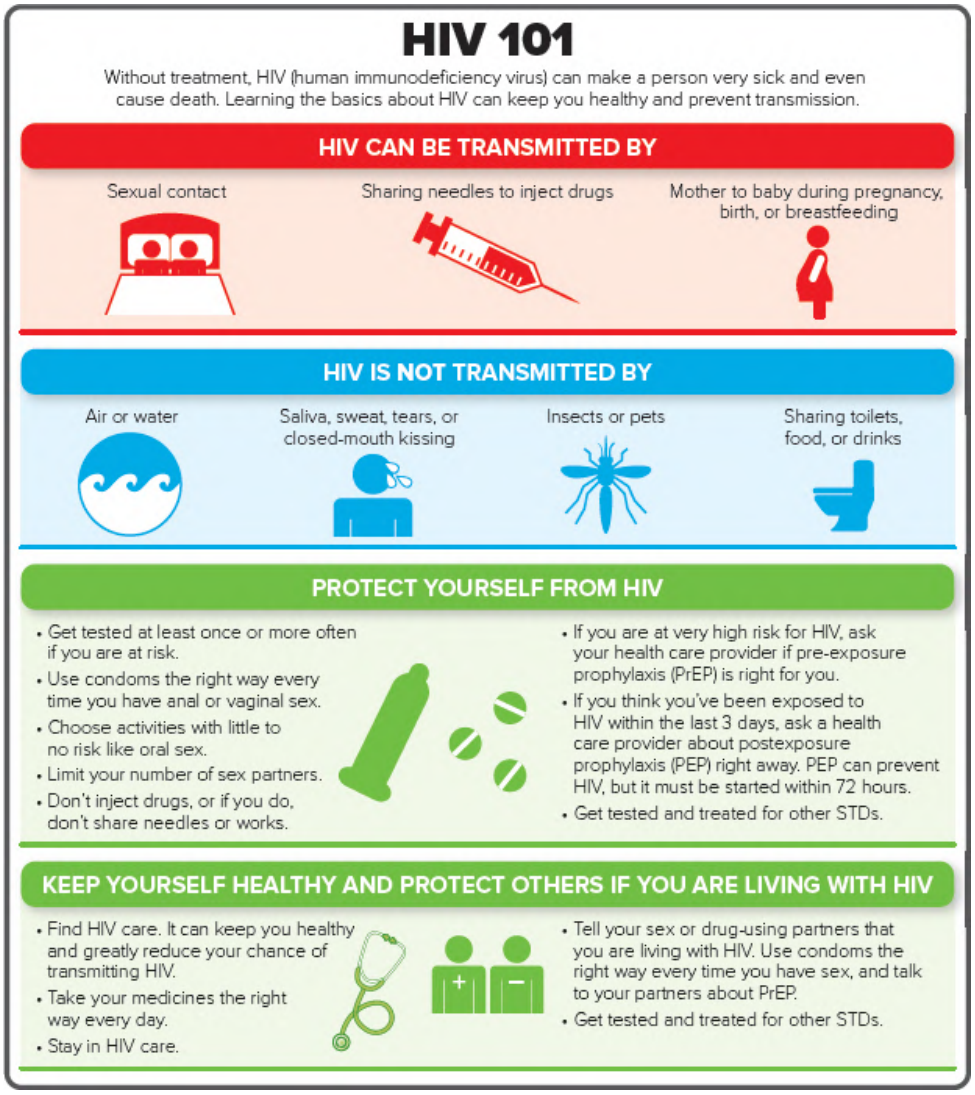


Figure 18.8 **HIV transmission**. Adapted from the Centers for Disease Control and Prevention. Source: CDC

United States. And, due to efforts of many global AIDS initiatives, many more people in the developing world are receiving lifesaving treatments.

Figure 18.9 **At-home HIV tests help increase awareness and promote early detection**.
©Kristoffer Tripplaar/Alamy Stock Photo

In most parts of the world, heterosexual intercourse is the primary mode of transmission. In the industrialized world, the overall rate of heterosexual infection has increased dramatically in the past several years, especially in adolescent and young adult women. In the United States, African-Americans have a disproportionate rate of infection. While they represent 12% of the population, they received 45% of new HIV diagnoses in 2015. Latinos/Hispanics represent 18% of the population, but accounted for 24% of the diagnoses.

We should note that not everyone who becomes infected or is antibody-positive develops AIDS. About 1% of people who are antibody-positive remain free of disease, indicating that functioning immunity to the virus can develop. Any person who remains healthy despite HIV infection is termed a *nonprogressor*. These people are the object of intense scientific study. Some have been found to lack the cytokine receptors that HIV requires. Others are infected by a weakened virus mutant.

Treatment of HIV-infected mothers with an anti-HIV drug has dramatically decreased the rate of maternal-to-infant transmission of HIV during pregnancy. Current treatment regimens result in a transmission rate of approximately 11%, with some studies of multidrug regimens claiming rates as low as 5%. Evidence suggests that giving mothers protease inhibitors can reduce the transmission rate to around 1%. (Untreated mothers pass the virus to their babies at the rate of 33%.) In 2015, Cuba became the first country to completely eliminate mother to child transmission of the virus, through comprehensive testing and treatment of mothers.

Culture and Diagnosis

A person is diagnosed as having HIV infection, as opposed to having AIDS, if he or she has tested positive for the human immunodeficiency virus. The U.S. Preventive Services Task Force recommends that all people between the ages of 13 and 64 be tested for HIV. People outside of that age group who are at high risk, as well as pregnant women, should also be tested. It is estimated that more than 153,000 people in the United States are currently infected but are unaware of it. This population is responsible for about 30% of the transmission of the virus.

Most initial testing is based on detection of antibodies specific to the virus in serum or other fluids **(Figure 18.9)**. Newer tests detect antibodies to the virus and viral antigens

simultaneously, and are also widely used.

False-negative results can occur when testing is performed too early in the infection. It takes a few weeks for antibodies to be detectable in body fluids, and the presence of viral antigen is also unpredictable in the early weeks (see table 18.2). Persons who test negative but feel they may have been exposed should be tested a second time 3 to 6 months later.

Positive tests always require follow-up with a more specific test. An antibody differentiation immunoassay determines whether the infection is with HIV-1 or HIV-2. Equivocal results are followed by an HIV nucleic acid test (NAT).

In the United States, people are diagnosed with AIDS, as opposed to HIV, if they meet the following criteria: (1) They are positive for the virus, *and* (2) they fulfill one of these additional criteria:

- They have a CD4 (helper T cell) count of fewer than 200 cells per microliter of blood.
- Their CD4 cells account for fewer than 14% of all lymphocytes.
- They experience one or more of a CDC-provided list of AIDS-defining illnesses (ADIs).

Prevention

Avoidance of sexual contact with infected persons is a cornerstone of HIV prevention. A sexually active person should consider every partner to be infected unless proven otherwise. Barrier protection (condoms) should be used when having sex with anyone whose HIV status is not known with certainty to be negative. Although

Medical Moment

Postexposure Prophylaxis

Health care workers are at risk of acquiring infectious disease from exposure to body fluids. The first line of defense is proper use of gloves and other personal protective equipment. Needles are designed with safety mechanisms to protect the user from inadvertent sticks. Despite these safety measures, exposure to patients' blood still occurs.

When a health care worker is exposed to body fluids, such as by puncture from a contaminated needle, they should seek medical attention as soon as possible. It is recommended that testing be run on the exposure source's blood to determine HIV status. Though the rate of transmission from occupational exposure is low, health care workers should be offered treatment with postexposure prophylaxis (PEP).

PEP is a 4-week antiretroviral regimen. It is effective in preventing HIV infection, though it does not offer complete protection. PEP must be started within 72 hours of exposure and the sooner treatment begins, the greater the likelihood that it will work. These medications also have some unpleasant side effects so it is important to discuss compliance with the drug regimen. Following PEP treatment, the health care worker should undergo HIV testing for 4 to 6 months after the exposure to monitor for infection.

Q. Health care workers are also vaccinated against what blood-borne virus that can be transmitted by needle sticks?

Answer in Appendix B.

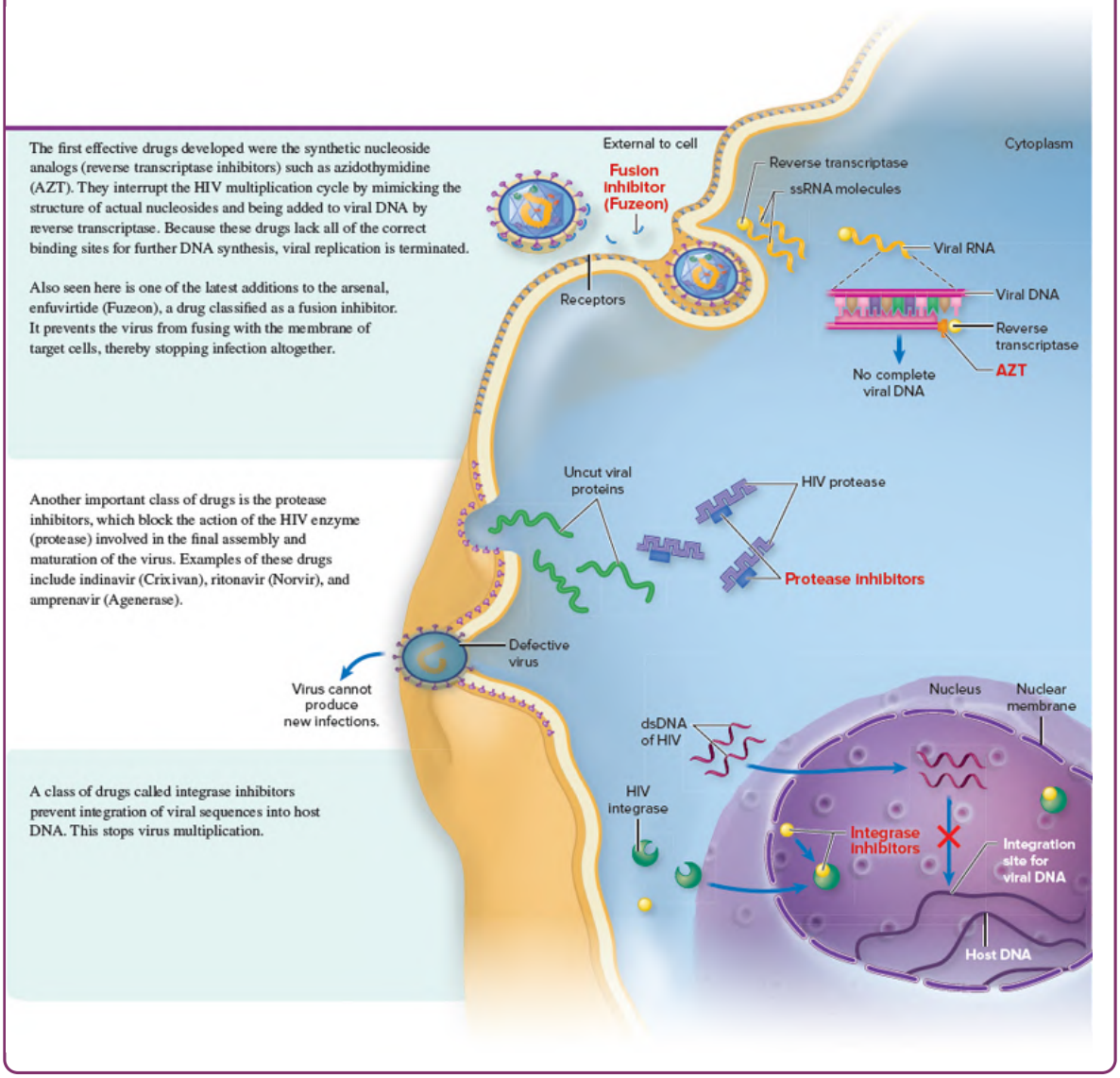
Source: cdc.gov

avoiding intravenous drugs is an important preventive measure, many drug addicts do not, or cannot, choose this option. In such cases, risk can be decreased by not sharing syringes or needles. Preexposure prophylaxis, called PrEP, is currently recommended for people who are high risk for becoming infected; for example, if their partner is positive and they are negative. It consists of a two-drug combination marketed as Truvada. It is designed to be used in combination with condoms, so that each is the fallback for the possible failure of the other.

Treatment

There is no cure for HIV. However, new drugs and new insights into their use have greatly improved the quality of life for some people living with HIV and AIDS.

Table 18.5 Mechanisms of Action of Anti-HIV Drugs



Unfortunately, much of the world is too poor to afford or properly use the latest medications. Two great challenges are (1) to make more widely available the drugs we do have and (2) to develop drugs and delivery mechanisms that are more affordable and practical.

Clear-cut guidelines exist for treating people who test HIV-positive. These guidelines are updated regularly. Treatment is referred to as antiretroviral therapy, or ART. Initially it is generally a combination of three drugs from two different classes **(table 18.5)**. It is adjusted when needed as a patient's response is monitored. The newer recommendations call for treatment to begin immediately after HIV diagnosis. In addition to antiviral chemotherapy, AIDS patients should receive a wide array of drugs to prevent or treat a variety of opportunistic infections and other ADIs such as wasting disease.

Disease Table 18.2 HIV Infection and AIDS

Causative Organism(s)	Human immunodeficiency virus 1 or 2 V
Most Common Modes of Transmission	Direct contact (sexual), parenteral (blood-borne), vertical (perinatal and via breast milk)
Virulence Factors	Attachment, syncytia formation, reverse transcriptase, high mutation rate
Culture/Diagnosis	Immunoassay to detect antibodies as well as HIV antigen
Prevention	Avoidance of contact with infected sex partner, contaminated blood, breast milk; pre-exposure prophylaxis (PrEP) for high-risk individuals
Treatment	Multiple simultaneous antiretroviral drugs
Epidemiological Features	Global infections: 37 million,2.1 million new infections in 2015. U.S. infections: approximately 1.2 million. Global deaths to date: 35 million.

Endocarditis

Endocarditis is an inflammation of the endocardium, or inner lining of the heart. Most of the time, endocarditis refers to an infection of the valves of the heart, often the mitral or aortic valve **(figure 18.10)**. Two variations of infectious endocarditis have been described: acute and subacute. Each has distinct groups of possible causative agents, primarily bacterial. On rare occasions, infections may be caused by fungi and perhaps viruses. Physical trauma can also cause such inflammation.

Patients with prosthetic valves can acquire acute endocarditis if bacteria are introduced during the surgical procedure, typically with high rates of morbidity and mortality. Alternatively, the prosthetic valves can serve as infection sites for the subacute form of endocarditis long after the surgical procedure.

Because the symptoms and the diagnostic procedures are similar for both forms of endocarditis, they are discussed first; then the specific aspects of acute and subacute endocarditis are addressed.

▶ Signs and Symptoms

The signs and symptoms are similar for both types of endocarditis, except that in the subacute condition they develop more slowly and are less pronounced than with the acute disease. Symptoms include fever, fatigue, joint pain, edema (swelling of feet, legs, and abdomen), weakness, anemia, abnormal heartbeat, and sometimes symptoms similar to myocardial infarction (heart attack), including shortness of breath or chills. Abdominal or side pain is sometimes reported. The patient may look very ill and may have petechiae (small red-to-purple

discolorations) over the upper half of the body and under the fingernails (splinter hemorrhages). Red, painless skin spots on the palms and soles (Janeway lesions) and small painful nodes on the pads of fingers and toes (Osler's nodes) may also be apparent on examination. In subacute cases, an enlarged spleen may have developed over time. Cases of extremely long duration (years) can lead to clubbed fingers and toes due to lack of oxygen in the blood.

Acute Endocarditis

Acute endocarditis is most often the result of an overwhelming bloodstream challenge with bacteria. Some of these bacteria seem to have the ability to colonize normal heart valves. Accumulations of bacteria on the valves (vegetations) hamper their function and can lead directly to cardiac malfunction and death. Alternatively, pieces of the bacterial vegetation can break off and create emboli (blockages) in vital organs. The bacterial biofilms can also provide a constant source of blood-borne bacteria, with the accompanying systemic inflammatory response and shock.

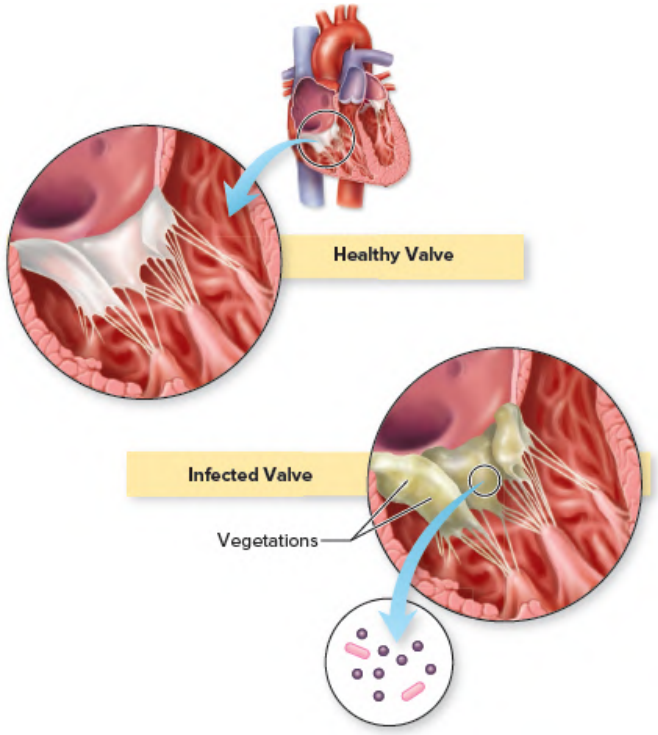


Figure 18.10 **Endocarditis**. Infected valves don't work properly.

Causative Agents

The acute form of endocarditis is most often caused by *Staphylococcus aureus*. Other agents that cause it are *Streptococcus pyogenes, Streptococcus pneumoniae*, and *Neisseria gonorrhoeae*,

as well as a host of other bacteria. Each of these bacteria is described elsewhere in this book. Physicians report that *Staphylococcus aureus* is the most dangerous cause of endocarditis.

Transmission and Epidemiology

The most common route of transmission for acute endocarditis is parenteral—that is, via direct entry into the body. Intravenous or subcutaneous drug users have been a growing risk group for the condition. Traumatic injuries and surgical procedures can also introduce the large number of bacteria required for the acute form of endocarditis. The heroin epidemic currently sweeping the United States has led to a large increase in the incidence of acute endocarditis, usually caused by *Staphylococcus aureus*. In many hospitals, on any given day, the majority of the infectious disease physician's list of consults is endocarditis secondary to injecting drug use. These patients often also suffer from epidural abscesses, caused by bacteria from the bloodstream forming abscesses along the spine.

Subacute Endocarditis

Subacute forms of this condition are almost always preceded by some form of damage to the heart valves or by congenital malformation. Irregularities in the valves encourage the attachment of bacteria, which then form biofilms and impede normal function, as well as provide an ongoing source of bacteria to the bloodstream. People who have suffered rheumatic fever and the accompanying damage to heart valves are particularly susceptible to this condition (see chapter 19 for a complete discussion of rheumatic fever).

Causative Agents

Most commonly, subacute endocarditis is caused by bacteria of low pathogenicity, often originating in the oral cavity. Alpha-hemolytic streptococci, such as *Streptococcus sanguis, S. oralis*, and *S. mutans*, are most often responsible, although normal biota from the skin and other bacteria can also colonize abnormal valves and lead to this condition.

Transmission and Epidemiology

Minor disruptions in the skin or mucous membranes, such as those induced by overly vigorous toothbrushing, dental procedures, or relatively minor cuts and lacerations, can introduce bacteria into the bloodstream and lead to valve colonization. The bacteria are not, therefore, transmitted from other people or from the environment.

Prevention

The practice of prophylactic antibiotic therapy in advance of surgical and dental procedures on patients with underlying valve irregularities has decreased the incidence of this infection.

Disease Table 18.3 Endocarditis

Disease	Acute Endocarditis B	Subacute Endocarditis B
Causative Organism(s)	*Staphylococcus aureus, Streptococcus pyogenes*, *S. pneumoniae, Neisseria gonorrhoeae*, others	Alpha-hemolytic streptococci, others
Most Common Modes of Transmission	Parenteral	Endogenous transfer of normal biota to bloodstream
Culture/Diagnosis	Blood culture	Blood culture
Prevention	Aseptic surgery, injections	Prophylactic antibiotics before invasive procedures

Disease	Acute Endocarditis B	Subacute Endocarditis B
Causative Organism(s)	*Staphylococcus aureus, Streptococcus pyogenes*, *S. pneumoniae, Neisseria gonorrhoeae*, others	Alpha-hemolytic streptococci, others
Treatment	Nafcillin or oxacillin +/− gentamicin or tobramycin OR vancomycin + gentamicin; surgery may be necessary. Be aware that several of these bacteria are on CDC "Threat" list for antibiotic resistance.	Surgery may be necessary
Distinctive Features	Acute onset, high fatality rate	Slower onset
Epidemiological Features	Three times more common in males than females—	

Septicemia (or Sepsis)

Septicemia occurs when organisms are actively multiplying in the blood. Many different bacteria (and a few fungi) can cause this condition. Patients suffering from these infections are sometimes described as "septic."

Disease Table 18.4 Septicemia

Causative Organism(s)	Bacteria or fungi, often MRSA
Most Common Modes of Transmission	Parenteral, endogenous transfer
Virulence Factors	Cell wall or membrane components
Culture/Diagnosis	Blood culture, deep sequencing
Prevention	—
Treatment	Broad-spectrum antibiotic until identification and susceptibilities tested
Epidemiological Features	In United States: 200,000 cases and 100,000 deaths per year

Signs and Symptoms

Fever is a prominent feature of septicemia. The patient appears very ill and may have an altered mental state, shaking chills, and gastrointestinal symptoms. Often an increased breathing rate is exhibited, accompanied by respiratory alkalosis (increased tissue pH due to breathing disorder). Low blood pressure is a hallmark of this condition and is caused by the inflammatory response to infectious agents in the bloodstream, which leads to a loss of fluid from the vasculature. This condition is the most dangerous feature of the disease, often culminating in death.

Causative Agents

The vast majority of septicemias are caused by bacteria, and they are approximately evenly divided between gram-positives and gram-negatives. MRSA is a very common cause. Perhaps 10% are caused by fungal infections. Polymicrobial bloodstream infections increasingly are being identified in which more than one microorganism is causing the infection.

Pathogenesis and Virulence Factors

Gram-negative bacteria multiplying in the blood release large amounts of endotoxin into the bloodstream, stimulating a massive inflammatory response mediated by a host of cytokines. This response invariably leads to a drastic drop in blood pressure, a condition called **endotoxic shock**.

Gram-positive bacteria can instigate a similar cascade of events when fragments of their cell walls are released into the blood.

Transmission and Epidemiology

In many cases, septicemias can be traced to parenteral introduction of the microorganisms via intravenous lines or surgical procedures. Other infections may arise from serious urinary tract infections or from renal, prostatic, pancreatic, or gallbladder abscesses. Patients with underlying spleen malfunction may be predisposed to multiplication of microbes in the bloodstream. Meningitis, osteomyelitis (bone infections), and pneumonia can all lead to sepsis. Hospitalization for sepsis has more than doubled in recent years. More alarming is the fact there is a 20% to 50% mortality rate, reflecting the high risks of this disease even when treatment is available. At least 200,000 cases occur each year in the United States, resulting in more than 100,000 deaths.

Culture and Diagnosis

Because the infection is in the bloodstream, a blood culture is the obvious route to diagnosis. Increasingly, deep sequencing techniques are being used for faster and more appropriate treatment. Empiric therapy should be started immediately before culture and susceptibility results are available. Also, different agents are more likely to be the causative agents in specific patient populations, such as neonates, injecting drug users, splenectomized patients, and recent international travelers.

Prevention and Treatment

Empiric therapy, which is begun immediately after blood cultures are taken, often begins with a broad-spectrum antibiotic. Once the organism is identified and its antibiotic susceptibility is known, treatment can be adjusted accordingly, as data show that rapid diagnosis and treatment are of paramount importance. **Disease Table 18.4**.

Plague

Although pandemics of plague have occurred since antiquity, the first one that was reliably chronicled killed an estimated 100 million people in the sixth century AD.

Prairie dogs in the southwest United States sometimes harbor *Yersinia pestis*.
©Corbis

Figure 18.11 A classic inguinal bubo of bubonic plague. This hard nodule is very painful and can rupture onto the surface.
Source: CDC

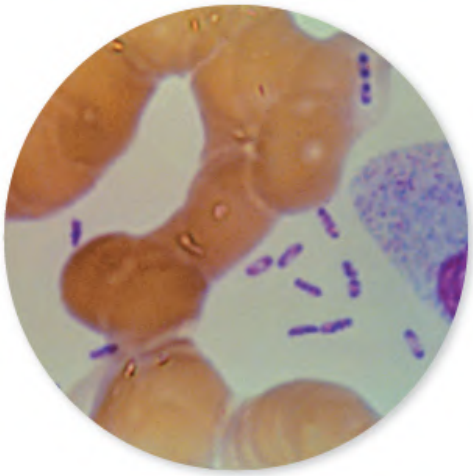


Figure 18.12 ***Yersinia pestis*****.** Note the more darkly stained poles of the bacterium, lending it a "safety pin" appearance.
©Science Source

Signs and Symptoms

Three possible manifestations of infection occur with the bacterium causing plague. **Pneumonic plague** is a respiratory disease, described in chapter 19. In **bubonic plague**, the bacterium, which is injected by the bite of a flea, enters the lymph and is filtered by a local lymph node. Infection causes inflammation and necrosis of the node, resulting in a swollen lesion called a **bubo**, usually in the groin or axilla **(figure 18.11)**. The incubation period lasts 2 to 8 days, ending abruptly with the onset of fever, chills, headache, nausea, weakness, and tenderness of the bubo. Mortality rates, even with treatment, can reach up to 15%.

These cases often progress to massive bacterial growth in the blood termed **septicemic plague**. The presence of the bacteria in the blood results in disseminated intravascular

coagulation, subcutaneous hemorrhage, and **purpura** that may degenerate into necrosis and gangrene. Mortality rates, once the disease has progressed to this point, are 30% to 50% with treatment and 100% without treatment. Because of the visible darkening of the skin, the plague has often been called the "Black Death."

Disease Table 18.5 Plague

Causative Organism(s)	*Yersinia pestis* B
Most Common Modes of Transmission	Vector, biological; also droplet contact (pneumonic) and direct contact with body fluids
Virulence Factors	Capsule, plasminogen activator
Culture/Diagnosis	Rapid genomic methods
Prevention	Flea and/or animal control; vaccine available for high-risk individuals
Treatment	Streptomycin or gentamicin
Epidemiological Features	United States: endemic in all western and southwestern states; internationally, 95% of human cases occur in Africa, including Madagascar; Category A Bioterrorism Agent

Causative Agent

The cause of this dreadful disease is a tiny gram-negative rod, *Yersinia pestis*, a member of the family *Enterobacteriaceae*. *Y. pestis* displays unusual bipolar staining that makes it look like a safety pin **(figure 18.12)**.

Pathogenesis and Virulence Factors

The number of bacteria required to initiate a plague infection is small—perhaps only 3 to 50 cells.

Transmission and Epidemiology

The plague bacterium resides in over 200 species of mammalian hosts. Some of these, such as mice and voles, serve as long-term endemic reservoirs, which are not affected by the disease. Other species, including rats and rabbits, are amplifying reservoirs, which get sick and tend to be closely connected to outbreaks of plague in humans.

The principal agents in the transmission of the plague bacterium are fleas. After a flea ingests a blood meal from an infected animal, the bacteria multiply in its gut. The bacterium promotes its spread by causing coagulation and blockage of the flea's esophagus. Being unable to feed properly, the ravenous flea jumps from animal to animal in a futile attempt to get nourishment. Regurgitated infectious material then is inoculated into the bite wound.

The distribution of plague is extensive. Although the incidence of disease has been reduced in the developed world, it has actually been increasing in Africa and other parts of the world. In the United States, sporadic cases (usually 10 to

20 per year) occur as a result of contact with wild and domestic animals. This disease is considered endemic in U.S. western and southwestern states. Persons most at risk for developing plague are veterinarians and people living and working near woodlands and forests. Dogs and cats can be infected with the plague, often from contact with infected wild animals such as prairie dogs. **Disease Table 18.5**.

Tularemia

Signs and Symptoms

After an incubation period ranging from a few days to 3 weeks, acute symptoms of headache, backache, fever, chills, coughing, and weakness appear. Further clinical manifestations are tied to the portal of entry. They include ulcerative skin lesions, swollen lymph glands, conjunctival inflammation, sore throat, intestinal disruption, and pulmonary involvement. Unfortunately, clinicians can misinterpret these signs and symptoms, delaying effective treatment. The death rate in the most serious forms of disease is 30%, but proper treatment with gentamicin or streptomycin reduces mortality to almost zero.

Causative Agent

The causative agent of tularemia is a facultative intracellular gram-negative bacterium called *Francisella tularensis*. It is a zoonotic disease of assorted mammals endemic to the Northern Hemisphere. Because it has been associated with outbreaks of disease in wild rabbits, it is sometimes called rabbit fever. It is currently listed as a Category A bioterrorism agent, along with anthrax, plague, and others.

Disease Table 18.6 Tularemia

Causative Organism(s)	*Francisella tularensis* (B)
Most Common Modes of Transmission	Biological vector (tick); also direct contact with body fluids from infected animal; airborne
Virulence Factors	Intracellular growth
Culture/Diagnosis	Culture dangerous to lab workers and not reliable; serology most often used
Prevention	Avoiding contact; postexposure prophylaxis
Treatment	Gentamicin or streptomycin
Epidemiological Features	United States: several hundred cases per year; internationally, 500,000 cases per year; Category A Bioterrorism Agent

Transmission and Epidemiology

Although rabbits and rodents (muskrats and ground squirrels) are the chief reservoirs, other wild animals (skunks, beavers, foxes, opossums) and some domestic animals are implicated as well. The chief route of transmission in the past had been through the activity of skinning rabbits, but with the decline of rabbit hunting, transmission via tick bites is more common. Ticks are the most frequent arthropod vector, followed by biting flies, mites, and mosquitoes. This has led to increased disease incidence during the summer.

With an estimated infective dose of between 10 and 50 organisms, *F. tularensis* is often considered one of the most infectious of all bacteria. Cases of tularemia have appeared in people who have accidentally run over rabbits while lawn mowing, presumably from inhaling aerosolized bacteria. In 2009, two different people in Alaska acquired tularemia after wresting infected rabbits from their dogs' mouths.

Prevention

Because the intracellular persistence of *F. tularensis* can lead to relapses, antimicrobial therapy must not be discontinued prematurely. Postexposure prophylaxis with doxycycline or ciprofloxacin can prevent the disease in lab workers or others who may have been exposed.

Laboratory workers and other occupationally exposed personnel must wear gloves, masks, and eyewear. **Disease Table 18.6**.

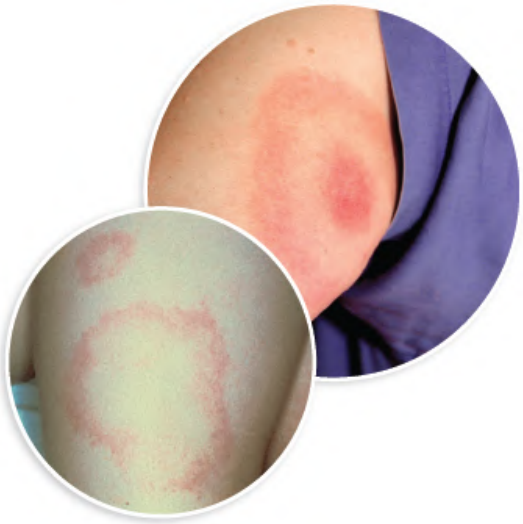


Figure 18.13 **Lesions of Lyme disease**. Two different presentations of the skin sign of Lyme disease. This obvious sign of infection does not always occur.
Source: CDC/James Gathany (arm); Source: CDC (leg)

Lyme Disease

Signs and Symptoms

Lyme disease is slow-acting, but it often evolves into a slowly progressive syndrome that mimics neuromuscular and rheumatoid conditions. An early symptom in some cases is a rash at the site of a tick bite. The lesion, called *erythema migrans*, can look something like a bull's eye, with a raised erythematous (reddish) ring that gradually spreads outward and a pale central region **(figure 18.13)**. Until recently, this lesion was thought to be the most common presentation of erythema migrans. But it only has this appearance in about 10% of cases. It can also be flat and scaly with no clear areas, or it can be pustular. It can mimic the appearance of ringworm. Clinicians now realize

Hunting and cleaning rabbits is a common cause of tularemia.
©Ingram Publishing/SuperStock (rabbit)

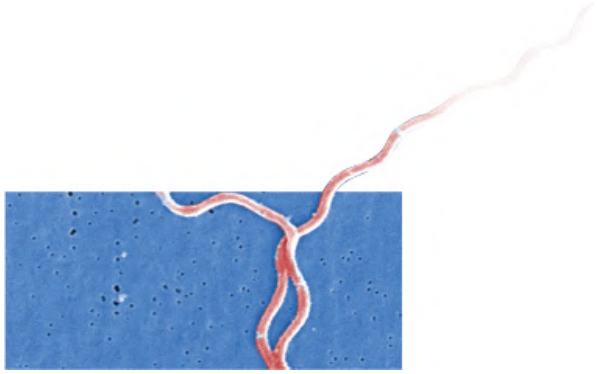


Figure 18.14 ***Borrelia*** **has 3 to 10 loose, irregular coils**.
Source: CDC/Claudia Molins

that many Lyme disease cases have been missed because of the unpredictable nature of the initial lesion. Other early symptoms are fever, headache, stiff neck, and dizziness. If not treated or if treated too late, the disease can advance to the second stage, during which cardiac and neurological symptoms, such as facial palsy, can develop. After several weeks or months, the third stage may occur. This involves a crippling arthritis; some people acquire chronic neurological complications that are severely disabling.

Causative Agent

Borrelia burgdorferi are considered the cause of Lyme disease. They are unusual spirochetes in that they are large, ranging from 0.2 to 0.5 μm in width and from 10 to 20 μm in length, and contain 3 to 10 irregularly spaced and loose coils **(figure 18.14)**.

Pathogenesis and Virulence Factors

The bacterium is a master of immune evasion. It changes its surface antigens while it is in the tick and again after it has been transmitted to a mammalian host. It provokes a strong humoral and cellular immune response, but this response is mainly ineffective, perhaps because of the bacterium's ability to switch its antigens. Indeed, it is possible that the immune response contributes to the pathology of the infection. Closely related *Borrelia burgdorferi*—like strains can also cause the disease.

Transmission and Epidemiology

B. burgdorferi is transmitted primarily by hard ticks of the genus *Ixodes*. In the northeastern part of the United States, *Ixodes scapularis* (the black-legged deer tick, **figure 18.15**) passes through a complex 2-year cycle that involves two principal hosts. In California, the transmission cycle involves *Ixodes pacificus*, another black-legged tick, and the dusky-footed woodrat as reservoir.

The greatest concentrations of Lyme disease are found in areas having large populations of both the intermediate and definitive hosts **(figure 18.16)**.

Culture and Diagnosis

Culture of the organism is not useful. Diagnosis in the early stages, if the rash is present, is usually accomplished based on symptoms and history of possible exposure to ticks, since the organism is not easily detectable at this stage. Acute and convalescent sera may be helpful. In late Lyme disease, ELISAs and/or Western blots can be used to detect antibodies to the organism in blood.

It is important to consider coinfection with *Anaplasma* or *Babesia* (see Nonhemmorhagic Fever Diseases), since these organisms are transmitted by the same kind of tick that transmits Lyme disease, and the tick may be coinfected. Lingering cases of Lyme disease may occasionally be due to failure to consider these microbes.

Prevention and Treatment

Anyone involved in outdoor activities should wear protective clothing, boots, leggings, and insect repellent containing DEET. Individuals exposed to the outdoors should routinely inspect their bodies for ticks and remove ticks gently without crushing, preferably with forceps or fingers protected with gloves, because it is possible to become infected by tick feces or body fluids.

Early, prolonged (2 weeks) treatment with doxycycline or amoxicillin is effective, and other antibiotics such as ceftriaxone and penicillin are used in late Lyme disease therapy. Unfortunately, 10% to 20% of treated patients develop a syndrome called posttreatment Lyme disease syndrome (PTLDS). This is a condition lasting weeks or months characterized by fatigue and muscle and joint pain. The medical community is still debating whether antibiotics given during this extended period improve the patient's condition. Many clinicians feel that the prolonged symptoms are lingering effects of the infection that persist after the spirochete has been eradicated by the initial course of antibiotics. **Disease Table 18.7**.

Medical Moment

Where Does the Fluid Go?

A characteristic feature of sepsis is the loss of fluid in the intravascular space, also known as "third spacing." Where is this third space? In homeostasis, fluid constantly moves between the intracellular and extracellular spaces. Intracellular fluid is found within cells. Extracellular fluid makes up approximately one-third of the body's fluid volume and includes plasma (fluid in the blood vessels) and interstitial fluid (fluid bathing body tissues and organs).

In sepsis, the capillaries become leaky, causing fluid volume to leave the blood vessels and move to the interstitial space. With depleted plasma volume, the patient experiences low blood pressure.

The rapid administration of intravenous fluid volume is often necessary to resuscitate a patient with sepsis. Medications may be started to help support the patient's blood pressure, but without adequate fluid volume in the intravascular space, these are ineffective. While treating the symptoms of capillary leak with fluid may stabilize the patient temporarily, excessive fluid loss may progress to multisystem organ failure. Timely administration of antibiotics is essential to halt this progression by treating the organism that is causing the capillary leak.

Q. What is the term for low blood pressure caused by loss of fluids in the vessels, when it is caused by a microorganism?

Answer in Appendix B.

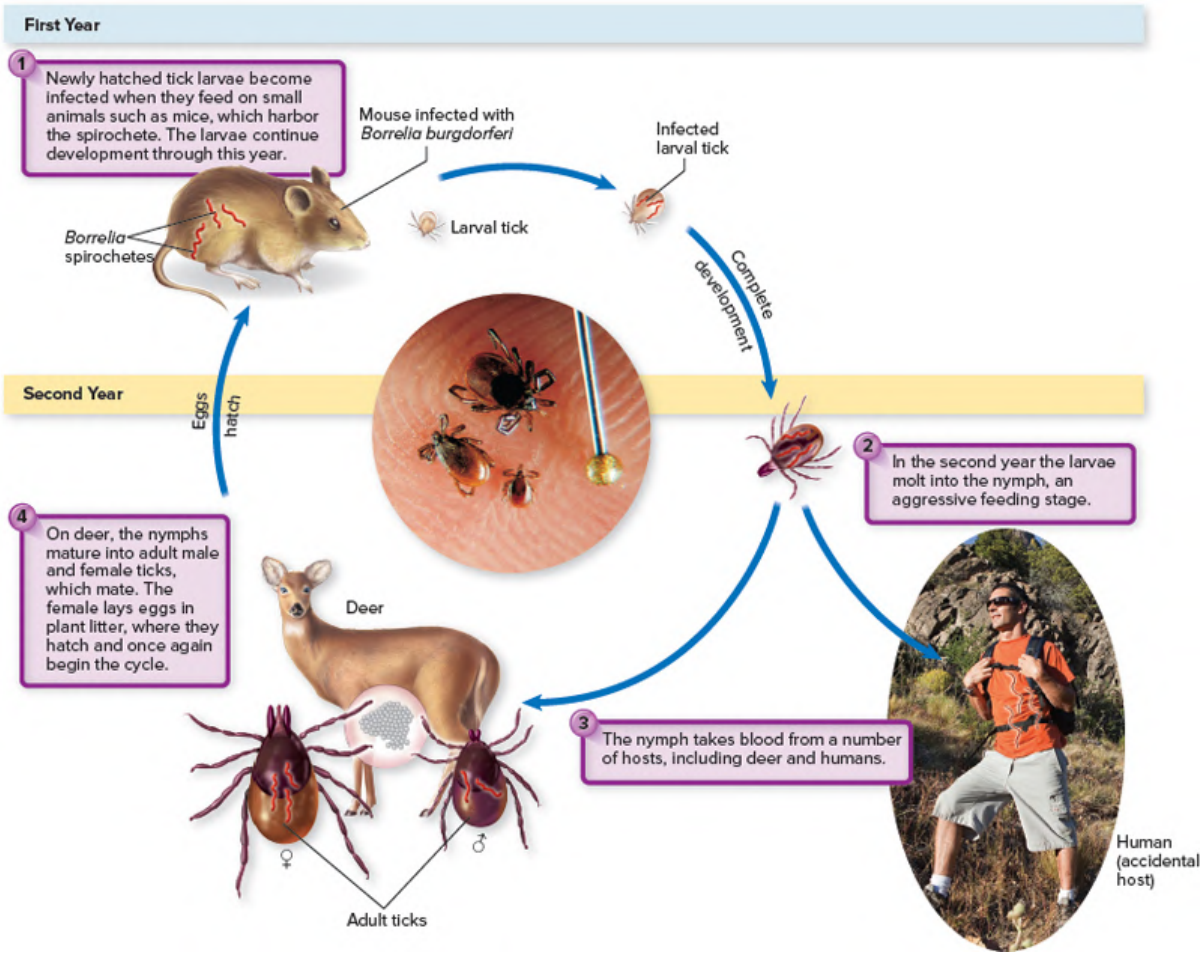


Figure 18.15 **The cycle of Lyme disease in the northeastern United States**. The disease is tied intimately into the life cycle of a tick vector, which generally is completed over a 2-year period. The exact hosts and species of tick vary from region to region but still display this basic pattern. The photograph in the center gives an idea of the actual size and proportion of the nymph and adult black-legged deer ticks displayed on a human finger. Many people may not realize how very small and difficult to detect the feeding nymph can be.

©Scott Camazine/Science Source (ticks); ©Image Source (hiker)

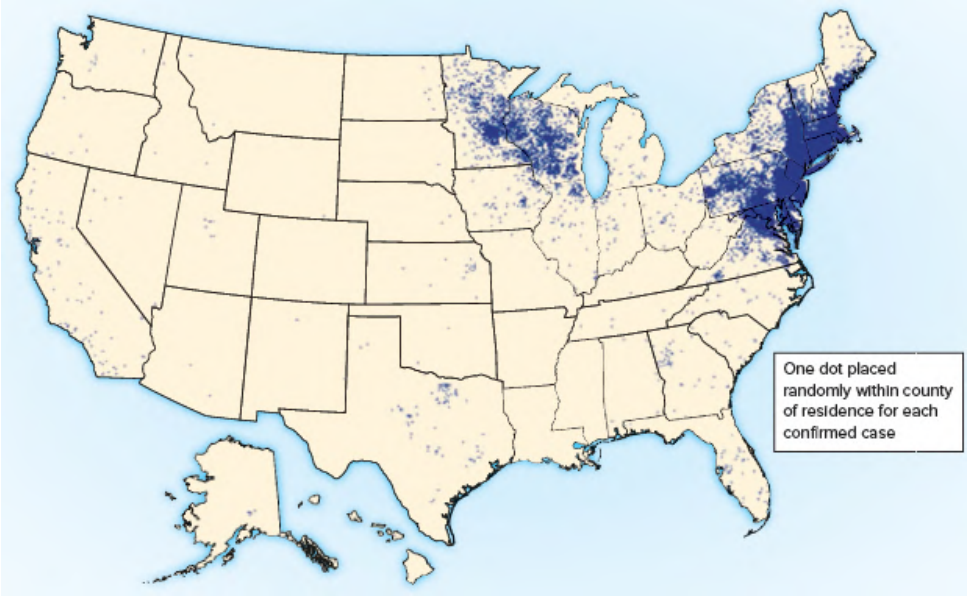


Figure 18.16 **A map of Lyme disease in the United States.**

Disease Table 18.7 Lyme Disease

Causative Organism(s)	*Borrelia burgdorferi* and closely related species (B)
Most Common Modes of Transmission	Biological vector (tick)
Virulence Factors	Antigenic shifting, adhesins
Culture/Diagnosis	ELISA for Ab, Western blot
Prevention	Tick avoidance
Treatment	Doxycycline and/or amoxicillin (2 weeks), also cephalosporins and penicillin
Epidemiological Features	Endemic in North America, Europe, and Asia; approximately 300,000 new cases/year in the United States; only 10% diagnosed/reported

Infectious Mononucleosis

This lymphatic system disease, which is often simply called "mono" or the "kissing disease," can be caused by a number of bacteria or viruses, but the vast majority of cases are caused by the **Epstein-Barr virus (EBV)**, a member of the herpesvirus family.

▶ Signs and Symptoms

The symptoms of mononucleosis are sore throat, high fever, and cervical lymphadenopathy, which develop after a long incubation period (30 to 50 days). Many patients also have a gray-white exudate in the throat, a skin rash, and enlarged spleen and liver. A notable sign of mononucleosis is sudden leukocytosis (an increase in number of white blood cells), consisting initially of infected B cells and later T cells. Fatigue is a hallmark of the disease. Patients remain fatigued for a period of weeks. During that time, they are advised not to engage in strenuous activity due to the possibility of injuring their enlarged spleen (or liver).

Eventually, the strong, cell-mediated immune response is decisive in controlling the infection and preventing complications. But after recovery, people usually remain chronically infected with

EBV.

Transmission and Epidemiology

More than 90% of the world's population is infected with EBV. In general, the virus causes no noticeable symptoms, but the time of life when the virus is first encountered seems to matter. In the case of EBV, infection during the teen years results in disease about 25% of the time, whereas infection before or after this period is usually asymptomatic.

Direct oral contact and contamination with saliva are the principal modes of transmission, although transfer through blood transfusions, sexual contact, and organ transplants is possible.

Prevention and Treatment

The usual treatments for infectious mononucleosis are directed at symptomatic relief of fever and sore throat. Hospitalization is rarely needed. Occasionally, rupture of the spleen necessitates immediate surgery to remove it.

Disease Table 18.8 Infectious Mononucleosis

Causative Organism(s)	Epstein-Barr virus (EBV) V
Most Common Modes of Transmission	Direct, indirect contact; parenteral
Virulence Factors	Latency, ability to incorporate into host DNA
Culture/Diagnosis	Differential blood count, monospot test for heterophile antibody, specific ELISA
Prevention	—
Treatment	Supportive
Distinctive Features	Most common in teens
Epidemiological Features	United States: 500 cases per 100,000 per year

Hemorrhagic Fever Diseases

A number of agents that infect the blood and lymphatics cause extreme fevers, some of which are accompanied by internal hemorrhaging. The presence of the virus in the bloodstream causes capillary fragility and disrupts the blood-clotting system, which leads to various degrees of pathology, including death. All of these viruses are RNA enveloped viruses, the distribution of which is restricted to their natural host's distribution.

The fast-spreading Ebola epidemic of 2014 demonstrates that diseases that may get minimal amounts of space in a textbook can suddenly become worldwide threats. While sporadic infections are still occurring, the Ebola epidemic has been contained through the often heroic efforts of medical professionals in Africa and many from around the world to help fight the disease. This was the biggest Ebola outbreak ever, and **figure 18.17** illustrates the quick and thorough response that brought the epidemic to an end. See also the Medical Moment about Ebola for specifics about the epidemic.

The diseases yellow fever, chikungunya, and dengue fever are all notable for the fact that they are spread by the *Aedes* genus of mosquito. All three diseases are common in South America and Africa; and dengue fever has exploded into the Americas in recent years. One of the highlight diseases in chapter 17 is Zika virus disease. Zika is also transmitted by the *Aedes* mosquito. When we discussed that disease we mentioned that Zika virus spread rapidly through the Americas, and continues to do so. **Figure 18.18** depicts the likely habitat of one common *Aedes* species, *Aedes*

aegypti. The vector *A. aegypti* used to be everywhere in the United States, bringing with it multiple diseases,

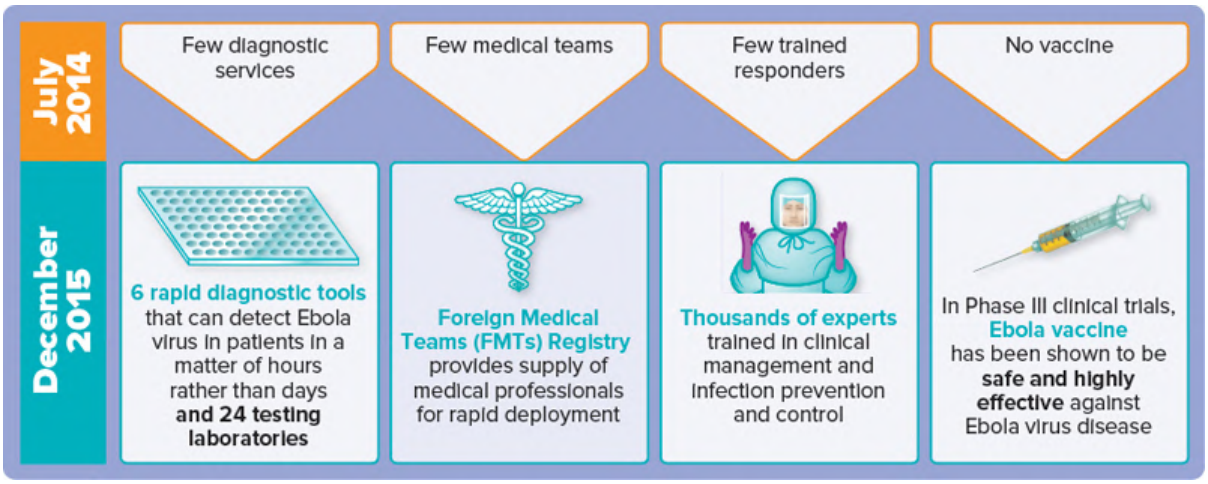


Figure 18.17 **Quick progress during the Ebola epidemic of 2014–2015**.
Source: WHO

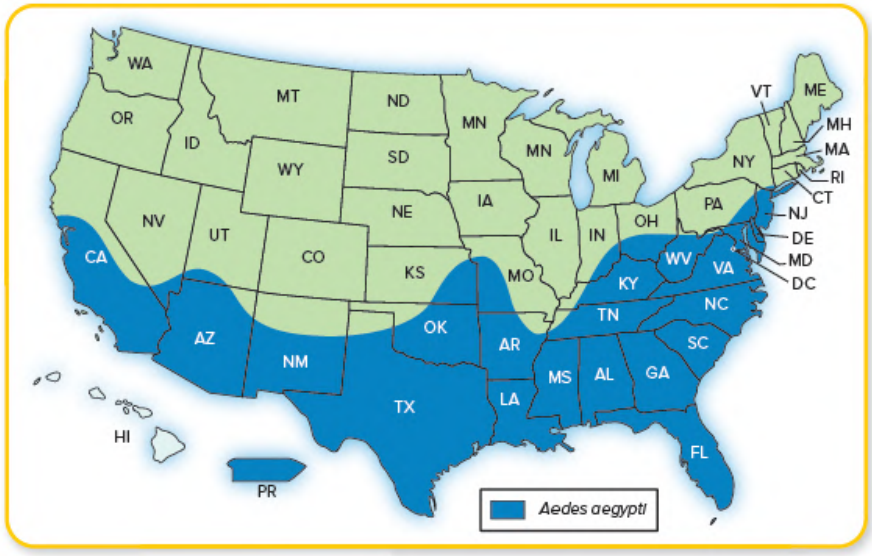


Figure 18.18 **Estimated range in United States of *Aedes aegypti* in 2016**.
Source: CDC

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4. A patient presents to his primary care provider with a tick bite, surrounded by an erythematic rash. He has been experiencing fever, lethargy, and headache. The nurse provides education for the patient regarding Lyme disease. Which of the following statements by the patient demonstrates a proper understanding of the teaching?

a. *"It is important that I take my antiviral medications as prescribed."*
b. *"My family is at risk of contracting Lyme disease from me."*

c. *"If the disease progresses, I may develop a facial droop."*
d. *"Lyme disease is preventable with a vaccination."*

notably malaria, but it was largely eliminated in temperate regions in the 1800s. Now it is back, as temperatures have allowed it into more northern regions. Changing temperatures inevitably lead to new disease patterns. By comparing the map in figure 18.18 to the map in figure 17.10, you can see the spread of the mosquito in one year.

In chapter 17 we also mentioned using sterile male mosquitoes to limit the transmission of Zika virus. There is a similar strategy being deployed against dengue. Currently in several places in the world, trials are being conducted in which male *A. aegypti* mosquitoes infected with the bacterium *Wolbachia* are being released in areas with heavy dengue burden. When these males mate with females, the resultant eggs are not delivered. Then the mosquito population is greatly reduced or

Medical Moment

Ebola Epidemic

Prior to 2014, sporadic small-scale outbreaks of Ebola had occurred from time to time in rural areas in Africa. Because the population density was low in those places, when health care workers descended into these areas to care for patients and prevent the spread of the disease, the outbreaks remained contained. In 2014 that all changed. The virus exploded in crowded urban areas of Liberia, Guinea, and Sierra Leone. In all, more than 28,000 people were infected, and more than 11,000 of them died. The epidemic hit the shores of North America when one man ill with the disease traveled to Dallas, Texas, and two health care workers who cared for him contracted the disease. An additional health care worker, an American volunteering with the organization Doctors Without Borders, returned from Africa, ill with the disease.

Source: CDC/Dr. Heidi Soeters

Ebola is an excellent example of the concepts of *communicability* and *deadliness* that we are illustrating in each disease chapter in this text. If you look at that figure at the end of this chapter you will see that Ebola is, indeed, high on the deadliness scale. Perhaps surprisingly, though, it is low on the communicability scale, despite how easily it seemed to spread during the epidemic. Most transmission occurs when a patient is in the final stages of the disease and hemorrhaging virus-filled fluids heavily. So caregivers at the bedside are at highest risk. Preparing bodies for burial is also a risky activity, which is why one of the first things public health officials do in an epidemic situation is to manage the handling and burial of corpses.

Q. Look at the "deadliness" of Ebola in the feature at the end of this chapter. Based on the largest epidemic to date (in 2014–2015), do you think the case fatality rate of Ebola might be revised?

Answer in Appendix B.

eliminated. This is viewed as a much better approach than killing mosquitoes with insecticide, a technique we have relied on for decades. Mosquitoes, like bacteria, acquire resistance to chemicals that are initially lethal to them.

Here is a quick run-down on the hemorrhagic fever diseases. Note that chikungunya and dengue fever have now spread to the Americas.

Yellow Fever Virus:	Endemic in Africa and South America; more frequent in rainy climates.	Carried by *Aedes* mosquitoes.
Dengue Fever:	Endemic in southeast Asia and Africa; epidemics have occurred in South America and Central America, and the Caribbean.	Carried by *Aedes* mosquitoes.
Chikungunya:	Endemic in Africa; arrived in Central America in 2013 and in U.S. and Europe in 2014; went from zero to over 1.7 million cases in the Americas in 3 years.	Carried by *Aedes* mosquitoes.
Ebola and Marburg	Endemic to Africa; capillary fragility is extreme and patients can bleed from their orifices and mucous membranes.	Bats thought to be natural reservoir

Fevers:		of Ebola.
Lassa Fever:	Endemic to West Africa. Asymptomatic in 80% of cases. In others, severe symptoms develop.	Reservoir of virus is multimammate rat.

These diseases are summarized in **Disease Table 18.9**.

Nonhemorrhagic Fever Diseases

In this section, we examine some infectious diseases that result in a syndrome characterized by high fever but without the capillary fragility that leads to hemorrhagic symptoms. All of the diseases in this section, except for Babesiosis, are caused by bacteria.

Brucellosis

This common disease goes by several other names, including Malta fever, undulant fever, and Bang's disease. Brucellosis often causes severe outbreaks of placental infections in livestock, which result in devastating economic impacts. The potential economic impact is one reason the CDC lists it as a possible bioterrorism agent.

▶ Signs and Symptoms

The *Brucella* bacteria responsible for this disease live in phagocytic cells. These cells carry the bacteria into the bloodstream, creating focal lesions in the liver, spleen, bone marrow, and kidney. The cardinal manifestation of human brucellosis is a fluctuating pattern of fever, which is the origin of the name *undulant fever*.

▶ Causative Agent

The bacterial genus *Brucella* contains tiny, aerobic, gram-negative coccobacilli. Several species can cause this disease in humans: *B. melitensis, B. abortus*, and *B. suis*. Even though a principal manifestation of the disease in animals is an infection of the placenta and fetus, human placentas do not become infected.

▶ Pathogenesis and Virulence Factors

Brucella enters through damaged skin or via mucous membranes of the digestive tract, conjunctiva, and respiratory tract. From there it is taken up by phagocytic cells. Because it is able to avoid destruction in the phagocytes, the bacterium is transported easily through the bloodstream and to various organs, such as the liver, kidney, breast tissue, or joints. Scientists suspect that the up-and-down nature of the fever is related to unusual properties of the bacterial lipopolysaccharide.

Q Fever

The name of this disease arose from the frustration created by not being able to identify its cause. The Q stands for "query." Its cause, a bacterium called *Coxiella burnetii*, was finally identified in the mid-1900s. The clinical manifestations of acute Q fever are abrupt onset of fever, chills, head and muscle ache, and, occasionally, a rash. The disease is sometimes complicated by pneumonitis

(30% of cases), hepatitis, and endocarditis. About a quarter of the cases are chronic rather than acute and result in vascular damage and endocarditis-like symptoms.

C. burnetii is a very small pleomorphic (variously shaped) gram-negative bacterium, and for a time it was considered a rickettsia, in the same genus as the bacterium that causes Rocky Mountain spotted fever (below). *C. burnetii* is apparently harbored by a wide assortment of vertebrates and arthropods, especially ticks, which play an essential role in transmission between wild and domestic animals. Ticks do not transmit the disease to humans, however. Humans acquire infection largely by means of environmental contamination and airborne spread. Birth products, such as placentas, of infected domestic animals contain large numbers of bacteria. Other sources of infectious material include urine, feces, milk, and airborne particles from infected animals. The primary portals of entry are the lungs, skin, conjunctiva, and gastrointestinal tract.

People at highest risk are farm workers, meat cutters, veterinarians, laboratory technicians, and consumers of raw milk products.

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Disease Table 18.9 Hemorrhagic Fevers

Disease	Yellow Fever	Dengue Fever	Chikungunya	Ebola and/or Marburg	Lassa Fever
Causative Organism(s)	Yellow fever virus (V)	Dengue fever virus (V)	Chikungunya virus (V)	Ebola virus, Marburg virus (V)	Lassa fever virus (V)

Disease	Yellow Fever	Dengue Fever	Chikungunya	Ebola and/or Marburg	Lassa Fever
Most Common Modes of Transmission	Biological vector; (*Aedes* mosquito)	Biological vector; (*Aedes* mosquito)	Biological vector; (*Aedes* mosquito)	Direct contact, body fluids	Droplet contact (aerosolized rodent excretions), direct contact with infected fluids
Virulence Factors	Disruption of clotting factors	Disruption of clotting factors	Disruption of clotting factors	Disruption of clotting factors	Disruption of clotting factors
Culture/Diagnosis	ELISA, PCR	Rise in IgM titers	PCR	PCR, viral culture (conducted at CDC)	ELISA
Prevention	Live attenuated vaccine available	—	—	New Ebola vaccine suitable for epidemic situations tested successfully in 2016	Avoiding rats, safe food storage
Treatment	Supportive	Supportive	Supportive	Supportive	Ribavirin
Distinctive Features	Accompanied by jaundice	"Breakbone fever"—so named due to severe pain in some forms	Arthritic symptoms	Massive hemorrhage; rash sometimes present	Chest pain, deafness as long-term sequelae
Epidemiological Features	United States: only sporadic cases in travelers; internationally, 200,000 cases annually, 30,000 deaths; 90% of cases in Africa	United States: only sporadic cases, small outbreaks. Most cases in Puerto Rico, the U.S. Virgin Islands, Samoa, and Guam; internationally, 50–300 million people infected every year and tens of thousands of deaths occur, mostly among children; Category A Bioterrorism Agent	First local transmission in United States in 2014; has exploded in the Americas since its arrival in 2013 with an estimated 1.7 million suspected cases	United States: only imported infections; internationally, sporadic outbreaks in Africa; major Ebola outbreak 2014–2016; Category A Bioterrorism Agent	United States: no reported cases; internationally, estimated 100,000–300,000 cases annually in West Africa; Category A Bioterrorism Agent

Cat-Scratch Disease

This disease is one of a group of diseases caused by different species of the small gram-negative rod *Bartonella. Bartonella* species are considered to be emerging pathogens. They are fastidious but not obligate intracellular parasites, so they will grow on blood agar. Two other species of *Bartonella* cause diseases known as trench fever and Carrión's disease, but cat-scratch fever is most common.

B. henselae is the agent of cat-scratch disease (CSD), an infection connected with being clawed or bitten by a cat. It is transmitted among cats by fleas. The pathogen is present in over 40% of cats, especially kittens. There are approximately 25,000 cases per year in the United States, 80% of them in children 2 to 14 years old. The symptoms start after 1 to 2 weeks, with a cluster of small papules at the site of inoculation. Most infections remain localized and resolve in a few weeks, but drugs such as azithromycin, erythromycin, and rifampin can be effective therapies. The disease can be prevented by flea control and by thorough antiseptic cleansing of a cat bite or scratch.

Ehrlichiosis

Ehrlichia is a small intracellular bacterium with a strict parasitic existence and association with ticks (*Ixodes* species). The species of tick varies with the geographic location in the United States and Europe. Its distribution can be seen in **figure 18.19**. The signs and symptoms include an acute febrile state resulting in headache, muscle

pain, and rigors. Most patients recover rapidly with no lasting effects, but about 5% of older, chronically ill patients can die.

Rapid diagnosis is done through PCR tests and indirect fluorescent antibody tests. It can be critical to differentiate or detect coinfection with Lyme disease *Borrelia*, which is carried by the same tick. Doxycycline will clear up most infections within 7 to 10 days.

Anaplasmosis

Anaplasma is another small intracellular bacterium. It shares lifestyle characteristics with *Ehrlichia* and causes nearly identical clinical manifestations. But the two bacteria have important differences in geographic distributions (see figure 18.19) and are carried by two different species of ticks. Treatment of anaplasmosis is also with doxycycline.

Babesiosis

Babesia is a protozoan that infects red blood cells. It produces similar symptoms to *Ehrlichia* and *Anaplasma*. It is also carried by ticks. It is found in the upper Great Lakes region as well as in the northeast United States. It is often diagnosed via a blood smear; the protozoan is visible inside red blood cells **(figure 18.20)**.

Since it is a protozoan, treatment is different than for *Ehrlichia* and *Anaplasma*. Combined therapy of either atovaquone (an antiprotozoal) + azithromycin, or clindamycin + quinine (another antiprotozoal) is recommended.

Rocky Mountain Spotted Fever (RMSF)

This disease is named for the region in which it was first detected in the United States—the Rocky Mountains of Montana and Idaho. In spite of its name, the disease occurs infrequently in the western United States. The majority of cases are concentrated in the Southeast and Eastern Seaboard regions. It also occurs in Canada and Central and South America. Infections occur most frequently in the spring and summer, when the tick vector is most active. The yearly rate of RMSF is 20 to 40 cases per 10,000 population, with fluctuations coinciding with weather and tick infestations.

RMSF is caused by a bacterium called *Rickettsia rickettsii* transmitted by hard ticks such as the wood tick, the American dog tick, and the Lone Star tick. The dog tick is probably most responsible for transmission to humans because it is the major vector in the southeastern United States.

After 2 to 4 days of incubation, the first symptoms are sustained fever, chills, headache, and muscular pain. A distinctive spotted rash usually comes on within 2 to 4 days after the prodrome **(figure 18.21)**, which usually appears first on the wrists, forearms, and ankles, before spreading. Early lesions are slightly mottled like measles, but later ones can change shape to look like other

types of rashes. In the most severe untreated cases, the enlarged lesions merge and can become necrotic, predisposing to gangrene of the toes or fingertips.

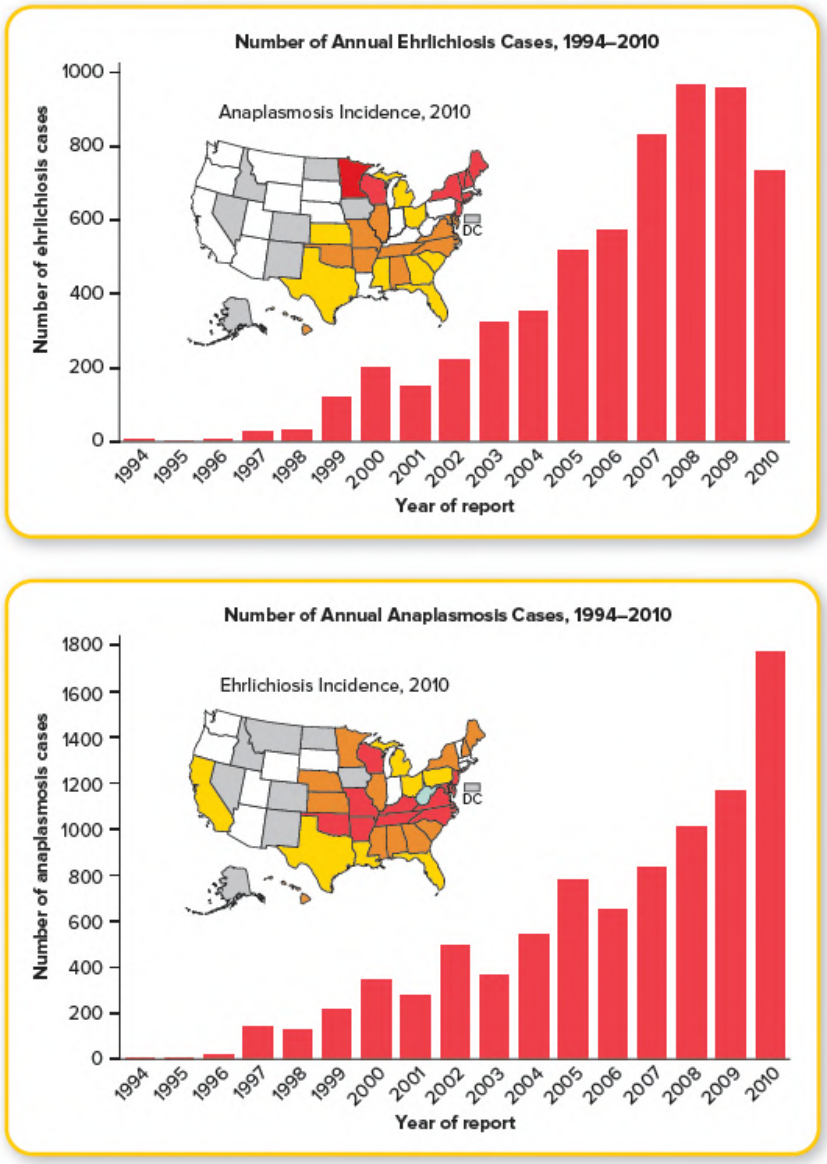

Figure 18.19 **Number of annual anaplasmosis and ehrlichiosis cases in the United States from 1994–2010.**
Source: CDC

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5. Early lesions in Rocky Mountain spotted fever may resemble the rash of
- **a.** *measles*.
- **b.** *chickenpox*.
- **c.** *rubella*.
- **d.** *Lyme disease*.

Although the spots are the most obvious symptom of the disease, the most serious manifestations are cardiovascular disruption, including hypotension, thrombosis, and hemorrhage. Conditions of restlessness, delirium, convulsions, tremor, and coma are signs of the often overwhelming effects on the central nervous system. Fatalities occur in an average of 20% of untreated cases and 5% to 10% of treated cases. **Disease Table 18.10**.

Chagas Disease

Chagas disease is sometimes called "the American trypanosomiasis." The causative agent is the flagellated protozoan *Trypanosoma cruzi*. A different trypanosome, *T. brucei*, causes sleeping sickness on the African continent. Chagas disease has been called "the new AIDS of the Americas," because it has a long incubation time and is very difficult to cure.

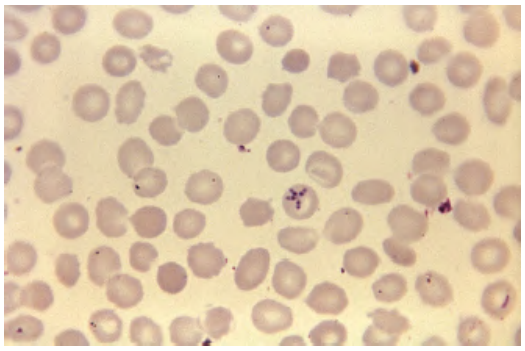

Figure 18.20 ***Babesia*** **cells inside red blood cells**.
Source: CDC

Signs and Symptoms

Once the trypanosomes are transmitted by a group of insects called the triatomines **(figure 18.22)**, they multiply in muscle and blood cells. From time to time, the blood cells rupture and large numbers of trypanosomes are released into the bloodstream. The disease manifestations are divided into acute and chronic phases. Soon after infection, the acute phase begins. Symptoms are relatively nondescript and range from mild to severe fever, nausea, and fatigue. A swelling called a "chagoma" at the site of the bug bite may be present. If the bug bite is close to the eyes, a distinct condition called Romana's sign, swelling of the eyelids, may appear. The acute phase lasts for weeks or months after which the condition becomes chronic, which is virtually asymptomatic for a period of years or indefinitely. Eventually, the trypanosomes are found in numerous sites around the body; in later years, this may lead to inflammation and disruption of function in organs such as the heart, the brain, and the intestinal tract.

Figure 18.21 **The rash in RMSF**. This case occurred in a child several days after the onset of fever.
Source: CDC (hand); ©PhotoLink/Getty Images (tick)

Transmission and Epidemiology

Estimates put the prevalence of this disease at 8 million people, 300,000 of whom live in the United States. Chagas disease is one of the five "Neglected Parasitic Infections" in the United States as designated by the CDC (see Medical Moment in chapter 17). Most U.S. cases were acquired in an endemic area by travelers or other people who have since immigrated to this country.

As already noted, the disease is transmitted through the bite of a triatomine bug. The trypanosome can also be transmitted vertically because it crosses the placenta, and via blood transfusion with infected blood. Argentina, Brazil, and Mexico are the top three countries in terms of numbers of cases. Recently, the United States began screening all donated blood for this disease.

Prevention

No vaccine exists for Chagas disease. In endemic areas, pesticides and improved building materials in houses are used to minimize the presence of the bug.

Treatment

Treatment is most successful if begun during the acute phase. However, it is often not accomplished because the acute phase of the disease is not necessarily suggestive of Chagas. Drugs for treatment are only available through the CDC. During the chronic phase of the disease, symptomatic treatment of cardiac and other problems may be indicated. **Disease Table 18.11**.

Anthrax

Anthrax causes disease in the lungs and in the skin, and is addressed elsewhere in this book. We discuss anthrax in this chapter because it multiplies in large numbers in the blood and because

septicemic anthrax is a possible outcome of all forms of anthrax.

Figure 18.22 **A representative triatomine bug, the carrier of *T. cruzi*.**
©The Natural History Museum/The Image Works

Disease Table 18.10 Nonhemorrhagic Fever Diseases

Disease	Brucellosis	Q Fever	Cat-Scratch Disease	Ehrlichiosis	Anaplasmosis	Babesiosis	Rocky Mountain Spotted Fever
Causative Organism(s)	*Brucella* species B G—	*Coxiella burnetii* B G —	*Bartonella henselae* B G —	*Ehrlichia* species B G —	*Anaplasma* species B G —	*Babesia* species P G —	*Rickettsia rickettsii* P G—
Most Common Modes of Transmission	Direct contact, airborne, parenteral (needlesticks)	Airborne, direct contact, foodborne	Parenteral (cat scratch or bite)	Biological vector (tick)	Biological vector (tick)	Biological vector (tick)	Biological vector (tick)
Virulence Factors	Intracellular growth; avoidance of destruction by phagocytes	Endospore-like structure	Endotoxin	—	—	—	Induces apoptosis in cells lining blood vessels
Culture/Diagnosis	Gram stain of biopsy material	Serological tests for antibody	Biopsy of lymph nodes plus Gram staining; ELISA (performed by CDC)	PCR, indirect antibody test	PCR, indirect antibody	Blood smear	Fluorescent antibody, PCR
Prevention	Animal control, pasteurization of milk	Vaccine for high-risk population	Clean wound sites, control fleas	Avoid ticks	Avoid ticks	Avoid ticks	Avoid ticks
Treatment	Doxycycline plus gentamicin or streptomycin	Doxycycline	Azithromycin	Doxycycline	Doxycycline	Combination therapy with antibacterial + antiprotozoal	Doxycycline

Disease	Brucellosis	Q Fever	Cat-Scratch Disease	Ehrlichiosis	Anaplasmosis	Babesiosis	Rocky Mountain Spotted Fever
Distinctive Features	Undulating fever, muscle aches	Airborne route of transmission, variable disease presentation	History of cat bite or scratch; fever not always present	Southeast, south central United States	Upper Midwest and northeastern United States	Northeastern and upper midwestern United States	Most common in eastern and southeastern United States
Epidemiological Features	United States: fewer than 100 cases per year; internationally, 500,000 cases per year	United States: number of cases increased from 21 in 1999 to 176 in 2014	United States: estimated incidence is 9.3 cases per 100,000; internationally, seroprevalence from 0.6% to 37% depending on cat population	Great increase in incidence since mid-1990s	Great increase in incidence since mid-1990s	—	Only in Americas

For centuries, anthrax has been known as a zoonotic disease of herbivorous livestock (sheep, cattle, and goats). It has an important place in the history of medical microbiology because Robert Koch used anthrax as a model for developing his postulates in 1877 and later, Louis Pasteur used the disease to prove the usefulness of vaccination.

Signs and Symptoms

As just noted, anthrax infection can exhibit its primary symptoms in various locations of the body: on the skin (cutaneous anthrax), in the lungs (pulmonary anthrax), in the gastrointestinal tract (acquired through ingestion of contaminated foods), and

Disease Table 18.11 Chagas Disease

Causative Organism	*Trypanosoma cruzi* (P)
Most Common Modes of Transmission	Biological vector (triatomine bug), vertical
Virulence Factors	Antioxidant enzymes, co-opting host antigens; induces autoimmunity
Culture/Diagnosis	Blood smear in acute phase; serological methods in later stages
Prevention	Consult CDC
Epidemiological Features	Endemic in Central and South America; 300,000 cases present in United States

in the central nervous system (anthrax meningitis). The cutaneous and pulmonary forms of the disease are the most common. In all of these forms, the anthrax bacterium gains access to the bloodstream, and death, if it occurs, is usually a result of an overwhelming septicemia. Pulmonary anthrax—and the accompanying pulmonary edema and hemorrhagic lung symptoms—can sometimes be the primary cause of death, although it is difficult to separate the effects of septicemia from the effects of pulmonary infection.

In addition to symptoms specific to the site of infection, septicemic anthrax results in headache, fever, and malaise. Bleeding in the intestine and from mucous membranes and orifices may occur in late stages of septicemia.

Causative Agent

Bacillus anthracis is a gram-positive endospore-forming rod that is among the largest of all bacterial pathogens. It is composed of block-shaped, angular rods 3 to 5 µm long and 1 to 1.2 µm wide. Central endospores develop under all growth conditions except in the living body of the host **(figure 18.23)**. Because the primary habitat of many *Bacillus* species, including *B. anthracis*, is the soil, endospores are continuously dispersed by means of dust into water and onto the bodies of plants and animals.

Pathogenesis and Virulence Factors

The main virulence factors of *B. anthracis* are its polypeptide capsule and what is referred to as a "tripartite" toxin—a protein complex composed of three separate exotoxins. The end result of exotoxin action is massive inflammation and initiation of shock. Clinicians have noticed over time that some people experience noticeably lighter symptoms than others when infected with *B. anthracis*. Researchers have discovered that some people have a gene that codes for a surface protein that makes it more difficult for the bacterium's toxins to enter the cell. This could provide a way to predict who would be most affected in a large outbreak, as well as novel treatment strategies.

Additional virulence factors for *B. anthracis* include hemolysins and other enzymes that damage host membranes.

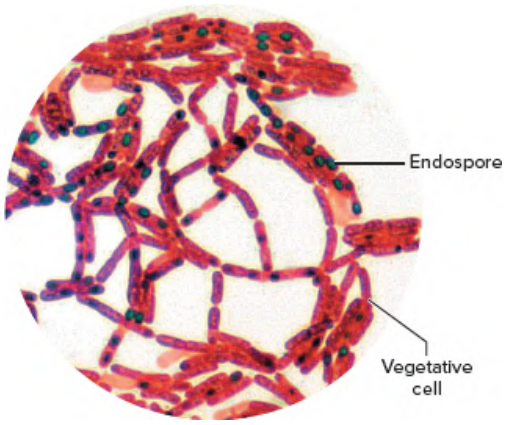

Figure 18.23 ***Bacillus anthracis*.** Note the centrally placed endospores and streptobacillus arrangement (600×).
Source: CDC/Courtesy Larry Stauffer, Oregon State Public Health Laboratory

Transmission and Epidemiology

The anthrax bacillus undergoes its cycle of vegetative growth and sporulation in the soil. Animals become infected while grazing on grass contaminated with endospores. When the pathogen is returned to the soil in animal excrement or carcasses, it can sporulate and become a long-term reservoir of infection for the animal population. The majority of natural anthrax cases are reported in livestock from Africa, Asia, and the Middle East. Most recent (natural) cases in the United States have occurred in

textile workers handling imported animal hair or hide or products made from them. Because of effective control procedures, the number of cases in the United States is extremely low (fewer

than 10 per year).

In 2001, envelopes containing freeze-dried endospores of *B. anthracis* were sent through the mail to two United States senators and several media outlets. It was an act of bioterrorism. During that attack, 22 people acquired anthrax and 5 people died.

Disease Table 18.12 Anthrax

Causative Organism(s)	*Bacillus anthracis* B G^+
Most Common Modes of Transmission	Vehicle (air, soil), indirect contact (animal hides), vehicle (food)
Virulence Factors	Triple exotoxin
Culture/Diagnosis	Culture, direct fluorescent antibody tests
Prevention	Vaccine for high-risk population
Treatment	In consultation with the CDC
Epidemiological Features	Internationally, 2,000–20,000 cases annually, most cutaneous; Category A Bioterrorism Agent

Culture and Diagnosis

Diagnosis requires a high index of suspicion. This means that anthrax must be present as a possibility in the clinician's mind or it is likely not to be diagnosed, because it is such a rare disease in the developed world and because, in all of its manifestations, it can mimic other infections that are not so rare. First-level (presumptive) diagnosis begins with culturing the bacterium on blood agar and performing a Gram stain. Ultimately, samples should be handled by the Centers for Disease Control and Prevention, which will perform confirmatory tests, usually involving direct fluorescent antibody testing and phage typing tests.

Prevention and Treatment

Humans should be vaccinated with the purified toxoid if they have occupational contact with livestock or products such as hides and bone. Military personnel may also receive the vaccine in certain circumstances. Effective vaccination requires five inoculations given over the space of a year, with annual boosters. The cumbersome nature of the vaccination has spurred research and development of more manageable vaccines. Persons who are suspected of being exposed to the bacterium are given prophylactic antibiotics, which seem to be effective at preventing disease even after exposure.

The recommended treatment for anthrax is usually doxycycline or ciprofloxacin. However, there is still debate about the best way to treat anthrax exposure, because antibiotic usage can sometimes worsen symptoms by releasing large amounts of toxin into the bloodstream. Treatment of human cases are conducted in consultation with the CDC. **Disease Table 18.12**.

18.3 LEARNING OUTCOMES—Assess Your Progress

4. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Diseases" malaria and HIV.
5. Discuss the epidemiology of malaria.
6. Describe the epidemiology of HIV infection in the developing world.
7. Discuss the important features of infectious cardiovascular conditions that have more than one possible cause. These are the two forms of endocarditis, septicemia,

hemorrhagic fever diseases, and nonhemorrhagic fever diseases.

8. Identify factors that distinguish hemorrhagic and nonhemorrhagic fever diseases.
9. Outline the series of events that may lead to septicemia and how it should be prevented and treated.
10. Discuss the important features of infectious cardiovascular diseases that have only one possible cause. These are plague, tularemia, Lyme disease, infectious mononucleosis, Chagas disease, and anthrax.
11. Describe what makes anthrax a good agent for bioterrorism, and list the important presenting signs to look for in patients.

CASE FILE WRAP-UP

©ZouZou/Shutterstock (health care worker)

Source: Library of Congress [LC-DIG-highsm-11900] (forest)

If Lyme disease is not promptly treated, neurological and cardiovascular complications can occur. As the infection spreads through the body, cranial nerves and the spinal cord may be affected. The patient may experience acute neurological changes, such as facial palsy, nerve pain, and sleep disturbances. The disease may also inhibit conduction of electrical activity in the heart.

The late stages of untreated Lyme disease involve chronic symptoms. Motor and sensory deficits lead to limited mobility and neuropathy. Cognitive abilities, memory, and personality may be affected. Patients may develop swollen joints and debilitating arthritis.

If patients are treated promptly with antibiotics, full recovery from Lyme disease is likely. In a small percentage of treated individuals, posttreatment Lyme disease syndrome (PTLDS) may occur. Fatigue, musculoskeletal pain, and cognitive problems persist for 6 months or longer after treatment. Damage to tissues and the immune system may be to blame for these lingering symptoms. Unfortunately long-term antibiotic therapy and corticosteroids have not shown to be beneficial. It is a lengthy recovery, but most patients with PTLDS eventually return to their original state of health.

Summing Up

Taxonomic Organization Summing up Microorganisms Causing Disease in the Cardiovascular and Lymphatic Systems

Microorganism	Pronunciation	Disease Table
Gram-positive endospore-forming bacteria		
Bacillus anthracis	buh-sill′-us an-thray′-sus	Anthrax, 18.12
Gram-positive bacteria		
Staphylococcus aureus	staf″-uh-lo-kok′-us are′-ee-us	Endocarditis, 18.3; septicemia, 18.4
Streptococcus pyogenes	strep″-tuh-kok′-us pie′-ah″-gen-eez	Endocarditis, 18.3
Streptococcus pneumoniae	strep″-tuh-kok′-us nu-mo′-nee-ay	Endocarditis, 18.3
Gram-negative bacteria		
Yersinia pestis	yur-sin′-ee-uh pes′-tiss	Plague, 18.5
Francisella tularensis	fran-si′-sell″-uh tew′-luh-ren″-sis	Tularemia, 18.6
Borrelia burgdorferi	bor-rill′-ee-ah berg-dorf ′-fur-eye	Lyme disease, 18.7

Microorganism	Pronunciation	Disease Table
Brucella abortus, B. suis	bru-sell′-uh uh-bort′-us	Nonhemorrhagic fever diseases, 18.10
Coxiella burnetii	cox-ee-ell′-uh bur-net′-tee-eye	Nonhemorrhagic fever diseases, 18.10
Bartonella henselae	bar-ton-nell′-uh hen′-sell-ay	Nonhemorrhagic fever diseases, 18.10
Ehrlichia species	air′-lick″-ee-uh	Nonhemorrhagic fever diseases, 18.10
Anaplasma species	an′-uh-plaz″-muh	Nonhemorrhagic fever diseases, 18.10
Neisseria gonorrhoeae	nye-seer″-ee-uh′ gon′-uh-ree″-uh	Endocarditis, 18.3
Rickettsia rickettsii	ri-ket′-see-uh ri-ket′-see-eye	Nonhemorrhagic fever diseases, 18.10
DNA viruses		
Epstein-Barr virus	ep′-steen bar ″ vie′-russ	Infectious mononucleosis, 18.8
RNA viruses		
Yellow fever virus	yel′-loh fee′-ver vie′-russ	Hemorrhagic fever diseases, 18.9
Dengue fever virus	den′-gay fee′-ver vie′-russ	Hemorrhagic fever diseases, 18.9
Ebola and Marburg viruses	ee-bowl′-uh and mar′-berg vie′-russ-suz′	Hemorrhagic fever diseases, 18.9
Lassa fever virus	lass′-sah fee′-ver vie′-russ	Hemorrhagic fever diseases, 18.9
Chikungunya virus	chick-un-goon′ yah vie′-russ	Hemorrhagic fever diseases, 18.9
Retroviruses		
Human immunodeficiency virus 1 and 2	hew′-mun im′-muh-noh-dee-fish″-shun-see vie′-russ	HIV infection and AIDS, 18.2
Protozoa		
Plasmodium falciparum, P. vivax, P. knowlesi, P. ovale, P. malariae	plas-moh′-dee-um fal-sip′-uh-rum, plas-moh′-dee-um vy′-vax, plas-moh′-dee-um nohles′-eye, plas-moh′-dee-um oh-val′-ee, plas-moh′-dee-um ma-lair′-ee-ay	Malaria, 18.1
Babesia species	buh-bee′-see-uh	Nonhemorrhagic fever diseases, 18.10
Trypanosoma cruzi	tri-pan″-uh-sohm′-uh krewz′-ee-eye	Chagas disease, 18.11

Does Our Gut Microbiome Train Our Immune System Not to Attack HIV?

Finding or creating an HIV vaccine that protects against infection is the best strategy for definitively ending this scourge. Since AIDS came on the scene in the 1980s, many scientists have devoted their lives to doing just that. A satisfactory vaccine has not yet emerged. But as frequently happens with research, even when it is not entirely successful, we are learning many things about the immune system and its response to HIV.

A recent clinical trial of an HIV vaccine candidate was halted because it did not protect against HIV infection. What seemed to be happening was that the antibodies being produced that responded to HIV seemed to also recognize healthy members of the microbiome. And they did not attack either the HIV or the healthy bacteria. This has been seen in natural HIV infection, in addition to in this vaccine experiment. Scientists speculate that HIV has learned to activate the immune system in such a way as to dampen a response, rather than induce one.

As one researcher said, "As soon as we enter the world . . . we're constantly exposed to microbes that we have to identify as non-self but also not overreact to, otherwise our bodies would be in a constant state of immune activation and battling the microbial communities that are all around and within us. There is this enormous impact that the microbiome has on the evolution of our immune system." If HIV is indeed piggy-backing on this mechanism, researchers should be able to tinker with the vaccine candidates to get around it.

One possible solution is to administer the vaccine not to adults but to infants, before their immune cells are trained not to respond to certain antigens.

Source: *Science*, 2015

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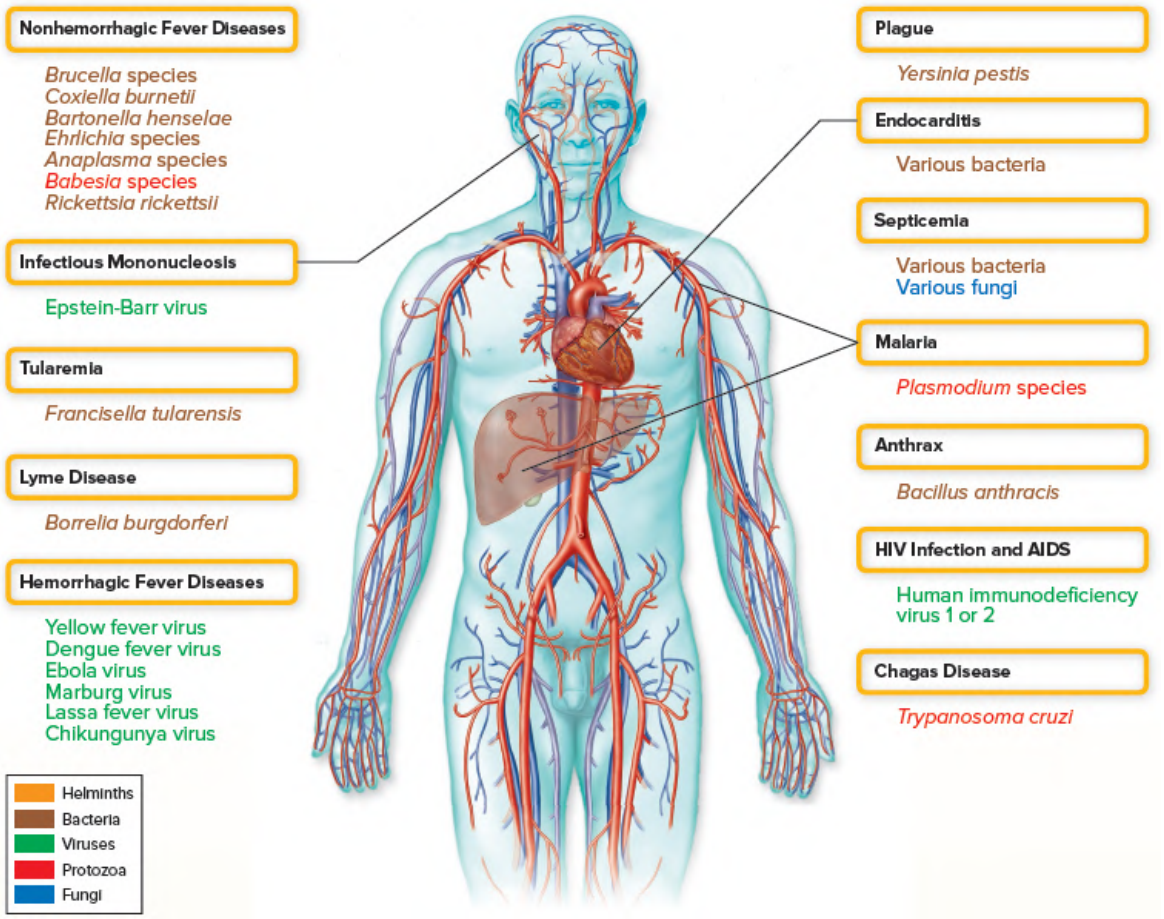
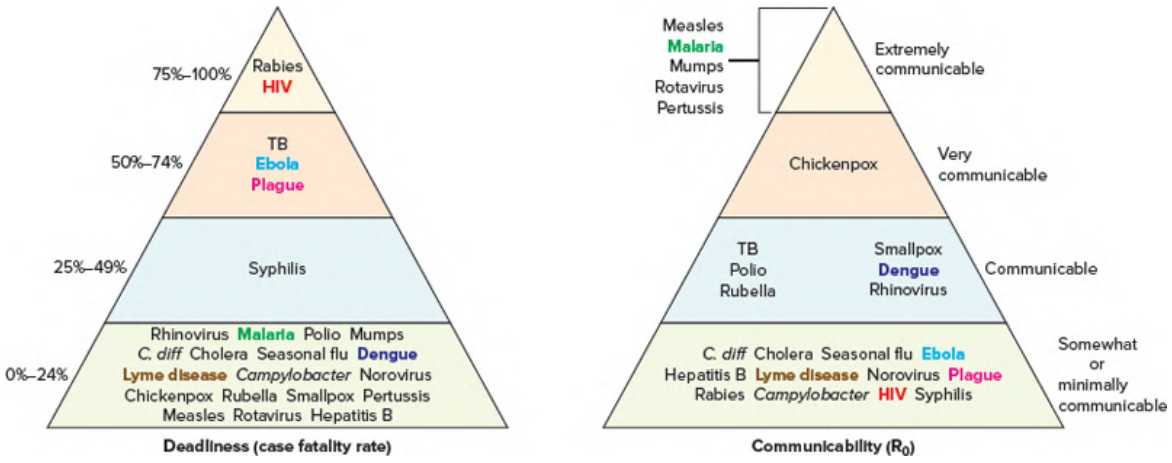
Infectious Diseases Affecting
The Cardiovascular and Lymphatic Systems



System Summary Figure 18.24

Deadliness and Communicability of Selected Diseases of the Cardiovascular and Lymphatic Systems



Chapter Summary

18.1 The Cardiovascular and Lymphatic Systems and Their Defenses

- The cardiovascular system is composed of the blood vessels and the heart.
- The lymphatic system is a one-way passage, returning fluid from the tissues to the cardiovascular system.
- The systems are highly protected from microbial infection, as they are not an open body system and they contain many components of the host's immune system.

18.2 Normal Biota of the Cardiovascular and Lymphatic Systems

- Recent research suggests that blood cells can contain "normal" microbiota.

18.3 Cardiovascular and Lymphatic System Diseases Caused by Microorganisms

- **Malaria:** Symptoms are malaise, fatigue, vague aches, and nausea, followed by bouts of chills, fever, and sweating. Causative organisms are *Plasmodium* species: *P. malariae, P. vivax, P. falciparum, P. knowlesi*, and *P. ovale*. Carried by *Anopheles* mosquito.
- **HIV Infection and AIDS:** Symptoms directly tied to the level of virus in the blood versus the level of T cells in the blood.

  - HIV is a retrovirus. Contains *reverse transcriptase*, which catalyzes the replication of double-stranded DNA from single-stranded RNA.
  - Destruction of T4 lymphocytes paves the way for invasion by opportunistic agents and malignant cells.
  - Transmission occurs mainly through sexual intercourse and transfer of blood or blood products.
- **Endocarditis:** Inflammation of the endocardium, usually due to infection of the valves of the heart.
  - **Acute Endocarditis:** Most often caused by *Staphylococcus aureus*, group A streptococci, *Streptococcus pneumoniae, and Neisseria gonorrhoeae*.
  - **Subacute Endocarditis:** Usually preceded by some form of damage to the heart valves or by congenital malformation. Alpha-hemolytic streptococci and normal biota most often responsible.
- **Septicemia:** Organisms actively multiplying in the blood. Most caused by bacteria, often MRSA, a lesser extent by fungi.
- **Plague:** *Pneumonic plague* is a respiratory disease; *bubonic plague* causes inflammation and necrosis of the lymph nodes; *septicemic plague* is the result of multiplication of bacteria in the blood. *Yersinia pestis* is the causative organism. Fleas are principal agents in transmission of the bacterium.
- **Tularemia:** Causative agent is *Francisella tularensis*, a facultative intracellular gram-negative bacterium. Disease is often called rabbit fever.

- **Lyme Disease:** Caused by *Borrelia burgdorferi*. Syndrome mimics neuromuscular and rheumatoid conditions. *B. burgdorferi* is a spirochete transmitted primarily by *Ixodes* ticks.
- **Infectious Mononucleosis:** Vast majority of cases are caused by the herpesvirus *Epstein-Barr virus (EBV)*.
- **Hemorrhagic Fever Diseases:** Extreme fevers often accompanied by internal hemorrhaging. Hemorrhagic fever diseases described here (Yellow fever, Dengue fever, Ebola and Marburg diseases, and Lassa fever) are caused by RNA enveloped viruses.
- **Nonhemorrhagic Fever Diseases:** Characterized by high fever without the capillary fragility that leads to hemorrhagic symptoms.

- **Brucellosis:** Also called Malta fever, undulant fever, Bang's disease. Multiple *Brucella* species cause this disease in humans.
- **Q Fever:** Caused by *Coxiella burnetii*, a small pleomorphic gram-negative bacterium and intracellular parasite. *C. burnetii* is harbored by a wide assortment of vertebrates and arthropods, especially ticks.
- **Cat-Scratch Disease:** Infection by *Bartonella henselae* connected with being clawed or bitten by a cat.
- **Ehrlichiosis:** *Ehrlichia* is a tick-borne intracellular pathogen, most common in midwestern United States and eastern seaboard.
- **Anaplasmosis:** *Anaplasma*, also tick-borne and intracellular, occurs most frequently in upper northeast United States and in the Great Lakes region.
- **Babesiosis:** *Babes* is a protozoan pathogen, also tick-borne; found in upper northeast and the Great Lakes region of the United States.
- **Rocky Mountain Spotted Fever:** Another tick-borne disease; causes a distinctive rash. Caused by *Rickettsia rickettsii*.

- **Chagas Disease:** *Trypanosoma cruzi* transmitted by insects; endemic in South and Central America; now frequent in the United States.
- **Anthrax:** Exhibits primary symptoms in various locations: skin (cutaneous anthrax), lungs (pulmonary anthrax), gastrointestinal tract, central nervous system (anthrax meningitis). Caused by *Bacillus anthracis*, gram-positive, endospore-forming rod found in soil.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. Which of the following microbes have evolved an intracellular life style? **a**. *Bacillus anthracis* **b**. *Coxiella burnetii* **c**. *MRSA* **d**. *two of these*	**2**. Why do you think that malarial infection is more often fatal in children than in adults in areas where it is endemic?	**3**. In chapter 17 you were asked to speculate on why a disease such as polio has become less virulent over time, while rabies has not. Malaria has also not become less virulent over time. There is evidence that HIV is becoming less virulent in humans. Is there a pattern you can discern in microbes that don't become less virulent over time?
Cell Structure and Function	**4**. Which of the following is a G^+ bacterium? **a**. *Staphylococcus aureus* **b**. *Borrelia burgdorferi* **c**. *Coxiella burnetii* **d**. *Trypanosoma cruzi*	**5**. What characteristic(s) of *Bacillus anthracis* make it a good candidate for bioterror?	**6**. Construct an immunological argument about why Lyme disease might continue to cause symptoms after the bacterium has been cleared.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Metabolic Pathways	**7**. Which of the following diseases is characterized by the formation of a biofilm? **a**. plague **b**. HIV **c**. endocarditis **d**. Chagas disease	**8**. Name some ways in which you think intracellular bacteria such as *Coxiella* and *Brucella might* resemble viruses.	**9**. Argue for the bloodstream being an advantageous environment for microbial pathogens. Now argue the opposite.
Information Flow and Genetics	**10**. Which of the following diseases is caused by a retrovirus? **a**. Lassa fever **b**. Ebola **c**. anthrax **d**. HIV	**11**. Discuss whether human genetics plays a role in HIV infection, providing at least one example to illustrate your position.	**12**. Use a unique characteristic of HIV to provide an explanation of why it is so easy for HIV to integrate into its host cell's DNA, and linger there for months/years.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Microbial Systems	**13**. The bite of a tick can cause **a**. ehrlichiosis. **b**. Lyme disease. **c**. anaplasmosis. **d**. all of these.	**14**. Discuss the merits and problems inherent in eliminating all mosquitoes in an effort to battle diseases such as yellow fever, dengue fever, and chikungunya.	**15**. This text frequently discusses the increase in infectious diseases in humans caused by the warming climate. Can you describe a hypothetical microbial pathogen that would cause less disease in a warming climate?

Impact of Microorganisms	**16**. Normal biota found in the oral cavity are most likely to cause **a**. acute endocarditis. **b**. subacute endocarditis. **c**. malaria. **d**. tularemia.	**17**. In the Middle Ages, during a massive plague epidemic, one of the control measures instituted was the quarantine of infected people. Why was this not successful?	**18**. For decades public health officials have been trying to eliminate malaria. There are some ecologists and anthropologists who suggest that eliminating malaria will lead to overpopulation and famine in "the malaria belt." Defend one of these viewpoints.
Scientific Thinking	**19**. Lyme disease is most likely to	**20**. Explain why the	**21**. When female mosquitoes mate with males infected

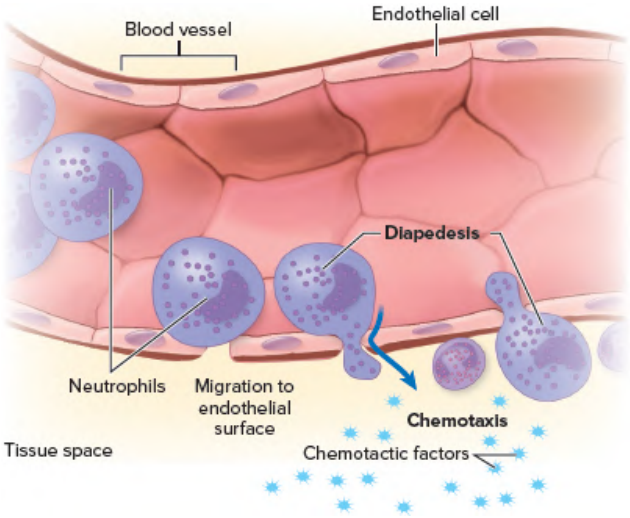
	occur in **a**. North Dakota. **b**. Connecticut. **c**. Oklahoma. **d**. Arkansas.	incidence of HIV infection in the United States has declined, while the prevalence of HIV^+ persons has increased.	with the bacterium *Wolbachia*, they no longer produce viable eggs. This is being tested as a control strategy for dengue fever and other mosquito-borne diseases. What questions would be important to answer before making this a widespread practice?

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. a. **From chapter 12, figure 12.9*a***. Imagine that the WBCs shown in this illustration are unable to control the microorganisms in the blood. Could the change that has occurred in the vessel wall help the organism spread to other locations? If so, how?
   b. If the organisms are able to survive phagocytosis, how could that impact the progress of this disease? Explain your answer.



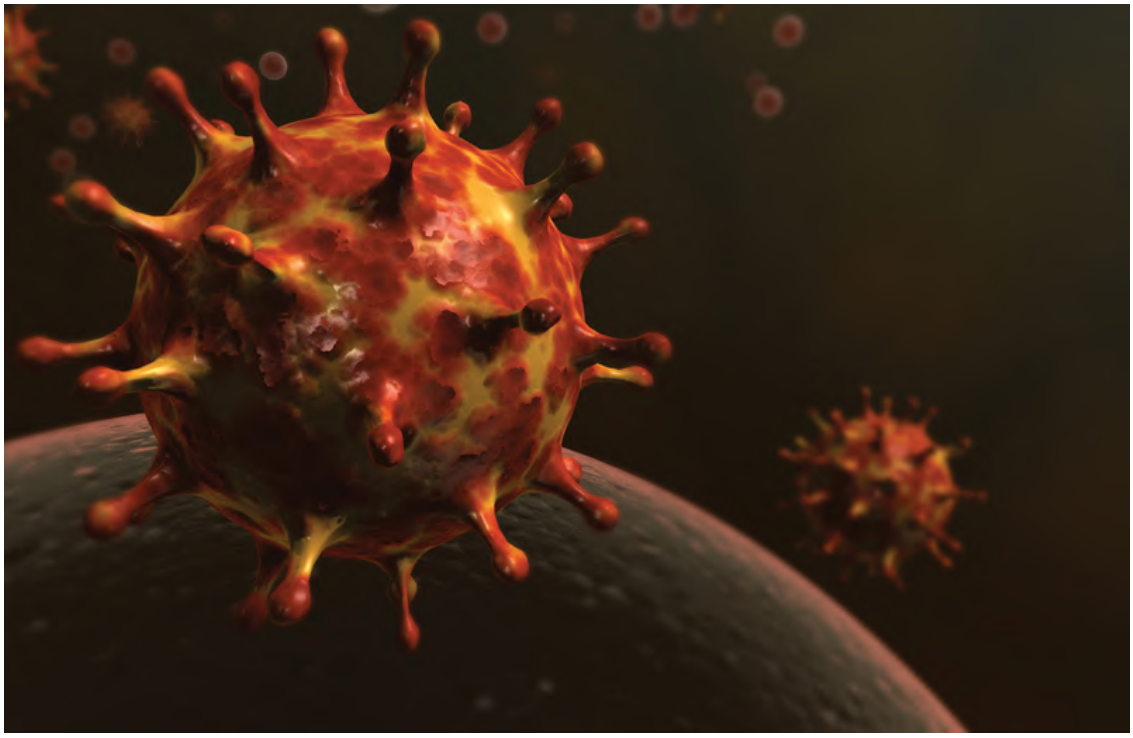
Source: CDC/Dr. Leanor Haley

connect Resources are available through Connect, including interactive learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

19

Infectious Diseases Affecting the Respiratory Systems



©Purestock/SuperStock (health care worker); ©MedicalRF.com (coronavirus); ©Allan Harris/Medical Images (throat); Source: CDC/Ronald W. Smithwick (stain)

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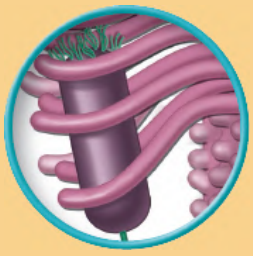
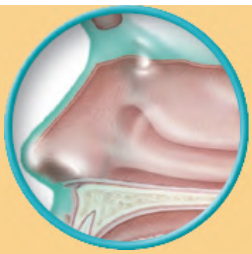


19.1 The Respiratory Tract and Its Defenses

1. Draw or describe the anatomical features of the respiratory tract.
2. List the natural defenses present in the respiratory tract.



19.2 Normal Biota of the Respiratory Tract

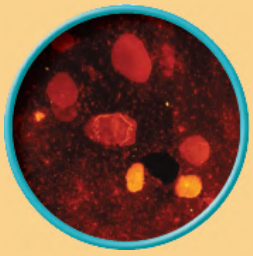
3. List the types of normal biota presently known to occupy the respiratory tract.

19.3 Upper Respiratory Tract Diseases Caused by Microorganisms

4. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Disease," pharyngitis.
5. Identify the most dangerous cause of pharyngitis and discuss possible sequelae of the infection.
6. Discuss important features of the other infectious diseases of the upper respiratory tract. These are the common cold, sinusitis, acute otitis media, and diphtheria.

19.4 Diseases Caused by Microorganisms Affecting Both the Upper and Lower Respiratory Tract

7. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Disease," influenza.
8. Compare and contrast *antigenic drift* and *antigenic shift* in influenza viruses.
9. Discuss important features of the other infectious diseases of the upper and lower respiratory tracts. These are pertussis and RSV disease.



19.5 Lower Respiratory Tract Diseases Caused by Microorganisms

10. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Disease," tuberculosis.
11. Discuss the problems associated with MDR-TB and XDR-TB.

12. Discuss important features of the other lower respiratory tract diseases, community-acquired and healthcare-associated pneumonia.

CASE FILE

Very Sick, Very Fast

I was a respiratory therapist student completing a rotation in pediatrics when I became involved in caring for Michael, a 6-month-old who was admitted to the pediatric unit with a respiratory infection.

Michael was a healthy infant who was born at term (39 weeks' gestation). He was current with all of his immunizations. Michael was the youngest of three children, with two older siblings who were school age. Michael attended day care on a part-time basis when his mother worked.

Michael and both his siblings had become ill during recent days with cold symptoms. At first, Michael had a runny nose and a slight cough. He was fussy and irritable and his mother stated he had been sleeping more than usual. Michael was breast-fed. However, when he became ill, he fussed at the breast and fed less often, even though his mother offered him the breast frequently to encourage fluid intake and provide comfort. Having three children, Michael's mother was well versed in caring for a sick child and was not prone to panic over a common cold.

However, on the third day of his illness, Michael took an alarming turn for the worse. Although he acted as though he was hungry, he would stop feeding almost as soon as he started. Michael had copious nasal discharge, and his mother rightly tried to clear his nostrils using a bulb syringe, but Michael still fed poorly. He had only had one slightly wet diaper over the course of the night and had not urinated at all in the morning. He was running a high fever of 39.6°C (103.3°F). His eyes appeared sunken in his face and there were no tears when he cried, both signs of dehydration. He was coughing frequently. Most concerning, Michael's respiratory rate was 48 breaths/minute. His nail beds were cyanosed (blue) and he was very pale in color. His oxygen saturation was 89% on room air.

Michael was seen in the emergency room and was immediately admitted to the pediatric unit by the physician on call, who was very concerned. My preceptor and I were called to assess Michael on the unit. When we arrived, Michael had a chest X ray and blood work done. He was lying in a crib with a nasal cannula supplying humidified oxygen taped to his face. His color was still very pale but his nail beds were pink. His oxygen saturation had increased to 95% with oxygen flow at 5 L/minute. I listened to Michael's chest and heard wheezes throughout Michael's lungs. After I assessed Michael, my preceptor asked me what I thought might be going on with Michael. Hedging my bets, I replied that all I could tell for certain was that Michael had a respiratory tract illness and a virus was the likely culprit. My preceptor promptly replied, "You're right that Michael has a viral illness of the respiratory tract, but which one?"

When I confessed that I did not know, my preceptor informed me that Michael's symptoms were consistent with respiratory syncytial virus (RSV). Michael's tests would prove him right.

- How is RSV spread?
- How might Michael have contracted the respiratory syncytial virus?

Case File Wrap-Up appears at the end of the chapter.

19.1 The Respiratory Tract and Its Defenses

The respiratory tract is the most common place for infectious agents to gain access to the body. We breathe 24 hours a day, and anything in the air we breathe passes at least temporarily into this organ system.

The structure of the system is illustrated in **figure 19.1*a***. Most clinicians divide the system into two parts, the *upper* and *lower respiratory tracts*. The upper respiratory tract includes the mouth, the nose, nasal cavity and sinuses above it, the throat or pharynx, and the epiglottis and larynx. The lower respiratory tract begins with the trachea, which feeds into the bronchi and bronchioles in the lungs. Attached to the bronchioles are small balloonlike structures called alveoli, which inflate and deflate with inhalation and exhalation. These are the sites of oxygen exchange in the lungs.

Several anatomical features of the respiratory system protect it from infection. As described in chapter 12, nasal hair serves to trap particles. Cilia **(figure 19.1*b*)** on the epithelium of the trachea and bronchi (the ciliary escalator) propel particles upward and out of the respiratory tract. Mucus on the surface of the mucous membranes lining the respiratory tract is a natural trap for invading microorganisms. Once the microorganisms are trapped, involuntary responses such as coughing, sneezing, and swallowing can move them out of sensitive areas. These are first-line defenses.

The second and third lines of defense also help protect the respiratory tract. Complement action, antimicrobial peptides, and increased levels of chemocytokines all help battle pathogens in the lungs. Macrophages inhabit the alveoli of the lungs and the clusters of lymphoid tissue (tonsils) in the throat. Secretory IgA against specific pathogens can be found in the mucus secretions as well.

19.1 LEARNING OUTCOMES—Assess Your Progress

1. Draw or describe the anatomical features of the respiratory tract.
2. List the natural defenses present in the respiratory tract.

19.2 Normal Biota of the Respiratory Tract

The latest research shows that a healthy upper respiratory system harbors thousands of commensal microorganisms and that even the lungs have a normal, if limited, biota. Part of this normal biota can cause serious disease, especially in immunocompromised individuals. These include *Streptococcus pyogenes, Haemophilus influenzae, Streptococcus pneumoniae, Neisseria meningitidis*, and *Staphylococcus aureus*. Yeasts, especially *Candida albicans*, also colonize the mucosal surfaces of the mouth in the upper respiratory tract. Other fungi can be found throughout the respiratory tract. The composition of the lung microbiome differs in patients suffering from lung disorders such as chronic obstructive pulmonary disease (COPD), asthma, and cystic fibrosis. Smokers and nonsmokers also appear to have different microbiota.

Medical Moment

Epiglottitis

The epiglottis is a small piece of cartilage that partially covers the larynx. Its job is to help prevent the inhalation of food and fluids into your lungs. Epiglottitis occurs when an infection or an injury causes the epiglottis to swell, which may result in the inability to draw air into the lungs.

Symptoms of epiglottitis may include fever, an extremely sore throat, a muffled or hoarse voice, stridor (a high-pitched sound that occurs during inhalation), and difficulty breathing and swallowing. Children may drool due to their inability to swallow oral secretions and may appear very anxious.

Epiglottitis is a medical emergency and may cause complete blockage of the airway. The most common infectious cause of epiglottitis historically was *Haemophilus influenzae* b. Because a vaccine for that bacterium is now in common use, the condition is less common, with less than 20,000 cases a year in the United States.

Q. Name two other serious infectious conditions that have decreased since the introduction of the Hib vaccine.

Answer in Appendix B.

Respiratory Tract Defenses and Normal Biota

	Defenses	Normal Biota
Upper Respiratory Tract	Nasal hair, ciliary escalator, mucus, involuntary responses such as coughing and sneezing, secretory IgA	Many species of bacteria and fungi, including *Neisseria meningitidis, Staphylococcus, Streptococcus, Aspergillus*, and other fungi
Lower Respiratory Tract	Mucus, alveolar macrophages, secretory IgA	Still unclear; low levels of colonization by multiple species probable

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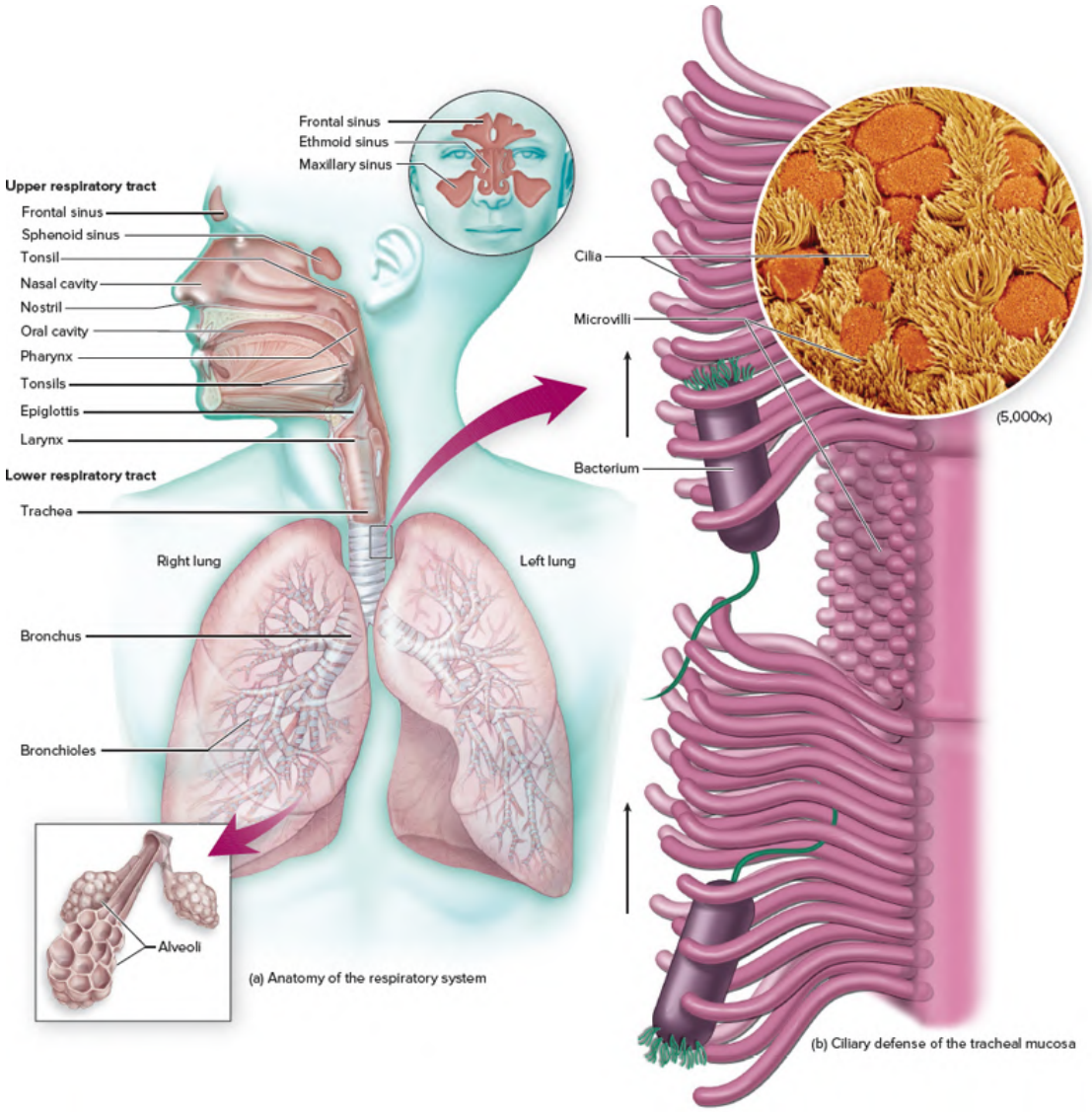

Figure 19.1 **The respiratory tract.** **(a)** Important structures in the upper and lower respiratory tract. The four pairs of sinuses are pictured in the inset. **(b)** Ciliary defense of the respiratory tract.
©Susumu Nishinaga/Science Source (micrograph)

In the respiratory system, as in some other organ systems, the normal biota performs the important function of microbial antagonism (see chapter 11). This reduces the chances of pathogens establishing themselves in the same area by competing with them for resources and space. To illustrate this point, *Lactobacillus sakei*, a known member of the sinus microbiome, can suppress the pathogenic potential of another normal biota organism, *Corynebacterium tuberculostearicum*, reducing the incidence of sinus infection.

19.2 LEARNING OUTCOMES—Assess Your Progress

3. List the types of normal biota presently known to occupy the respiratory tract.

Figure 19.2 The appearance of the throat in pharyngitis and tonsillitis. The pharynx and tonsils become bright red and suppurative. Whitish pus nodules may also appear on the tonsils.
©Allan Harris/Medical Images

19.3 Upper Respiratory Tract Diseases Caused by Microorganisms

Highlight Disease

Pharyngitis

▶ Signs and Symptoms

The name says it all—this is an inflammation of the throat, which the host experiences as pain and swelling. The severity of pain can range from moderate to severe, depending on the causative agent. Viral sore throats are generally mild and sometimes lead to hoarseness. Sore throats caused by bacteria are generally more painful than those caused by viruses, and they are more likely to be accompanied by fever, headache, and nausea.

Clinical signs of a sore throat are reddened mucosa, swollen tonsils, and sometimes white packets of inflammatory products visible on the walls of the throat, especially in streptococcal disease **(figure 19.2)**. The mucous membranes may be swollen, affecting speech and swallowing. Often pharyngitis results in foul-smelling breath. The incubation period for most sore throats is generally 2 to 5 days.

▶ Causative Agents

The same viruses causing the common cold are those most often causing a sore throat, which can also accompany other diseases, such as infectious mononucleosis (described in chapter 18). Pharyngitis may simply be the result of mechanical irritation from prolonged shouting or from drainage of an infected sinus cavity. The most serious cases of pharyngitis are caused by *Streptococcus pyogenes*, a group A streptococcus.

Streptococcus pyogenes

S. pyogenes is a gram-positive coccus that grows in chains. It does not form endospores, is nonmotile, and forms capsules and slime layers. *S. pyogenes* is a facultative anaerobe that ferments a variety of sugars. It does not produce catalase, but it does have a peroxidase system for inactivating hydrogen peroxide, which allows its survival in the presence of oxygen.

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NCLEX® PREP

1. What information should be included in health promotion classes for parents of school-age children with regard to the treatment of sore throats?
   - **a.** *As long as the child does not have a fever, there is no need to seek medical treatment.*
   - **b.** *Provide fluids as tolerated and keep the child well hydrated.*
   - **c.** *Parents should take their child to a health care provider so that a rapid throat culture can be obtained.*
   - **d.** *If the child can swallow with minimal pain, all that is needed is increased fluid.*

Pathogenesis

Untreated streptococcal throat infections can occasionally result in serious complications, either right away or days to weeks after the throat symptoms subside. The post-streptococcal conditions can be caused by the presence of an extra toxin (as in scarlet fever), or by the deposition of antigen-antibody complexes in the body (as in glomerulonephritis). Another cause of post-streptococcal complications is immune system attack of self tissues triggered by streptococcal "superantigens" that cause immune activation to similar or even unrelated (human) proteins. This is responsible for rheumatic fever and for some types of obsessive-compulsive disorder (brain tissue is targeted) and psoriasis (skin is targeted). More details about scarlet fever and rheumatic fever are presented here.

NCLEX® PREP

2. A nurse at a medical call center is consulting with a mother regarding her 8-year-old daughter. The child is experiencing throat pain and fever. The nurse educates the mother about a condition associated with untreated pharyngitis that may result in damage to heart valves and cause joint pain. What condition is the nurse referencing?
   - **a.** *diphtheria*
   - **b.** *pertussis*
   - **c.** *influenza*
   - **d.** *rheumatic fever*

Scarlet Fever Scarlet fever is the result of infection with an *S. pyogenes* strain that is itself infected with a bacteriophage. This lysogenic virus gives the streptococcus the ability to produce erythrogenic toxin, described in the section on virulence. Scarlet fever is characterized by a sandpaper-like rash, most often on the neck, chest, elbows, and inner surfaces of the thighs. High fever accompanies the rash. It most often affects school-age children, and was a source of great suffering in the United States in the

early part of the 20th century. In epidemic form, the disease can have a fatality rate of up to 95%. Although the disease had all but disappeared in the last 100 years, an international study in 2015 documented a resurgence in cases in the United Kingdom and in parts of Asia.

Rheumatic Fever Rheumatic fever is thought to be due to an immunologic cross-reaction between the streptococcal M protein and heart muscle. This means that the lymphocyte clones activated by the M protein also react with an epitope on the heart muscle. It tends to occur approximately 3 weeks after pharyngitis has subsided. It can result in permanent damage to heart valves. Other symptoms include arthritis in multiple joints and the appearance of nodules over bony surfaces just under the skin.

Virulence Factors

The virulence of *S. pyogenes* is a result of two main phenomena: the ability of its surface antigens to mimic host proteins, and its possession of superantigens. Superantigens were described in chapter 12.

Streptococci display numerous surface antigens **(figure 19.3)**. Specialized polysaccharides on the surface of the cell wall help to protect the bacterium from being dissolved by the lysozyme of the host. Lipoteichoic acid (LTA) contributes to the adherence of *S. pyogenes* to epithelial cells in the pharynx. A spiky surface projection called *M protein* contributes to virulence by resisting phagocytosis and possibly by contributing to adherence. A capsule made of hyaluronic acid (HA) is formed by most *S. pyogenes* strains. It probably contributes to the bacterium's adhesiveness.

Extracellular Toxins Group A streptococci owe some of their virulence to the effects of hemolysins called **streptolysins**. The two types are streptolysin O (SLO) and streptolysin S (SLS). Both types cause beta-hemolysis of sheep blood agar (see "Culture and Diagnosis"). Both hemolysins rapidly injure many cells and tissues, including leukocytes and liver and heart muscle (in other forms of streptococcal disease).

A key toxin in the development of scarlet fever is **erythrogenic** (eh-rith′-roh-jen′-ik) **toxin**. This toxin is responsible for the bright red rash typical of this disease, and it also induces fever by acting upon the temperature regulatory center in the brain. Only lysogenic strains of *S. pyogenes* that contain

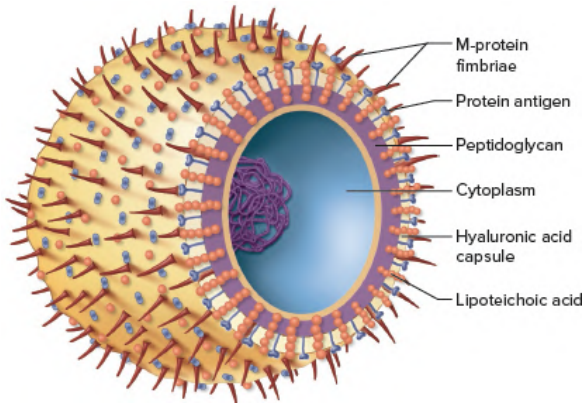

Figure 19.3 **Cutaway view of group A *Streptococcus*.**

genes from a temperate bacteriophage can synthesize this toxin. (For a review of the concept of lysogeny, see chapter 5.)

Transmission and Epidemiology

Physicians estimate that 30% of sore throats may be caused by *S. pyogenes*, adding up to several million cases each year. Most transmission of *S. pyogenes* is via respiratory droplets or direct contact with mucus

secretions. This bacterium is carried as "normal" biota by 15% of the population, but transmission from this reservoir is less likely than from a person who is experiencing active disease from the infection because of the higher number of bacteria present in the disease condition. It is less common but possible to transmit this infection via fomites. Humans are the only significant reservoir of *S. pyogenes*.

More than 80 serotypes of *S. pyogenes* exist, and thus people can experience multiple infections throughout their lives because immunity is serotype-specific. Even so, only a minority of encounters with the bacterium result in disease. An immunocompromised host is more likely to suffer from strep pharyngitis as well as serious sequelae of the throat infection.

Culture and Diagnosis

The failure to recognize group A streptococcal infections can have devastating effects. Rapid cultivation and diagnostic techniques to ensure proper treatment and prevention measures are essential. Several different rapid diagnostic test kits are used in clinics and doctors' offices to detect group A streptococci from pharyngeal swab samples. These tests are based on antibodies that react with the outer carbohydrates of group A streptococci **(figure 19.4*a*)**. Because the rapid tests have a significant possibility of returning a false-negative result, guidelines call for confirming the negative finding with a culture, which can be read the following day.

A culture is generally taken at the same time as the rapid swab and is plated on sheep blood agar. *S. pyogenes* displays a beta-hemolytic pattern due to its streptolysins (and hemolysins) **(figure 19.4*b*)**. If the pharyngitis is caused by a virus, the blood agar dish will show a variety of colony types, representing the normal bacterial biota. Active infection with *S. pyogenes* will yield a plate with a majority of beta-hemolytic colonies. Group A streptococci are by far the most common beta-hemolytic isolates in human diseases, but lately an increased number of infections by group B streptococci (also beta-hemolytic), as well as the existence of beta-hemolytic enterococci, have made it important to use differentiation tests. A positive bacitracin disc test (figure 19.4*b*) provides additional evidence for group A.

Prevention

No vaccine exists for group A streptococci, although many researchers are working on the problem. A vaccine against this bacterium would also be a vaccine against rheumatic fever, and thus it is in great demand. In the meantime, infection can be prevented by good hand washing, especially after coughing and sneezing and before preparing foods or eating.

Treatment

The antibiotic of choice for *S. pyogenes* is penicillin. In patients with penicillin allergies, a first-generation cephalosporin, such as cephalexin, is prescribed. Although most sore throats caused by *S. pyogenes* can resolve on their own, they should be treated with antibiotics because serious sequelae are a distinct possibility.

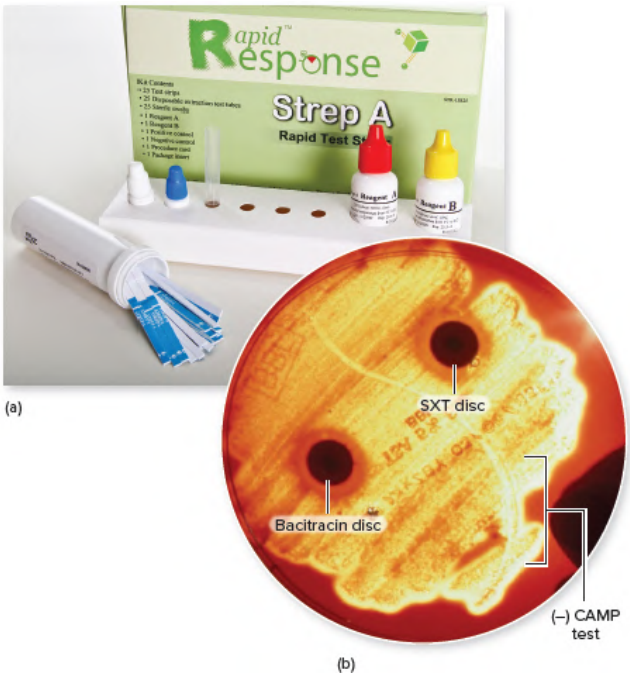

Figure 19.4 **Streptococcal tests.** **(a)** A rapid, immunologic test for diagnosis of group A infections. **(b)** Bacitracin disc test. With very few exceptions, only *Streptococcus pyogenes* is sensitive to a minute concentration (0.02 µg) of bacitracin. Any zone of inhibition around the B disc is interpreted as a presumptive indication of this species. (Note: Group A streptococci are negative for sulfamethoxazole-trimethoprim [SXT] sensitivity and the CAMP test.)
©Science Source (a); ©Van Bucher/SPL/Science Source (b)

Disease Table 19.1 Pharyngitis

Causative Organism(s)	*Streptococcus pyogenes* (B) G+	Viruses (V)	Other Bacteria to Consider
Most Common Modes of Transmission	Droplet or direct contact	All forms of contact	Bacteria: *Mycoplasma pneumoniae, Arcanobacterium, Fusobacterium, Neisseria gonorrhoeae*
Virulence Factors	LTA, M protein, hyaluronic acid capsule, SLS and SLO, superantigens, induction of autoimmunity	–	
Culture/Diagnosis	Beta-hemolytic on blood agar, sensitive to bacitracin, rapid antigen tests	Goal is to rule out *S. pyogenes*, further diagnosis usually not performed	
Prevention	Hygiene practices	Hygiene practices	
Treatment	Penicillin, cephalexin in penicillin-allergic	Symptom relief only	
Distinctive Features	Generally more severe than viral pharyngitis	Hoarseness frequently accompanies viral pharyngitis	
Epidemiological Features	United States: 20%–30% of all cases of pharyngitis	Ubiquitous; responsible for 40%–60% of all pharyngitis	Account for remaining percentage of pharyngitis cases

The Common Cold

Everyone is familiar with the symptoms of a common cold: sneezing, scratchy throat, and runny nose, which usually begin 2 or 3 days after infection. An uncomplicated cold generally is not accompanied by fever, although children can experience low fevers (less than 102°F). The incubation period is usually 2 to 5 days.

People with asthma and other underlying respiratory conditions, such as chronic obstructive pulmonary disease (COPD), often suffer more severe symptoms triggered by the common cold.

The common cold is caused by one of over 200 different kinds of viruses. The most common type of virus leading to rhinitis is the group called rhinoviruses, of which there are 99 serotypes. Coronaviruses and adenoviruses are also major causes. Also, the respiratory syncytial virus (RSV) causes colds in most people it infects, but in some, especially infants and children, they can lead to more serious respiratory tract symptoms (discussed later in the chapter). **Disease Table 19.2**.

Sinusitis

Commonly called a *sinus infection*, this inflammatory condition of any of the four pairs of sinuses in the skull (see figure 19.1*c*) can actually be caused by allergy (most common), infections, or simply by structural problems such as narrow passageways or a deviated nasal septum. The infectious agents that may be responsible for the condition commonly include a variety of viruses or bacteria and, less commonly, fungi. **Disease Table 19.3**.

Acute Otitis Media (Ear Infection)

Viral infections of the upper respiratory tract lead to inflammation of the eustachian tubes and the buildup of fluid in the middle ear, which can lead to bacterial multiplication in those fluids. Although the middle ear normally has no biota, bacteria can migrate along the eustachian tube from the upper respiratory tract **(figure 19.5)**.

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Disease Table 19.2 The Common Cold

Causative Organism(s)	Approximately 200 viruses V
Most Common Modes of Transmission	Indirect contact, droplet contact
Virulence Factors	Attachment proteins; most symptoms induced by host response
Culture/Diagnosis	Not necessary
Prevention	Hygiene practices
Treatment	For symptoms only
Epidemiological Features	Highest incidence among preschool and elementary schoolchildren with average of three to eight colds per year; adults and adolescents: two to four colds per year

Disease Table 19.3 Sinusitis

Causative Organism(s)	Viruses V	Various bacteria, often mixed infection B	Various fungi F
Most Common Modes of Transmission	Direct contact, indirect contact	Endogenous (opportunism)	Introduction by trauma or opportunistic overgrowth

Virulence Factors	—	—	—
Culture/Diagnosis	Culture not usually performed; diagnosis based on clinical presentation.	Culture not usually performed; diagnosis based on clinical presentation, occasionally X rays or other imaging technique used	Same
Prevention	Hygiene	—	—
Treatment	None	Broad-spectrum antibiotics or none	Physical removal of fungus; in severe cases, antifungals used
Distinctive Features	Viral and bacterial much more common than fungal	Viral and bacterial much more common than fungal	Suspect in immunocompromised patients
Epidemiological Features	—	United States: affects 1 of 7 adults; between 12 and 30 million diagnoses per year	Fungal sinusitis varies with geography; in United States, more common in SE and SW; internationally: more common in India, North Africa, Middle East

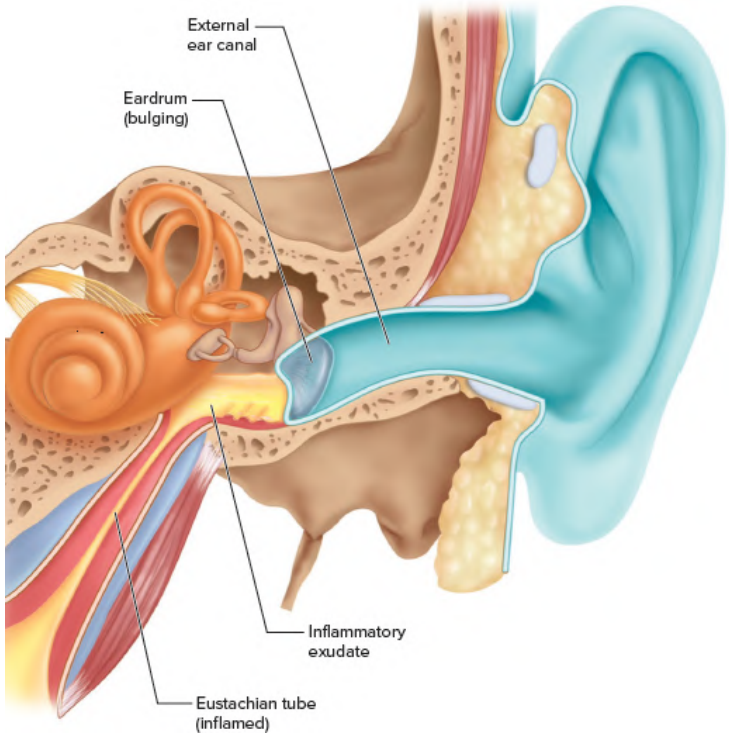

Figure 19.5 **An infected middle ear**.

When bacteria encounter mucus and fluid buildup in the middle ear, they multiply rapidly. Their presence increases the inflammatory response, leading to pus production and continued fluid secretion. This fluid is referred to as *effusion*.

Another condition, known as chronic otitis media, occurs when fluid remains in the middle ear for indefinite periods of time. Until recently, physicians considered it to be the result of a noninfectious immune reaction because they could not culture bacteria from the site and because antibiotics were not effective. New data suggest that this form of otitis media is caused by a mixed biofilm of bacteria that is attached to the mucosa of the middle ear. Biofilm bacteria generally are less susceptible to antibiotics (as discussed in chapter 3), and their presence in biofilm form would explain the inability to culture them from ear fluids. Scientists now believe that the majority of acute and chronic otitis media cases are mixed infections with viruses and bacteria acting together.

The single most common bacterium seen in acute otitis media is *Streptococcus pneumoniae*. A vaccine against *S. pneumoniae* has been a part of the recommended childhood vaccination schedule since 2000. The vaccine (Prevnar) is a conjugated vaccine (see chapter 13). It contains polysaccharide capsular material from

13 different strains of the bacterium complexed with a chemical that makes it more antigenic. It is distinct from another vaccine for the same bacterium (Pneumovax), which is primarily targeted to the older population to prevent pneumococcal pneumonia.

The current treatment recommendation for uncomplicated acute otitis media with a fever below 104°F is "watchful waiting" for 72 hours to allow the body to clear the infection, avoiding the use of antibiotics. When antibiotics are used, antibiotic resistance must be considered. Children who experience frequent recurrences of ear infections sometimes have small tubes placed through the tympanic membranes into their middle ears to provide a means of keeping fluid out of the site when inflammation occurs.

Disease Table 19.4 Otitis Media

Causative Organism(s)	*Streptococcus pneumoniae* B **G+**	*Haemophilus influenzae* B **G-**	Other bacteria/viruses
Most Common Modes of Transmission	Endogenous (may follow upper respiratory tract infection by *S. pneumoniae* or other microorganisms)	Endogenous (follows upper respiratory tract infection)	Endogenous
Virulence Factors	Capsule, hemolysin	Capsule, fimbriae	–
Culture/Diagnosis	Usually relies on clinical symptoms and failure to resolve within 72 hours	Same	Same
Prevention	Pneumococcal conjugate vaccine	Hib vaccine	None
Treatment	Wait for resolution; if needed, amoxicillin (high rates of resistance) or amoxicillin + clavulanate or cefuroxime; in **Serious Threat** category in CDC Antibiotic Resistance Report	Same as for *S. pneumoniae*	Wait for resolution; if needed, a broad-spectrum antibiotic (azithromycin) may be used in absence of etiologic diagnosis
Distinctive Features	–	–	Suspect if fully vaccinated against other two
Epidemiological Features	United States: 70% of children experience at least one case before age 2; in developing world: chronic otitis media results in significant hearing loss in 100s of millions and death in approx. 30,000 per year (in absence of treatment)		

Diphtheria

For hundreds of years, diphtheria was a significant cause of morbidity and mortality, but in the last 50 years, the number of cases steadily has declined throughout the world. However, as soon as dips occur in the vaccination rate, this disease resurfaces. For example, during a period of civil unrest in Venezuela in 2017, health care was disrupted and hundreds of children got diphtheria.

The disease is caused by *Corynebacterium diphtheriae*, a non-endospore-forming, gram-positive club-shaped bacterium. The most striking symptom of this disease is a characteristic membrane, usually referred to as a pseudomembrane, that forms on the tonsils or pharynx **(figure 19.6)**. It can eventually cut off the airway, leading to death.

The major virulence factor is an exotoxin encoded by a bacteriophage of *C. diphtheriae*. Strains of the bacterium that are not lysogenized by this phage do not cause serious disease. The release of diphtheria toxin in the blood leads to complications in distant organs, especially myocarditis and neuritis. Neuritis affects motor nerves and may result in temporary paralysis of limbs, the soft palate, and even the diaphragm, a condition that can predispose a patient to other lower respiratory tract infections.

Figure 19.6 **Diagnosing diphtheria**. The clinical appearance in diphtheria infection includes gross inflammation of the pharynx and tonsils marked by grayish patches (a pseudomembrane) and swelling over the entire area.
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Disease Table 19.5 Diphtheria

Causative Organism(s)	*Corynebacterium diphtheriae* (B) G+
Most Common Modes of Transmission	Droplet contact, direct contact or indirect contact with contaminated fomites
Virulence Factors	Exotoxin: diphtheria toxin
Culture/Diagnosis	Tellurite medium—gray/black colonies, club-shaped morphology on Gram stain; treatment begun before definitive identification
Prevention	Diphtheria toxoid vaccine (part of DTaP, Tdap, and Td)
Treatment	Antitoxin plus penicillin or erythromycin
Epidemiological Features	United States: 5 cases in last 10 years; internationally: +/– 5,000 cases per year, even though there is 83% vaccine coverage

19.3 LEARNING OUTCOMES—Assess Your Progress

4. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Disease," pharyngitis.
5. Identify the most dangerous cause of pharyngitis and discuss possible sequelae of the infection.
6. Discuss important features of the other infectious diseases of the upper respiratory tract. These are the common cold, sinusitis, acute otitis media, and diphtheria.

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3. A mother brings her child into the clinic setting and tells the nurse the "baby has an ear infection and I want the doctor to prescribe antibiotics." After the client sees the provider, no prescription is given. The nurse then provides discharge instructions to the client. The mother is visibly upset and states, "I don't know why I even came to the doctor if he is not going to treat my baby." What is the nurse's best response?
   a. *Ask the client why she feels this way and provide empathetic listening.*
   b. *Explain the concepts of "watchful waiting" and developing antibiotic resistance.*
   c. *Tell the client to wait, relate the concern to the provider, and have the provider go back and talk to the client.*
   d. *Tell the client that her baby's symptoms will go away in a few days and if they do not, then the provider will prescribe antibiotic therapy.*

19.4 Diseases Caused by Microorganisms Affecting Both the Upper and Lower Respiratory Tract

A number of infectious agents affect both the upper and lower respiratory tract regions. We address the more well-known diseases in this section. They are influenza, whooping cough, and respiratory syncytial virus (RSV).

HIGHLIGHT DISEASE

Influenza

The "flu" is a very important disease to study for several reasons. First of all, everyone is familiar with the cyclical increase of influenza infections occurring during the winter months in the United States. Second, many conditions are erroneously termed the "flu," while in fact only diseases caused by influenza viruses are actually the flu. Third, the way that influenza viruses evolve provides an excellent illustration of the way other viruses can, and do, change to cause more serious diseases than they did previously.

Influenzas that occur every year are called "seasonal" flus. Often these are the only flus that circulate each year. Occasionally another flu strain appears, one that is new and may cause worldwide pandemics. In some years, such as in 2009, both of these flus were issues. They may have different symptoms, affect different age groups, and have separate vaccine protocols.

▶ Signs and Symptoms

Influenza begins in the upper respiratory tract but in serious cases may also affect the lower respiratory tract. There is a 1- to 4-day incubation period, after which symptoms begin very quickly. These include headache, chills, dry cough, body aches, fever, stuffy nose, and sore throat. Even the sum of all these symptoms can't describe how a person actually feels: lousy. The flu is known to "knock you off your feet." Extreme fatigue can last for a few days or even a few weeks. An infection with influenza can leave patients vulnerable to secondary infections, often bacterial. Influenza infection occasionally leads to a pneumonia that can cause rapid death, even in young healthy adults.

▶ Causative Agent

All influenza is caused by one of three influenza viruses: A, B, or C. They belong to the family *Orthomyxoviridae*. They are spherical particles with an average diameter of 80 to 120 nanometers. Each virus has a lipoprotein envelope that is studded with glycoprotein spikes acquired during viral maturation **(figure 19.7)**. Also note that the envelope contains proteins that form a channel for ions into

©Getty Images

the virus. The two glycoproteins that make up the spikes of the envelope and contribute to virulence are called hemagglutinin (H) and neuraminidase (N). The name hemagglutinin is derived from this glycoprotein's agglutinating action on red blood cells, which is the basis for viral assays used to identify the viruses. Hemagglutinin contributes to infectivity by binding to host cell receptors of the respiratory mucosa, a process that facilitates viral penetration. Neuraminidase breaks down the protective mucous coating of the respiratory tract, assists in viral budding and release, keeps viruses from sticking together, and participates in host cell fusion.

The ssRNA genome of the influenza virus is known for its extreme variability. It is subject to constant genetic changes that alter the structure of its envelope glycoproteins. This constant mutation of the glycoproteins is called **antigenic drift**—the antigens gradually change their amino acid composition, resulting in decreased ability of host memory cells to recognize them. Antigenic drift is the reason that a new vaccine is required for each year. This vaccine is called the seasonal vaccine.

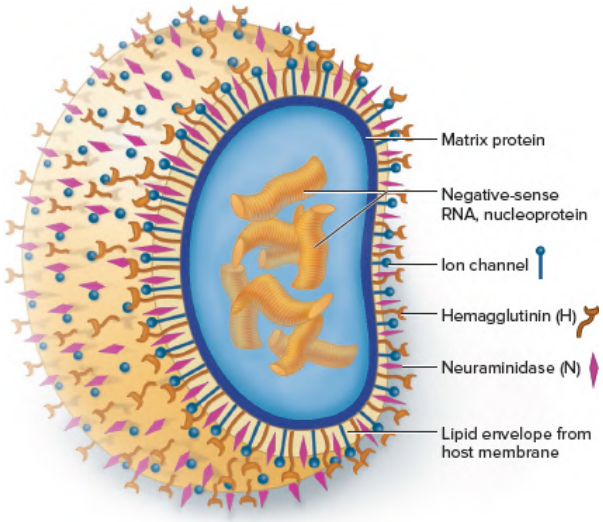


Figure 19.7 **Schematic drawing of influenza virus.**

An even more serious phenomenon is known as **antigenic shift**. The genome of the virus consists of just 10 genes, encoded on 8 separate RNA strands. Antigenic shift is the swapping out of one of those genes or strands with a gene or strand from a different influenza virus. Some explanation is in order. For example, we know that certain influenza viruses infect both humans and swine. Other influenza viruses infect birds and swine. All of these viruses have 10 genes coding for the same important influenza proteins (including H and N)—but the actual sequence of the genes is different in the different types of viruses. Second, when the two viruses just described infect a single swine host, with both virus types infecting the same host cell, the viral packaging step can accidentally produce a human influenza virus that contains seven human influenza virus RNA strands plus a single duck influenza virus RNA strand **(figure 19.8)**. When that virus infects a human, no immunologic recognition of the protein that came from the duck virus occurs. Experts have traced the flu pandemics of 1918, 1957, 1968, 1977, and 2009 to strains of a virus that came from pigs (swine flu). Influenza A viruses are named according to the different types of H and N spikes they display on their surfaces. In 2009, a swine flu called H1N1 caused a limited pandemic. It reappeared in 2014. In 2013, the main circulating virus was H3N2. This strain—along with H5N1 and H7N9—is called bird flu, because it originates in poultry.

Some people express frustration that public health officials seem to raise the alarm about new flu threats that don't pan out. Now that you have learned how easily a new flu strain can become lethal to a new species (i.e., humans), and knowing that when that happened in 1918 the flu killed 20–50 million people, you can appreciate the science—and the necessary precaution—behind these warnings. The warnings are usually accompanied by extra measures of preparation, such as additional vaccine, antivirals, and diagnostic kits being made available. "Better safe than sorry" is the thinking. Influenza B viruses are not divided into subtypes because they are thought to undergo only antigenic drift and not antigenic shift. Influenza C viruses are thought to cause only minor respiratory disease and are probably not involved in epidemics.

Pathogenesis and Virulence Factors

The influenza virus binds primarily to ciliated cells of the respiratory mucosa. Infection causes the rapid shedding of these cells along with a load of viruses. Stripping the respiratory epithelium to the basal layer eliminates protective ciliary clearance. Sometimes the immune system responds too aggressively in a phenomenon called a "cytokine storm," and this leads to more severe irritation and inflammation in the

lungs. The illness is further aggravated by fever, headache, and the other symptoms just described. The viruses tend to remain in the respiratory tract rather than spread to the bloodstream. As the normal ciliated epithelium is restored in a week or two, the symptoms subside.

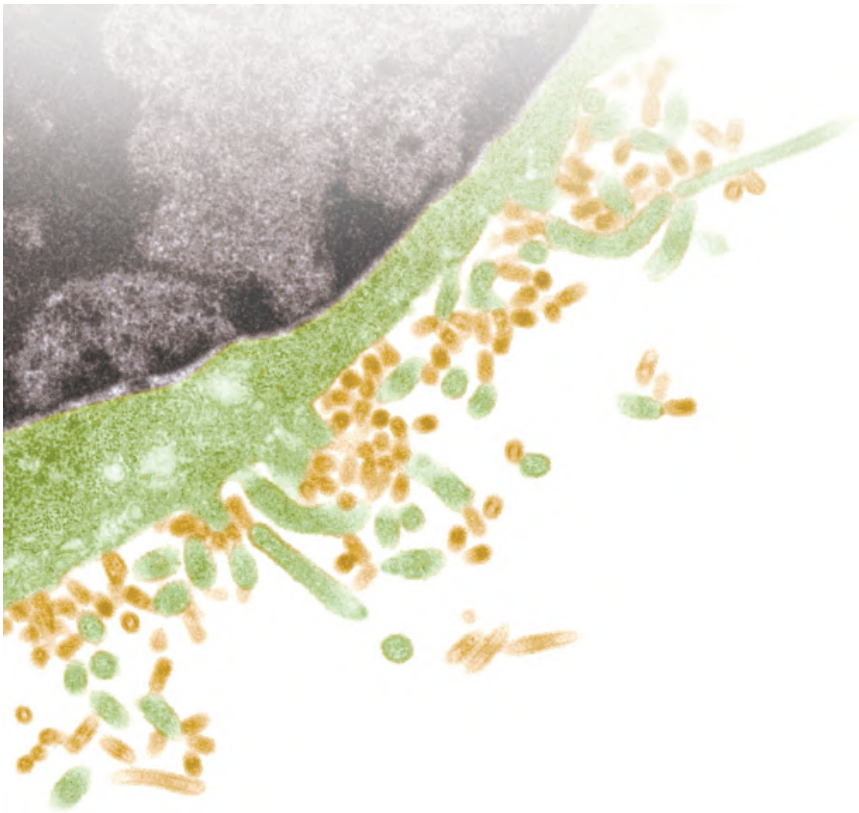
As just noted, the glycoproteins and their structure are important virulence determinants. First of all, they mediate the adhesion of the virus to host cells. Second, they change gradually and sometimes suddenly, evading immune recognition.

Transmission and Epidemiology

Inhalation of virus-laden aerosols and droplets constitutes the major route of influenza infection, although fomites can play a secondary role. Transmission is greatly facilitated by crowding and poor ventilation in classrooms, barracks, nursing homes, dormitories, and military installations in the late fall and winter. The drier air of winter facilitates the spread of the virus, as the moist particles expelled by sneezes and coughs become dry very quickly, helping the virus remain airborne for longer periods of time. In addition, the dry cold air makes respiratory tract mucous membranes more brittle, with microscopic cracks that facilitate invasion by viruses.

Influenza is highly contagious and affects people of all ages. Annually, deaths from seasonal influenza and its complications (usually pneumonia) occur mainly among the very young and the very

old. Numbers range from 12,000 to 60,000, depending on the year. The number of deaths each year depends on several factors, including the relative virulence of the virus circulating that season, the percentage of the population



Influenza viruses (orange) budding from a host cell (83,300×)
©Science Photo Library/Getty Images

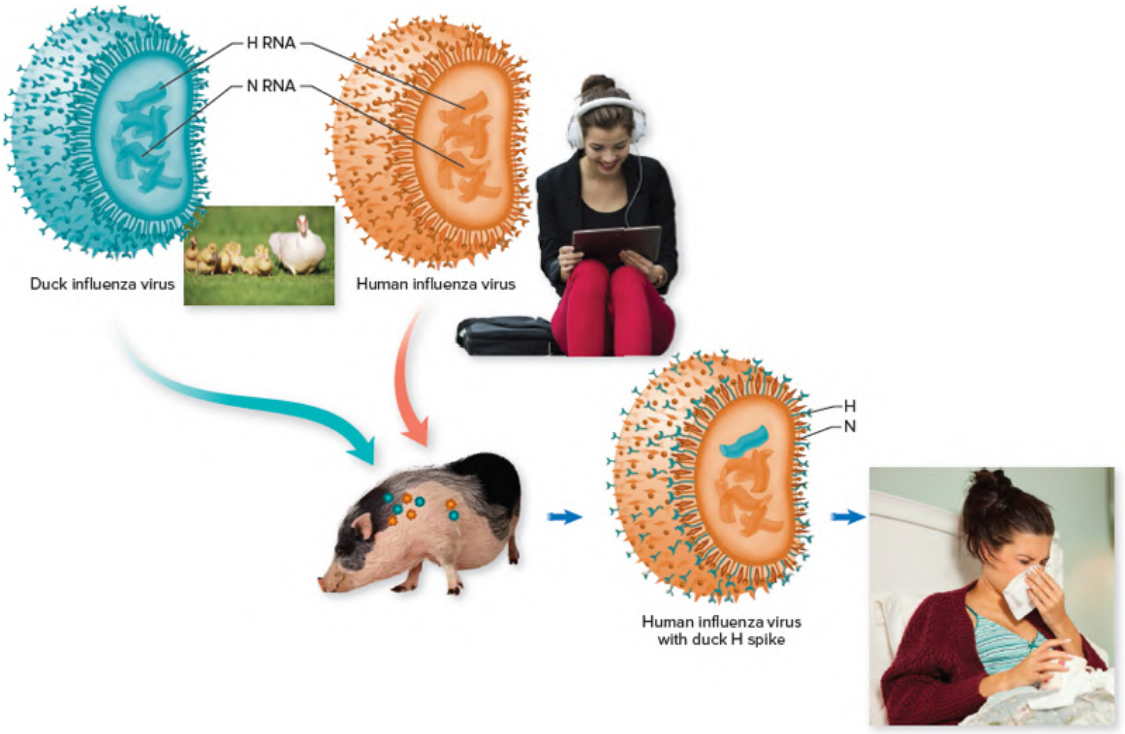


Figure 19.8 **Antigenic shift event**. Where ducks and swine and humans live close together, the swine can serve as a melting pot for creating "hybrid" influenza viruses that are not recognized by the human immune system.
©Westend61/Getty Images (healthy woman); ©Image Source/Getty Images (sick woman); ©G.K. & Vikki Hart/Getty Images (pig); ©Image Source (ducks)

that received vaccination, and how well the vaccine strains matched the actual circulating strains **(figure 19.9)**.

The most recent year in which a pandemic strain circulated was during the 2009–2010 flu season. In that season a new H1N1 virus appeared in the United States in April. It was called a quadruple reassortment virus, meaning that its genes came from four different influenza virus sources (including swine flu, avian flu, and human flu). The World Health Organization declared the emergence of this virus a "Public Health Emergency of International Concern." In hindsight, the pandemic was kept in check, but it could have gone differently.

Keep in mind that the annual vaccine had already been chosen and was in preparation for the beginning of the flu season in the fall. That vaccine (called the seasonal vaccine) did not contain the new H1N1 virus. Many people in the United States received the seasonal vaccine, and then heard the news that they should get an additional, different vaccine (the H1N1). (The international public health community had worked at record pace to create the new vaccine.) This led to frustration and confusion among the public. Influenza was just being influenza: unpredictable. As it happened, the vast majority of cases of influenza in the 2009–2010 season were caused by the new H1N1. So the first vaccine was of little use but the second one was exactly right. If you are going to work in health care, it will be your job to explain the intricacies of influenza vaccines to a public that can be confused and frustrated.

Culture and Diagnosis

There is a wide variety of culture-based and non-culture-based methods to diagnose the infection. RT-PCR is by far the preferred method for diagnosis. Cultures are not typically performed at the point of care; they must be sent to diagnostic laboratories, and they require up to 10 days for results. In 2009,

officials did not often test for H1N1 but tested for influenza A or B virus, assuming if it was A then it was H1N1, since the

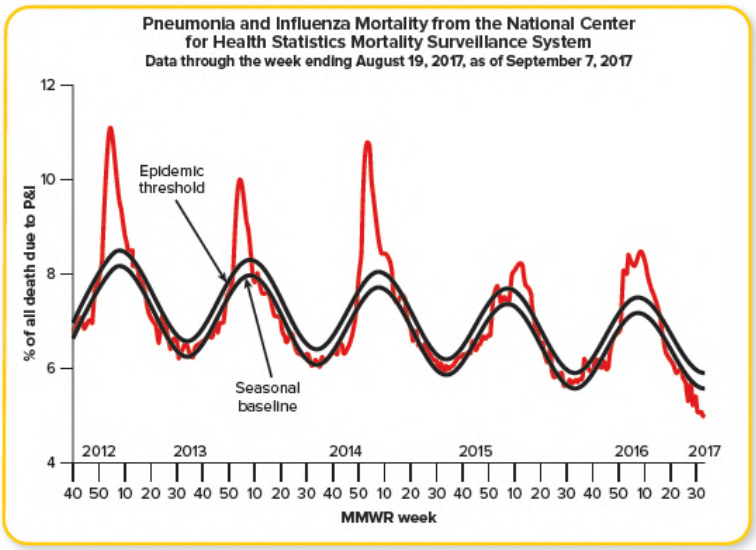


Figure 19.9 **Epidemic curve for deaths from influenza/pneumonia**. The horizontal axis is marked in weeks of the year. Clearly influenza peaks in mid-winter. The bottom black line in the graph indicates the seasonal baseline number of cases calculated from years of data. The top black line is the threshold beyond which influenza is declared an epidemic. You can see that in the first three years depicted in the graph there were epidemic levels of influenza, but in the 2015/2016 and 2016/2017 flu seasons the infection levels, while technically an epidemic, were much lower than in previous years.
Source: CDC

circulating seasonal virus was influenza B. When specimens were tested, 100% of the influenza A isolates were in fact the H1N1.

Prevention

Preventing influenza infections and epidemics is one of the top priorities for public health officials. There are currently several types of vaccines—all of them injectable. Some are trivalent (containing three viruses) and some are quadrivalent (containing four). In previous years an intranasal vaccine that contained live virus had been used but in the 2016 season it was no longer recommended. The CDC recommends that everyone over the age of 6 months receive one of these vaccinations. Because of the changing nature of the antigens on the viral surface, annual vaccination is considered the best way to avoid infection.

One of the most promising new vaccine prospects is a vaccine that would protect against *all* flu viruses and not need to be given every year. This vaccine, still experimental, targets epitopes that most flu viruses share in common. This discovery has the possibility of revolutionizing influenza prevention.

Treatment

Influenza is one of the first viral diseases for which effective antiviral drugs became available. The drugs must be taken early in the infection, preferably by the second day. This requirement is an inherent difficulty because most people do not realize until later that they may have the flu. Amantadine and rimantadine can be used to treat and prevent some influenza type A infections, but they do not work against influenza type B viruses.

Zanamivir (Relenza) is an inhaled drug that works against influenza A and B. Oseltamivir (Tamiflu) is available in capsules or as a powdered mix to be made into a drink. It can also be used for prevention

of influenza A and B. The CDC website should always be consulted for up-to-date treatment recommendations. In 2008, more than 98% of the H1N1 strains were resistant to Tamiflu. In the next year, more than 98% of them were sensitive to the same drug. As we know with all antimicrobials, the more we use them, the more quickly we lose them (the more quickly they lose their effectiveness). **Disease Table 19.6**.

Disease Table 19.6 Influenza

Causative Organism(s)	Influenza A, B, and C viruses V
Most Common Modes of Transmission	Droplet contact, direct contact, or indirect contact
Virulence Factors	Glycoprotein spikes, overall ability to change genetically, ability to slow down immune system
Culture/Diagnosis	Gold standard is RT-PCR
Prevention	Annual vaccination with one of several types of inactivated vaccines
Treatment	Oseltamivir or zanamivir
Epidemiological Features	For seasonal flu, deaths vary from year to year. United States: range from 12,000–60,000; internationally: range from 250,000–500,000

Whooping Cough (Pertussis)

This disease has two distinct symptom phases called the catarrhal and paroxysmal stages, which are followed by a long recovery (or convalescent) phase, during which a patient is particularly susceptible to other respiratory infections. After an incubation period of from 3 to 21 days, the **catarrhal** stage begins when bacteria present in the respiratory tract cause what appear to be cold symptoms, most notably a runny nose. This stage lasts 1 to 2 weeks. The disease worsens in the second **(paroxysmal)** stage, which is characterized by severe and uncontrollable coughing (a *paroxysm* can be thought of as a convulsive attack). The common name for the disease comes from the whooping sound a patient makes as he or she tries to grab a breath between uncontrollable bouts of coughing.

As in any disease, the convalescent phase is the time when numbers of bacteria are decreasing and no longer cause ongoing symptoms.

Over the the past 25 years, pertussis has been on the rise in the United States **(figure 19.10)**, and this appears to be due to three factors. First, the vaccine may not provide lifelong immunity, and so adults are contracting it—usually without symptoms—and spreading it to infants. All of the deaths from pertussis in 2013 were in babies younger than 6 months, who had not yet been fully vaccinated. Second, the bacterium appears to be evolving, rendering the older vaccine less effective. Last, some parents have the mistaken notion that the vaccine is more dangerous than pertussis

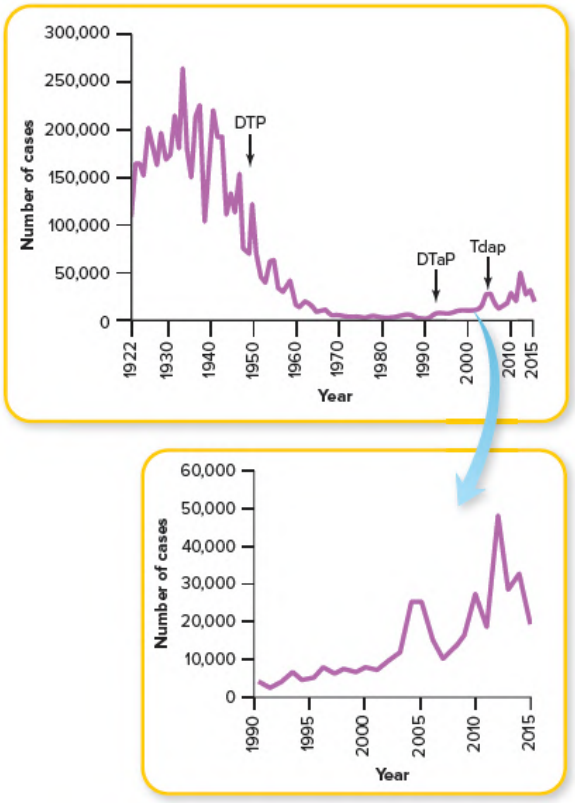


Figure 19.10 Number of cases of pertussis in the United States from 1922 to 2015. The inset graph magnifies the last 25 years, and illustrates the rise in cases.
Source: CDC

itself and so choose to leave their children unvaccinated and unprotected. **Disease Table 19.7**.

Disease Table 19.7 Pertussis (Whooping Cough)

Causative Organism(s)	*Bordetella pertussis* (B) G—
Most Common Modes of Transmission	Droplet contact
Virulence Factors	Fimbrial hemagglutinin (adhesion), pertussis toxin and tracheal cytotoxin, endotoxin
Culture/Diagnosis	PCR or grown on B-G, charcoal, or potato-glycerol agar; diagnosis can be made on symptoms
Prevention	Acellular vaccine (DTaP), azithromycin, sulfamethoxazole for contacts
Treatment	Azithromycin or erythromycin
Epidemiological Features	Resurging in United States and other countries; internationally: similar pattern of gradual increase

Respiratory Syncytial Virus Infection

As its name indicates, respiratory syncytial virus (RSV) infects the respiratory tract and produces giant multinucleated cells (syncytia). Outbreaks of droplet-spread RSV disease occur regularly throughout the world, with peak incidence in the winter and early spring. Children 6 months of age or younger, as well as premature babies, are especially susceptible to serious disease caused by this virus. RSV is the most prevalent cause of respiratory infection in the newborn age group, and nearly all children have experienced it by age 2. An estimated 100,000 children are hospitalized with RSV infection each year in the United States. Infection in older children and adults usually manifests as a cold.

The virus is highly contagious and is transmitted through droplet contact but also through fomite contamination. Diagnosis of RSV infection is more critical in babies than in older children or adults. The afflicted child is conspicuously ill, with signs typical of pneumonia and bronchitis.

There is no RSV vaccine available yet, but an effective passive antibody preparation is used as prevention in high-risk children and babies born prematurely. **Disease Table 19.8**.

Disease Table 19.8 RSV Disease

Causative Organism(s)	Respiratory syncytial virus (RSV) V
Most Common Modes of Transmission	Droplet and indirect contact
Virulence Factors	Syncytia formation
Culture/Diagnosis	RT-PCR preferred
Prevention	Passive antibody (humanized monoclonal) in high-risk children
Treatment	Ribavirin in severe cases
Epidemiological Features	United States: general population, less than 1% mortality rates, 3% to 5% mortality in premature infants or those with congenital heart defects; internationally: 7 times higher fatality rate in children in developing countries

19.4 LEARNING OUTCOMES—Assess Your Progress

7. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Disease," influenza.
8. Compare and contrast *antigenic drift and antigenic shift* in influenza viruses.
9. Discuss important features of the other infectious diseases of the upper and lower respiratory tracts. These are pertussis and RSV disease.

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19.5 Lower Respiratory Tract Diseases Caused by Microorganisms

In this section, we consider microbial diseases that affect the lower respiratory tract primarily—namely, the bronchi, bronchioles, and lungs, with minimal involvement of the upper respiratory tract. Our discussion focuses on tuberculosis and pneumonia.

Highlight Disease

Tuberculosis

Mummies from the Stone Age, ancient Egypt, and Peru provide evidence that tuberculosis (TB) is an ancient human disease. In fact, historically it has been such a prevalent cause of death that it was called "Captain of the Men of Death" and "White Plague." After the discovery of streptomycin in 1943, the rates of tuberculosis in the developed world declined rapidly. But since the mid-1980s, it has reemerged as a serious threat. Worldwide, 2.5 billion people are currently infected. That is about one-third of the world's population! The cause of tuberculosis is primarily the bacterial species *Mycobacterium*

tuberculosis, informally called the tubercle bacillus. In this discussion, we will first address the general aspects of the infection and then turn to its most troubling form today, drug-resistant tuberculosis.

Signs and Symptoms

A clear-cut distinction can be made between infection with the TB bacterium and the disease it causes. In general, humans are rather easily infected with the bacterium but are resistant to the disease. Estimates project that only about 5% to 10% of infected people actually develop a clinical case of tuberculosis. The majority (85%) of TB cases are contained in the lungs, even though disseminated TB bacteria can result in tuberculosis in any organ of the body. Clinical tuberculosis is divided into primary tuberculosis, secondary (reactivation or reinfection) tuberculosis, and disseminated or extrapulmonary tuberculosis.

Primary Tuberculosis The minimum infectious dose for lung infection is low, around 10 bacterial cells. Alveolar macrophages phagocytose these cells, but they are not killed and continue to multiply inside the macrophages. This period of hidden infection is asymptomatic or is accompanied by mild fever. Some bacteria escape from the lungs into the blood and lymphatics. After 3 to 4 weeks, the immune system mounts a complex, cell-mediated assault against the bacteria. The large influx of mononuclear cells into the lungs plays a part in the formation of specific

infection sites called **tubercles**. Tubercles are granulomas that consist of a central core containing TB bacteria in enlarged macrophages and an outer wall made of fibroblasts, lymphocytes, and macrophages **(figure 19.11)**. Although this response further checks spread of infection and helps prevent the disease, it also carries a potential for damage. Frequently, as neutrophils come on the scene and release their enzymes, the centers of tubercles break down into necrotic **caseous** (kay′-see-us) **lesions** that gradually heal by *calcification*—normal lung tissue is replaced by calcium deposits. The response of T cells to *M. tuberculosis* proteins also causes a cell-mediated immune response evident in the skin test called the **tuberculin reaction**, a valuable diagnostic and epidemiological tool.

TB infection outside of the lungs is more common in immunosuppressed patients and young children. Organs most commonly involved in **extrapulmonary TB** are the regional lymph nodes, kidneys, long bones, genital tract, brain, and meninges. Because of the debilitation of the patient and the high load of TB bacteria, these complications are often fatal.

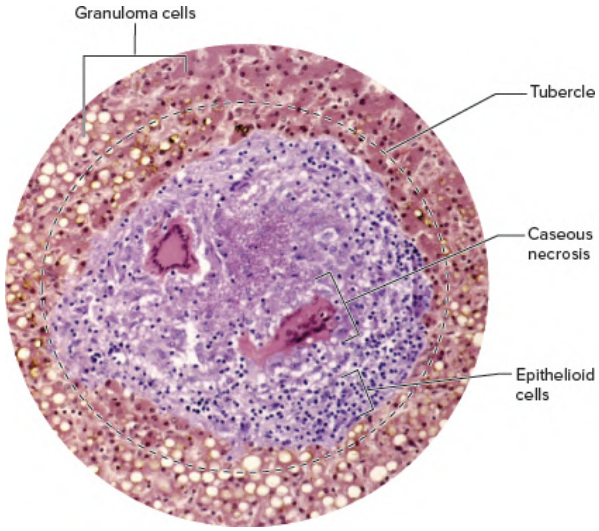


Figure 19.11 **Tubercle formation**. Photomicrograph of a tubercle. The massive granuloma infiltrate has obliterated the alveoli and set up a dense collar of fibroblasts, lymphocytes (granuloma cells), and epithelioid cells. The core of this tubercle is a caseous (cheesy) material containing the bacilli.

©Steve Gschmeissner/Science Source

Secondary (Reactivation) Tuberculosis Although the majority of adequately treated TB patients recover more or less completely from the primary episode of infection, live bacteria can remain dormant and become reactivated weeks, months, or years later, especially in people with weakened immunity. In chronic tuberculosis, tubercles filled with masses of bacteria expand, cause cavities in the lungs, and drain into the bronchial tubes and upper respiratory tract. The patient gradually experiences more severe symptoms, including violent coughing, greenish or bloody sputum, low-grade fever, anorexia, weight loss, extreme fatigue, night sweats, and chest pain. It is the gradual wasting of the body that accounts for an older name for tuberculosis—*consumption*. Untreated secondary disease has nearly a 60% mortality rate.

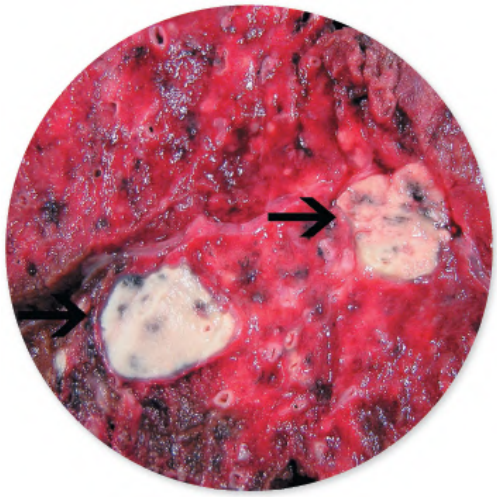
Causative Agents

M. tuberculosis, the cause of tuberculosis in most patients, is an acid-fast rod, long and thin. It is a strict aerobe, and technically speaking, there is still debate about whether it is a gram-positive or a gram-negative organism. It is rarely called gram anything, however, because its acid-fast nature is much more relevant in a clinical setting. It grows very slowly. With a generation time of 15 to 20 hours, a period of up to 6 weeks is required for colonies to appear in culture. (Note: The prefix *Myco-* might make you think of fungi, but this is a bacterium. The prefix in the name came from the mistaken impression that colonies growing on agar resembled fungal colonies. Be sure to differentiate this bacterium from *Mycoplasma*—they are unrelated.)

Robert Koch identified that *M. tuberculosis* often forms serpentine cords while growing, and he called the unknown substance causing this style of growth *cord factor*. Cord factor appears to be associated with virulent strains, and it is a lipid component of the mycobacterial cell wall. All mycobacterial species have walls that have a very high content of complex lipids, including mycolic acid and waxes. This chemical characteristic makes them relatively impermeable to stains and difficult to decolorize (acid-fast) once they are stained. The lipid wall of the bacterium also influences its virulence and makes it resistant to drying and disinfectants.

In recent decades, tuberculosis-like conditions caused by *Mycobacterium avium* and related mycobacterial species (sometimes referred to as the *M. avium* complex, or MAC) have been found in AIDS patients and other immunocompromised people. In this section, we consider only *M. tuberculosis*.

A positive skin test for tuberculosis
Source: CDC/Donald Kopanoff



Caseous necrosis (white areas) of the lung seen on biopsy.
©McGraw-Hill Education

Pathogenesis and Virulence Factors

The course of the infection—and all of its possible variations—was described under "Signs and Symptoms." Important characteristics of the bacterium that contribute to its virulence are its waxy surface (contributing both to its survival in the environment and its survival within macrophages) and its ability to stimulate a strong cell-mediated immune response that contributes to the pathology of the disease.

Transmission and Epidemiology

Mycobacterium tuberculosis is transmitted almost exclusively by fine droplets of respiratory mucus suspended in the air. The TB bacterium is highly resistant and can survive for 8 months in fine aerosol particles. Although larger particles become trapped in mucus and are expelled, tinier ones can be inhaled into the bronchioles and alveoli. This effect is especially pronounced among people sharing small closed rooms with limited access to sunlight and fresh air. In 2016 scientists published a paper showing that acquiring tuberculosis from animals is much more common than had previously been appreciated. In those cases the bacterium is *Mycobacterium bovis*, which may be resistant to first-line antibiotics used for *M. tuberculosis*. Estimates of how many cases come from animals vary widely, from 9% in one study, to 45% in another.

The epidemiological patterns of *M. tuberculosis* infection vary with the living conditions in a community or an area of the world. Factors that significantly affect people's susceptibility to tuberculosis are inadequate nutrition, debilitation of the immune system, poor access to medical care, lung damage, and their own genetics. Put simply, TB is an infection of poverty. People in developing countries are often infected as infants and harbor the microbe for many years until the disease is reactivated in young adulthood. In 2015, there were 10.4 million new cases of TB, and approximately 1.8 million people died from TB, or almost 5,000 people every day. People who are HIV+ are at particular risk for TB infection. In 2015, 11% of new TB diagnoses were in persons living with HIV.

TB infection rates have been falling in the United States since a high in the early 1990s. In 2015 there was a small but significant increase in cases. Some populations are at higher risk of infection or of developing life-threatening forms of the disease. For instance, about 60% of cases in the United States are among foreign-born persons. People who work or live in certain communities such as nursing homes, hospitals, or jails are also among those at greater risk. This is important to know as a health care provider so you can be alert for TB in certain populations.

Culture and Diagnosis

You are probably familiar with several methods of detecting tuberculosis in humans. Clinical diagnosis of tuberculosis relies on three techniques: (1) tuberculin testing, (2) interferon-gamma release assays (IGRAs) performed on a blood sample, and (3) cultural isolation and antimicrobial susceptibility testing. Acid-fast staining is used as a supplement to these techniques.

Tuberculin Sensitivity and Testing Because infection with the TB bacillus can lead to delayed hypersensitivity to tuberculoproteins, testing for hypersensitivity has been an important way to screen populations for tuberculosis infection and disease. Although there are newer methods available, the most widely used test is still the tuberculin skin test, called the **Mantoux test**. It involves local injection of purified protein derivative (PPD), a standardized solution taken from culture fluids of *M. tuberculosis*. The injection is done intradermally into the forearm to produce an immediate small bleb. After 48 and 72 hours, the site is observed for a red wheal called an **induration**, which is measured and interpreted as positive or negative according to size **(figure 19.12)**.

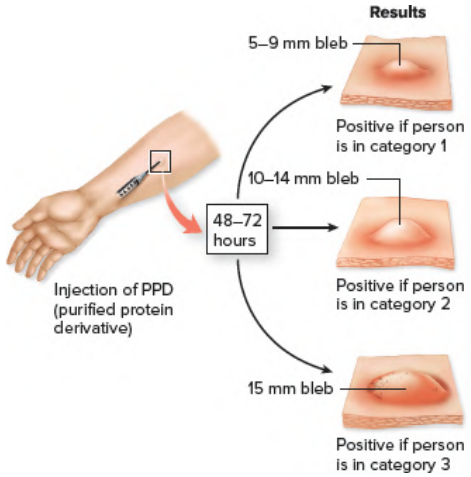


Figure 19.12 **Skin testing for tuberculosis**. The Mantoux test. Tuberculin is injected into the dermis. A small bleb from the injected fluid develops but will be absorbed in a short time. After 48 to 72 hours, the skin reaction is rated by the degree (or size) of the raised area. The surrounding red area is *not* counted in the measurement. People in different categories have different thresholds for their reactions being counted as positive. People are categorized on the basis of factors such as their HIV status, whether they are recent immigrants from TB-endemic areas, or if they have no risk factors for TB.

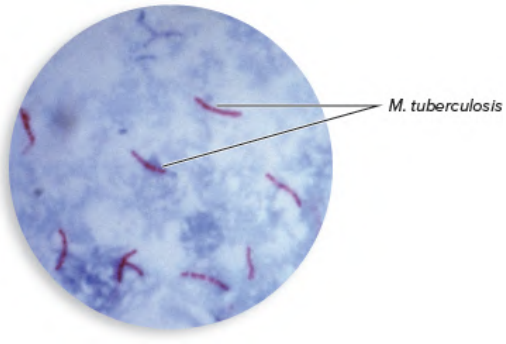


Figure 19.13 **Ziehl-Neelsen staining of *Mycobacterium tuberculosis* in sputum**.
Source: CDC/Dr. George P. Kubica

Blood Testing Because the main immune response to *Mycobacterium* comes from T cells, the blood test for tuberculosis looks for T-cell activities in the form of cytokine release. The assay is called

interferon gamma release assay (IGRA). It uses whole blood and can be completed within 24 hours.

Culture Treatment and isolation should be instituted presumptively until culture results are available. Antibiotic sensitivities can be determined after the culture has grown. Even though newer cultivation schemes exist that shorten the incubation period from 6 weeks to several days, this delay is unacceptable for beginning treatment or isolation precautions. But a culture still must be performed because growing colonies are required to determine antibiotic sensitivities.

Acid-Fast Staining The diagnosis of tuberculosis in people with positive skin tests or X rays can be backed up by acid-fast staining of sputum or other specimens. Several variations on the acid-fast stain are currently in use. The Ziehl-Neelsen stain produces bright red acid-fast bacilli (AFB) against a blue background **(figure 19.13)**. Fluorescence staining shows luminescent yellow-green bacteria against a dark background.

▶ Prevention

Preventing TB in the United States is accomplished by limiting exposure to infectious airborne particles. Extensive precautions, such as isolation in negative-pressure rooms, are used in health care settings when a person with active TB is identified. Vaccine is generally not used in the United States, although an attenuated vaccine called BCG is used in many countries. BCG stands for Bacille Calmette-Guerin, named for two French scientists who created the vaccine in the early 1900s. It is a live strain of a bovine tuberculosis bacterium that has been made avirulent by long passage through artificial media. Remember that persons vaccinated with BCG may respond positively to a tuberculin skin test.

In the past, prevention in the context of tuberculosis referred to preventing a person with latent TB from experiencing reactivation. This strategy is more accurately referred to as treatment of latent infection and is considered in the next section, "Treatment."

▶ Treatment

Treatment of latent TB infection is effective in preventing full-blown disease in persons who have positive skin tests and who are at risk for reactivated TB. Treatment of latent TB is with three drugs: isoniazid, rifampin, and rifapentine. The rifampin and rifapentine are taken for 4 and 3 months, respectively, and the isoniazid is continued for 9 months.

Treatment of active TB infection occurs in two phases. In the first, four drugs—rifampin, isoniazid, ethambutol, and pyrazinamide—are used for 2 months. The second phase uses only rifampin and isoniazid and lasts either 4 or 7 months, decided on a case-by-case basis.

One of the biggest problems with TB therapy is noncompliance on the part of the patient. It is very difficult, even under the best of circumstances, to keep to a regimen of multiple antibiotics daily for months—and most TB patients are living under conditions that are far from the best of circumstances. Failure to adhere to the antibiotic regimen leads to antibiotic resistance in the slow-growing microorganism; in fact, many *M. tuberculosis* isolates are now found to be **MDR-TB**, or multidrug-resistant TB.

Multidrug-Resistant Tuberculosis (MDR-TB)

Multidrug-resistant tuberculosis is defined as being resistant to at least isoniazid and rifampin. It requires treatment of 18 to 24 months with four to six drugs. It is particularly common in people who have been previously treated for tuberculosis. In 2015, approximately 500,000 new cases of MDR-TB were diagnosed. Of these, 45% were in India, China, and the Russian Federation.

People with MDR-TB are generally sicker and have higher mortality rates than those infected with non-MDR-TB. This is true even when they are being treated, as

Medical Moment

Tuberculosis—A Lesson in Inadequate Treatment

Tuberculosis is such a slow-growing bacterium that to be effective, antibiotic treatment has to continue uninterrupted for months, even in an uncomplicated case. Because of the demographics of TB infection (i.e., the countries hardest hit, the socioeconomic status of individuals who become infected), sustained treatment has always been very difficult to achieve. That is how the first MDR-TB strains arose. When a patient stops taking antibiotics prematurely, the bacteria that have not yet been killed are the ones that are least sensitive to the antibiotics. Those bacteria can then spread to other people, and if some of those people are also noncompliant with the rigorous treatment schedule, the bacterium can become even more resistant. Repeat this pattern hundreds of thousands of times and you can see why we have MDR-TB and XDR-TB.

Q. In addition to patient noncompliance with the lengthy antibiotic regimen, can you think of other factors that might have led to the development of resistant strains?

Answer in Appendix B.

Source: CDC/Ronald W. Smithwick

Disease Table 19.9 Tuberculosis

Causative Organism(s)	*Mycobacterium tuberculosis* B	MDR-TB and XDR-TB B
Most Common Modes of Transmission	Vehicle (airborne)	
Virulence Factors	Lipids in wall, ability to stimulate strong cell-mediated immunity (CMI)	
Culture/Diagnosis	Rapid methods plus culture; initial tests are skin testing, positive blood culture, chest X ray	
Prevention	Avoiding airborne *M. tuberculosis;* BCG vaccine in other countries	
Treatment	Isoniazid, rifampin, and pyrazinamide + ethambutol or streptomycin for varying lengths of time (always lengthy)	Multiple-drug regimen, which may include bedaquiline; in **Serious Threat** category in CDC Antibiotic Resistance Report
Distinctive Features	Remains airborne for long periods; extremely slow-growing which has implications for diagnosis and treatment.	Much higher fatality rate over shorter duration
Epidemiological Features	United States: approx. 10,000 cases/year, 16% of cases Whites, 84% ethnic minorities; internationally: 1.8 million deaths in 2015	United States: fewer than 100/year; worldwide: approximately 500,000 new cases MDR-TB in 2015

the multiple-drug combination has severe side effects. Even with treatment, about half of these patients die.

Extensively Drug-Resistant Tuberculosis (XDR-TB)

MDR-TB strains with resistance to two additional drugs are called **XDR-TB**. These strains have been reported in patients in 117 countries. Worldwide, it is estimated that 9% of the MDR-TB cases also qualify as XDR-TB. Patients with XDR-TB have few treatment options and their mortality rate is estimated to be about 70% within months of diagnosis. **Disease Table 19.9**.

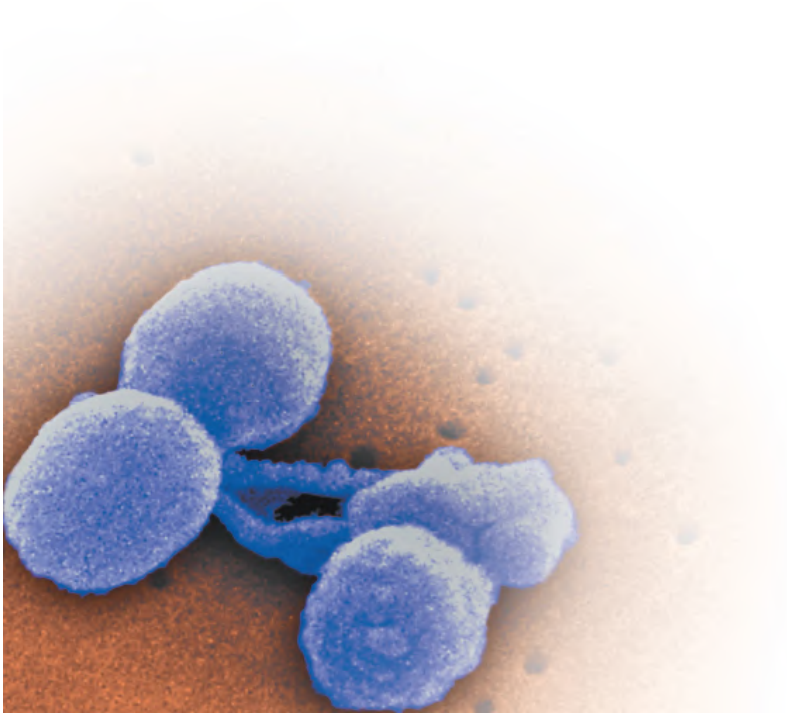
Pneumonia

Pneumonia is a classic example of a disease characterized by an *anatomical diagnosis*. It is defined as an inflammatory condition of the lung in which fluid fills the alveoli. The set of symptoms that we call pneumonia can be caused by a wide variety of different microorganisms. In a sense, the microorganisms need only to have appropriate characteristics to allow them to circumvent the host's defenses and to penetrate and survive in the lower respiratory tract. In particular, the microorganisms must avoid being phagocytosed by alveolar macrophages, or at least avoid being killed once inside the macrophage. Bacteria, fungi, and a wide variety of viruses can cause pneumonias, and there is a lot of variation in the virulence of different pathogenic agents. Pneumonia can be deadly, and across the globe, more children under the age of 5 die from pneumonia than any other infectious disease. In the United States, residents experience 2 to 3 million cases of pneumonia and more than 45,000 deaths due to this condition every year. It is much more common in the winter.

Even though all pneumonias have similar kinds of symptoms, physicians often distinguish between two forms of pneumonia, characterized by different modes of transmission and pathogenic agents. *Community-acquired pneumonia (CAP)* is experienced by persons in the general population. *Healthcare-associated pneumonia (HCAP)* develops in individuals receiving treatment at health care facilities, including hospitals.

Causative Agents of Community-Acquired Pneumonia

Streptococcus pneumoniae accounts for up to 40% of community-acquired bacterial pneumonia cases. It causes more lethal pneumonia cases than any other microorganism.



Streptococcus pneumoniae
Source: CDC/Janice Haney Carr

Figure 19.14 Signature tents in Yosemite National Park. These tent types harbored hantavirus in the 2012 Yosemite outbreak. ©Jebb Harris/ZUMA Press/Newscom

Legionella is a less common but also serious cause of the disease. *Haemophilus influenzae* had been a major cause of community-acquired pneumonia, but the introduction of the Hib vaccine in 1988 has reduced its incidence. A number of bacteria cause a milder form of pneumonia that is often referred to as "walking pneumonia." Two of these are *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* (formerly known as *Chlamydia pneumoniae*). *Histoplasma capsulatum* is a fungus that infects many people but causes a pneumonia-like disease in relatively few. One virus causes a type of pneumonia that can be very serious: hantavirus, which emerged in 1993 in the United States. (Be aware that *Bacillus anthracis*, covered in chapters 18 and 20, can also cause a pneumonic form of anthrax.) Also, recall that many, if not most, deaths stemming from influenza are caused by pneumonia, either from the original virus or from secondary infection.

Streptococcus pneumoniae This bacterium, which is often simply called the pneumococcus, is a small gram-positive flattened coccus that often appears in pairs, lined up end to end. It is alpha-hemolytic on blood agar. Factors that favor the ability of the pneumococcus to cause disease are old age, the season (rate of infection is highest in the winter), underlying viral respiratory disease, diabetes, and chronic abuse of alcohol or narcotics. Healthy people commonly inhale this and other microorganisms into the respiratory tract without serious consequences because of the host defenses present there.

Because the pneumococcus is such a frequent cause of pneumonia in older adults, this population is encouraged to seek immunization with the older pneumococcal polysaccharide vaccine PPSV23 or with PCV13.

Legionella pneumophila *Legionella* is a weakly gram-negative bacterium that has a range of shapes, from coccus to filaments. Several species or subtypes have been characterized, but *L. pneumophila* ("lung-loving") is the one most frequently isolated from infections.

Legionella's ability to survive and persist in natural habitats has been something of a mystery, yet it appears to be widely distributed in aqueous habitats as diverse as tap water, cooling towers, spas, ponds, and other freshwaters. It is resistant to chlorine. The bacterium can live in close association with free-living amoebas **(figure 19.14)**. It is released during aerosol formation and can be carried for long distances. Cases have been traced to supermarket vegetable sprayers, hotel fountains,

air-conditioning vents, and even the fallout from the Mount St. Helens volcano eruption in 1980. In the summer of 2015, a cluster of Legionnaire's cases occurred in the Bronx in New York City. Cooling towers were suspected early on, and the health commissioner ordered all New York City cooling towers to be

disinfected. In all, 133 people became ill and 16 died. Eventually one cooling tower was identified as the source of the outbreak.

In 2016 the CDC reported that cases of Legionnaires' disease (as it is often called) had nearly quadrupled in the period 2000–2014.

Atypical Pneumonias Pneumonias caused by *Mycoplasma* (as well as those caused by *Chlamydophila* and some other microorganisms) are often called atypical pneumonia—atypical in the sense that the symptoms do not resemble those of pneumococcal or other severe pneumonias. *Mycoplasma* pneumonia is transmitted by aerosol droplets among people confined in close living quarters, especially families, students, and the military. Lack of acute illness in most patients has given rise to the name "walking pneumonia."

Hantavirus In 1993, hantavirus suddenly burst into the American consciousness. A cluster of unusual cases of severe lung edema among healthy young adults arose in the Four Corners area of New Mexico. Most of the patients died within a few days. They were later found to have been infected with hantavirus, an agent that had previously only been known to cause severe kidney disease and hemorrhagic fevers in other parts of the world. The new condition was named hantavirus pulmonary syndrome (HPS). Since 1993, the disease has occurred sporadically, but it has a mortality rate of at least 33%.

Very soon after the initial cases in 1993, it became clear that the virus was associated with the presence of mice in close proximity to the victims. Investigators eventually determined that the virus, an enveloped virus of the *Bunyaviridae* family, is transmitted via airborne dust contaminated with the urine, feces, or saliva of infected rodents. Deer mice and other rodents can carry the virus with few apparent symptoms. Small outbreaks of the disease are usually correlated with increases in the local rodent population. In 2012, hantavirus caused a localized outbreak among visitors to Yosemite National Park. Visitors staying in "signature tent cabins" were exposed to mouse droppings containing the virus. Three people died, and at least 10 were sickened. The signature tent cabins were of a new double-walled design that was meant to be safer, but the space between the walls turned out to be a perfect nesting space for the mice.

Histoplasma capsulatum This organism is endemically distributed on all continents except Australia. Its highest rates of incidence occur in the eastern and central regions of the United States, especially in the Ohio Valley. This fungus appears to grow most abundantly in moist soils high in nitrogen content, especially those supplemented by bird and bat droppings **(figure 19.15)**.

A useful tool for determining the distribution of *H. capsulatum* is to inject a fungal extract into the skin and monitor for allergic reactions (much like the TB skin test). Application of this test has verified the extremely widespread distribution of the fungus. In high-prevalence areas such as southern Ohio, Illinois, Missouri, Kentucky, Tennessee, Michigan, Georgia, and Arkansas, 80% to 90% of the population show signs of prior infection.

Pneumocystis (carinii) jirovecii Although the fungus *Pneumocystis jirovecii* (formerly called *P. carinii*) was discovered in 1909, it remained relatively obscure until it was suddenly propelled into clinical prominence as the agent of *Pneumocystis* pneumonia. PCP is one of the most frequent opportunistic infections in AIDS patients, most of whom will develop one or more episodes during their lifetimes. Cancer patients and others with extreme immunosuppresssion are also at risk for this disease. There is some debate about how *Pneumocystis* is acquired, but inhalation of spores is probably common, and healthy people may even harbor it as "normal biota" in their lungs.

Figure 19.15 **Sign in wooded area in Kentucky**. The sign is covered in bird droppings. Up to 90% of the population in the Ohio Valley show evidence of past infection with *Histoplasma*.
©Tom Volk

Disease Table 19.10 Community-Acquired Pneumonia

Causative Organism(s)	*Streptococcus pneumoniae* B **G+**	*Legionella* species B **G**	*Mycoplasma pneumoniae* B	*Hantavirus* V	*Histoplasma capsulatum* F	*Pneumocystis jirovecii* F	Respiratory viruses V
Most Common Modes of Transmission	Droplet contact or endogenous transfer	Vehicle (water droplets)	Droplet contact	Vehicle—airborne virus emitted from rodents	Vehicle—inhalation of contaminated soil	Droplet contact	Droplet contact or endogenous transfer
Virulence Factors	Capsule	–	Adhesins	Ability to induce inflammatory response	Survival in phagocytes	–	–
Culture/Diagnosis	Gram stain often diagnostic, alpha-hemolytic on blood agar	Requires selective charcoal yeast extract agar; serology unreliable	PCR; rule out other etiologic agents	Serology (IgM), PCR identification of antigen in tissue	Rapid antigen tests, microscopy	PCR	Failure to find bacteria or fungi
Prevention	Pneumococcal polysaccharide vaccine	–	No vaccine, no permanent immunity	Avoid mouse habitats and droppings	Avoid contaminated	Antifungals given to AIDS patients to prevent this	Hygiene

	(PPSV23) or the conjugate vaccinne PCV13				soil/bat, bird droppings		
Treatment	Cefotaxime, ceftriaxone, with or without vancomycin; in **Serious Threat** category in CDC Antibiotic Resistance Report	Levofloxacin, azithromycin, moxifloxacin	Azithromycin	Supportive	Amphotericin B; itraconazole	Trimethoprim-sulfamethoxazole	None
Distinctive Features	Patient usually severely ill	Mild pneumonias in healthy people; can be severe in elderly or immunocompromised	Usually mild; "walking pneumonia"	Rapid onset; high mortality rate	Many infections asymptomatic	Vast majority occur in AIDS patients	Usually mild
Epidemiological Features	40% of CAP cases; in 2009 influenza epidemic, 29% of fatalities were coinfected with this bacterium	United States: +/− 5,000 cases per year; internationally: 2 million cases per year	20%–40% of CAP cases	United States: 10–40 cases per year; fatality rate 25%–75%	In United States, 250,000 infected per year; only 5%–10% have symptoms	Almost exclusively in severely immunocompromised patients.	30% of CAP cases

Traditional antifungal drugs are ineffective against *Pneumocystis* pneumonia because the chemical makeup of the organism's cell wall differs from that of most fungi.

Respiratory Viruses Viruses are very common causes of community-acquired pneumonia. They are viruses that are either residents in the upper respiratory tract or acquired through our daily activities. Viral pneumonias are generally mild. Recently a very common cause of respiratory infections, including pneumonia, was identified. Human metapneumovirus can now be tested for via RT-PCR or immunoassay. **Disease Table 19.10**.

A Note About SARS and MERS

In 2003, a virus in the *Coronavirus* family, previously known to cause only coldlike symptoms, burst onto the world stage as it started to cause pneumonias and death in Hong Kong. The SARS epidemic ended nearly as quickly as it started, and since 2004 new cases of SARS have not been detected anywhere on the planet. In 2013, another new coronavirus emerged from Saudi Arabia. It was named MERS (Middle East Respiratory Syndrome). It has a 30% fatality rate and while it is transmissible human-to-human, the rate at which it does so seems to be low. Because that could change, MERS is one of the three emerging infections targeted in the new initiative by the Coalition for Epidemic Preparedness Innovation (see chapter 12).

©Travel Ink/Getty Images

Disease Table 19.11 Healthcare-Associated Pneumonia

Causative Organism(s)	Gram-negative and gram-positive bacteria from upper respiratory tract or stomach; environmental contamination of ventilator
Most Common Modes of Transmission	Endogenous (aspiration)
Virulence Factors	–
Culture/Diagnosis	Culture of lung fluids
Prevention	Elevating patient's head, preoperative education, care of respiratory equipment
Treatment	Broad-spectrum antibiotics
Epidemiological Features	United States: 300,000 cases per year; occurs in 0.5%–1.0% of admitted patients; mortality rate in United States and internationally is 20%–50%

Healthcare-Associated Pneumonia

Up to 1% of hospitalized or institutionalized people experience the complication of pneumonia. Together with surgical site infections, it is the most common healthcare-associated infection. It is most commonly associated with mechanical ventilation, via an endotracheal or tracheostomy tube. This is sometimes labeled "ventilator-associated pneumonia," or VAP. The mortality rate is quite high—between 30% and 50%. The most frequent cause of all forms of healthcare-associated pneumonia today are MRSA strains of *Staphylococcus aureus*, as well as nonresistant strains. MRSA is on the CDC's list as a Serious Threat. After MRSA, gram-negative bacteria are most common. These include *Klebsiella pneumoniae, Enterobacter, E. coli, Pseudomonas aeruginosa*, and *Acinetobacter*. Some *Enterobacter* strains are highly antibiotic-resistant. When they are resistant to a last-line antibiotic (carbapenem), they are designated CRE and are in the Urgent Threat category from the CDC. Likewise, *Acinetobacter* is likely to be multidrug-resistant. In those cases it is in the Serious Threat category. Further complicating matters, many healthcare-associated pneumonias

appear to be polymicrobial in origin—meaning that there are multiple microorganisms multiplying in the alveolar spaces.

Culture of sputum or of tracheal swabs is not very useful in diagnosing healthcare-associated pneumonia, because the condition is usually caused by normal biota. Cultures of fluids obtained through endotracheal tubes or from bronchoalveolar lavage provide better information but are fairly intrusive. It is also important to remember that if the patient has already received antibiotics, culture results will be affected.

Because most healthcare-associated pneumonias are caused by microorganisms aspirated from the upper respiratory tract, measures that discourage the transfer of microbes into the lungs are very useful for preventing the condition. Elevating patients' heads to a 30- to 45-degree angle helps reduce aspiration of secretions. Good preoperative education of patients about the importance of deep breathing and frequent coughing can reduce postoperative infection rates. Proper care of mechanical ventilation and respiratory therapy equipment is essential as well.

Studies have shown that delaying antibiotic treatment of suspected healthcare-associated pneumonia leads to a greater likelihood of death. Even in this era of conservative antibiotic use, empiric therapy should be started as soon as healthcare-associated pneumonia is suspected, using multiple antibiotics that cover both gram-negative and gram-positive organisms. **Disease Table 19.11**.

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4. A nurse is developing a health promotion class on identification and prevention of tuberculosis (TB). Which clients would the nurse include in TB testing? Select all that apply.

a. *client who is HIV-positive*
b. *male, age 38, who works as a prison guard*
c. *nursing student with no comorbid conditions*
d. *migrant farm worker with limited health access who presents to the clinic for information on diabetes*
e. *female, age 26, who recently traveled abroad visiting endemic areas*

19.5 LEARNING OUTCOMES—Assess Your Progress

10. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Disease," tuberculosis.
11. Discuss the problems associated with MDR-TB and XDR-TB.
12. Discuss important features of the other lower respiratory tract diseases, community-acquired and healthcare-associated pneumonia.

CASE FILE WRAP-UP

©Purestock/SuperStock (health care worker); ©MedicalRF.com (coronavirus)

Respiratory syncytial virus (RSV) is prevalent during the winter months and is a common cause of lower respiratory tract infection during infancy and childhood. Older children and adults generally experience mild disease, but infants ages 6 months and younger may develop bronchiolitis (inflammation of the smaller airways) or pneumonia as a consequence of the infection. Premature infants are particularly at risk and may develop life-threatening disease. There is no vaccine for RSV, but premature infants and/or infants born with cardiac or lung disease may be given a series of injections of the monoclonal antibody palivizumab (Synagis), which has proven to be moderately effective at preventing the disease.

RSV spreads easily by droplet contact. It can also survive for hours on inanimate objects and surfaces such as tables. Michael, the infant in the case file at the beginning of this chapter, may have contracted RSV at his day care, or he may have been exposed to the virus from his school-aged siblings. By 2 to 3 years of age, most children in the United States will have encountered RSV. Unfortunately, there is no vaccine for RSV, but researchers are getting closer to developing one.

Summing Up

Taxonomic Organization Microorganisms Causing Disease in the Respiratory Tract

Microorganism	Pronunciation	Disease Table
Gram-positive bacteria		
Streptococcus pneumoniae	strep′-tuh-kok″-us nu- moh′ nee-ay	Otitis media, 19.4 Pneumonia, 19.10
Streptococcus pyogenes	strep′-tuh-kok″-us pie-ah′-gen-eez	Pharyngitis, 19.1
Corynebacterium diphtheriae	cor-eye′-nee-back-teer″-e-em dip-theer′-e-ay	Diphtheria, 19.5
Gram-negative bacteria		
Haemophilus influenzae	huh-mah′-fuh-luss in′-floo-en″-zay	Otitis media, 19.4
Bordetella pertussis	bor′-duh-tell′-uh per-tuss′-is	Whooping cough, 19.7
Legionella spp.	lee″-juhn-el′-uh	Pneumonia, 19.10
Pseudomonas aeruginosa	sue′-doe-moe″-ness air-roo′-gi-no″-sa	Healthcare-associated pneunomia, 19.11
Acinetobacter baumannii	a′-ci-nay′-toe-bak″-ter bow-mah″-nee-i	Healthcare-associated pneunomia, 19.11
Other bacteria		
*Mycobacterium tuberculosis**	my″-co-back-teer′-ee-em tuh-ber′-cue-loh-sis	Tuberculosis, 19.9
Mycoplasma pneumoniae	my″-co-plazz′-muh nu-moh′-nee-ay	Pneumonia, 19.10
RNA viruses		
Respiratory syncytial virus	ress″-pur-uh-tor′-ee sin-sish′-ull vie′-russ	RSV disease, 19.8
Influenza virus A, B, and C	in″-floo-en′-zuh vie′-russ	Influenza, 19.6
Hantavirus	haun″-tuh vie′-russ	Pneumonia, 19.10
Fungi		
Pneumocystis jirovecii	new-moh-siss′-tiss yee″-row-vet′-zee	Pneumonia, 19.10
Histoplasma capsulatum	hiss″-toe-plazz′-muh cap″-sue-lah′-tum	Pneumonia, 19.10

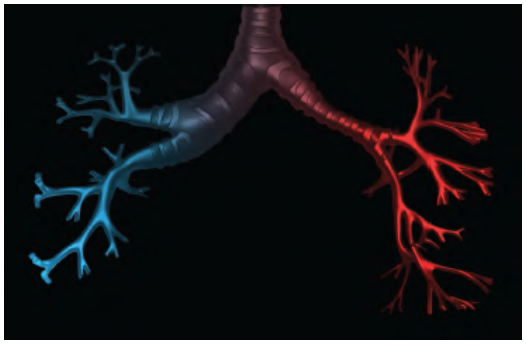
*There is some debate about the gram status of the genus *Mycobacterium;* it is generally not considered gram-positive or gram-negative.

Gut Bacteria Involved in Deadly Lung Disease?

Acute respiratory distress syndrome (ARDS) is a dangerous condition with sudden onset that affects more than 200,000 people a year in the United States. It has a case fatality rate of nearly 50%. In 2016 a group of researchers from the University of Michigan found a new clue about the pathological process of this disease.

They studied 68 patients with the condition, and compared their lung microbiome to the lung microbiome of healthy volunteers. They found microbes in the ill patients' lungs that are generally only found in the GI tract. This led them to hypothesize that treatments for the severe condition were missing an important component. They characterize ARDS as a "vicious cycle," in which critically ill patients develop systemic inflammation, which can lead to tight junctions in the intestines becoming leaky, allowing gut bacteria into the bloodstream. In the bloodstream they would gain access possibly to the lungs. This, of course, will increase inflammation and the whole system becomes amped up, resulting in injury to lung tissue and other inflammatory effects.

They point out that even before the importance of the microbiome was appreciated, critical care teams recognized that the gut microbiome influenced the outcome of a critical illness. In some European countries, patients in intensive care units—even for noninfectious reasons—are treated with antibiotics, a practice believed to decrease organ system failure. This technique is called selective decontamination of the digestive tract. The new findings from the Michigan team might provide a reason that the practice works.



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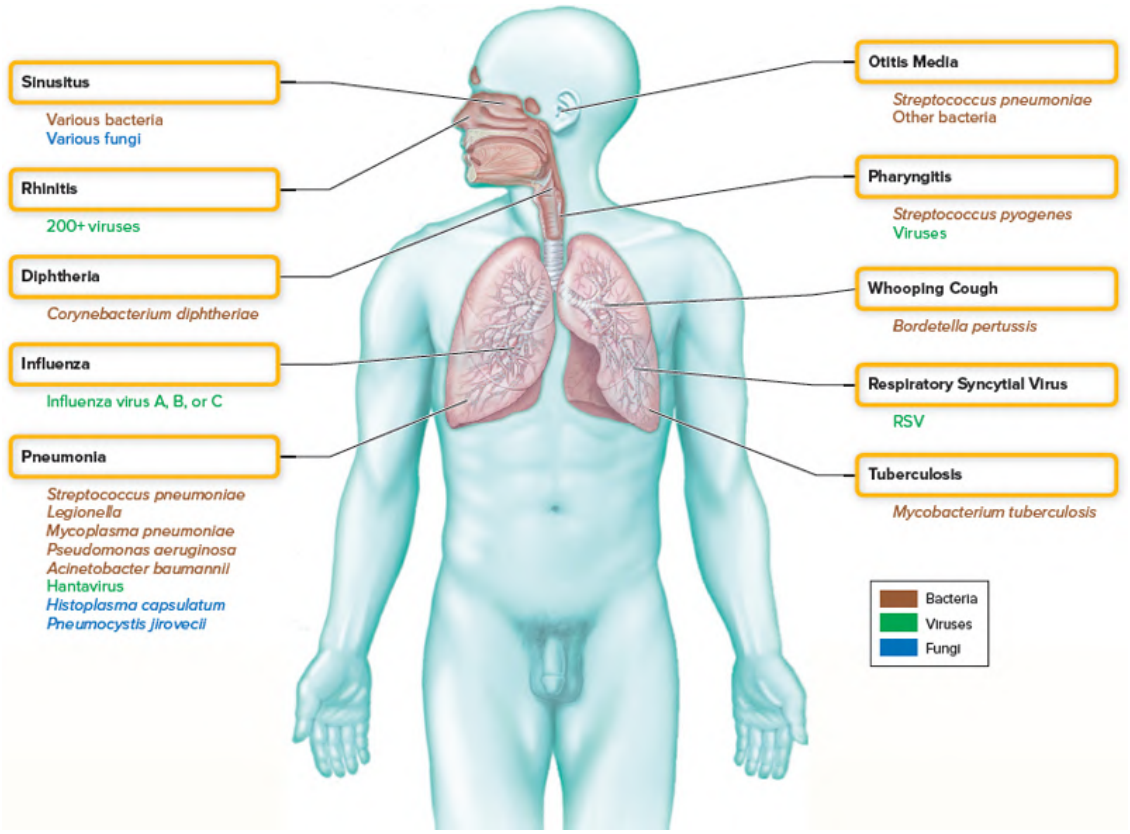
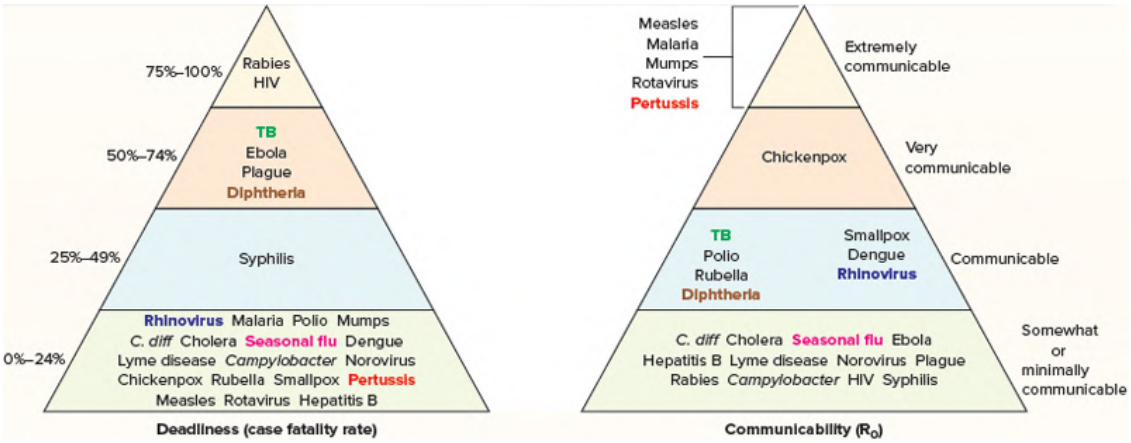


System Summary Figure 16.17

Deadliness and Communicability of Selected Diseases of the Respiratory System



Chapter Summary

19.1 The Respiratory Tract and Its Defenses

- The upper respiratory tract includes the mouth, the nose, nasal cavity and sinuses above it, the throat or pharynx, and epiglottis and larynx.
- The lower respiratory tract consists of the trachea, the bronchi, bronchioles, and alveoli in the lungs.
- The ciliary escalator, mucus on the surface of the mucous membranes, and involuntary responses such as coughing, sneezing, and swallowing are structural defenses. Alveolar macrophages, and secretory IgA are also helpful.

©Thinkstock/Jupiterimages

19.2 Normal Biota of the Respiratory Tract

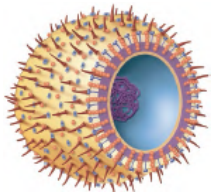
- Normal biota include *Streptococcus pyogenes, Haemophilus influenzae, Streptococcus pneumoniae, Neisseria meningitidis, Staphylococcus aureus, Lactobacillis sakei, Corynebacterium tuberculostearicum*, and *Candida albicans*.

19.3 Upper Respiratory Tract Diseases Caused by Microorganisms

- **Pharyngitis:** Viruses are common cause of pharyngitis. However, *Streptococcus pyogenes* is a more serious bacterial cause.
- **The Common Cold:** Caused by one of over 200 different kinds of viruses, most commonly the rhinoviruses, followed by the coronaviruses. Respiratory syncytial virus (RSV) causes colds in many people, but in some, especially children, they can lead to more serious respiratory tract symptoms.
- **Sinusitis:** Inflammatory condition of the sinuses in the skull, most commonly caused by allergy or infections by a variety of viruses or bacteria and, less commonly, fungi.
- **Acute Otitis Media (Ear Infection):** Most common cause is *Streptococcus pneumoniae*, though multiple organisms are usually present in infections.
- **Diphtheria:** Caused by *Corynebacterium diphtheriae*, a non-endospore-forming, gram-positive club-shaped bacterium. An important exotoxin is encoded by a bacteriophage of *C. diphtheriae*.

19.4 Diseases Caused by Microorganisms Affecting Both the Upper and Lower Respiratory Tract

- **Influenza:** The ssRNA genome of the influenza virus is subject to constant genetic changes that alter the structure of its envelope glycoprotein. *Antigenic drift* refers to constant mutation of this glycoprotein. *Antigenic shift*, where the eight separate RNA strands are involved in the swapping out of one of those genes or strands with a gene or strand from a different influenza virus, is even more serious.
- **Whooping Cough:** Causative agent, *Bordetella pertussis*, releases multiple exotoxins *pertussis toxin* and *tracheal cytotoxin* that damage ciliated respiratory epithelial cells and cripple other components of the host defenses.
- **Respiratory Syncytial Virus Infection:** RSV infects the respiratory tract and produces giant multinucleated cells (syncytia). RSV is the most prevalent cause of respiratory infection in the newborn age group.



19.5 Lower Respiratory Tract Diseases Caused by Microorganisms

- **Tuberculosis:** Cause is primarily the bacterial species *Mycobacterium tuberculosis*. Multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) are growing problems and have higher fatality rates.
- **Pneumonia:** Inflammatory condition of the lung in which fluid fills the alveoli. Caused by a wide variety of different microorganisms.
  - *Streptococcus pneumoniae:* The main agent for community-acquired bacterial pneumonia cases. *Legionella* is a less common but serious cause of the disease. Other bacteria: *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae. Histoplasma capsulatum* is a fungus that can cause a pneumonia-like disease. A hantavirus causes a pneumonia-like condition named hantavirus pulmonary syndrome (HPS).
- **Healthcare-Associated Pneumonia:** Many cases are ventilator-associated. HAP is commonly caused by *S. aureus*, often MRSA strains. Furthermore, many healthcare-associated pneumonias appear to be polymicrobial in origin.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. Which of the following organisms is most problematic with respect to its resistance to antibiotics? **a**. *Streptococcus pyogenes* **b**. *Haemophilus influenzae* **c**. *Mycobacterium tuberculosis* **d**. *Bordetella pertussis*	**2**. Explain why an antigenic shift event in the influenza virus can lead to a catastrophic pandemic.	**3**. It has recently been established that the lower respiratory tract—the lungs—has a microbiome. Use what you know about human evolution to explain why this is not surprising, and how it is probably highly advantageous to health.
Cell Structure and Function	**4**. Which of these microbes has an unusual, waxy, wall structure that contributes to its virulence? **a**. *Streptococcus pyogenes* *Haemophilus influenzae* *Mycobacterium tuberculosis* *Bordetella pertussis* **d**.	**5**. What cell structure of *Streptococcus pneumoniae* makes it such a successful and versatile pathogen, in your estimation? Defend your answer.	**6**. Scientists have worked for years to create a vaccine for the common cold. One effort attempts to create a vaccine that is targeted to the host epithelial cell instead of viral antigens. What is the logic behind this approach? What antigens would you include in such a vaccine?

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Metabolic Pathways	**7**. The beta-hemolysis of blood agar observed with *Streptococcus pyogenes* is due to the presence of **a**. streptolysin. **b**. M protein. **c**. hyaluronic acid. **d**. capsule.	**8**. Would you expect the microbiome in the lung to be aerobes, strict anaerobes, or facultative anaerobes? Explain your answer.	**9**. In some diseases and outbreaks of respiratory diseases caused by certain strains of hantavirus and influenza, healthy young adults suffer the most pathology and mortality. This is an unusual pattern; it is more common for the younger and older vulnerable patients to suffer the worst effects of infectious diseases. Speculate on what is happening when the most robust suffer the most pathology.

Information Flow and Genetics	**10**. Which of the following pathogens uses the product of a bacteriophage to cause disease? **a**. *Streptococcus pneumoniae* **b**. *Mycobacterium tuberculosis* **c**. *Bordetella pertussis* **d**. *Corynebacterium diphtheriae*	**11**. Explain why we are advised to get a flu vaccine every year. Then explain why there may be years when we are advised to get two flu vaccines.	**12**. The vaccine for pertussis is an acellular vaccine. We have learned that current circulating strains of *B. pertussis* may "match" the vaccine less well than previous strains. Suggest some molecular events that would have led to this phenomenon.
Microbial Systems	**13**. Which of the following infections often has/have a polymicrobial cause? **a**. otitis media **b**. healthcare-associated pneumonia **c**. sinusitis **d**. all of these	**14**. Outline how a progression from influenza infection to pneumonia to death might occur, focusing on the microbes responsible and the symptoms that ensue.	**15**. Explain why it is unlikely that we will ever see a vaccine for sinusitis.
Impact of	**16**. The vast majority of	**17**. A distraught mother	**18**. Conducting your own

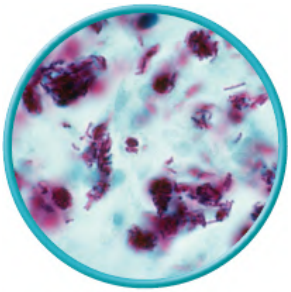
Microorganisms	pneumonias caused by this organism occur in AIDS patients. **a**. *hantavirus* **b**. *Histoplasma capsulatum* **c**. *Pneumocystis jirovecii* **d**. *Mycoplasma pneumoniae*	recently posted on Facebook that although she had not been sick since the birth of her child 3 months ago, she was responsible for her baby being in the ICU with whooping cough. Explain this (accurate) assessment.	research, describe the total impact on human population from the two diseases, malaria and tuberculosis.
Scientific Thinking	**19**. "Watchful waiting" is the recommended response to which infection? **a**. otitis media **b**. tuberculosis **c**. diphtheria **d**. healthcare-associated pneumonia	**20**. Why might it be inadvisable to use tuberculin skin testing to determine the TB status of a person who is HIV+?	**21**. Explain everything we can learn from figure 19.9, the influenza surveillance graph. Be sure to explain the meaning of the black lines as well as the red.

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From chapter 2, figure 2.18*b***. Although there are many different organisms present in the respiratory tract, an acid-fast stain of sputum like the one shown here along with patient symptoms can establish a presumptive diagnosis of tuberculosis. Explain why.



Acid-fast stain
Reddish-purple cells are acid-fast.
Blue cells are non-acid-fast.

©Richard J. Green/Science Source

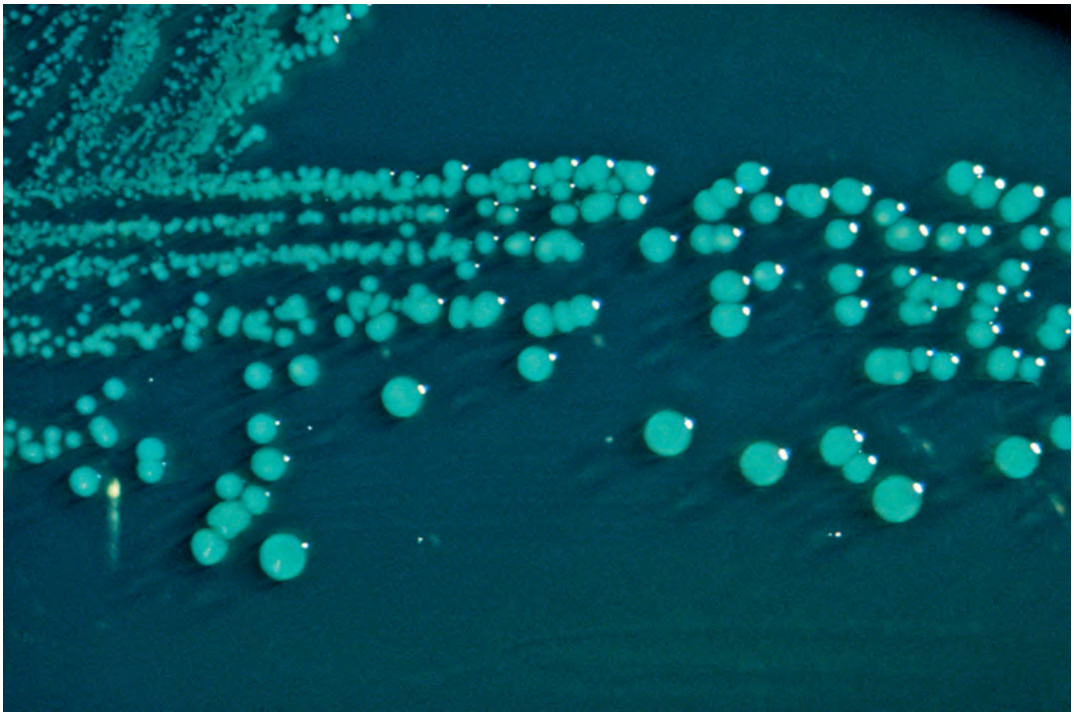
connect Resources are available through Connect, including interactive learning tools,

animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

20

Infectious Diseases Affecting the Gastrointestinal Tract

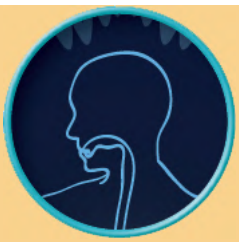


Source: CDC (*Shigella*); ©Anna Smirnova/Alamy Stock Vector (normal biota); ©Moredun Animal Health Ltd./Science Source (helminthic disease); ©Eric Grave/SPL/Getty Images (helminth); ©Purestock/SuperStock (health care worker)

IN THIS CHAPTER…

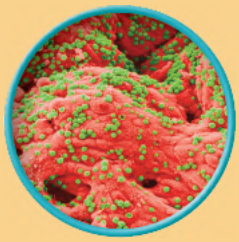
20.1 The Gastrointestinal Tract and Its Defenses

1. Draw or describe the anatomical features of the gastrointestinal tract.
2. List the natural defenses present in the gastrointestinal tract.



20.2 Normal Biota of the Gastrointestinal Tract

3. List the types of normal biota presently known to occupy the gastrointestinal tract.
4. Describe how our view has changed of normal biota present in the stomach.



20.3 Gastrointestinal Tract Diseases Caused by Microorganisms (Nonhelminthic)

5. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Disease" acute diarrhea.
6. Discuss important features of food poisoning and chronic diarrhea.
7. Discuss important features of the two categories of oral conditions: dental caries and periodontal diseases.
8. Identify the most important features of mumps, gastritis, and gastric ulcers.
9. Differentiate among the main types of hepatitis, and discuss the causative agents, mode of transmission, diagnostic techniques, prevention, and treatment of each.

20.4 Gastrointestinal Tract Diseases Caused by Helminths

10. Describe some distinguishing characteristics and commonalities seen in helminthic infections.
11. List four helminths that cause primarily intestinal symptoms, and identify which life cycle they follow and one unique fact about each one.
12. Explain reasons for and the consequences of the following statement, "Cysticercosis is underdiagnosed in the United States."

CASE FILE

"Blood and Guts"

I was working as an LPN in a rural facility when Peter came into the emergency room one evening. My supervising RN and I realized almost immediately that Peter was very ill, and we worked together quickly to obtain Peter's history and vital signs.

Peter stated that he had been ill since the morning, when he woke up earlier than normal with diarrhea. He stated that he had been well the night before when he went to bed and had awoken in the morning with severe abdominal cramping. The diarrhea started almost immediately after the cramps began. Peter estimated that he had had approximately 20 bouts of watery, foul-smelling diarrhea throughout the day. He also stated that there was blood present in his stool.

I took Peter's vital signs. He had a fever of 38.4°C (101°F). He was tachycardic with a heart rate of 118 beats per minute. His blood pressure was low at 100/50 mmHg. He was pale and diaphoretic (sweaty) and complained of chills and a headache. He also complained of intermittent severe cramping. His abdomen was tender in all four quadrants on palpation and his bowel sounds were hyperactive on auscultation. His mucous membranes were dry and he complained of thirst. His skin turgor was poor.

My supervising RN called the physician to report Peter's symptoms and vital signs. The doctor stated that he would be coming in right away and to notify the lab. He asked us to start an intravenous on Peter.

We inserted a large-bore IV and started an infusion of normal saline at 250 mL/hour to rehydrate Peter. The physician and lab technician arrived almost simultaneously, and blood was drawn by the lab tech while the physician examined Peter and asked about his symptoms. Peter was asked to provide a stool sample for culture and an ova and parasites test (O&P test), which he was able to provide in a short time. The physician asked if Peter's wife and daughter were ill and if they had eaten the same food as Peter had in the last few days.

Peter's blood work came back. His white blood cell count was elevated and his potassium level was low. Peter was admitted to the hospital for rehydration and was started on broad-spectrum intravenous antibiotics for full coverage of potential pathogens while we awaited the stool culture results.

Peter's stool cultures came back, revealing that Peter had *Shigella*, which was sensitive to sulfamethoxazole/trimethoprim and ciprofloxacin. Peter had an allergy to ciprofloxacin, so he was started on sulfamethoxazole/trimethoprim in oral form twice a day and IV fluids were continued. After 5 days in the hospital, Peter had recovered enough to go home. He continued the antibiotic therapy for another week and eventually fully recovered. The source of the infection was never determined.

- How is *Shigella* transmitted?
- How can *Shigella* be prevented?

Case File Wrap-Up appears at the end of the chapter.

©Kate Mitchell/Corbis

20.1 The Gastrointestinal Tract and Its Defenses

The gastrointestinal (GI) tract can be thought of as a long tube, extending from mouth to anus. It is a very sophisticated delivery system for nutrients, composed of eight main sections and augmented by four accessory organs. The eight sections are the mouth, pharynx, esophagus, stomach, small intestine, large intestine, rectum, and anus. Along the way, the salivary glands, liver, gallbladder, and pancreas add digestive fluids and enzymes to assist in digesting and processing the food we take in **(figure 20.1)**. The GI tract is often called the *digestive tract* or the *enteric tract*.

The GI tract has a very heavy load of microorganisms, and it encounters millions of new ones every day. Because of this, defenses against infection are extremely important. All intestinal surfaces are coated with a layer of mucus, which confers mechanical protection. Secretory IgA can also be found on most intestinal surfaces. The muscular walls of the GI tract keep food (and microorganisms) moving through the system through the action of peristalsis. Various fluids in the GI tract have antimicrobial properties. Saliva contains the antimicrobial proteins lysozyme and lactoferrin. The stomach fluid is antimicrobial by virtue of its extremely high acidity. Bile is also antimicrobial.

The entire system is outfitted with cells of the immune system, collectively called gut-associated lymphoid tissue (GALT). The tonsils and adenoids in the oral cavity and pharynx, small areas of lymphoid tissue in the esophagus, Peyer's patches in the small intestine, and the appendix are all packets of lymphoid tissue consisting of

T and B cells as well as cells of nonspecific immunity. One of their jobs is to produce IgA, but they perform a variety of other immune functions.

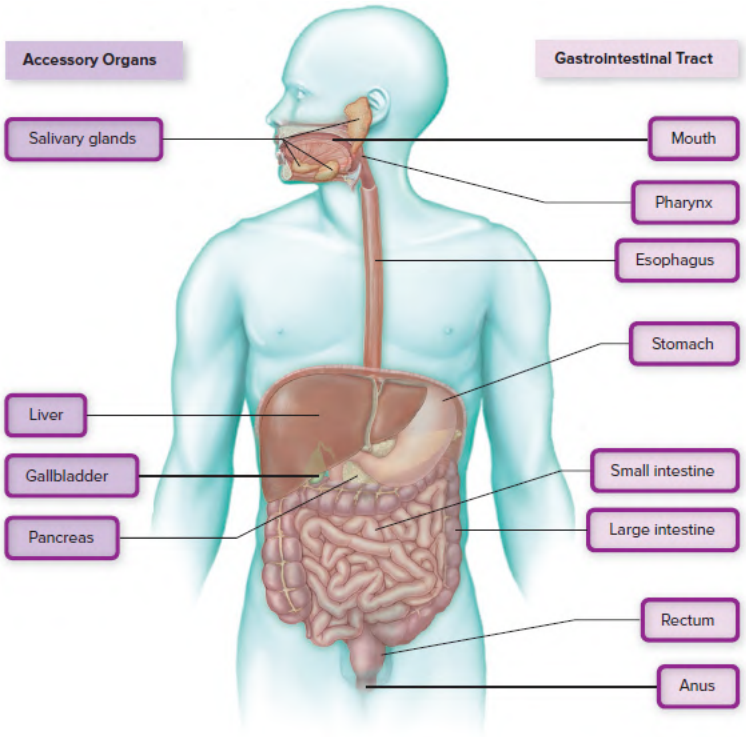


Figure 20.1 **Major organs of the digestive system**.

A huge population of commensal organisms lives in this system, especially in the large intestine. They avoid immune destruction through various mechanisms, including cloaking themselves with host sugars they find on the intestinal walls.

20.1 LEARNING OUTCOMES—Assess Your Progress

1. Draw or describe the anatomical features of the gastrointestinal tract.
2. List the natural defenses present in the gastrointestinal tract.

NCLEX® PREP

1. Gut-associated lymphoid tissue (GALT) includes
   a. *Peyer's patches in the small intestine*.
   b. *the appendix*.
   c. *tonsils*.
   d. *adenoids*.
   e. *all of the above*.
   f. *none of the above*.

20.2 Normal Biota of the Gastrointestinal Tract

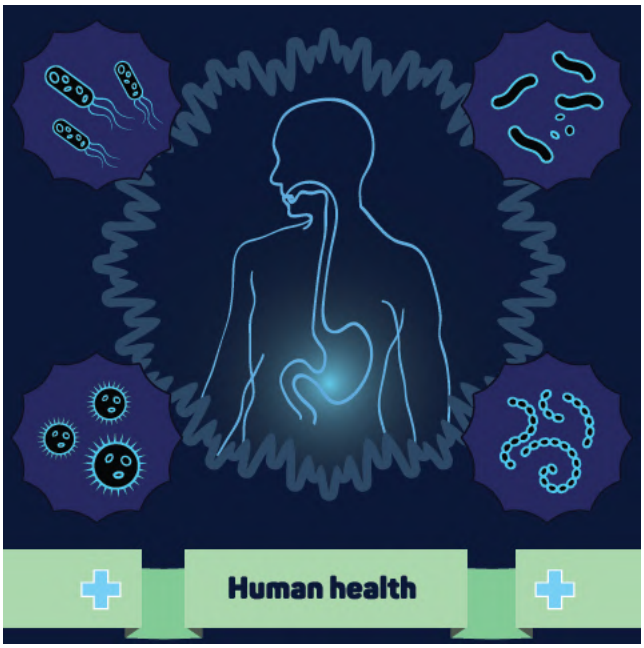
As just mentioned, the GI tract is home to a large variety of normal biota. The oral cavity alone is populated by more than 600 known species of microorganisms, including *Actinomyces, Lactobacillis, Neisseria, Prevotella, Streptococcus, Treponema*, and *Veillonella* species. Fungi such as *Candida albicans* are also numerous. A few protozoa (*Trichomonas tenax, Entamoeba gingivalis*) also call the mouth "home." Bacteria live on the teeth as well

as the soft structures in the mouth. Numerous species of normal biota bacteria live on the teeth in a synergistic community called dental plaque, which is a type of biofilm (see chapter 3). Bacteria are held in the biofilm by specific recognition molecules. Alpha-hemolytic streptococci are generally the first colonizers of the tooth surface after it has been cleaned. The streptococci attach specifically to proteins in the **pellicle**, a mucinous glycoprotein covering on the tooth. Then other species attach specifically to proteins or sugars on the surface of the streptococci, and so on.

The pharynx contains a variety of microorganisms, many of which were described in chapter 19. Although the stomach was previously thought to be sterile due to its very low pH, researchers have found the molecular signatures of 128 different species of microorganisms in the stomach. Though many of these are likely to be "just passing through," a few, including *Bacillus, Clostridium, Staphylococcus*, and *Streptococcus*, are permanent residents. The large intestine has always been known to be a haven for billions of microorganisms (10^{11} per gram of contents), including the bacteria *Bacteroides, Bifidobacterium, Clostridium, Enterobacter, Escherichia, Fusobacterium, Lactobacillus, Peptostreptococcus, Staphylococcus*, and *Streptococcus;* the fungus *Candida;* and several protozoa as well. Researchers have also found archaea species there.

You recall from chapter 17 that it is now clear the gut microbiome influences the development of the nervous system, including portions of the brain, and can have effects on behavior and neurological wellness. Within the gut itself, the normal biota in the gut provide a protective function, but they perform other jobs as well. Some of them help with digestion. Some provide nutrients that we can't produce ourselves. *E. coli*, for instance, synthesizes vitamin K. Its mere presence in the large intestine seems to be important for the proper formation of epithelial cell structure. And the normal biota in the gut plays an important role in "teaching" our immune system to react to microbial antigens. As you have seen throughout this book, we now know that the composition of the gut microbiome has far-reaching consequences on many aspects of human health. The coming years will bring us a more precise understanding, and the possibility of "healing" the microbiome, or manipulating it in ways that improve various disease conditions.

The accessory organs (salivary glands, gallbladder, liver, and pancreas) are free of microorganisms, just as all internal organs are.



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20.2 LEARNING OUTCOMES—Assess Your Progress

3. List the types of normal biota presently known to occupy the gastrointestinal tract.

4. Describe how our view has changed of normal biota present in the stomach.

Chickens and their eggs can be colonized by *Salmonella*.
©Pixtal/AGE Fotostock

20.3 Gastrointestinal Tract Diseases Caused by Microorganisms (Nonhelminthic)

In this section, we first address microbes that cause diarrhea of various types. Then we discuss oral diseases (dental caries and periodontitis), stomach conditions (gastritis and gastric ulcers), and the spectrum of hepatitis infections.

Highlight Disease

Acute Diarrhea

Diarrhea—usually defined as three or more loose stools in a 24-hour period—needs little explanation. In recent years, on average, citizens of the United States experienced 1.2 to 1.9 cases of diarrhea per person per year, and among children that number is twice as high. In developing countries, children may experience more than 10 episodes of diarrhea a year. In fact, more than 700,000 infants a year die from diarrhea, mostly in developing countries. In developing countries, the high mortality rate is not the only issue. Children who survive dozens of bouts with diarrhea during their developmental years are likely to have permanent physical and cognitive effects. Diarrheal illnesses are often accompanied by fever, abdominal pain and/or cramping, nausea, vomiting, and dehydration.

In the United States, up to a third of all acute diarrhea is transmitted by contaminated food. Accurate numbers are hard to come by, but the CDC estimates that 48 million people are sickened each year by a foodborne illness, and 3,000 of them die. Dozens of microbes are capable of causing foodborne illness. The most common culprits in the United States are *Salmonella*, norovirus, *Campylobacter, E. coli* STEC strains, and *Clostridium perfringens*. **Figure 20.2** highlights the work done by CDC to investigate dozens of foodborne outbreaks every year.

Although most diarrhea episodes are self-limiting and therefore do not require treatment, others (such as *E. coli* O157:H7) can have devastating effects. In most diarrheal illnesses, antimicrobial treatment is contraindicated (inadvisable), but some, such as shigellosis, call for quick treatment with antibiotics. For public

health reasons, it is important to know which agents are causing diarrhea in the community, but in many cases identification of the agent is not performed.

Some species of bacteria are subdivided into serotypes or subtypes. Many gram-negative enteric bacteria are named and designated according to the following antigens: H, the flagellar antigen; K, the capsular antigen; and O, the cell wall antigen. Not all enteric bacteria carry the H and K antigens, but all have O, the polysaccharide portion of the lipopolysaccharide implicated in endotoxic shock (see chapter 18). Most species of gram-negative enterics exhibit a variety of subspecies, variants, or serotypes caused by slight variations in the chemical structure of the HKO antigens. Some bacteria in this chapter (e.g., *E. coli* O157:H7) are named according to their surface antigens; however, we use Latin variant names for *Salmonella*.

Medical Moment

Dehydration

Dehydration is a common symptom experienced by individuals suffering from conditions affecting the gastrointestinal tract. Recognizing and treating dehydration is a high-priority task of health care workers.

Dehydration can be thought of as an imbalance of fluids resulting from excessive fluid loss (as in vomiting and diarrhea) or inadequate fluid intake (due to nausea or loss of appetite).

Health care workers classify dehydration as mild, moderate, or severe—based on the estimated amount of weight lost. This is often expressed in percentages. Capillary refill, skin turgor, and breathing are considered to be the most accurate signs in estimating degree of dehydration in adults.

Q. Dehydration is most dangerous for babies and young children. Why do you think that is?

Answer in Appendix B.

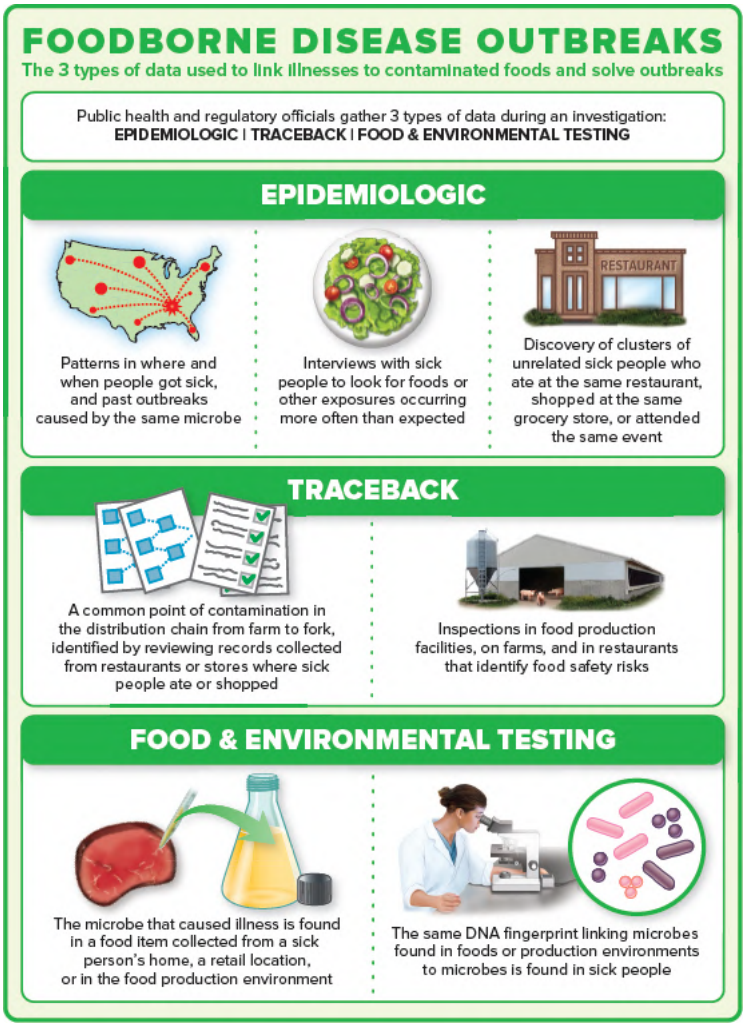


Figure 20.2 **Approaches used to investigate foodborne disease outbreaks**.
Source: CDC

In this section, we describe acute diarrhea with infectious agents as the cause. In the sections following this one, we discuss acute diarrhea and vomiting caused by toxins, commonly known as food poisoning, and chronic diarrhea and its causes.

Pet reptiles have occasionally been the source of *Salmonella* infections in humans.
©Ryan McVay/Getty Images

Salmonella

A decade ago, one of every three chickens destined for human consumption was contaminated with *Salmonella*, but the rate is now about 10%. Other poultry, such as ducks and turkeys, are also affected. Eggs are infected as well because the bacteria may actually enter the egg while the shell is being formed in the chicken. *Salmonella* is a very large genus of bacteria but with a complicated nomenclature. The disease condition in which we are interested is caused by *Salmonella enterica* subspecies *enterica*. This subspecies is further subdivided into serogroups that have formal names

such as "*Salmonella enterica* subspecies *enterica* serovar Typhi." In clinical situations, this latter organism is usually just called "*Salmonella typhi*."

Salmonellae are motile; they ferment glucose with acid and sometimes gas. Most, too, produce hydrogen sulfide (H_2S) but not urease. They grow readily on most laboratory media and can survive outside the host in inhospitable environments such as freshwater and freezing temperatures. These pathogens are resistant to chemicals such as bile and dyes, which are the basis for isolation on selective media.

▶ Signs and Symptoms

The genus *Salmonella* causes a variety of illnesses in the GI tract and beyond. Until fairly recently, its most severe manifestation was typhoid fever. Since the mid-1900s, a milder disease usually called salmonellosis has been much more common. Sometimes the condition is also called enteric fever or gastroenteritis. Whereas typhoid fever is caused by the Typhi serotype, gastroenteritises are generally caused by the serotypes known as Typhimurium, Enteritidis, Heidelberg, Newport, and Javiana. Most of these strains come from animals, unlike the Typhi serotype, which infects humans exclusively. *Salmonella* bacteria are normal intestinal biota in cattle, poultry, rodents, and reptiles and each has been a documented source of infection and disease in humans.

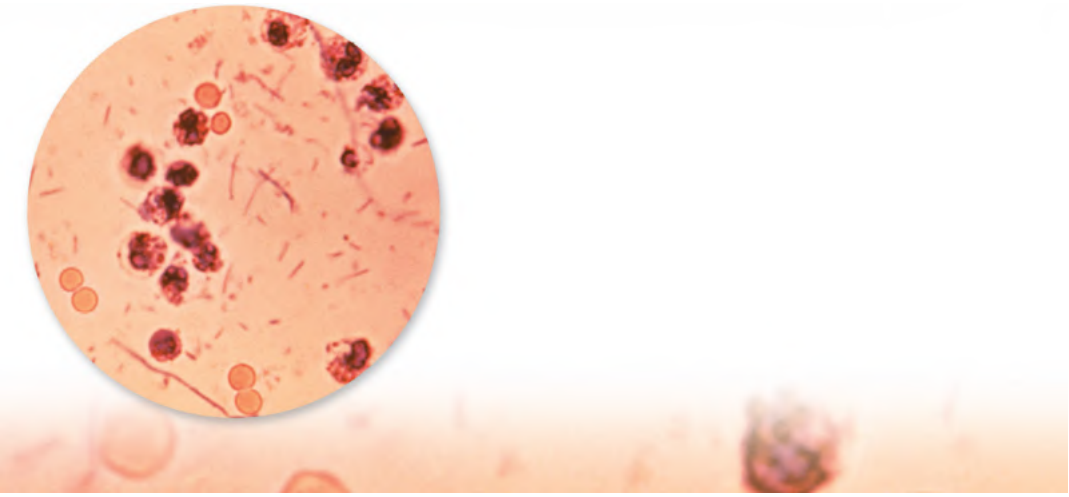
Salmonellosis can be relatively severe, with an elevated body temperature and septicemia as more prominent features than GI tract disturbance. But it can also be fairly mild, with gastroenteritis—vomiting, diarrhea, and mucosal irritation—as its major feature. Blood can appear in the stool. In otherwise healthy adults, symptoms spontaneously subside after 2 to 5 days. Death is infrequent except in debilitated persons.

Pathogenesis and Virulence Factors

Salmonella serotypes vary in their virulence due to genetic differences in their ability to adhere to the gut mucosa, and also to evade the immune system. Endotoxin, a lipopolysaccharide component of the outer membrane of gram-negative bacteria, is an important virulence factor for *Salmonella*.

Transmission and Epidemiology

The CDC estimates that *Salmonella* causes approximately 1.2 million infections a year in the United States. An important factor to consider in all diarrheal pathogens is how many organisms must be ingested to cause disease (their ID_{50}). It varies widely. *Salmonella* has a high ID_{50}, meaning a lot of organisms have to be ingested in order for disease to result. Animal products such as meat and milk can be readily contaminated with *Salmonella* during slaughter, collection, and processing. As you see in **Figure 20.3**, *Salmonella* is transmitted in a variety of foods.



Fecal smear from patient with *Shigella* infection shows the bacteria (the small red rods), red blood cells (the light red discs), and white blood cells (the larger cells with intracellular granules).
Source: CDC

***Salmonella* Outbreaks**

- Multistate Outbreak of *Salmonella* Virchow Infections Linked to Garden of Life RAW Meal Organic Shake & Meal Products
- Multistate Outbreak of *Salmonella* Paratyphi B variant L(+) tartrate(+) Infections Linked to JEM Raw Brand Sprouted Nut Butter Spreads
- Two Multistate Outbreaks of Human *Salmonella* Infections Linked to Small Turtles
- Multistate Outbreak of *Salmonella* Poona Infections Linked to Imported Cucumbers

Figure 20.3 **Screenshot of CDC *Salmonella* information from 2016.**

Most cases are traceable to a common food source such as milk or eggs. Some cases may be due to poor sanitation. In one outbreak, about 60 people became infected after visiting the Komodo dragon exhibit at the Denver zoo. They picked up the infection by handling the rails and fence of the dragon's cage.

Prevention and Treatment

The only prevention for salmonellosis is avoiding contact with the bacterium. Uncomplicated cases of salmonellosis are treated with fluid and electrolyte replacement. If the patient has underlying immunocompromise or if the disease is severe, trimethoprim-sulfamethoxazole is recommended.

Shigella

The *Shigella* bacteria are gram-negative rods, nonmotile and non-endospore-forming. They do not produce urease or hydrogen sulfide. Knowing this helps in their identification. They are primarily human parasites, though they can infect apes. All produce a similar disease that can vary in intensity. These bacteria resemble some types of pathogenic *E. coli* very closely.

Signs and Symptoms

The symptoms of shigellosis include frequent, watery stools, as well as fever, and often intense abdominal pain. Nausea and vomiting are common. Stools often contain obvious blood, and even more often are found to have occult (not visible to the naked eye) blood. Diarrhea containing blood is also called **dysentery**. Mucus from the GI tract will also be present in the stools.

Pathogenesis and Virulence Factors

Shigellosis is different from many GI tract infections in that *Shigella* invades the villus cells of the large intestine rather than the small intestine. In addition, it is not as invasive as *Salmonella* and does not perforate the intestine or invade the blood. It enters the intestinal mucosa by means of special cells in Peyer's patches. Once in the mucosa, *Shigella* instigates an inflammatory response that causes extensive tissue destruction. The release of endotoxin causes fever. **Enterotoxin**, an exotoxin that affects the enteric (or GI) tract, damages the mucosa and villi. Local areas of erosion give rise to bleeding and heavy secretion of mucus **(figure 20.4)**. *Shigella dysenteriae* (and perhaps some of the other species) produces a heat-labile exotoxin called **Shiga toxin**, which seems to be responsible for the more serious damage to the intestine as well as any systemic effects, including injury to nerve cells.

Transmission and Epidemiology

In addition to the usual oral route, shigellosis is also acquired through direct person-to-person contact, largely because of the small infectious dose required (from 10 to 200 bacteria). The disease is mostly associated with lax sanitation, malnutrition, and crowding. It is also spread epidemically in day care centers, prisons, mental institutions, nursing homes, and military camps. *Shigella* can establish a chronic carrier condition in some people that lasts several months.

Prevention and Treatment

The only prevention of this and most other diarrheal diseases is good hygiene and avoiding contact with infected persons. Unlike other acute diarrheas, all cases of shigellosis should be treated with antibiotics: trimethoprim/sulfamethoxazole if it is sensitive to it, and ciprofloxacin in resistant cases of shigellosis.

Note About "Enterotoxin"

You have already learned about two broad classes of toxins: exotoxins and endotoxins. (Do you remember the definitions?) In this chapter you learn the term *enterotoxin*. It sounds similar to the other two terms. But enterotoxin is a descriptive term for a particular type of exotoxin: one that targets the enteric—or the gastrointestinal—tract. It might be worth making a note to yourself!

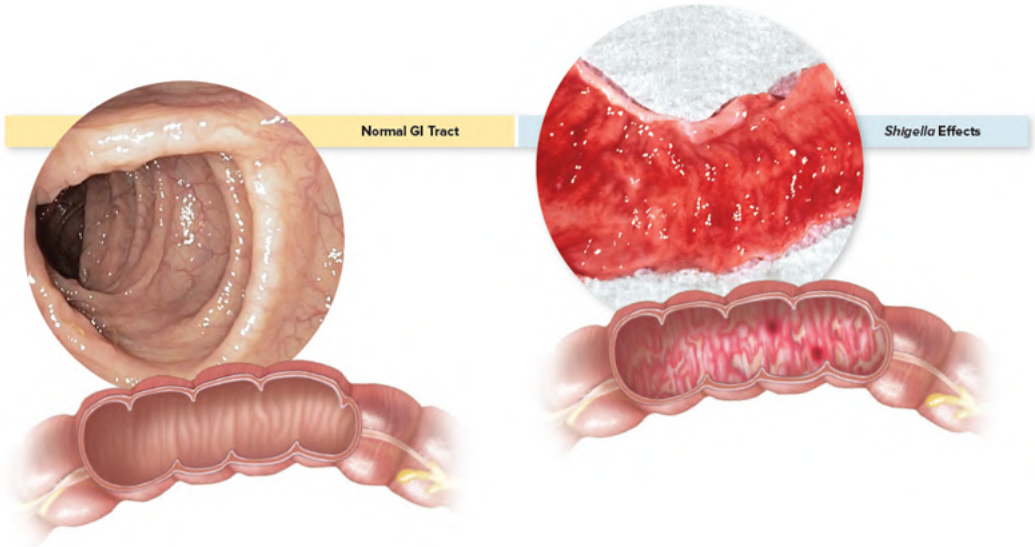


Figure 20.4 **The appearance of the large intestinal mucosa in *Shigella* dysentery**. Note the patches of blood and mucus, the erosion of the lining, and the absence of perforation.
©Gastrolab/Science Source (normal); Source: CDC (diseased)

Shiga-Toxin-Producing *E. coli* (STEC)

Dozens of different strains of *E. coli* exist, most of which cause no disease at all. A handful of them cause various degrees of intestinal symptoms, as described in this and the following section. Some of them cause urinary tract infections (see chapter 21). *E. coli* O157:H7 was the first representative of this group in the news, having caused an outbreak originating in a chain of Jack-in-the-Box restaurants in the Pacific Northwest. Now there are other "numbered" *E. coli* strains that produce Shiga toxins; all of them are dangerous pathogens.

Signs and Symptoms

Shiga-toxin-producing *E. coli* is the agent of a spectrum of conditions, ranging from mild gastroenteritis with fever to bloody diarrhea. A minority of patients will develop **hemolytic uremic syndrome (HUS)**, a severe hemolytic anemia that can cause kidney damage and failure. Neurological symptoms such as blindness, seizure, and stroke (and long-term debilitation) are also possible. These serious manifestations are most likely to occur in children younger than age 5 and in elderly people.

Pathogenesis and Virulence Factors

These *E. coli* owe much of their virulence to Shiga toxins (so named because they are identical to the Shiga exotoxin secreted by virulent *Shigella* species). Shiga toxin genes are present on prophage genes donated by bacteriophage in *E. coli* but are on the chromosome of *Shigella dysenteriae*, suggesting that the *E. coli* acquired the virulence factor through phage-mediated transfer (transduction). As described for *Shigella*, the Shiga toxin interrupts protein synthesis in its target cells. It seems to be responsible especially for the systemic effects of this infection.

Another important virulence determinant for STEC is the ability to efface (rub out or destroy) enterocytes, which are gut epithelial cells. The net effect is a lesion in the gut (effacement), usually in the large intestine. The microvilli are lost from the gut epithelium, and the lesions produce bloody diarrhea.

Transmission and Epidemiology

The most common mode of transmission for STEC is the ingestion of contaminated and undercooked beef, although other foods and beverages can be contaminated as well.

In 2015 the Chipotle chain of restaurants was the source of two distinct *E. coli* O26 outbreaks in the United States. In 2016 there was a multistate outbreak of two STEC strains, *E. coli* O12 and *E. coli* O26, which were traced to flour manufactured by General Mills in Kansas City, Missouri. By early 2017, 63 people in 24 states had become ill, although those numbers are expected to continue to grow, since flour has such a long shelf life. The CDC reminds consumers not to consume raw dough, or even to taste batters before they are cooked.

Culture and Diagnosis

Infection with this type of *E. coli* should be confirmed with stool culture and also tested for Shiga toxin. Positive specimens should be sent to state or local health departments for detailed characterization and outbreak monitoring.

Prevention and Treatment

The best prevention for this disease is good food hygiene. The Shiga toxin is heat-labile and the *E. coli* is killed by heat as well.

No vaccine exists for *E. coli* O157:H7 or other STEC strains. However, some countries vaccinate cattle against *E. coli* O157:H7 as a means to protect human populations.

Antibiotics may be contraindicated for this infection, as they may increase the pathology by releasing more toxin, leading to HUS. Supportive therapy, including plasma transfusions to dilute toxin in the blood, is a good option.

Other *E. coli*

At least five other categories of *E. coli* can cause diarrheal diseases. Scientists call these **enterotoxigenic** *E. coli* (ETEC), **enteroinvasive** *E. coli* (EIEC), **enteropathogenic** *E. coli* (EPEC), diffusely adherent *E. coli* (DAEC), and **enteroaggregative** *E. coli* (EAEC). In clinical practice, most physicians are interested in differentiating Shiga-toxin-producing *E. coli* (STEC) from all the others. In **Disease Table 20.1**, the non-Shiga-toxin-producing *E. coli* are grouped together in one column.

Campylobacter

Although you may never have heard of *Campylobacter*, it is one of the most common bacterial causes of diarrhea in the United States. The symptoms of campylobacteriosis are frequent watery stools, fever, vomiting, headaches, and abdominal pain. The symptoms may last longer than most acute diarrheal episodes, sometimes extending beyond 2 weeks. They may subside and then recur over a period of weeks.

Campylobacter jejuni is the most common cause, although there are other pathogenic *Campylobacter* species. Campylobacters are slender, curved, or spiral gram-negative bacteria propelled by polar flagella at one or both poles, often appearing in S-shaped or gull-winged pairs. These bacteria tend to be microaerophilic inhabitants of the intestinal tract, genitourinary tract, and oral cavity of humans and animals. Transmission of this pathogen takes place via the ingestion of contaminated beverages and food, especially water, milk, meat, and chicken.

Hand washing is a very effective way to reduce transmission of fecal bacteria.
©BananaStock/PunchStock

Once ingested, *C. jejuni* cells reach the mucosa at the last segment of the small intestine (ileum) near its junction with the colon. They adhere, burrow through the mucus, and multiply. Symptoms commence after an incubation period of 1 to 7 days. The mechanisms of pathology appear to involve a heat-labile enterotoxin that stimulates a secretory diarrhea like that of cholera. In a small number of cases, infection with this bacterium can lead to a serious neuromuscular paralysis called Guillain-Barré syndrome.

Guillain-Barré syndrome (GBS) (pronounced gee″-luhn-buh-ray′) is the leading cause of acute paralysis in the United States since the eradication of polio. The good news is that many patients recover completely from this paralysis. The condition is still mysterious in many ways, but it seems to be an autoimmune reaction that can be brought on by infection with viruses and bacteria, by vaccination in rare cases, and even by surgery. The single most common precipitating event for the onset of GBS is *Campylobacter* infection. Twenty to forty percent of GBS cases are preceded by infection with *Campylobacter*. The reasons for this are not clear. (Note that even though 20% to 40% of GBS cases are preceded by *Campylobacter* infection, only about 1 in 1,000 cases of *Campylobacter* infection results in GBS.)

Resolution of *Campylobacter* infection occurs in most instances with simple, nonspecific rehydration and electrolyte balance therapy. In more severely affected patients, it may be necessary to administer erythromycin or azithromycin. Antibiotic resistance is increasing in these bacteria, in large part due to the use of fluoroquinolones in the poultry industry. Because vaccines are yet to be developed, prevention depends on rigid sanitary control of water and milk supplies and care in food preparation.

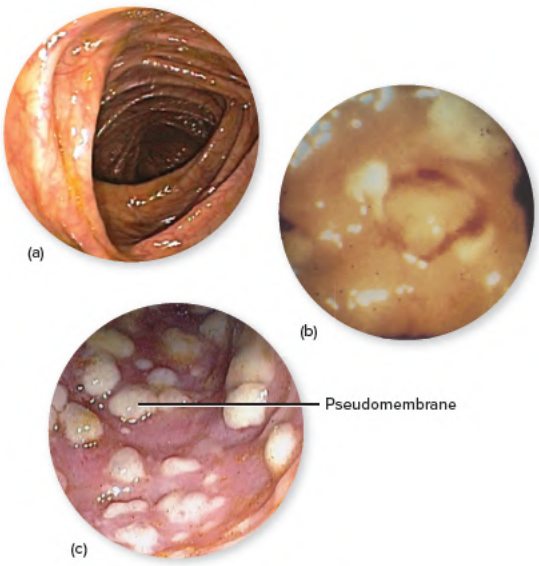


Figure 20.5 **Antibiotic-associated colitis**. **(a)** Normal colon. **(b)** A mild form of colitis with diffuse, inflammatory patches. **(c)** Heavy yellow plaques, or pseudomembranes, typical of more severe cases. Photographs were made by a sigmoidoscope, an instrument capable of photographing the interior of the colon.
(a) ©David Musher/Science Source; (b) Courtesy Fred Pittman; (c) ©David M. Martin, M.D./Science Source

Clostridium difficile

Clostridium difficile is a gram-positive endospore-forming rod found as normal biota in the intestine. It was once considered relatively harmless but now is known to cause a condition called pseudomembranous colitis, also called antibiotic-associated colitis. In most cases, this infection seems to be precipitated by therapy with broad-spectrum antibiotics such as ampicillin, clindamycin, or cephalosporins. Although *C. difficile* is relatively noninvasive, it is able to superinfect the large intestine when drugs have disrupted the normal biota. It produces two enterotoxins, toxins A and B, that cause areas of necrosis in the wall of the intestine. More severe cases exhibit abdominal cramps, fever, and leukocytosis. The colon is inflamed and gradually sloughs off loose, membranelike patches called pseudomembranes consisting of fibrin and cells **(figure 20.5)**. If the condition is not stopped, perforation of the cecum and death can result.

The epidemiology of *C. diff* is changing. In the most comprehensive study conducted to date in the United States in 2011, an estimated 453,000 people acquired *C. diff*. Of these U.S. cases, 66% were healthcare-associated, even though 24% of infections did not occur until after the patient had gone home. If a patient is receiving clindamycin, ceftriaxone, or a fluoroquinolone for a different infection and displays *C. diff* symptoms, the first step is to withdraw the offending antibiotic. In mild *C. diff* infections, metronidazole should be administered. If it is more severe, the drug of choice is vancomycin. It can be very difficult to eradicate, and stubborn infections can significantly degrade a patient's quality of life.

A key point to remember is that *C. diff* releases endospores, which contaminate the environment. Hospitalized patients must be put in isolation conditions, and constant attention to disinfection and infection control is required.

Vibrio cholerae

Cholera has been a devastating disease for centuries. It is not an exaggeration to say that the disease has shaped a good deal of human history in Asia and Latin America, where it has been endemic. These days we have come to expect outbreaks of cholera to occur

after natural disasters, war, or large refugee movements, especially in underdeveloped parts of the world.

These bacteria are rods with a single polar flagellum. They belong to the family *Vibrionaceae*. A freshly isolated specimen of *Vibrio cholerae* contains quick, darting cells that slightly resemble a comma. *Vibrio* shares many characteristics with members of the *Enterobacteriaceae* family. Vibrios are fermentative and grow

on ordinary or selective media containing bile at 37°C. They possess unique O and H antigens and membrane receptor antigens that provide some basis for classifying members of the family. There are two major types, called classic and *El Tor*.

Signs and Symptoms

After an incubation period of a few hours to a few days, symptoms begin abruptly with vomiting, followed by copious watery feces called secretory diarrhea. The intestinal contents are lost very quickly, leaving only secreted fluids. This voided fluid contains flecks of mucus—hence, the description "rice-water stool." Fluid losses of nearly 1 liter per hour have been reported in severe cases, and an untreated patient can lose up to 50% of body weight during the course of this disease. The diarrhea causes loss of blood volume, acidosis from bicarbonate loss, and potassium depletion, which manifest in muscle cramps, severe thirst, flaccid skin, sunken eyes, and, in young children, coma and convulsions. Secondary circulatory consequences can include hypotension, tachycardia, cyanosis, and collapse from shock within 18 to 24 hours. If cholera is left untreated, death can occur in less than 48 hours, and the mortality rate is between 55% and 70%.

Pathogenesis and Virulence Factors

V. cholerae has a relatively high infectious dose (10^8 cells). At the junction of the duodenum and jejunum, the vibrios penetrate the mucous barrier using their flagella, adhere to the microvilli of the epithelial cells, and multiply there. The bacteria never enter the host cells or invade the mucosa. The virulence of *V. cholerae* is due to an enterotoxin called cholera toxin (CT), which disrupts the normal physiology of intestinal cells. Under the influence of this system, the cells shed large amounts of electrolytes into the intestine, an event accompanied by profuse water loss.

Transmission and Epidemiology

The pattern of cholera transmission and the onset of epidemics are greatly influenced by the season of the year and the climate. Cold, acidic, dry environments inhibit the migration and survival of *Vibrio*, whereas warm, monsoon, alkaline, and saline conditions favor them. The bacteria survive in water sources for long periods of time. Recent outbreaks in several parts of the world have been traced to giant cargo ships that pick up ballast water in one port and empty it in another elsewhere in the world. Cholera ranks among the top seven causes of morbidity and mortality, affecting several million people in endemic regions of Asia and Africa. See some interesting history about this disease in chapter 22.

In nonendemic areas such as the United States, the microbe is spread by water and food contaminated by asymptomatic carriers, but it is relatively uncommon.

Vibrio cholerae
Source: CDC/Janice Haney Carr

Prevention and Treatment

Effective prevention is contingent on proper sewage treatment and water purification. Vaccines are available for travelers and people living in endemic regions. There are two vaccines approved by the World Health Organization that are not available in the United States; their effectiveness is between 60% and 67%. In 2016 a new vaccine was approved for use in the United States for people traveling to cholera-endemic areas. It is more effective than the previous vaccines in early months, but its long-term efficacy is not yet known.

The key to cholera therapy is prompt replacement of water and electrolytes, because their loss accounts for the severe morbidity and mortality. This therapy can be accomplished by various rehydration techniques that replace the lost fluid and electrolytes. One of these, oral rehydration therapy (ORT), is incredibly simple and astonishingly effective. Until the 1970s, the treatment, if one could access it, was rehydration through an IV drip. This treatment usually required traveling to the nearest clinic, often miles or days away. Most affected children received no treatment at all, and 3 million of them died every year. Then scientists tested a simple sugar-salt solution that patients could drink.

The relatively simple solution, developed by the World Health Organization (WHO), consists of a mixture of the electrolytes sodium chloride, sodium bicarbonate, potassium chloride, and glucose or sucrose dissolved in water. When administered early in amounts ranging from 100 to 400 milliliters per hour, the solution can restore patients in 4 hours, often bringing them literally back from the brink of death. This therapy has several advantages, especially for countries with few resources. It does not require medical facilities, high-technology equipment, or complex medication protocols. It also eliminates the need for clean needles, which is a pressing issue in many parts of the world.

Disease Table 20.1 Acute Diarrhea

Bacterial Causes

Bacterial Causes							
Causative Organism(s)	*Salmonella* B **G−**	*Shigella* B **G−**	Shiga-toxin-producing *E. coli* (STEC) B **G−**	Other *E. coli* (non-Shiga-toxin producing) B **G−**	*Campylobacter* B **G−**	*Clostridium difficile* B **G+**	*Vibrio cholerae* **G−**
Most Common Modes of Transmission	Vehicle (food, beverage), fecal-oral	Fecal-oral, direct contact	Vehicle (food, beverage), fecal-oral	Vehicle, fecal-oral	Vehicle (food, water), fecal-oral	Endogenous (normal biota)	Vehicle (water an some foo fecal-oral
Virulence Factors	Adhesins, endotoxin	Endotoxin, enterotoxin, Shiga toxins in some strains	Shiga toxins; proteins for attachment, secretion, effacement	Various: proteins for attachment, secretion, effacement; heat-labile and/or heat-stable exotoxins; invasiveness	Adhesins, exotoxin, induction of autoimmunity	Enterotoxins A and B	Cholera toxin (CT
Culture/Diagnosis	Stool culture, not usually necessary	Stool culture; antigen testing for Shiga toxin	Stool culture, antigen testing for Shiga toxin; involve state or local health department	Stool culture not usually necessary in absence of blood, fever	Stool culture not usually necessary; dark-field microscopy	Stool culture, PCR, ELISA demonstration of toxins in stool	Clinical diagnosis, microscop technique serologica detection antitoxin
Prevention	Food hygiene and personal hygiene	Food hygiene and personal hygiene	Avoid live *E. coli* (cook meat, wash vegetables, avoid raw flour)	Food hygiene and personal hygiene	Food hygiene and personal hygiene	—	Water hygiene
Treatment	Rehydration; no antibiotic for uncomplicated disease; in **Serious Threat** category in CDC Antibiotic Resistance Report	Trimethoprim/sulfamethoxazole or ciprofloxacin; rehydration; in **Serious Threat** category in CDC Antibiotic Resistance Report	Antibiotics may be contraindicated, supportive measures	Rehydration, antimotility agent	Rehydration, erythromycin or azithromycin in severe cases (antibiotic resistance rising); in **Serious Threat** category in CDC Antibiotic Resistance Report	Withdrawal of antibiotic; in severe cases metronidazole or fidaxomicin (Dificid); in **Urgent Threat** category in CDC Antibiotic Resistance Threat Report	Rehydrati in severe cases, doxycycli
Fever Present?	Usually	Often	Often	Sometimes	Usually	Sometimes	No
Blood in Stool?	Sometimes	Often	Usually	Sometimes	No	Not usually; mucus prominent	No
Distinctive Features	Often associated with chickens, reptiles	Very low ID_{50}	Hemolytic uremic syndrome	EIEC, ETEC, EPEC, EAEC	Guillain-Barré syndrome	Antibiotic-associated diarrhea	Rice-wate stools
Epidemiological Features	United States: +/− 1.2 million cases per year; 20% of all cases require hospitalization; death rate of 0.6%	United States: estimated 450,000 cases per year; internationally: 165 million cases per year	Internationally: causes HUS in 10% of patients; 25% of HUS patients suffer neurological complications; 50% have chronic renal sequelae	—	United States: 2.4 million cases per year; internationally: 400 million cases per year	United States: 3 million cases per year	Global estimate: 21,000–143,000 deaths annually

In the United States and other developed countries oral antibiotics, such as doxycycline, are given orally along with rehydration. These can shorten periods of both diarrhea and bacterial excretion.

Non-cholera *Vibrio* Species

In the United States, infection with non-cholera *Vibrio* species is more likely. These infections are called vibrioses, as opposed to cholera. Two species are most prominent, *V. vulnificus* and *V. parahaemolyticus*. Infection can be from exposure to sea-water but more often is associated with eating contaminated shellfish. It is a relatively rare infection but very much on the increase. Scientists suspect that the increase is due to three factors: (1) the increased demand for raw oysters; (2) increased awareness, meaning that more people are diagnosed; and (3) global warming, causing a wider habitat for these bacteria in bodies of water. In people who are immunocompromised, the infections can be fatal, especially in the case of *V. vulnificus*. These infections, along with those by *V. cholerae*, are nationally notifiable diseases.

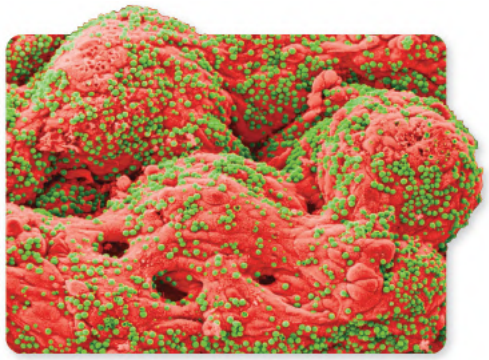


Figure 20.6 **Scanning electron micrograph of *Cryptosporidium* (green) attached to the intestinal epithelium**.
©Moredun Animal Health Ltd./Science Source

Cryptosporidium

Cryptosporidium is an intestinal protozoan of the apicomplexan type (see chapter 4) that infects a variety of mammals, birds, and reptiles. For many years, cryptosporidiosis was considered an intestinal ailment exclusive to calves, pigs, chickens, and other poultry, but it is clearly a zoonosis as well. The organism's life cycle includes a hardy intestinal oocyst as well as a tissue phase. Humans accidentally ingest the oocysts with water or food that has been contaminated by feces from infected animals. The oocyst "excysts" once it reaches the intestines and releases sporozoites that attach to the epithelium of the small intestine **(figure 20.6)**. The organism penetrates the intestinal cells and lives intracellularly in them. It undergoes asexual and sexual reproduction in these cells, produces more oocysts, which are released into the gut lumen, excreted from the host, and after a short time become infective again. The oocysts are highly infectious and extremely resistant to treatment with chlorine and other disinfectants.

The prominent symptoms mimic other types of gastroenteritis, with headache, sweating, vomiting, severe abdominal cramps, and diarrhea. AIDS patients may experience chronic persistent cryptosporidial diarrhea that can be used as a criterion to help diagnose AIDS. The agent can be detected in fecal samples or in biopsies using ELISA or acid-fast staining. Stool cultures should be performed to rule out other (bacterial) causes of infection.

Half of the outbreaks of diarrhea associated with swimming pools are caused by *Cryptosporidium*. Because chlorination is not entirely successful in eradicating the cysts, most treatment plants use filtration to remove them, but even this method can fail.

Treatment is not usually required for otherwise healthy patients. Antidiarrheal agents (antimotility drugs) may be used. Although no curative antimicrobial agent exists for *Cryptosporidium*, physicians will often try nitazoxanide, which can be effective against protozoa in immunocompetent patients.

NCLEX® PREP

2. A nurse is preparing to assess a patient admitted for persistent diarrhea. It was reported that the patient has experienced a 6% weight loss in the past 24 hours. Which of the following clinical manifestations may be expected? Select all that apply.

a. *increased respiratory rate*
b. *hypertension*
c. *headache*
d. *extreme thirst*
e. *sustained tachycardia*

Rotavirus

Rotavirus is a member of the *Reovirus* group, which consists of an unusual double-stranded RNA genome with both an inner and an outer capsid. Globally, rotavirus is the primary viral cause of morbidity and mortality resulting from diarrhea, accounting for nearly 50% of all cases. Before the rotavirus vaccine was introduced in 2006, 1 million cases of rotavirus infection occurred in the United States every year, leading to 70,000 hospitalizations. The virus gets its name from its physical appearance, which is said to resemble a spoked wheel.

The virus is transmitted by the fecal-oral route, including through contaminated food, water, and fomites. For this reason, disease is most prevalent in areas of the world with poor sanitation.

The effects of infection vary with the age, nutritional state, general health, and living conditions of the patient. Babies from 6 to 24 months of age lacking maternal antibodies have the greatest risk for fatal disease. These children present symptoms of watery diarrhea, fever, vomiting, dehydration, and shock. The intestinal mucosa can be damaged in a way that chronically compromises nutrition, and long-term or repeated infections can retard growth. Newborns seem to be protected by maternal antibodies. Adults can also acquire this infection, but it is generally mild and self-limiting.

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Norovirus

Noroviruses are the second most common cause of hospitalizations from foodborne diseases in the United States. However, overall they cause about five times as much foodborne illness as *Salmonella*, the most common cause of hospitalization from foodborne disease. It's just that many fewer norovirus cases require hospitalization.

Transmission is fecal-oral or via contamination of food and water **(figure 20.7)**. Viruses generally cause a profuse, watery diarrhea of 3 to 5 days' duration. Vomiting may accompany the disease, especially in the early phases. Mild fever is often seen.

Treatment of these infections always focuses on rehydration. **Disease Table 20.1**.

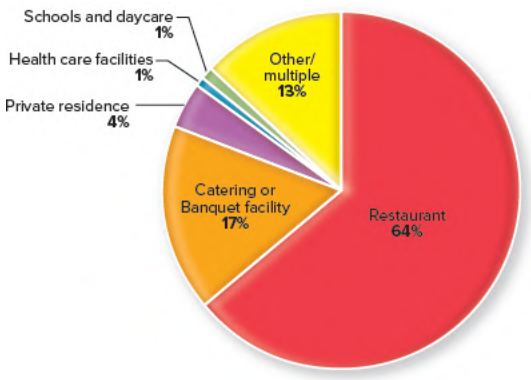

Figure 20.7 **Norovirus transmission in food**. The pie chart shows where foodborne transmission of the virus occurs. Adapted from the Centers for Disease Control and Prevention
Source: CDC

Food Poisoning

If a patient presents with severe nausea and frequent vomiting accompanied by diarrhea, and reports that companions with whom he or she shared a recent meal (within the last 1 to 6 hours) are suffering the same fate, food poisoning should be suspected. **Food poisoning** refers to symptoms in the gut that are caused by a preformed toxin of some sort. In many cases, the toxin comes from *Staphylococcus aureus*. In others, the source of the toxin is *Bacillus cereus* or *Clostridium perfringens*. The toxin occasionally comes from nonmicrobial sources such as fish, shellfish, or mushrooms. In any case, if the symptoms are violent and the incubation period is very short, this condition, which is an *intoxication* (the effects of a toxin) rather than an *infection*, should be considered.

Staphylococcus aureus Exotoxin

This illness is associated with eating foods such as custards, sauces, cream pastries, processed meats, chicken salad, or ham that have been contaminated by handling and then left unrefrigerated for a few hours. Because of the high salt tolerance of *S. aureus*, even foods containing salt as a preservative are not exempt. The toxins produced by the multiplying bacteria do not noticeably alter the food's taste or smell. The exotoxin (which is an enterotoxin) is heat-stable; inactivation requires 100°C for at least 30 minutes. Thus, heating the food after toxin production may not prevent disease. The ingested toxin acts upon the gastrointestinal epithelium and stimulates nerves, with acute symptoms of cramping, nausea, vomiting, and diarrhea. Recovery is also rapid, usually within 24 hours. Most often, the *S. aureus* comes from a food handler's skin or nose.

This condition is almost always self-limiting, and antibiotics are definitely not warranted.

Bacillus cereus Exotoxin

Bacillus cereus is a sporulating gram-positive bacterium that is naturally present in soil. As a result, it is a common resident on vegetables and other products in close contact with soil. It produces two exotoxins, one of which causes a diarrheal-type disease, the other of which causes an **emetic** (ee-met′-ik) or vomiting disease. The type of disease that takes place is influenced by the type of food that is contaminated by the bacterium. The emetic form is most frequently linked to fried rice, especially when it has been cooked and kept warm for long periods of time. These conditions are apparently ideal for the expression of the low-molecular-weight, heat-stable exotoxin having an emetic effect. The diarrheal form of the disease is usually associated with cooked meats or vegetables that are held at a warm temperature for long periods of time. These conditions apparently favor the production of the high-molecular-weight, heat-labile exotoxin. The symptom in these cases is a watery, profuse diarrhea that lasts only for about 24 hours.

In both cases, the only prevention is the proper handling of food.

Clostridium perfringens Exotoxin

Another sporulating gram-positive bacterium that causes intestinal symptoms is *Clostridium perfringens*. Endospores from *C. perfringens* can contaminate many kinds of foods. Those most frequently implicated in disease are animal flesh (meat, fish) and vegetables such as beans that have not been cooked thoroughly enough to destroy endospores. When these foods are cooled, endospores germinate, and the germinated cells multiply, especially if the food is left unrefrigerated. If the food is eaten without adequate reheating, live *C. perfringens* cells enter the small intestine and release exotoxin. The toxin, acting upon epithelial cells, initiates acute abdominal pain, diarrhea, and nausea in 8 to 16 hours. Recovery is rapid, and deaths are extremely rare.

C. perfringens also causes an enterocolitis infection similar to that caused by *C. difficile*. This infectious type of diarrhea is acquired from contaminated food, or it may be transmissible by inanimate objects. **Disease Table 20.2**.

Disease Table 20.2 Food Poisoning

Causative Organism(s)	*Staphylococcus aureus* exotoxin B G+	*Bacillus cereus* B G+	*Clostridium perfringens* B G+
Most Common Modes of Transmission	Vehicle (food)	Vehicle (food)	Vehicle (food)
Virulence Factors	Heat-stable exotoxin	Heat-stable toxin, heat-labile toxin	Heat-labile toxin
Culture/Diagnosis	Usually based on epidemiological evidence	Microscopic analysis of food or stool	Detection of toxin in stool
Prevention	Proper food handling	Proper food handling	Proper food handling
Treatment	Supportive	Supportive	Supportive
Fever Present	Not usually	Not usually	Not usually
Blood in Stool	No	No	No
Distinctive Features	Suspect in foods with high salt or sugar content	Two forms: emetic and diarrheal	Acute abdominal pain
Epidemiological Features	United States: estimated 240,000 cases per year	United States: estimated 63,000 cases per year	United States: estimated 966,000 cases per year

Chronic Diarrhea

Chronic diarrhea is defined as lasting longer than 14 days. It can have infectious causes or can reflect noninfectious conditions. Most of us are familiar with diseases that present a constellation of bowel syndromes, such as irritable bowel syndrome and ulcerative colitis, neither of which is directly caused by a microorganism as far as we know. Increasing evidence suggests that a chronically disrupted intestinal biota (from long-term use of antibiotics, for example) can predispose people to these conditions.

People suffering from AIDS frequently suffer from chronic diarrhea. Most of the patients who are not taking antiretroviral drugs have diarrhea caused by a variety of opportunistic microorganisms, including *Cryptosporidium, Mycobacterium avium*, and so forth. A patient's HIV status should be considered if he or she presents with chronic diarrhea.

Here we examine a few of the microbes that can be responsible for chronic diarrhea in otherwise healthy people.

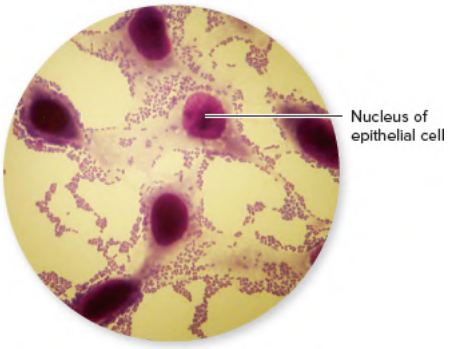

Figure 20.8 **Enteroaggregative *E. coli* adhering to epithelial cells.**
©Iruka Okeke

Enteroaggregative *E. coli* (EAEC)

In the section on acute diarrhea, you read about the various categories of *E. coli* that can cause disease in the gut. One type, the enteroaggregative *E. coli* (EAEC), is particularly associated with chronic disease, especially in children. This bacterium is distinguished by its ability to adhere to human cells in aggregates rather than as single cells **(figure 20.8)**. Its presence appears to stimulate secretion of large amounts of mucus in the gut, which may be part of its role in causing chronic diarrhea.

Cyclospora

The first case of human disease diagnosed as being caused by the protozoan *Cyclospora cayetanensis* was in 1979. But since 1990, the disease has become very common. Its mode of transmission is fecal-oral, and most cases have been associated with consumption of fresh produce and water presumably contaminated with feces. This disease occurs worldwide, and although primarily of human origin, it is not spread directly from person to person. This is due to its life cycle. When it is shed in the feces of infected people it is not in its infective stage but rather in a noninfective oocyst stage. It takes days or weeks for the oocyst to sporulate and become infective to humans. Outbreaks have been traced to imported raspberries, salad made with fresh greens, and drinking water. An outbreak in 2014, linked to cilantro imported from Mexico, sickened more than 300 people.

Giardia

Giardia lamblia (also known as *Giardia intestinalis*) is a pathogenic flagellated protozoan first observed by Antonie van Leeuwenhoek in his own feces. In fact, it is the

most common flagellate isolated in clinical specimens. Observed straight on, the trophozoite has a unique symmetrical heart shape with organelles positioned in such a way that it resembles a face **(figure 20.9)**. Four pairs of flagella emerge from the ventral surface, which is concave and acts like a suction cup for attachment to a substrate. *Giardia* cysts are small and compact and contain four nuclei.

Typical symptoms include diarrhea of long duration, abdominal pain, and flatulence. Stools have a greasy, foul-smelling quality to them. Fever is usually not present.

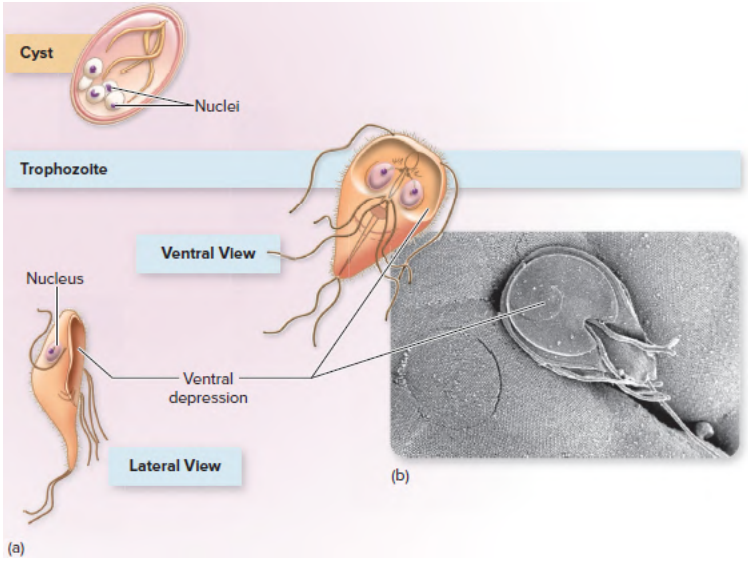

Figure 20.9 ***Giardia lamblia* trophozoite**. **(a)** Schematic drawing. **(b)** Scanning electron micrograph of intestinal surface, revealing (on the left) the lesion left behind by adhesive disc of a *Giardia* that has detached. The trophozoite on the right is lying on its "back" and is revealing its adhesive disc.
(b) Source: CDC/Dr. Stan Erlandsen

©McGraw-Hill Education/Barry Barker, photographer

Transmission and Epidemiology of Giardiasis

Giardiasis has a complex epidemiological pattern. The protozoan has been isolated from the intestines of beavers, cattle, coyotes, cats, and human carriers, but the precise reservoir is unclear at this time. Although both trophozoites and cysts escape in the stool, the cysts play a greater role in transmission. Unlike other pathogenic flagellates, *Giardia* cysts can survive for 2 months in the environment. Cysts are usually ingested with water and food or swallowed after close contact with infected people or contaminated objects. Infection can occur with a dose of only 10 to 100 cysts.

Prevention and Treatment

There is a vaccine against *Giardia* that can be given to animals, including dogs. No human vaccine is available. Avoiding drinking from freshwater sources is the major preventive measure that can be taken.

Treatment is with tinidazole or nitazoxanide.

Entamoeba

Amoebas are widely distributed in aqueous habitats and are frequent parasites of animals, but only a small number of them have the necessary virulence to invade tissues and cause serious pathology. One of the most significant pathogenic amoebas is *Entamoeba histolytica* (en″-tah-mee′-bah his″-toh-lit′-ih-kuh). The relatively simple life cycle of this parasite alternates between a large trophozoite that is motile by means of pseudopods and a smaller, compact, nonmotile cyst **(figure 20.10*a*–*c*)**.

The trophozoite lacks most of the organelles of other eukaryotes, and it has a large single nucleus that contains a prominent nucleolus called a *karyosome*. The mature cyst is encased in a thin yet tough wall and contains four nuclei as well as distinctive cigar-shaped bodies called *chromatoidal bodies*, which are actually dense clusters of ribosomes.

Signs and Symptoms

Clinical amoebiasis exists in intestinal and extraintestinal forms. The initial targets of intestinal amoebiasis are the cecum, appendix, colon, and rectum. The amoeba secretes enzymes that dissolve tissues, and it actively penetrates deeper layers of the mucosa, leaving erosive ulcerations **(figure 20.10*d*)**. This phase is marked by dysentery (bloody, mucus-filled stools), abdominal pain, fever, diarrhea, and weight loss. The most life-threatening manifestations of intestinal infection are hemorrhage, perforation, appendicitis, and tumorlike growths called amoebomas. Lesions in the mucosa of the colon have a characteristic flasklike shape.

Extraintestinal infection occurs when amoebas invade the viscera of the peritoneal cavity. The most common site of invasion is the liver. Here, abscesses containing necrotic tissue and trophozoites develop and cause amoebic hepatitis. Another rarer complication is pulmonary amoebiasis. Other infrequent targets of infection are the spleen, adrenals, kidney, skin, and brain. Severe forms of the disease result in about a 10% fatality rate.

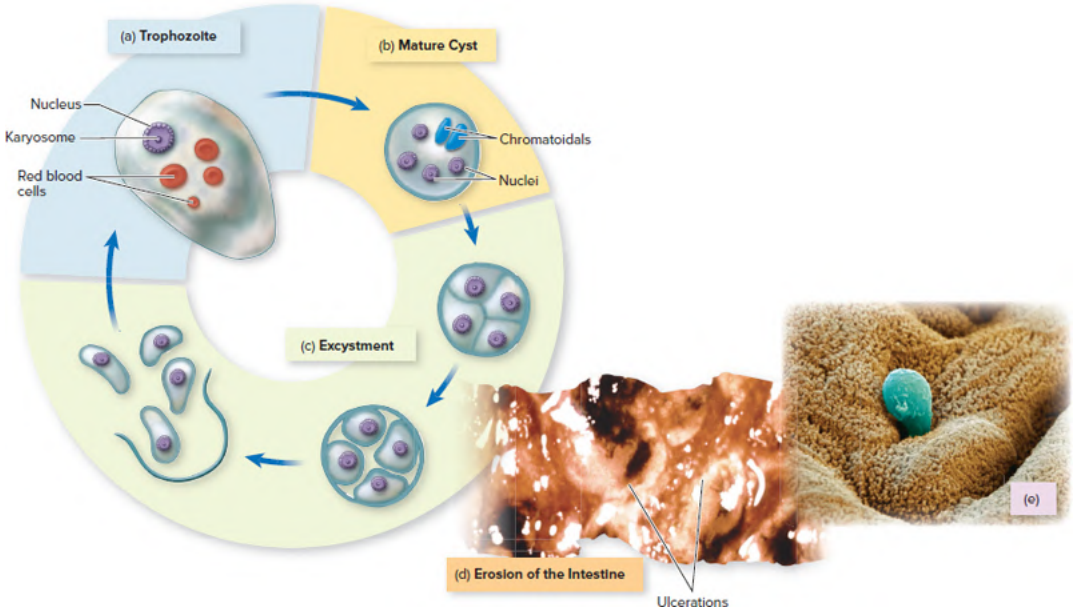

Figure 20.10 ***Entamoeba histolytica*. (a)** A trophozoite containing a single nucleus, a karyosome, and red blood cells. **(b)** A mature cyst with four nuclei and two blocky chromatoidals. **(c)** Stages in excystment. Divisions in the cyst create four separate cells, or metacysts, that differentiate into trophozoites and are released. **(d)** Intestinal amoebiasis and dysentery of the cecum. Red patches are sites of amoebic damage to the intestinal mucosa. **(e)** Trophozoite of *Entamoeba histolytica*. Note the fringe of very fine pseudopods it uses to invade and feed on tissue.

(d) Source: CDC; (e) ©Eye of Science/Science Source

Transmission and Epidemiology of Amoebiasis

Entamoeba is harbored by chronic carriers whose intestines favor the encystment stage of the life cycle. Cyst formation cannot occur in active dysentery because the feces are so rapidly flushed from the body; but after recuperation, cysts are continuously shed in feces.

Infection is usually acquired by ingesting food or drink contaminated with cysts released by an asymptomatic carrier. The amoeba is thought to be carried in the intestines of one-tenth of the world's population, and it kills up to 100,000 people a year. Occurrence is highest in tropical regions (Africa, Asia, and Latin America), where "night soil" (human excrement) or untreated sewage is used to fertilize crops, and sanitation of water and food can be substandard. Although the prevalence of the disease is lower in the United States, as many as 10 million people could harbor the agent.

Prevention and Treatment

Prevention of the disease relies on purification of water because no vaccine currently exists. Because regular chlorination of water supplies does not kill cysts, more rigorous methods such as boiling or iodine are required.

Disease Table 20.3 Chronic Diarrhea

Causative Organism(s)	Enteroaggregative *E. coli* (EAEC) B G−	*Cyclospora cayetanensis* P	*Giardia lamblia* P	*Entamoeba histolytica* P
Most Common Modes of Transmission	Vehicle (food, water), fecal-oral	Fecal-oral, vehicle	Vehicle, fecal-oral, direct and indirect contact	Vehicle, fecal-oral
Virulence Factors	?	Invasiveness	Attachment to intestines alters mucosa	Lytic enzymes, induction of apoptosis, invasiveness
Culture/Diagnosis	Difficult to distinguish from other *E. coli*	Stool examination, PCR	Stool examination, ELISA	Stool examination, ELISA, serology
Prevention	?	Washing, cooking food, personal hygiene	Water hygiene, personal hygiene	Water hygiene, personal hygiene
Treatment	None, or ciprofloxacin	TMP-SMZ	Tinidazole, nitazoxanide	Metronidazole or chloroquine
Fever Present	No	Usually	Not usually	Yes
Blood in Stool	Sometimes, mucus also	No	No, mucus present (greasy and malodorous)	Yes
Distinctive Features	Chronic in the malnourished	–	Frequently occurs in backpackers, campers	–
Epidemiological Features	Developing countries: 87% of chronic diarrhea in children >2 years old	United States: estimated 16,000 cases per year; internationally: endemic in 27 countries, mostly tropical	United States: estimated 1.2 million cases per year; internationally: prevalence rates from 2% to 5% in industrialized world; 40–50 million cases per year	Internationally: 40,000–100,000 deaths annually

Effective treatment usually involves the use of metronidazole (Flagyl) or chloroquine. Dehydroemetine is used to control symptoms, but it will not cure the disease. Other drugs are given to relieve diarrhea and cramps, while lost fluid and electrolytes are replaced by oral or intravenous therapy. Infection with *E. histolytica* provokes antibody formation against several antigens, but permanent immunity is unlikely and reinfection can occur. **Disease Table 20.3**.

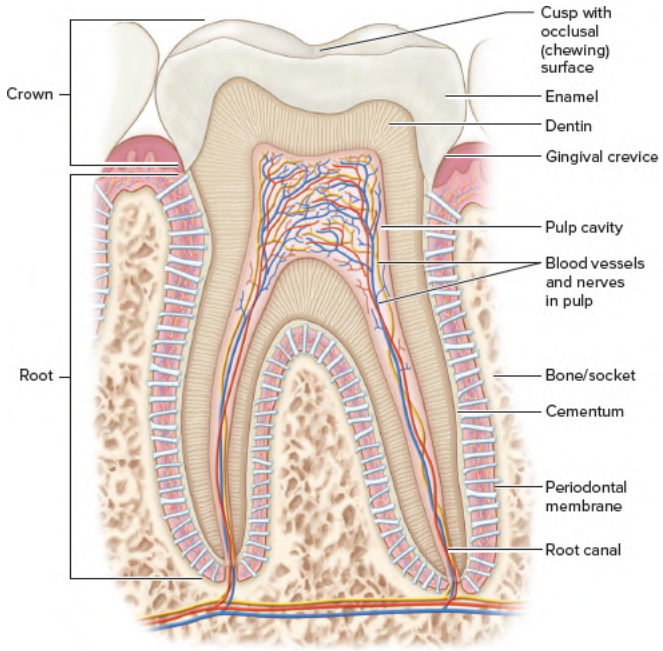

Figure 20.11 **The anatomy of a tooth.**

Tooth and Gum Infections

It is difficult to pinpoint exactly when the "normal biota biofilm" described for the oral environment becomes a "pathogenic biofilm." If left undisturbed, the biofilm structure eventually contains anaerobic bacteria that can damage the soft tissues and bones (referred to as the periodontium) surrounding the teeth. Also, the introduction of carbohydrates to the oral cavity can result in breakdown of hard tooth structure due to the production of acid by certain oral streptococci in the biofilm. These two separate circumstances are discussed here.

Dental Caries (Tooth Decay)

Dental caries is the most common infectious disease of human beings. The process involves the dissolution of solid tooth surface due to the metabolic action of bacteria. (**Figure 20.11** depicts the structure of a tooth.) The symptoms are often not noticeable but range from minor disruption in the outer (enamel) surface of the tooth to complete

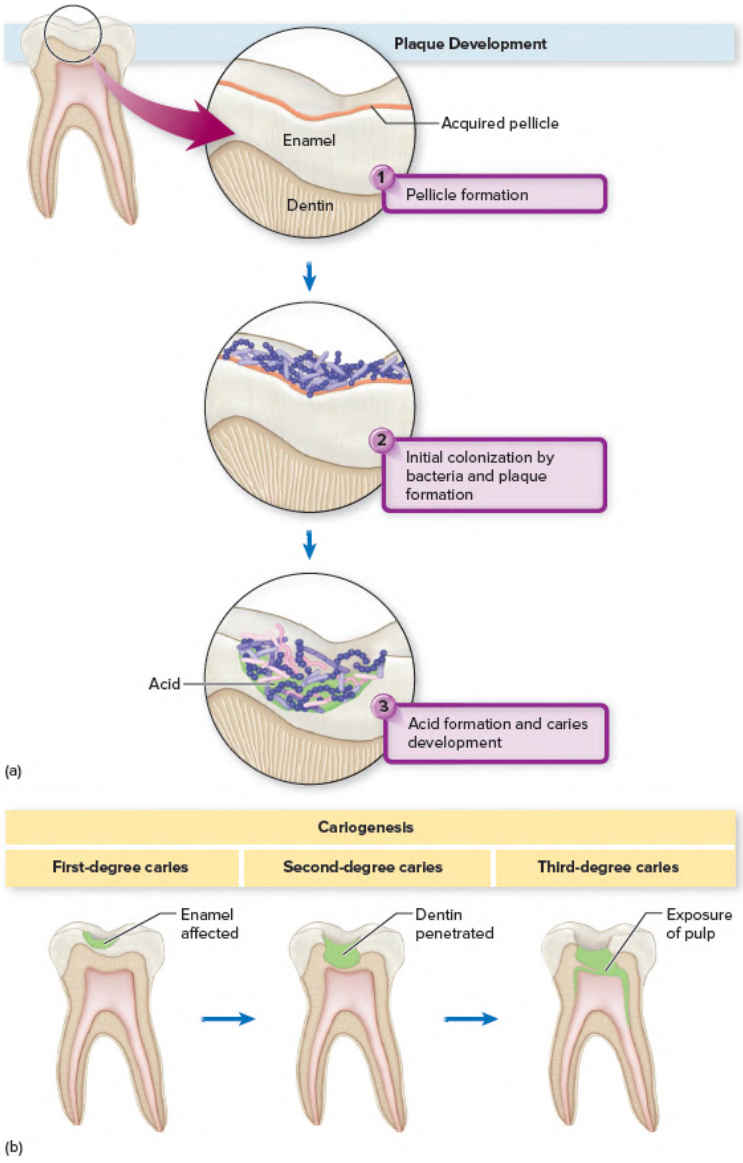

Figure 20.12 **Stages in plaque development and cariogenesis**. **(a)** A microscopic view of pellicle and plaque formation, acidification, and destruction of tooth enamel. **(b)** Progress and degrees of cariogenesis.

destruction of the enamel and then destruction of deeper layers **(figure 20.12)**. Deeper lesions can result in infection to the soft tissue inside the tooth, called the pulp, which contains blood vessels and nerves. These deeper infections lead to pain, referred to as a "toothache."

Disease Table 20.4 Dental Caries

Causative Organism(s)	A polymicrobial mixture of acid-producing bacteria
Most Common Modes of Transmission	Direct contact
Virulence Factors	Adhesion, acid production
Culture/Diagnosis	–
Prevention	Oral hygiene, fluoride supplementation
Treatment	Removal of diseased tooth material
Epidemiological Features	Globally, 60%–90% prevalence in school-age children

Causative Agent

An oral alpha-hemolytic streptococcus, *Streptococcus mutans*, was long thought to be the primary cause of dental caries. More recent techniques analyzing the oral

microbiome in caries points to a polymicrobial consortium of bacteria that contributes to the destructive acid production. Note that in the absence of dietary carbohydrates, bacteria do not cause decay.

Pathogenesis and Virulence Factors

In the presence of sucrose and, to a lesser extent, other carbohydrates, *S. mutans* and other streptococci produce sticky polymers of glucose called fructans and glucans. These adhesives help bind them to the smooth enamel surfaces and contribute to the sticky bulk of the plaque biofilm **(figure 20.13)**. If mature plaque is not removed from sites that readily trap food, it can result in a carious lesion. This is due to the action of the streptococci and other bacteria that produce acid as they ferment the carbohydrates. If the acid is immediately flushed from the plaque and diluted in the mouth, it has little effect. However, in the denser regions of plaque, the acid can accumulate in direct contact with the enamel surface and lower the pH to below 5, which is acidic enough to begin to dissolve (decalcify) the calcium phosphate of the enamel in that spot. This initial lesion can remain localized in the enamel and can be repaired with various inert materials (fillings). Once the deterioration has reached the level of the dentin, tooth destruction speeds up and the tooth can be rapidly destroyed.

Figure 20.13 The microscopic and macroscopic appearance of plaque. Disclosing tablets containing vegetable dye stain heavy plaque accumulations at the junction of the tooth and gingiva and other parts of the tooth. The blown-up image is a scanning electron micrograph

of the plaque biofilm with long filamentous forms and “corn cobs” that are mixed bacterial aggregates.
©David Scharf/Science Source (micrograph); ©BSIP SA/Alamy Stock Photo (tooth)

Transmission and Epidemiology

The bacteria that cause dental caries are transmitted to babies and children by their close contacts, especially the mother or closest caregiver. There is evidence for transfer of oral bacteria between children in day care centers, as well.

Culture and Diagnosis

Dental professionals diagnose caries based on the tooth condition. Culture of the lesion is not routinely performed.

Prevention and Treatment

The best way to prevent dental caries is through dietary restriction of sucrose and other refined carbohydrates. Regular brushing and flossing to remove plaque are also important. Most municipal communities in the United States add trace amounts of fluoride to their drinking water, because fluoride, when incorporated into the tooth structure, can increase tooth (as well as bone) hardness. Fluoride can also encourage the remineralization of teeth that have begun the demineralization process. The CDC estimates that the rate of tooth decay is decreased by 25% by the addition of fluoride to drinking water. Nearly 75% of people in the United States using public drinking water supplies are using fluoridated water. The World Health Organization recommends the use of fluoride in many different forms for all countries. Fluoride is also added to toothpastes and mouth rinses and can be applied in gel form.

Treatment of a carious lesion involves removal of the affected part of the tooth (or the whole tooth in the case of advanced caries), followed by restoration of the tooth structure with an artificial material. **Disease Table 20.4**.

Disease Table 20.5 Periodontitis

Causative Organism(s)	Polymicrobial community including some or all of: *Tannerella forsythia, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis*, others
Most Common Modes of Transmission	–
Virulence Factors	Induction of inflammation, enzymatic destruction of tissues
Culture/Diagnosis	–
Prevention	Oral hygiene
Treatment	Removal of plaque and calculus, gum reconstruction, possibly anti-inflammatory treatments
Epidemiological Features	United States: smokers = 11%, nonsmokers = 2%; internationally: 10%–15% of adults

Periodontal Diseases

Periodontal disease is so common that 97% to 100% of the population has some manifestation of it by age 45. Most kinds are due to bacterial colonization and varying degrees of inflammation that occur in response to gingival damage.

Periodontitis

The initial stage of periodontal disease is **gingivitis**, the signs of which are swelling, loss of normal contour, patches of redness, and increased bleeding of the gums (gingiva). Spaces or pockets of varying depth also develop between the tooth and

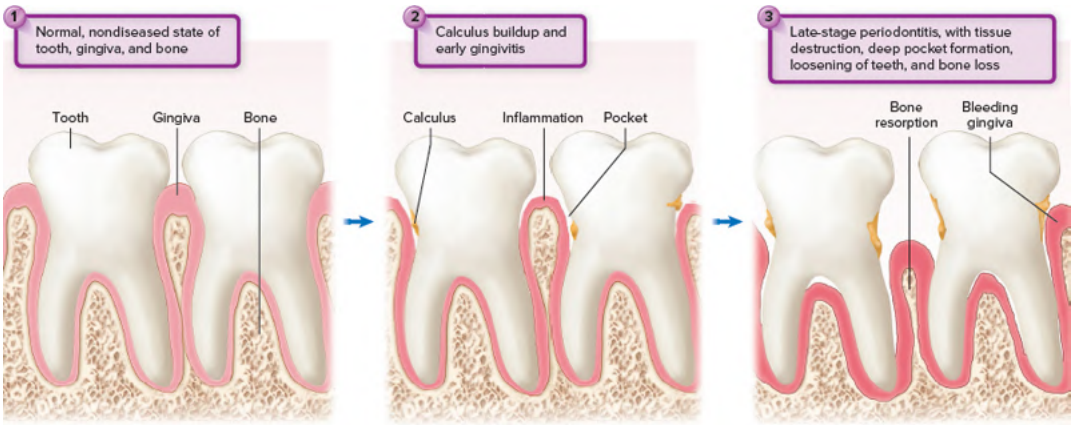

Figure 20.14 **Stages in soft-tissue infection, gingivitis, and periodontitis**.
©danielzgombic/iStock/Getty Images

the gingiva. If this condition persists, a more serious disease called periodontitis results. The deeper involvement increases the size of the pockets and can cause bone resorption severe enough to loosen the tooth in its socket. If the condition is allowed to progress, the tooth can be lost **(figure 20.14)**.

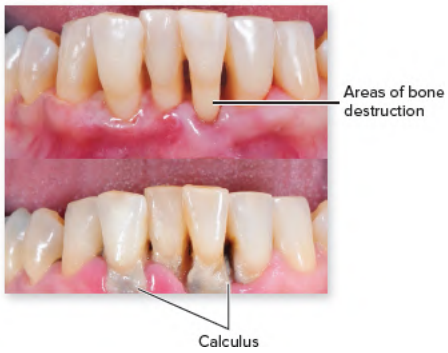

Figure 20.15 **The effect of calculus**. The bottom picture shows the lower front teeth before cleaning. Note the deposits of calculus on the root surfaces. After removal of the calculus the extent of bone destruction and gum recession, caused by the calculus, can be seen.

Causative Agents

Data from the Human Microbiome Project reveal that the composition of the microbial *community*, rather than single organisms, relates directly to risk of dental caries or periodontitis. When the polymicrobial biofilms consist of the right combination of bacteria, such as the anaerobes *Tannerella forsythia* (formerly *Bacteroides forsythus*), *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis*, and perhaps *Fusobacterium* and spirochete species, the periodontal destruction process begins. The most common predisposing condition occurs when the plaque becomes mineralized (calcified) with calcium phosphate crystals. This process produces a hard, porous substance called **calculus** above and below the gingival margin (edge) that can induce varying degrees of periodontal damage **(figure 20.15)**. The presence of calculus leads to a series of inflammatory events that characterize the disease.

Recent research has also shown that some of these microbes can escape into the bloodstream and increase the risk of coronary diseases, autoimmune diseases, and even meningitis.

Most periodontal disease is treated by removal of calculus and plaque and maintenance of good oral hygiene. Often, surgery to reduce the depth of periodontal pockets is required. Antibiotic therapy, either systemic or applied in periodontal packings, may also be utilized. **Disease Table 20.5**.

Mumps

The word *mumps* is Old English for “lump” or “bump.” The symptoms of this viral disease are so distinctive that Hippocrates clearly characterized it in the fifth century BC as a self-limited, mildly epidemic illness associated

with painful swelling at the angle of the jaw **(figure 20.16)**.

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3. A client has been diagnosed with Guillain-Barré syndrome (GBS). Which observation found in the client's past medical history is relevant?

a. *congestive heart failure*
b. *Campylobacter infection*
c. *dehydration*
d. *diabetes mellitus*

Signs and Symptoms

After an average incubation period of 2 to 3 weeks, symptoms of fever, nasal discharge, muscle pain, and malaise develop. These may be followed by inflammation of the salivary glands (especially the parotids), producing the classic gopherlike swelling of the cheeks on one or both sides. Swelling of the gland is called parotitis, and it can cause considerable discomfort. Viral multiplication in salivary glands is followed by invasion of other organs, especially the testes, ovaries, thyroid gland, pancreas, meninges, heart, and kidney. Despite the invasion of multiple organs, the prognosis of most infections is complete, uncomplicated recovery with permanent immunity.

Complications in Mumps In 20% to 30% of young adult males, mumps infection localizes in the epididymis and testis, usually on one side only. The resultant syndrome of orchitis and epididymitis may be rather painful, but no permanent damage usually occurs.

Transmission and Epidemiology of Mumps Virus

Humans are the exclusive natural hosts for the mumps virus. It is communicated primarily through salivary and respiratory secretions. Most cases occur in children under the age of 15, and as many as 40% are subclinical. Because lasting immunity follows any form of mumps infection, no long-term carrier reservoir exists in the population. The incidence of mumps in the United States spiked in 2016, with more than 5,000 cases reported. In that year and the few years preceding it, there were notable outbreaks on college campuses.

Figure 20.16 **The external appearance of swollen parotid glands in mumps (parotitis).**
Source: CDC

Prevention and Treatment

The general pathology of mumps is mild enough that symptomatic treatment to relieve fever, dehydration, and pain is usually adequate. Vaccine recommendations call for a dose of MMR at 12 to 15 months and a second dose at 4

to 6 years. Health care workers and college students who haven't already had both doses are advised to do so.

Disease Table 20.6 Mumps

Causative Organism(s)	Mumps virus (genus *Paramyxovirus*) V
Most Common Modes of Transmission	Droplet contact
Virulence Factors	Spike-induced syncytium formation
Culture/Diagnosis	Clinical, fluorescent Ag tests, ELISA for Ab
Prevention	MMR live attenuated vaccine
Treatment	Supportive
Epidemiological Features	United States: fluctuates between a few hundred cases a year and several thousand; internationally: are epidemic peaks every 2–5 years

Gastritis and Gastric Ulcers

The curved cells of *Helicobacter* were first detected by J. Robin Warren in 1979 in stomach biopsies from ulcer patients. He and a partner, Barry J. Marshall, isolated the microbe in culture and even served as guinea pigs by swallowing a large inoculum to prove that it would cause gastric ulcers. Warren and Marshall won the Nobel Prize in Medicine in 2005 for their discovery.

Signs and Symptoms

Gastritis is experienced as sharp or burning pain emanating from the abdomen. Gastric or peptic ulcers are actual lesions in the mucosa of the stomach (gastric ulcers) or in the uppermost portion of the small intestine (duodenal ulcers). Severe ulcers can be accompanied by bloody stools, vomiting, or both. The symptoms are often worse at night, after eating, or under conditions of psychological stress.

The fifth most common cancer in the world is stomach cancer, and ample evidence suggests that long-term infection with *Helicobacter pylori* is a major contributing factor.

Causative Agent

Helicobacter pylori is a curved gram-negative rod, closely related to *Campylobacter*, which we studied earlier in this chapter.

Pathogenesis and Virulence Factors

Once the bacterium passes into the gastrointestinal tract, it bores through the outermost mucous layer that lines the stomach epithelial tissue. Then it attaches to specific binding sites on the cells and entrenches itself. It produces an enzyme, urease, that breaks down urea in its vicinity to form carbon dioxide and ammonia. In this way it can neutralize the highly acidic environment of the stomach.

Before the bacterium was discovered, spicy foods, high-sugar diets (which increase acid levels in the stomach), and psychological stress were considered to be the cause of gastritis and ulcers. Now it appears that these factors merely aggravate the underlying infection.

Disease Table 20.7 Gastritis and Gastric Ulcers

Causative Organism(s)	*Helicobacter pylori* B **G−**
Most Common Modes of Transmission	Direct contact, fecal oral
Virulence Factors	Adhesins, urease
Culture/Diagnosis	Endoscopy, urea breath test, stool antigen test
Prevention	None
Treatment	Amoxicillin followed by clarithromycin + tinidazole
Epidemiological Features	United States: infection (not disease) rates at 35% of adults; internationally: infection rates at 50%

Transmission and Epidemiology

The bacterium has probably been in human stomachs for tens of thousands of years. However, there is evidence that the percentage of people with *Helicobacter* in their stomachs is decreasing with each generation. Scientists

suspect that this is due to our more sanitary environment and, of course, the use of antibiotics. While you might think this is a good thing, in light of the fact that it can cause ulcers and stomach cancer, there is evidence to suggest that its absence leads to higher incidences of acid reflux and even asthma. It should not surprise us by now that a single microbe can have both positive and negative consequences, depending on the circumstances. It should also not surprise us that obliterating a longstanding member of our microbiome in a short period of (evolutionary) time could lead to health imbalances and consequences.

H. pylori is probably transmitted from person to person by the oral-oral or fecal-oral route. It seems to be acquired early in life and carried asymptomatically unless its activities begin to damage the digestive mucosa. Because other animals are also susceptible to *H. pylori* and even develop chronic gastritis, it has been proposed that the bacterium is a zoonosis transmitted from an animal reservoir. The bacterium has also been found in water sources, suggesting that perhaps proper sanitation may reduce transmission.

Prevention and Treatment

The only preventive approaches available currently are those that diminish some of the aggravating factors just mentioned. Many over-the-counter remedies offer symptom relief; most of them act to neutralize stomach acid. The best treatment is a course of antibiotics augmented by acid suppressors. The antibiotics most prescribed are clarithromycin or metronidazole. **Disease Table 20.7**.

Medical Moment

Assessing Jaundice

Jaundice is caused by elevated levels of bilirubin in the blood. How is jaundice recognized in a patient? Jaundice progresses caudally from face to trunk and extremities, so you should begin by looking at the patient's face. In some patients, jaundice will be easily recognized, because the patient's sclerae (the white portions of the eye) will be yellow—sometimes startlingly so! The skin may also have a yellowish discoloration. In dark-skinned individuals, especially older adults, the sclerae may not be reliable indicators as the sclerae may have a yellowish tint even in patients without jaundice. You can assess the palms and soles of the feet in patients with dark skin. Blanching the skin (applying pressure to the skin with your fingertip) also helps to reveal the underlying skin color. The mucous membranes of affected individuals will also reveal a yellow discoloration.

Q. Why are the effects of jaundice so widespread in the body?

Answer in Appendix B.

Hepatitis

When certain viruses infect the liver, they cause **hepatitis**, an inflammatory disease marked by necrosis of hepatocytes and an inflammatory response that swells and disrupts the liver architecture. This pathologic change interferes with the liver's excretion of bile pigments such as bilirubin into the intestine. When bilirubin, a greenish-yellow pigment, accumulates in the blood and tissues, it causes **jaundice**, a yellow tinge in the skin and eyes. The condition can be caused by a variety of different viruses, including cytomegalovirus, Epstein-Barr virus, and a virus known as GBV-C. The others are all called "hepatitis viruses" but only because they all can cause this inflammatory condition in the liver. They are quite different from one another. While there are some recently discovered hepatitis viruses, they are not yet well characterized so we will cover the five that are well understood, named hepatitis A–E.

Note that noninfectious conditions can also cause inflammation and disease in the liver, including some autoimmune conditions, drugs, and alcohol overuse.

Hepatitis A and E Viruses

Hepatitis A virus (HAV) and hepatitis E virus (HEV) are both single-stranded nonenveloped RNA viruses. These two viruses are considered together since they are both transmitted through the fecal-oral route, and both

cause relatively minor, self-limited hepatitis. The important exception to this is when HEV infects pregnant women, in whom it causes a 10% to 30% fatality rate. It can also be dangerous to people who have received an organ transplant and are on immunosuppressive therapy.

Signs and Symptoms

Most infections by these viruses are either subclinical or accompanied by vague, flulike symptoms. In more overt cases, the presenting symptoms may include jaundice and swollen liver. The viruses are not oncogenic (cancer causing), and in most everyone besides pregnant women and transplant patients, complete uncomplicated recovery results.

Transmission and Epidemiology

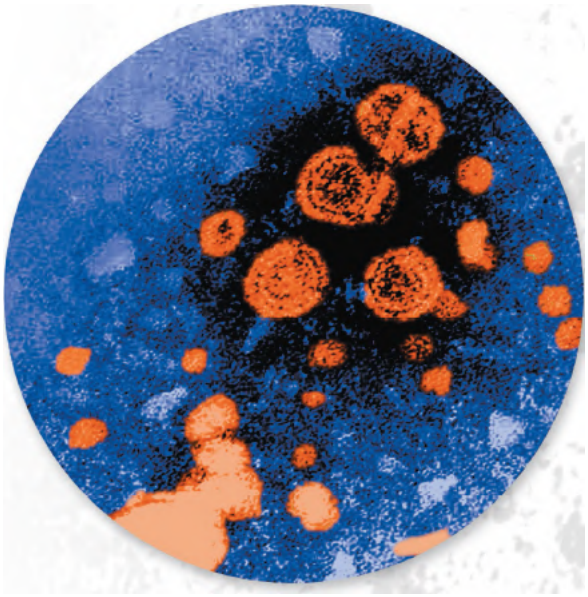
In general, the disease is associated with deficient personal hygiene and lack of public health measures. In countries with inadequate sewage control, most outbreaks are associated with fecally contaminated water and food. Most infections result from close institutional contact, unhygienic food handling, eating infected shellfish, sexual transmission, or travel to other countries.

Hepatitis A occasionally can be spread by blood or blood products, but this is the exception rather than the rule. In developing countries, children are the most common victims, because exposure to the virus tends to occur early in life, whereas in North America and Europe, more cases appear in adults. Because the virus is not carried chronically, the principal reservoirs are asymptomatic, short-term carriers (often children) or people with clinical disease.

Prevention and Treatment

Prevention of hepatitis A is based primarily on immunization. An inactivated viral vaccine (Havrix) has been in use since the mid-1990s. Short-term protection can be conferred by passive immune globulin. When people are exposed to infection via contact with an infected person or with a restaurant that was a source of a known outbreak, passive immunoglobulin or the vaccine can be administered. Administering a combined hepatitis A/hepatitis B vaccine, called Twinrix, is recommended for people who may be at risk for both diseases, such as people with chronic liver dysfunction, intravenous drug users, and anyone engaging in anal-oral intercourse. Travelers to areas with high rates of both diseases should obtain vaccine coverage as well. Hepatitis E has no vaccine.

No specific medicine is available for hepatitis A or E once the symptoms begin. Drinking lots of fluids and avoiding liver irritants such as aspirin or alcohol will speed recovery.



Electron micrograph of hepatitis B virions.
Source: CDC/Dr. Erskine Palmer

Hepatitis B Virus (and Hepatitis D)

Hepatitis B virus (HBV) is an enveloped DNA virus in the family *Hepadnaviridae*. Intact viruses are often called Dane particles. The genome is partly double-stranded and partly single-stranded. **Hepatitis D** is an enveloped RNA virus. It is actually a *subvirus satellite* of HBV; that is, it can propagate only in the presence of HBV.

Signs and Symptoms

In addition to the direct damage to liver cells just outlined, the spectrum of hepatitis disease may include fever, chills, malaise, anorexia, abdominal discomfort, diarrhea, and nausea. Rashes may appear and arthritis may occur. Hepatitis B infection can be very serious, even life-threatening. A small number of patients develop glomerulonephritis and arterial inflammation. Complete liver regeneration and restored function occur in most patients; however, a small number of patients develop chronic liver

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4. A patient with hepatitis is admitted to an inpatient medical unit. The nurse educates her about the clinical manifestations that accompany the disease. All of the following findings may be associated with jaundice, except:

a. *yellow sclerae*.
b. *pruritus*.
c. *clay-colored stools*.
d. *decreased bilirubin levels*.
e. *dark-colored urine*.

disease in the form of necrosis or **cirrhosis** (permanent liver scarring and loss of tissue). In some cases, chronic HBV infection can lead to liver cancer.

Patients who become infected as children have significantly higher risks of long-term infection and disease. In fact, 90% of neonates infected at birth develop chronic infection, as do 30% of children infected between the ages of 1 and 5, but only 6% of persons infected after the age of 5. This finding is one of the major justifications for the routine vaccination of children. Also, infection becomes chronic more often in men than in women. The mortality rate is 15% to 25% for people with chronic infection.

When patients are infected with both HBV and hepatitis D virus (HDV), the disease can become more severe and is more likely to progress to permanent liver damage.

Pathogenesis and Virulence Factors

The hepatitis B virus enters the body through a break in the skin or mucous membrane or by injection into the bloodstream. Eventually, it reaches the liver cells (hepatocytes) where it multiplies and releases viruses into the blood during an incubation period of 4 to 24 weeks (7 weeks average). Surprisingly, the majority of those infected exhibit few overt symptoms and eventually develop an immunity to HBV, but some people experience the symptoms described earlier.

Transmission and Epidemiology

An important factor in the transmission pattern of hepatitis B virus is that it multiplies exclusively in the liver, which continuously seeds the blood with viruses. Electron microscopic studies have revealed up to 10^7 virions per milliliter of infected blood. Even a minute amount of blood (a *millionth* of a milliliter) can transmit infection. The abundance of circulating virions is so high and the minimal dose so low that such simple practices as sharing a toothbrush or a razor can transmit the infection. HBV can be transmitted via semen and vaginal secretions. Vertical transmission is possible, and it predisposes the child to development of the carrier state and increased risk of liver cancer. It is sometimes known as *serum hepatitis*.

This virus is one of the major infectious concerns for health care workers. Needlesticks can easily transmit the virus, and therefore most workers are required to have the full series of HBV vaccinations. Unlike the more notorious HIV, HBV remains infective for days in dried blood, for months when stored in serum at room temperature, and for decades if frozen. Although it is not inactivated after 4 hours of exposure to 60°C, boiling for

the same period can destroy it. Disinfectants containing chlorine, iodine, and glutaraldehyde show potent anti-hepatitis B activity.

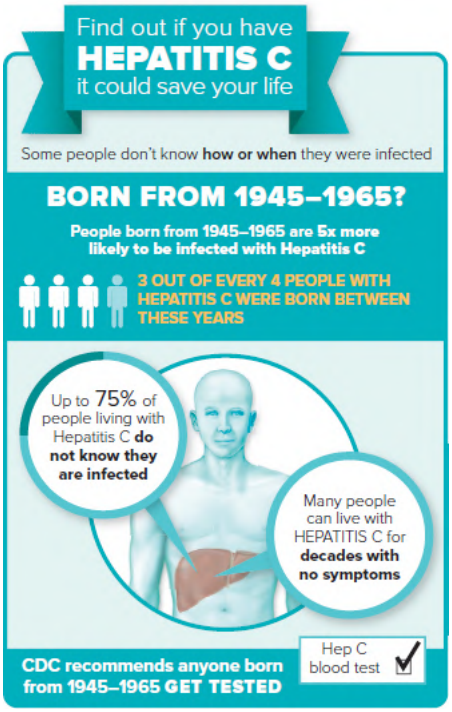
Culture and Diagnosis

Serological tests can detect either virus antigen or antibodies. Radioimmunoassay and ELISA testing are used to detect the important surface antigen (S antigen) of HBV very early in infection. Antibody tests are most valuable in patients who are negative for the antigen.

Prevention and Treatment

The primary prevention for HBV infection is vaccination. The most widely used vaccines are recombinant, containing the pure surface antigen cloned in yeast cells. Vaccination is a must for medical and dental workers and students, patients receiving multiple transfusions, immunodeficient persons, and cancer patients. The vaccine is also now strongly recommended for all newborns as part of a routine immunization schedule. As just mentioned, a combined vaccine for HAV/HBV may be appropriate for certain people.

Passive immunization with hepatitis B immune globulin (HBIG) gives significant immediate protection to people who have been exposed to the virus through needle puncture, broken blood containers, or skin and mucosal contact with blood. Another group for whom passive immunization is highly recommended is neonates born to infected mothers.



Source: CDC

Disease Table 20.8 Hepatitis

Causative Organism(s)	Hepatitis A or E virus (V)	Hepatitis B virus (V)	Hepatitis C virus (V)
Most Common Modes of Transmission	Fecal-oral, vehicle	Parenteral (blood contact), direct contact (especially sexual), vertical	Parenteral (blood contact), vertical
Virulence Factors	–	Latency	Core protein suppresses immune function?
Culture/Diagnosis	IgM serology	Serology (ELISA,	Serology, also PCR

		radioimmunoassay)	
Prevention	Hepatitis A vaccine or combined HAV/HBV vaccine	HBV recombinant vaccine	–
Treatment	HAV: hepatitis A vaccine or immune globulin; HEV: immune globulin	Interferon, tenofovir, or entecavir	Sofosbuvir/ledipasvir combination drug
Incubation Period	2–4 weeks	1–6 months	2–8 weeks
Epidemiological Features	Hepatitis A, United States: 20,000 cases annually and 40% of adults show evidence of prior infection; internationally: 1.4 million cases per year; hepatitis E, internationally: 20 million infections per year; 60% in East and Southeast Asia	United States: 19,000 new cases per year; 800,000 to 2.2 million have chronic infection; internationally: 240 million	United States: estimated 17,000 new cases per year; 3–4 million with chronic HCV; internationally: 150 million chronically infected

Mild cases of hepatitis B may be managed by symptomatic treatment and supportive care. Severe or chronic infection can be controlled with recombinant human interferon, tenofovir, or entevir. Each of these can help to slow virus multiplication and prevent liver damage in many but not all patients. None of the drugs is considered curative.

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Hepatitis C Virus

Hepatitis C virus (HCV) is sometimes referred to as the "silent epidemic" because more than 3.5 million Americans are infected with the virus, but it takes many years to cause noticeable symptoms. In the United States, its incidence fell between 1992 and 2003, but no further decreases have been seen since then. Liver failure from hepatitis C is one of the most common reasons for liver transplants in this country. Hepatitis C is an RNA virus in the *Flaviviridae* family. It used to be known as "non-A non-B" virus. It is usually diagnosed with a blood test for antibodies to the virus.

Signs and Symptoms

People have widely varying experiences with this infection. It shares many characteristics of hepatitis B disease, but it is much more likely to become chronic. Of those infected, 75% to 85% will remain infected indefinitely. (In contrast, only about 6% of persons who acquire hepatitis B after the age of 5 will be chronically infected.) With HCV infection, it is possible to have severe symptoms without permanent liver damage, but it is more common to have chronic liver disease even if there are no overt symptoms. Cancer may also result from chronic hepatitis C virus (HCV) infection. Worldwide, HBV infection is the most common cause of liver cancer, but in the United States it is more likely to be caused by HCV. In 2012, the CDC recommended that all baby boomers (those born between 1945 and 1965) be tested for HCV.

Transmission and Epidemiology

This virus is acquired in similar ways to HBV. It is more commonly transmitted through blood contact (both "sanctioned," such as in blood

transfusions, and "unsanctioned," such as needle sharing by injecting drug users) than through transfer of other body fluids. Vertical transmission is also possible.

Anyone with a history of exposure to blood products or organs before 1992 (when effective screening became available) is at higher risk for this infection, as is anyone with a history of injecting drug use.

Prevention and Treatment

There is currently no vaccine for hepatitis C. In 2013 a two-drug regimen became available that has excellent results, including complete cure. Sofosbuvir is a nucleotide analog that "fools" the DNA polymerase of the virus; ledipasvir acts as a protease inhibitor. **Disease Table 20.8**.

20.3 LEARNING OUTCOMES—Assess Your Progress

5. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Disease" acute diarrhea.
6. Discuss important features of the conditions food poisoning and chronic diarrhea.
7. Discuss important features of the two categories of oral conditions: dental caries and periodontal diseases.
8. Identify the most important features of mumps, gastritis, and gastric ulcers.
9. Differentiate among the main types of hepatitis, and discuss the causative agents, mode of transmission, diagnostic techniques, prevention, and treatment of each.

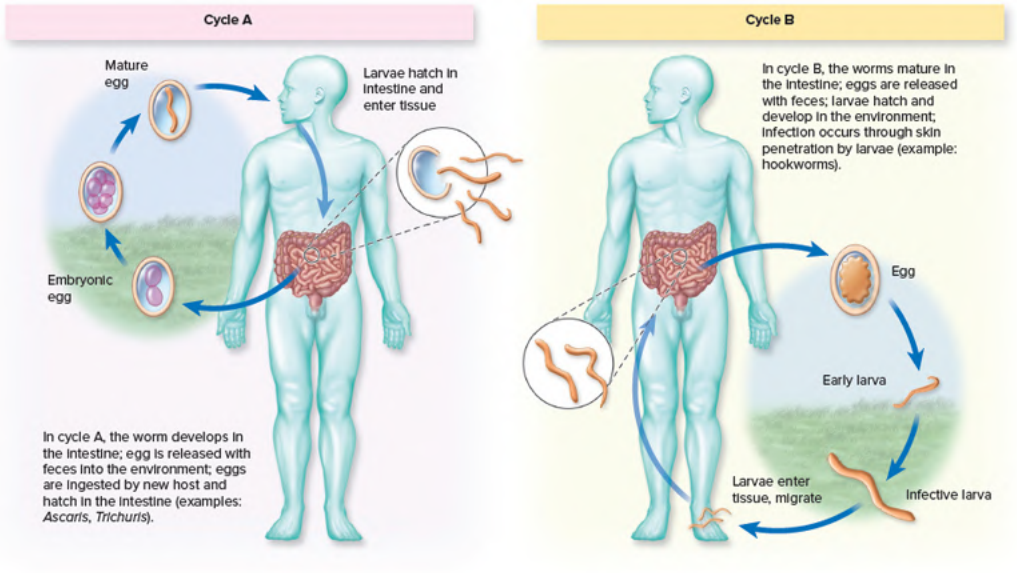


Figure 20.17 **Four basic helminth life and transmission cycles**.

20.4 Gastrointestinal Tract Diseases Caused by Helminths

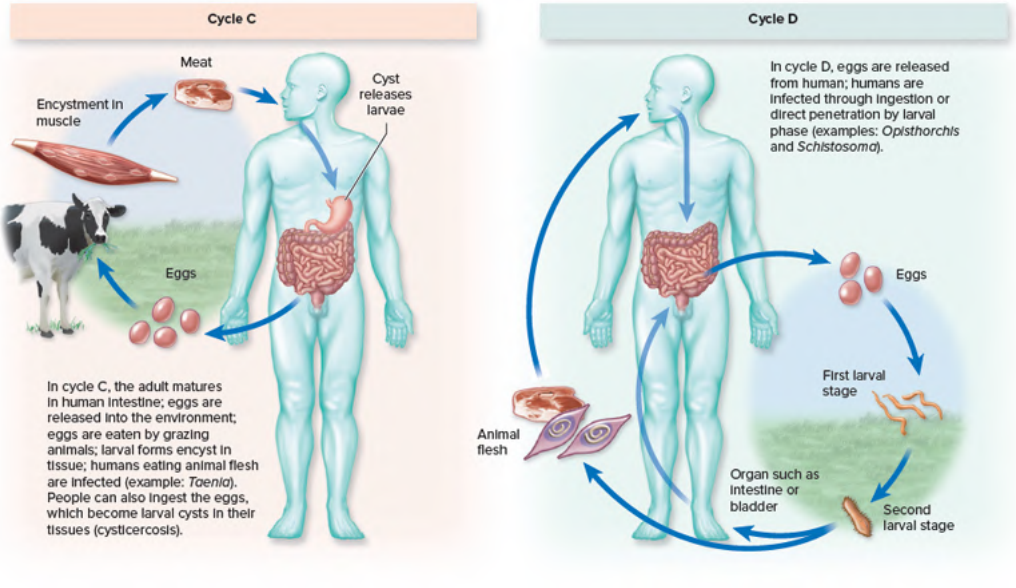
Helminths that parasitize humans are amazingly diverse, ranging from barely visible roundworms (0.3 mm) to huge tapeworms (25 m long). In the introduction to these organisms in chapter 4, we grouped them into three categories: nematodes (roundworms), trematodes (flukes), and cestodes (tapeworms), and we discussed basic characteristics of each group. You may want to review those sections before continuing. In this section, we examine the intestinal diseases caused by helminths. Although they can cause symptoms that may be mistaken for some of the diseases discussed elsewhere in this chapter, helminthic diseases are usually accompanied by an additional set of symptoms that arise from the host response to helminths. Worm infection usually provokes an increase in granular leukocytes called eosinophils, which have a specialized capacity to destroy multicellular parasites. This increase, termed **eosinophilia**, is a hallmark of helminthic infection and is detectable in blood counts. If the following symptoms occur coupled with eosinophilia, helminthic infection should be suspected.

Helminthic infections may be acquired through the fecal-oral route or through penetration of the skin, but most of these organisms spend part of their lives in the intestinal tract. **Figure 20.17** depicts the four different types of life cycles of the helminths. While the worms are in the intestines, they can produce a gamut of intestinal symptoms. Some of them also produce symptoms outside of the intestines.

Clinical Considerations

We start with diagnosis, pathogenesis and prevention, and treatment of the helminths as a group in the next subsections. We'll then highlight one of the most common forms of helminthic disease, and finish with summaries of the others.

Trained sushi chefs are skilled at detecting helminth infection in the raw fish they prepare.
©Stockbyte/Getty Images



Pathogenesis and Virulence Factors in General

Helminths have numerous adaptations that allow them to survive in their hosts. They have specialized mouthparts for attaching to tissues and for feeding, enzymes with which they liquefy and penetrate tissues, and a cuticle or other covering to protect them from host defenses. In addition, their organ systems are usually reduced to the essentials: getting food and processing it, moving, and reproducing. The damage they cause in the host is very often the result of the host's response to the presence of the invader.

Many helminths have more than one host during their lifetimes. If this is the case, the host in which the *adult* worm is found is called the definitive host (usually a vertebrate). Sometimes the actual definitive host is not the

host usually used by the parasite but an accidental bystander. Humans often become the accidental definitive hosts for helminths whose normal definitive host is a cow, pig, or fish. *Larval* stages of helminths are found in intermediate hosts. Humans can serve as intermediate hosts, too. Helminths may require no intermediate host at all or may need one or more intermediate hosts for their entire life cycle.

Diagnosis in General

Diagnosis of almost all helminthic infections follows a similar series of steps. The most common diagnostic method is the "O&P test," which is microscopic examination of a stool sample to look for ova or parasites. A differential blood count showing increased eosinophils and serological tests indicating sensitivity to helminthic antigens all provide indirect evidence of worm infection. A history of travel to the tropics or immigration from those regions is also helpful, even if it occurred years ago, because some flukes and nematodes persist for decades. The worms are sufficiently distinct in morphology that positive identification can be based on any stage, including eggs. That said, not all of these diseases result in eggs or larval stages that can easily be found in stool.

Prevention and Treatment in General

There are no vaccines for the helminthic infections described here. In populations in which the infections are common, prophylactic treatment twice a year with antihelminthic drugs has been shown to keep people healthy.

Although several useful antihelminthic medications exist, the cellular physiology of the eukaryotic parasites resembles that of humans, and drugs toxic to them can also be toxic to us. Some antihelminthic drugs suppress a metabolic process that is more important to the worm than to the human. Others inhibit the worm's movement and prevent it from maintaining its position in a certain organ. Note that some helminths have developed resistance to the drugs used to treat them. In some cases, surgery may be necessary to remove worms or larvae.

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5. Blood in the stool may occur in which of the following?

a. *Salmonella infection*
b. *rotavirus infection*
c. *Clostridium difficile infection*
d. *Shigella infection*
e. *b, c, and d*
f. *a, c, and d*

HIGHLIGHT DISEASE

Helminthic Infections: Intestinal Distress as the Primary Symptom

Both tapeworms and roundworms can infect the intestinal tract in such a way as to cause primary symptoms there.

Enterobius vermicularis

This nematode is often called the pinworm, or seatworm. It is the most common worm disease of children in temperate zones. The transmission of this roundworm is of the cycle A type. Freshly deposited eggs have a sticky coating that causes them to lodge

beneath the fingernails and to adhere to fomites. Upon drying, the eggs become airborne and settle in house dust. Eggs are ingested from contaminated food or drink and from self-inoculation from one's own fingers. Eggs hatch in the small intestine and release larvae that migrate to the large intestine. There the larvae mature into adult worms and mate.

The hallmark symptom of this condition is pronounced anal itching when the mature female emerges from the anus and lays eggs. Although infection is not fatal, and most cases are asymptomatic, the afflicted child can

suffer from disrupted sleep and sometimes nausea, abdominal discomfort, and diarrhea. A simple rapid test can be performed by pressing a piece of transparent adhesive tape against the anal skin and then applying it to a slide for microscopic examination. When one member of the family is diagnosed, the entire family should be tested and/or treated because it is likely that multiple members are infected. Eggs are not present in stool, so standard "O&P" is not useful.

Other Helminths Responsible for Intestinal Distress

Trichuris trichiura, the whipworm, follows the cycle A lifestyle. Trichuriasis has its highest incidence in areas of the tropics and subtropics that have poor sanitation. Symptoms of this infection may include localized hemorrhage of the bowel caused by worms burrowing and piercing intestinal mucosa. This can also provide a portal of entry for secondary bacterial infection. Heavier infections can cause dysentery, loss of muscle tone, and rectal prolapse, which can prove fatal in children.

The tapeworm *Diphyllobothrium latum* has an intermediate host in fish. It follows cycle C. It is common in the Great Lakes, Alaska, and Canada. Mammals, including humans, act as definitive hosts. It develops in the intestine and can cause long-term symptoms. It can be transmitted in raw food such as sushi and sashimi made from salmon. It is the largest human tapeworm known, growing up to 30 feet.

Hymenolepis species are small tapeworms and are the most common human tapeworm infections in the world. They follow cycle C. There are two species: *Hymenolepis nana*, known as the dwarf tapeworm because it is only 15 to 40 mm in length, and *H. diminuta*, the rat tapeworm, which is usually 20 to 60 cm in length as an adult.

Disease Table 20.9 Intestinal Distress

Causative Organism(s)	*Enterobius vermicularis* (pinworm) (H)	*Trichuris trichiura* (whipworm) (H)	*Diphyllobothrium latum* (fish tapeworm) (H)	*Hymenolepis nana* and *H. diminuta* (H)
Most Common Modes of Transmission	Cycle A: vehicle (food, water), fomites, self-inoculation	Cycle A: vehicle (soil), fecal-oral	Cycle C: vehicle (seafood)	Cycle C: vehicle (ingesting insects), fecal-oral
Virulence Factors	–	Burrowing and invasiveness	Vitamin B_{12} usage	–
Culture/Diagnosis	Adhesive tape + microscopy	Blood count, serology, egg or worm detection	Blood count, serology, egg or worm detection	Blood count, serology, egg or worm detection
Prevention	Hygiene	Hygiene, sanitation	Cook meat	Hygienic environment
Treatment	Albendazole (for whole family)	Mebendazole	Praziquantel	Praziquantel
Distinctive Features	Common in United States	Humans sole host	Large tapeworm; anemia	Most common tapeworm infection
Epidemiological Features	Most common nematode infection in United States; 40 million cases per year	United States: prevalence approx. 0.1%; internationally: prevalence as high as 80% in Southeast Asia, Africa, the Caribbean, and Central and South America	Estimated 20 million infections worldwide	United States: prevalence approximately 0.4%; internationally: the single most prevalent tapeworm infection

Helminthic Infections: Intestinal Distress Accompanied by Migratory Symptoms

A diverse group of helminths enter the body as larvae or eggs, mature to the worm stage in the intestine, and then migrate into the circulatory and lymphatic systems, after which they travel to the heart and lungs, migrate up the respiratory tree to the throat, and are swallowed. This journey puts the mature worms in the intestinal tract where they then take up residence. All of these conditions, in addition to causing symptoms in the digestive tract, may induce inflammatory reactions along their migratory routes, resulting in eosinophilia and, during their lung stage, pneumonia. Examples of this type of infection appear in **Disease Table 20.10**.

Cysticercosis

Taenia solium is a tapeworm. Adult worms are usually around 5 meters long and have a scolex with hooklets and suckers to attach to the intestine **(figure 20.18)**. This helminth follows cycle C in figure 20.17, in which humans are infected by eating animal flesh that contains the worm eggs, or even the worms themselves.

Disease caused by *T. solium* (the pig tapeworm) is distributed worldwide but is mainly concentrated in areas where humans live in close proximity with pigs or eat undercooked pork. The cycle starts in the pigs, when the eggs hatch in the small intestine and the released larvae migrate throughout the organs. Ultimately, they encyst in the pig's muscles, becoming *cysticerci*, young tapeworms that are the infective stage for humans. When humans ingest a live cysticercus in pork, the coat is digested and the organism is flushed into the intestine, where it firmly attaches by the scolex and develops into an adult tapeworm. Infection with *T. solium* can take another form when humans ingest the tapeworm eggs rather than infected meat. Then the human, instead of the tapeworm in the gut, becomes the host of the encysted larvae, or cysticerci, leading to a condition called **cysticercosis**, one of the five neglected parasitic infections (NPIs) in the United States **(figure 20.19)**. It is estimated that tens of thousands of Latinos living in the United States are affected by cysticercosis, but it is not

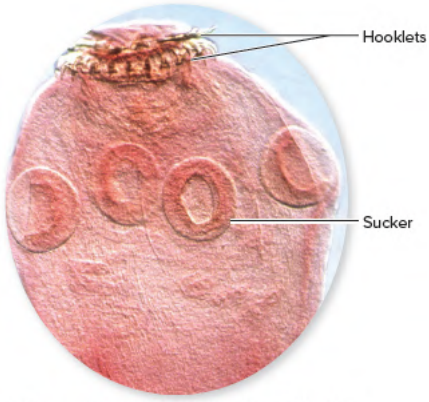
Disease Table 20.10 Intestinal Distress Plus Migratory Symptoms

Causative Organism(s)	*Toxocara* species (H)	*Ascaris lumbricoides* (intestinal roundworm) (H)	*Necator americanus* and *Ancylostoma duodenale* (hookworms) (H)
Most Common Modes of Transmission	Cycle A: dog or cat feces	Cycle A: vehicle (soil/fecal-oral), fomites, self-inoculation	Cycle B: vehicle (soil), fomite
Virulence Factors	–	Induction of hypersensitivity, adult worm migration, abdominal obstruction	Induction of hypersensitivity, adult worm migration, abdominal obstruction
Culture/Diagnosis	Blood count, serology, egg or worm detection	Blood count, serology, egg or worm detection	Blood count, serology, egg or worm detection
Prevention	Hygiene	Hygiene	Sanitation
Treatment	Albendazole	Albendazole	Albendazole
Distinctive Features	Can cause migration symptoms or blindness	Most cases mild, unnoticed	Penetrates skin, serious intestinal symptoms
Epidemiological Features	Nearly 100% of newborn puppies in United States infected; 14% of people in United States have been infected; designated a "neglected parasitic infection" in the United States by the CDC	Internationally: up to 25% prevalence, 80,000–100,000 deaths per year	United States: widespread in Southeast until early 1900s; internationally: 800 million infected

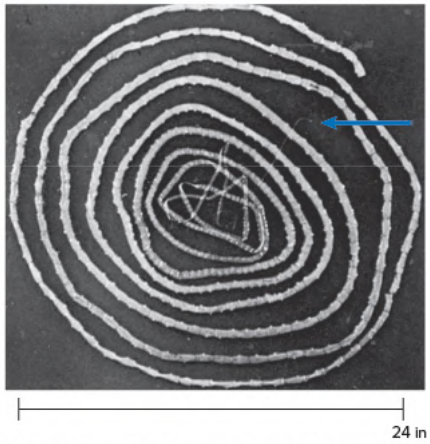
Disease Table 20.11 Cysticercosis

Causative Organism(s)	*Taenia solium* (pork tapeworm) (H)
Most Common Modes of Transmission	Cycle C: vehicle (pork), fecal-oral
Virulence Factors	–
Culture/Diagnosis	Blood count, serology, egg or worm detection
Prevention	Cook meat, avoid pig feces
Treatment	Praziquantel
Distinctive Features	Ingesting larvae embedded in pork leads to intestinal tapeworms: ingesting eggs (fecal-oral route) causes cysticercosis, larval cysts embedded in tissue of new host
Epidemiological Features	United States: a common cause of seizures; designated a "neglected parasitic infection" in the United States by the CDC; internationally: very common in Latin America and Asia

often recognized because American physicians may not know to look for it. A particularly nasty form of this condition is neurocysticerosis, in which the larvae encyst in the brain. It is estimated to be responsible for 10% of seizures requiring emergency room visits in some U.S. cities. **Disease Table 20.11**.



(a) Tapeworm scolex showing sucker and hooklets.



(b) Adult tapeworm. The arrow points to the (barely visible) scolex; the remainder of the tape, called the strobila, has a total length of 5 meters.

Figure 20.18 **Tapeworm characteristics**.
(a) ©Eric Grave/SPL/Getty Images; (b) Courtesy Dickson Despommier

Schistosomiasis: Liver Disease

When liver swelling or malfunction is accompanied by eosinophilia, **schistosomiasis** should be suspected. Schistosomiasis has afflicted humans for thousands of years. The disease described here is caused by the blood flukes *Schistosoma mansoni* or *S. japonicum*, species that are morphologically and geographically distinct but share similar life cycles, transmission methods, and general disease manifestations. It is one of the few infectious agents that can invade intact skin.

Another species called *Schistosoma haematobium* causes disease in the bladder.

Signs and Symptoms

The most severe consequences associated with chronic infection are hepatomegaly, liver disease, and splenomegaly. Occasionally, eggs from the worms are carried into the central nervous system and heart, and create a severe granulomatous response. Adult flukes can live for many years and, by eluding the immune defenses, cause a chronic affliction.

Causative Agent

Schistosomes are trematodes, or flukes (see chapter 4), but they are more cylindrical than flat **(figure 20.20)**. They are often called blood flukes. Humans are the definitive hosts for the blood fluke, and snails are the intermediate host.

Pathogenesis and Virulence Factors

This parasite is clever indeed. Once inside the host, it coats its outer surface with proteins from the host's bloodstream, basically "cloaking" itself from the host defense system. This coat reduces its surface antigenicity and allows it to remain in the host indefinitely.

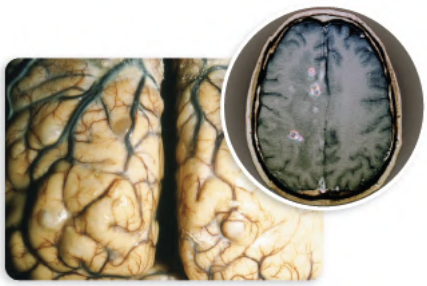


Figure 20.19 **Cysticerci in the brain caused by *Taenia solium*.**
©PR Bouree/age fotostock (brain); ©ZEPHYR/Getty Images (scan)

Disease Table 20.12 Schistosomiasis Liver Disease

Causative Organism(s)	*Schistosoma mansoni, S. japonicum*
Most Common Modes of Transmission	Cycle D: vehicle (contaminated water)
Virulence Factors	Antigenic "cloaking"
Culture/Diagnosis	Identification of eggs in feces, scarring of intestines detected by endoscopy
Prevention	Avoiding contaminated vehicles
Treatment	Praziquantel
Distinctive Features	Penetrates skin, lodges in blood vessels of intestine, damages liver
Epidemiological Features	Internationally: 230 million new infections per year by these and the urinary schistosome

Transmission and Epidemiology

The life cycle of the schistosome is of the "D" type, and is very complex (see figure 20.20). The cycle begins when infected humans release eggs into irrigated fields or ponds, either by deliberate fertilization with excreta or by defecating or urinating directly into the water.

The disease is endemic to 74 countries located in Africa, South America, the Middle East, and the Far East. Schistosomiasis (including the urinary tract form) is the second most prominent parasitic disease after malaria, probably affecting 200 million people at any one time worldwide.

20.4 LEARNING OUTCOMES—Assess Your Progress

10. Describe some distinguishing characteristics and commonalities seen in helminthic infections.
11. List four helminths that cause primarily intestinal symptoms, and identify which life cycle they follow and one unique fact about each one.
12. Explain reasons for and the consequences of the following statement, "Cysticercosis is underdiagnosed in the United States."

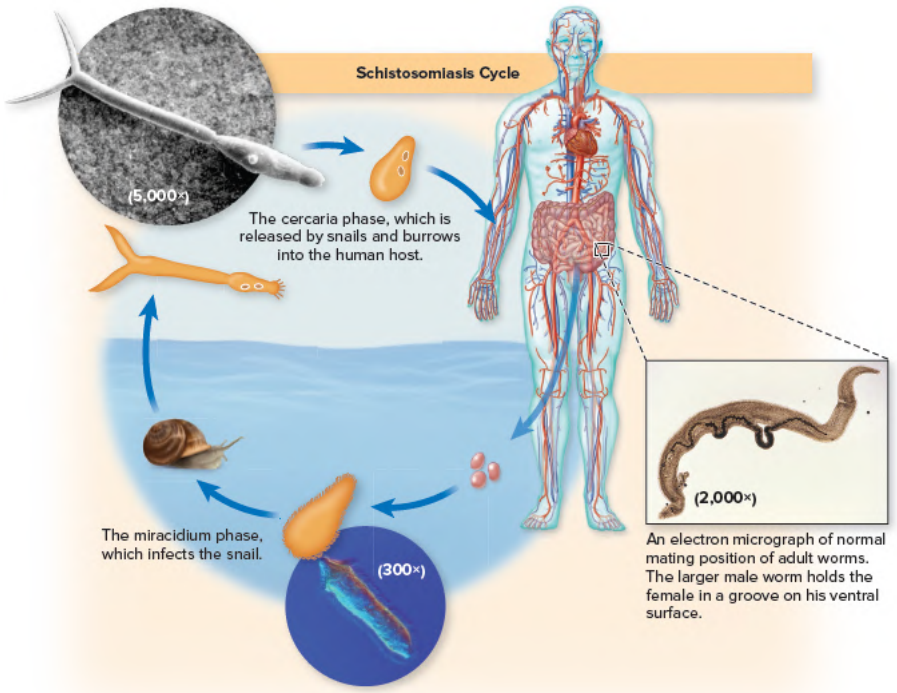


Figure 20.20 **Stages in the life cycle of *Schistosoma*.**
©Dr. Harvey Blankespoor (cercaria); Source: CDC/Dr. Shirley Maddison (mating); ©Sinclair Stammers/Science Source (miracidium)

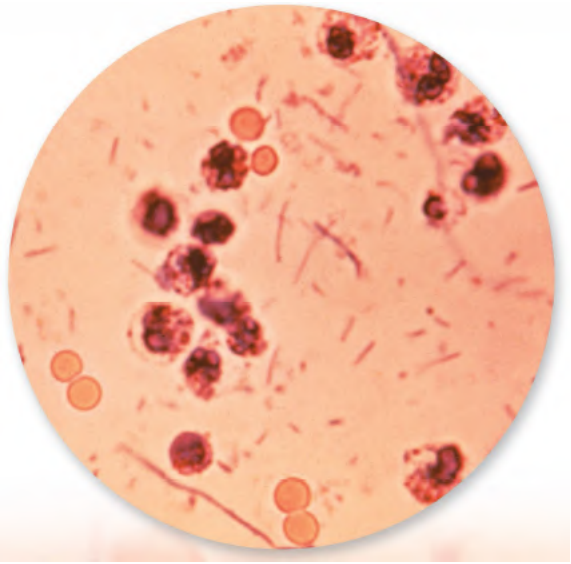
CASE FILE WRAP-UP

©Purestock/SuperStock (health care worker);
Source: CDC (Shigella)

Shigellosis is the disease caused by a group of bacteria called *Shigella*. Symptoms of *Shigella* include fever, abdominal cramps, and diarrhea that may be bloody. Some people with shigellosis are asymptomatic. Illness requiring hospitalization is uncommon—most people recover without treatment within a week. However, one form of *Shigella* (*Shigella dysenteriae* type 1) causes deadly epidemics in developing countries.

Shigellosis is spread through direct contact with someone who is carrying the bacteria. Poor hygiene is often a factor. *Shigella* may also be found in contaminated food and water. Proper food handling can help prevent shigellosis, as can frequent hand washing, especially following a trip to the bathroom. Peter, the patient diagnosed with *Shigella* in the case file at the beginning of this chapter, may have come in contact with

someone who was infected and did not wash his or her hands after a trip to the bathroom; or he may have been exposed to contaminated food, although no one else in his family fell ill.



Source: CDC

Summing Up

Taxonomic Organization Microorganisms Causing Disease in the GI Tract Microorganism

Microorganism	Pronunciation	Disease Table
Gram-positive endospore-forming bacteria		
Clostridium difficile	klos-trid″-ee-um dif ″-i-sil	Acute diarrhea, 20.1
Clostridium perfringens	klos-trid″-ee-um purr-frinj′-unz	Food poisoning, 20.2
Bacillus cereus	buh-sill′-us seer′-ee-uhs	Food poisoning, 20.2
Gram-positive bacteria		
Streptococcus species	strep″-tuh-kok′-us	Dental caries, 20.4
Staphylococcus aureus	staf″-uh-lo-kok′-us are′-ee-us	Food poisoning, 20.2
Gram-negative bacteria		
Campylobacter jejuni	cam″-plo-bac′-ter juh-june′-ee	Acute diarrhea, 20.1
Helicobacter pylori	heel″-i-coe-back′-tur pie-lor′-ee	Gastritis/gastric ulcers, 20.7
Shiga-toxin-producing *Escherichia coli*	esh′-shur-eesh″ ee -uh-col′-eye	Acute diarrhea 20.1
Other *E. coli*		Acute diarrhea, 20.1 Chronic diarrhea, 20.3
Salmonella	sal′-muh-nel″-luh	Acute diarrhea, 20.1
Shigella	shi-gel′-luh	Acute diarrhea, 20.1
Vibrio cholerae	vib′-ree-oh col′-er-ee	Acute diarrhea, 20.1

Microorganism	Pronunciation	Disease Table
Non-cholera *Vibrio*	vib′-ree′-oh	Acute diarrhea, 20.1
Tannerella forsythia, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema vincentii, Prevotella intermedia, Fusobacterium	tan-er-rel′-ah for-sye′-thee-ah, ag-gruh-ga″-ti-bac′-tur ack-tin′-oh-my-see″-tem-cah′-mi-tans, porfuhr′-oh-moan″-as jin-ji-vall′-is, trep′-oh-nee-ma vin-cen′-tee-eye, prev′-oh-tell-ah in-ter-meed′-ee-ah, few′-zo-bac-teer′-ee′-um	Periodontal disease, 20.5
DNA viruses		
Hepatitis B virus	hep-uh-tie′-tis B vie′-russ	Hepatitis, 20.8
RNA viruses		
Hepatitis A virus	hep-uh-tie′-tis A vie′-russ	Hepatitis, 20.8
Hepatitis C virus	hep-uh-tie′-tis C vie′-russ	Hepatitis, 20.8
Hepatitis E virus	hep-uh-tie′-tis E vie′-russ	Hepatitis, 20.8
Mumps virus	mumps vie′-russ	Mumps, 20.6
Rotavirus	ro′-ta-vie′-russ	Acute diarrhea, 20.1
Norovirus	no″-row-vie′-russ	Acute diarrhea, 20.1
Protozoa		
Entamoeba histolytica	en″-tah-mee′-ba his″-toh-lit′-ih-kuh	Chronic diarrhea, 20.3
Cryptosporidium	crip′-toe-spor-id″-ee-um	Acute diarrhea, 20.1
Cyclospora	Sie″-clo-spor′-ah	Chronic diarrhea, 20.3
Giardia lamblia	jee-ar′-dee-ah lam′-blee-ah	Chronic diarrhea, 20.3
Helminths—nematodes		
Ascaris lumbricoides	a-scare′-is lum′-bri-coi″-dees	Intestinal distress plus migratory symptoms, 20.10
Enterobius vermicularis	en′-ter-oh″-bee-us ver-mick″-u-lar′-is	Intestinal distress, 20.9
Trichuris trichiura	tri-cur′-is trick-ee-ur′-ah	Intestinal distress, 20.9
Necator americanus and *Ancylostoma duodenale*	neh-cay′-ter a-mer′-i-can″-us, an′-sy-lo-sto″-mah dew-ah′-den-al″-ee	Intestinal distress plus migratory symptoms, 20.10
Toxocara species	tox′-uh-kair″-uh	Intestinal distress plus migratory symptoms, 20.10
Helminths—cestodes		
Hymenolepis	hie′-men-oh″-lep-is	Intestinal distress, 20.9
Taenia solium	te′-ne-ah so′-lee-um	Cysticercosis, 20.11
Diphyllobothrium latum	dif′-oh-lo-both″-ree-um lah′-tum	Intestinal distress, 20.9
Helminths—trematodes		
Schistosoma mansoni and *S. japonicum*	shis′-toh-so-my″-a-sis man-sohn″-ee, ja-pawn′-i-cum	Helminthic liver disease, 20.12

Crohn's Disease and the Gut Microbiome

Throughout this book, you have read about the importance of the gut microbiome and its possible influence on mood, body weight, autoimmune diseases, and many other things. Let us examine for a moment the role the gut microbiome might play in a mostly gut-focused disease: Crohn's disease.

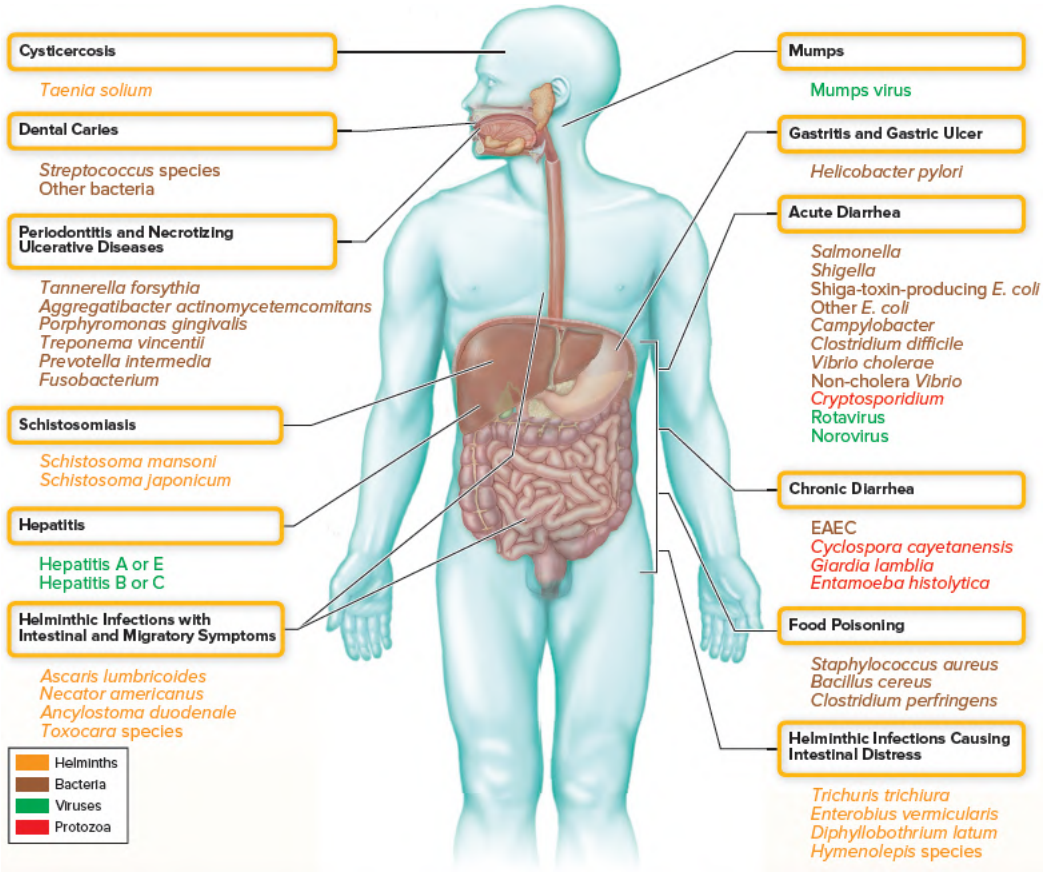
Physicians consider Crohn's to be idiopathic, meaning they are not sure what causes it. Genetic analysis of Crohn's sufferers' DNA compared to that of healthy subjects shows some difference in some Crohn's patients, but not all. It would not be unreasonable to expect the microbiome to have some influence on gut health.

The important question is, if there *is* a difference in microbiota between Crohn's patients and healthy people, is that what causes the Crohn's? Or does Crohn's pathology lead to a different microbiome?

Many studies have been conducted in an effort to characterize the microbiome in these two circumstances (Crohn's and health). So far, what has been found is that there are fewer different types of microbes in Crohn's patients than in healthy guts. This decrease in diversity is seen in many types of deviations from health, but in very few of them has it been found to *precede* the condition. So the question remains. And all we can say is that there is an *association*—a term that does not imply which causes which.

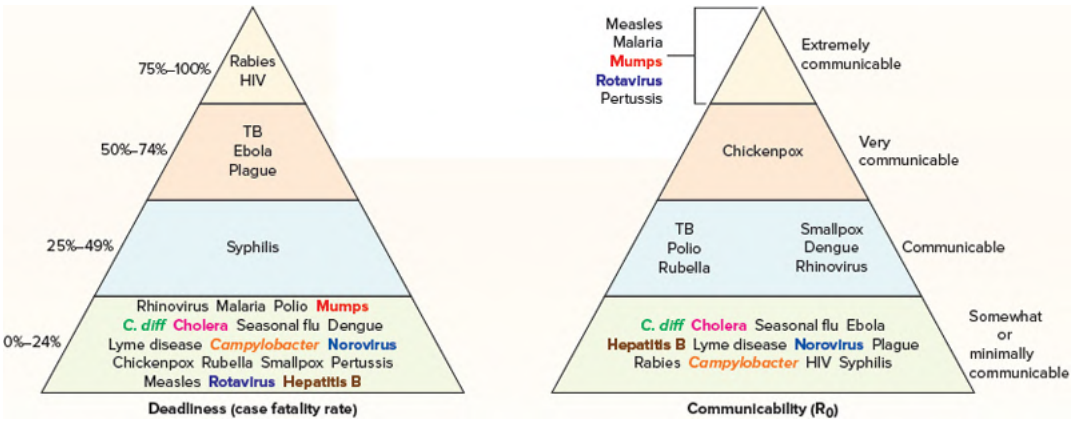
©Roger Harris/Science Source

Infectious Diseases Affecting
The Gastrointestinal Tract



System Summary Figure 20.21

Deadliness and Communicability of Selected Diseases of the Gastrointestinal Tract



Chapter Summary

20.1 The Gastrointestinal Tract and Its Defenses

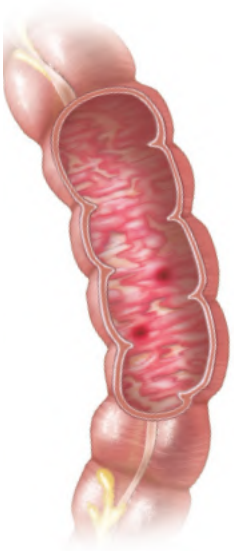
- The GI tract has a very heavy load of microorganisms, and it encounters millions of new ones every day. There are significant mechanical, chemical, and antimicrobial defenses to combat microbial invasion.

20.2 Normal Biota of the Gastrointestinal Tract

- Bacteria abound in all of the eight main sections of the gastrointestinal tract. Even the highly acidic stomach is colonized.
- The composition of the gut microbiome has a huge influence on many aspects of human health.

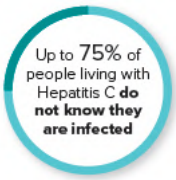
20.3 Gastrointestinal Tract Diseases Caused by Microorganisms (Nonhelminthic)

- **Acute Diarrhea:** In the United States, a third of all acute diarrhea is transmitted by contaminated food.
  - *Salmonella:* The *Salmonella* species that causes gastrointestinal disease is divided into many serotypes, based on major surface antigens.
  - *Shigella:* Frequent, watery, bloody stools, fever, and often intense abdominal pain. The bacterium *Shigella dysenteriae* produces a heat-labile exotoxin called Shiga toxin.
  - Shiga-Toxin-Producing *E. coli* (STEC): This group contains *E. coli* O157:H7 and other virulent strains that cause hemolytic uremic syndrome and other debilitating effects.
  - Other *E. coli:* Enterotoxigenic *E. coli* (traveler's diarrhea), enteroinvasive *E. coli*, enteropathogenic *E. coli*, diffusely adherent *E. coli*, and enteroaggregative *E. coli* also cause diarrhea.
  - *Campylobacter:* Frequent, watery stools, fever, vomiting, headaches, and severe abdominal pain. Infrequently, infection can lead to serious neuromuscular paralysis called *Guillain-Barré syndrome*.
  - *Clostridium difficile:* Pseudomembranous colitis. Known as "C. diff," a serious problem in hospitals and other health care facilities.
  - *Vibrio cholerae:* Symptoms of secretory diarrhea and severe fluid loss can lead to death in less than 48 hours.
  - *Cryptosporidium:* Intestinal waterborne protozoan that infects mammals, birds, and reptiles.
  - Rotavirus: Primary viral cause of morbidity and mortality resulting from diarrhea, accounting for 50% of all cases.
  - Norovirus: Second most common cause of U.S. hospitalizations from foodborne diseases.



- **Food Poisoning:** Refers to symptoms in the gut that are caused by a preformed toxin. Caused most often by exotoxins from *Staphylococcus aureus, Bacillus cereus*, and *Clostridium perfringens*.
- **Chronic Diarrhea:** Caused most often by enteroaggregative *E. coli* (EAEC), or the protozoa *Cyclospora cayetanensis* or *Entamoeba histolytica*.
- **Tooth and Gum Infections**
  - Dental Caries: A polymicrobial infection resulting in acid production and dissolution of tooth enamel.

- Periodontal Diseases: The anaerobic bacteria *Tannerella forsythia, Aggregatibacter actinomycetemcomitans, Porphyromonas, Fusobacterium*, and spirochete species are part of the polymicrobial anaerobic consortium leading to this condition.
- **Mumps:** Caused by an enveloped, single-stranded RNA virus (mumps virus) from the genus *Paramyxovirus*.
- **Gastritis and Gastric Ulcers:** *Helicobacter pylori*, a curved gram-negative rod, is the causative agent.
- **Hepatitis:** Inflammatory disease marked by damage to hepatocytes and a mononuclear response that swells and disrupts the liver, causing **jaundice**. Can be caused by a variety of different viruses.



- Hepatitis A virus (HAV): A nonenveloped, single-stranded RNA enterovirus of low virulence. Spread through fecal-oral route. Inactivated vaccine available.
- Hepatitis B virus (HBV): Enveloped DNA virus in the family *Hepadnaviridae*. Can be very serious, even life-threatening; some patients develop chronic liver disease in the form of necrosis, cirrhosis, or liver cancer. Transmitted by blood and other bodily fluids. Virus is major infectious concern for health care workers.
- *Hepatitis C Virus:* RNA virus in *Flaviviridae* family. Shares characteristics of hepatitis B disease but is much more likely to become chronic. More commonly transmitted through blood contact than through other body fluids.

20.4 Gastrointestinal Tract Diseases Caused by Helminths

- **Helminthic Infections: Intestinal Distress as the Primary Symptom**
  - *Enterobius vermicularis:* "Pinworm"; most common worm disease of children in temperate zones. Not fatal, and most cases are asymptomatic.
  - Other common helminthic causes of intestinal distress are *Trichuris trichiura, Diphyllobothrium latum*, and *Hymenolepis* species.
- **Helminthic Infections: Intestinal Distress Accompanied by Migratory Symptoms:** *Toxocara* **is categorized as a "neglected parasitic infection" by the CDC**. *Ascaris lumbricoides* **can obstruct the GI tract or leave the GI tract and migrate throughout the body. Two worms known as hookworms**, *Necator americanus* **and** *Anclyostoma duodenale*, **penetrate intact skin and travel through the bloodstream to the lungs and then to the GI tract**.
- **Cysticercosis:** Larval tapeworm cysts embedded in brain and other tissues, often causing seizures. Also a "neglected parasitic infection."
- **Schistosomiasis: Liver Disease:** *Schistosomiasis* in intestines is caused by blood flukes *Schistosoma mansoni* and *S. japonicum*. Symptoms include fever, chills, diarrhea, and liver and spleen disease.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. Which of the following causes of acute diarrhea is of the most concern to the CDC due to its antibiotic resistance characteristics? **a**. *Salmonella* *Clostridium difficile* *Cryptosporidium* *Shigella* **d**.	**2**. Healthy teeth are important for good nutrition and overall human health. Yet despite the fact that humans and their oral microbiome have coevolved over thousands of years, there was a vast increase in the incidence of human dental caries with the advent of Western civilization. What factor(s) are likely responsible for that?	**3**. Considering that *Helicobacter* has probably been colonizing human stomachs for thousands of years, would you suspect that its primary role is as a pathogen or as normal biota? Support your answer.
Cell Structure and Function	**4**. This endospore-forming bacterium contaminates meat and vegetables, and in other situations causes gas gangrene. **a**. *Bacillus cereus* **b**. *Clostridium perfringens* **c**. *Shigella* **d**. *Staphylococcus aureus*	**5**. Why is heating food contaminated with *Staphylococcus aureus* no guarantee that potential food poisoning will be prevented?	**6**. Several pathogens in this chapter have the ability to form endospores or cysts. Create an argument, using what you know about transmission mechanisms, for why these organisms are particularly suited for GI tract disease.
Metabolic Pathways	**7**. Which of the following bacteria produces an enzyme that breaks down urea? **a**. *Helicobacter pylori* **b**. *Salmonella* **c**. *Shigella* **d**. Shiga-toxin-producing *E. coli*	**8**. You probably think of the oral cavity as an aerobic environment. Considering the anatomy of the teeth and gums, where would you expect the anaerobes that colonize the mouth to survive?	**9**. *Vibrio cholerae* can survive in a wide range of aquatic conditions, from brackish water to seawater, and in habitats that have scarce nutrients. It readily forms biofilms on submerged surfaces, including other organisms such as phytoplankton. How would living in a biofilm protect *Vibrio* in a low-nutrient environment?

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Information Flow and Genetics	**10**. Which of these organisms has an unusual double-stranded RNA genome? **a**. *norovirus* **b**. *Schistosoma* **c**. *hepatitis C* **d**. *rotavirus*	**11**. Why is it thought that the Shiga toxin in Shiga-toxin-producing *E. coli* originated in *Shigella*, and not the other way around? Also, explain how this characteristic was transferred between the two species.	**12**. Conduct research on how the hepatitis B vaccine is manufactured, and write a paragraph responding to a patient who is unhappy about receiving a recombinant vaccine.
Microbial Systems	**13**. The normal biota of the GI tract is most diverse in the **a**. *pharynx*. **b**. *stomach*. **c**. *small intestine*. **d**. *large intestine*.	**14**. The normal biota of the GI tract seems to include a lot of disease-causing organisms. How can that be? That is, if they are "normal," why are they also potential pathogens?	**15**. The *Salmonella* strain causing typhoid fever is transmitted human-to-human. The *Salmonella* strains causing gastroenteritis generally are transmitted from animals. Sketch a scenario for why gastroenteritis is now the more common outcome of *Salmonella* infection, and not typhoid fever.

Impact of Microorganisms	**16**. Which of these microorganisms is associated with Guillain-Barré syndrome? **a**. *E. coli* **b**. *Salmonella* **c**. *Campylobacter* **d**. *Shigella*	**17**. Describe the populations that are most at risk for hepatitis C, and why.	**18**. Use what you know about inflammation to explain how periodontal organisms might be responsible for diseases elsewhere in the body, such as the heart.
Scientific Thinking	**19**. You are examining a patient who is a recent immigrant from Southeast Asia. She is complaining of diarrhea and vomiting. Which of these would you add to your differential	**20**. An outbreak of cholera occurred in Haiti following a devastating earthquake in 2010. It is still ongoing. Based on your knowledge of the bacterium involved, discuss the factors that probably favored this outbreak.	**21**. Consider the function of the liver. Then speculate on why so many different types of viruses can cause pathology there.

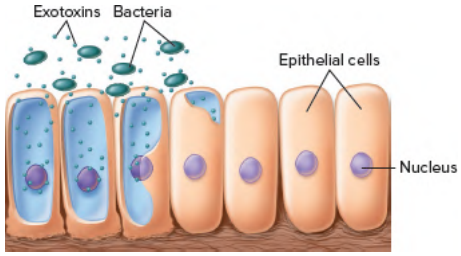
	diagnosis in light of her history? **a**. *Vibrio cholerae* **b**. *Salmonella* **c**. *Shigella* **d**. *Campylobacter*		

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From chapter 11, figure 11.2*a***. Imagine for a minute that the organism in this illustration is a Shiga-toxin-producing *E. coli*. What would be one reason not to treat a patient having this infection with powerful antibiotics?

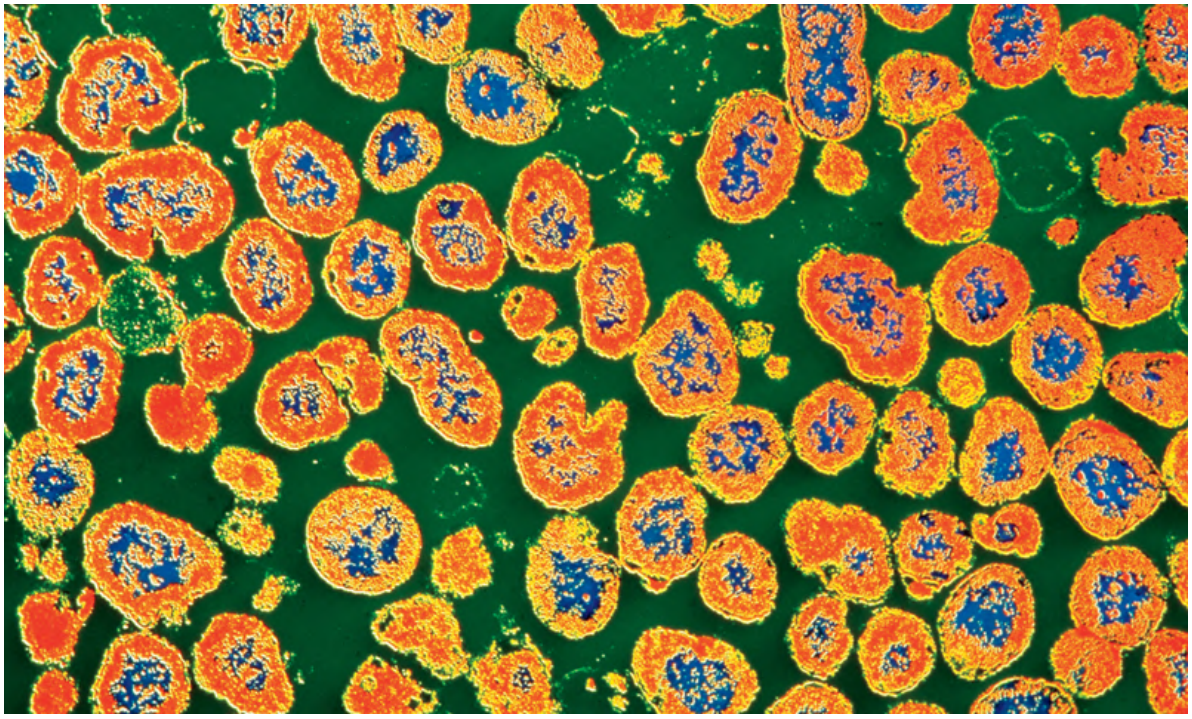


Resources are available through Connect, including interactive learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

21

Infectious Diseases Affecting the Genitourinary System

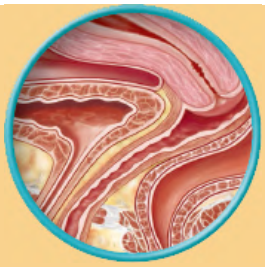


©Meredun Animal Health Ltd/Science Source (*Neisseria gonorrhoeae*); Source: CDC (*Leptospira*); Source: CDC (*Treponema pallidum*);©Syda Productions/Shutterstock (*health care worker*)

IN THIS CHAPTER…

21.1 The Genitourinary Tract and Its Defenses

1. Draw or describe the anatomical features of the genitourinary tracts of both sexes.
2. List the natural defenses present in the genitourinary tracts.



21.2 Normal Biota of the Genitourinary Tract

3. List the types of normal biota presently known to occupy the genitourinary tracts of both sexes.

21.3 Urinary Tract Diseases Caused by Microorganisms

4. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Disease," urinary tract infection.
5. Discuss important features of leptospirosis.

21.4 Reproductive Tract Diseases Caused by Microorganisms

6. List the possible causative agents, modes of transmission, virulence factors, and prevention/treatment for the "Highlight Diseases," gonorrhea and *Chlamydia* infection.
7. Distinguish between vaginitis and vaginosis.
8. Discuss prostatitis.
9. Name three diseases that result in genital ulcers, and discuss their important features.
10. Differentiate between the two diseases causing warts in the reproductive tract.
11. List at least three reasons the HPV vaccine is so strongly recommended for young adolescents.
12. Identify the most important risk group for group B *Streptococcus* infection and explain why that group is important.

CASE FILE

It's All in the Walk

One of the most disturbing and gut-wrenching cases I was ever involved in concerned a 10-year-old girl who was brought to the emergency room by her mother, who was afraid her daughter had appendicitis. What her daughter turned out to have was far more troubling.

The patient had developed a high fever (39.4°C [103°F]) and was complaining of severe lower abdominal pain. Upon questioning, the patient also reported painful urination. As I led her into an examining room, I noticed that she walked taking very small steps. She did not lift her feet off the floor and seemed reluctant or unable to straighten up fully. Her odd gait raised a red flag in my mind.

After performing a quick abdominal examination and obtaining vital signs, I found the physician on call and told her about the patient's symptoms. I also described how the patient moved with an odd shuffling gait. Although I could not recall what I had learned about the symptom, I knew that it was a diagnostic feature. The physician asked me how old the patient was and asked if I had taken the opportunity to ask the patient whether she was sexually active. I had not done so due to the patient's age. To my surprise, the physician asked me to prepare for an internal (vaginal) exam in another room while she examined the patient and spoke to her mother.

- What condition did the physician suspect?
- Is the condition reportable? If so, to whom would you report it?

Case File Wrap-Up appears at the end of the chapter.

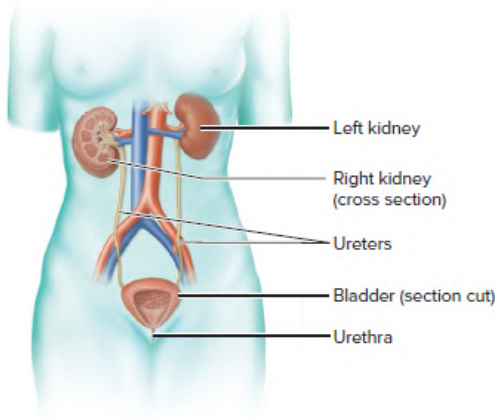


Figure 21.1 **The urinary system**.

21.1 The Genitourinary Tract and Its Defenses

As suggested by the name, the structures considered in this chapter are really two distinct organ systems. The *urinary tract* has the job of removing substances from the blood, regulating certain body processes, and forming urine and transporting it out of the body. The *genital system* has reproduction as its major function. It is also called the *reproductive system*.

The urinary tract includes the kidneys, ureters, bladder, and the urethra **(figure 21.1)**. The kidneys remove metabolic wastes from the blood, acting as a sophisticated filtration system. Ureters are tubular organs extending from each kidney to the bladder. The bladder is a collapsible organ that stores urine and empties it into the urethra, which is the conduit of urine to the exterior of the body. In males, the urethra is also the terminal organ of the reproductive tract, but in females the urethra is separate from the vagina, which is the outermost organ of the reproductive tract.

The most obvious defensive mechanism in the urinary tract is the flushing action of the urine flowing out of the system. The flow of urine also encourages the desquamation (shedding) of the epithelial cells lining the urinary tract. For example, each time a person urinates, he or she loses hundreds of thousands of epithelial cells! Any microorganisms attached to them are also shed, of course. Probably the most common microbial threat to the urinary tract is the group of microorganisms that comprise the normal biota in the gastrointestinal tract, because the two organ systems are in close proximity. But the cells of the epithelial lining of the urinary tract have different chemicals on their surfaces than do those lining the GI tract. For that reason, most (but not all) bacteria that are adapted to adhere to the chemical structures in the GI tract cannot gain a foothold in the urinary tract.

Urine, in addition to being acidic, also contains two antibacterial proteins, lysozyme and lactoferrin. You may recall that lysozyme is an enzyme that breaks down peptidoglycan. Lactoferrin is an iron-binding protein that inhibits bacterial growth. Finally, secretory IgA specific for previously encountered microorganisms can be found in the urine.

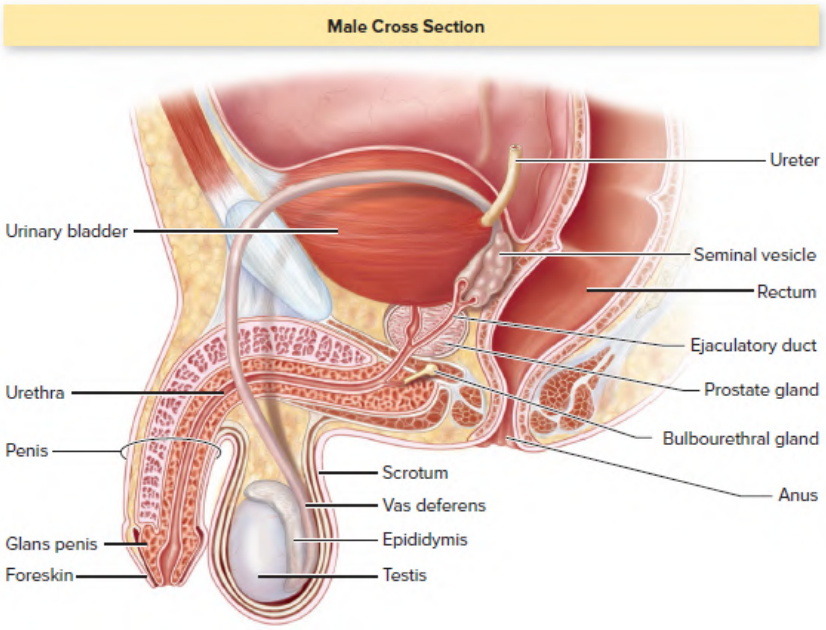


Figure 21.2 **The male reproductive system**.

The male reproductive system produces, maintains, and transports sperm cells and is the source of male sex hormones. It consists of the *testes*, which produce sperm cells and hormones, and the *epididymides*, which are coiled tubes leading out of the testes. Each epididymis terminates in a *vas deferens*, which combines with the seminal vesicle and terminates in the ejaculatory duct **(figure 21.2)**. The contents of the ejaculatory duct empty into the urethra during ejaculation. The *prostate gland* is a walnut-shaped structure at the base of the urethra. It also contributes to the released fluid (semen). The external organs are the scrotum, containing the testes, and the *penis*, a cylindrical organ that houses the urethra. As for its innate defenses, the male reproductive system also benefits from the flushing action of the urine, which helps move microorganisms out of the system.

The female reproductive system consists of the *uterus*, the *fallopian tubes* (also called uterine tubes), *ovaries*, and *vagina* **(figure 21.3)**. One very important tissue of the female reproductive tract is the *cervix*, which is the lower one-third of the uterus and the part that connects to the vagina. The cervix serves as the opening to the uterus. The cervix is a common site of infection in the female reproductive tract.

The natural defenses of the female reproductive tract vary over the lifetime of the woman. The vagina is lined with mucous membranes and, thus, has the protective covering of secreted mucus. During childhood and after menopause, this mucus is the major nonspecific defense of this system. Secretory IgA antibodies specific for any previously encountered infections will also be present on these surfaces. During a woman's reproductive years, a major portion of the defense is provided by changes in the pH of the vagina brought about by the release of estrogen. This hormone stimulates the vaginal mucosa to secrete glycogen, which certain bacteria can ferment into acid, lowering the pH of the vagina to between 4.2 and 5.0. Before puberty, a girl produces little estrogen and little glycogen and has a vaginal pH of about 7. The change in pH beginning in adolescence results in a vastly different normal biota in the vagina, described later. The biota of women in their childbearing years is thought to prevent the establishment and invasion of microbes that might have the potential to harm a developing fetus.

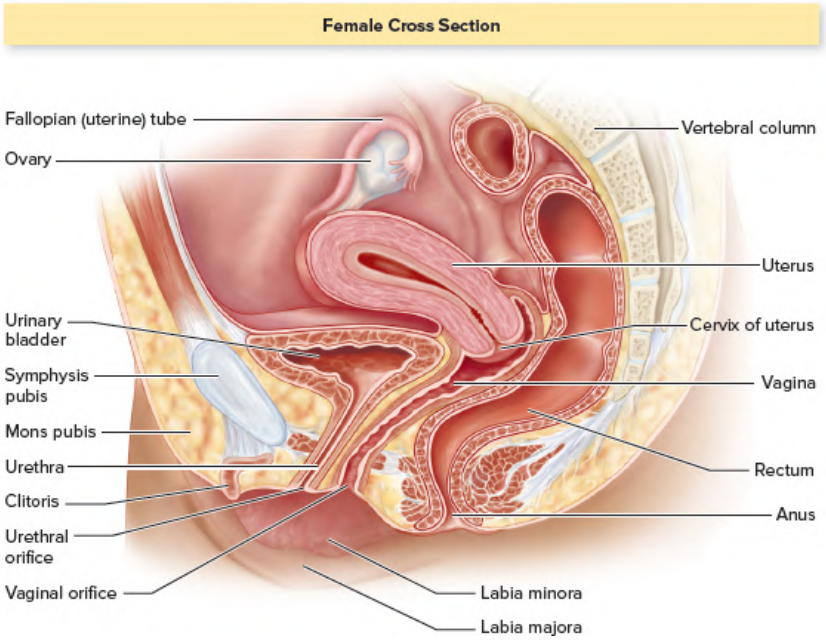


Figure 21.3 **The female reproductive system**.

©Monika Bender/Getty Images

21.1 LEARNING OUTCOMES—Assess Your Progress

1. Draw or describe the anatomical features of the genitourinary tracts of both sexes.
2. List the natural defenses present in the genitourinary tracts.

Normal biota in the female genital tract is different in women of childbearing age than in younger or older women.
©Ariel Skelley/Blend Images

21.2 Normal Biota of the Genitourinary Tract

As with all other organ systems, recent research is showing that the urinary system harbors a more diverse microbiota than we used to think. The lower urethra has a well-established microbiota, while the upper urinary tract appears to have fewer types and lower abundance. The exact microbial composition varies between men and women—and also among individuals. Because the urethra in women is so short (about 3.5 cm long) and is in such close proximity to the anus, it can act as a pipeline for bacteria from the GI tract to the bladder, resulting in urinary tract infections. The outer surface of the penis is colonized by *Pseudomonas* and *Staphylococcus* species—aerobic bacteria. In an uncircumcised penis, the area under the foreskin is colonized by anaerobic gram-negatives.

Normal Biota of the Male Genital Tract

Because the terminal "tube" of the male genital tract is the urethra, the normal biota of the male genital tract (i.e., in the urethra) is comprised of the same residents just described. However, after sexual activity begins, microbes associated with sexually transmitted infections (STIs) can sometimes become long-term residents. Circumcision has a large effect on the "normal" biota of the penis. The uncircumcised penis hosts a larger, more diverse population of microbes, and more anaerobic species.

Normal Biota of the Female Genital Tract

Like other body systems we have studied, the most internal portions, in this case the uterus and above, were long thought to be sterile. Next-generation sequencing has suggested otherwise. We don't know for sure how much and what kind of microbes colonize the upper female reproductive tract, but there are almost certainly either occasional "trespassers" or possibly more permanent residents. We do know that, before puberty and after menopause, the pH of the vagina is close to neutral and the vagina harbors a biota that is similar to that found in the urethra. After the onset of puberty, estrogen production leads to glycogen release in the vagina,

Genitourinary Tract Defenses and Normal Biota

	Defenses	Normal Biota
Urinary Tract (Both Sexes)	Flushing action of urine; specific attachment sites not recognized by most nonnormal biota; shedding of urinary tract epithelial cells, secretory IgA, lysozyme, and lactoferrin in urine	Nonhemolytic *Streptococcus, Staphylococcus, Corynebacterium, Lactobacillus, Prevotella, Veillonella, Gardnerella*
Female Genital Tract (Childhood and Postmenopause)	Mucus secretions, secretory IgA	Same as for urinary tract
Female Genital Tract (Childbearing Years)	Acidic pH, mucus secretions, secretory IgA	Variable, but often *Lactobacillus* predominates; also *Prevotella, Sneathia, Streptococcus*, and *Candida albicans*
Male Genital Tract	Same as for urinary tract	Urethra: same as for urinary tract Outer surface of penis: *Pseudomonas* and *Staphylococcus* Sulcus of uncircumcised penis: anaerobic gram-negatives

resulting in an acidic pH. *Lactobacillus* species thrive in the acidic environment and contribute to it, converting sugars to acid. Their predominance in the vagina, combined with the acidic environment, discourages the growth of many microorganisms. The estrogen-glycogen effect continues, with minor disruptions, throughout the childbearing years until menopause, when the biota gradually returns to a mixed population similar to that of prepuberty. Note that the fungus *Candida albicans* is also present at low levels in the healthy female reproductive tract. When the normal vaginal microbiota is altered and is unable to keep *Candida albicans* in check, an overgrowth of the fungus may occur, resulting in a symptomatic yeast infection.

21.2 LEARNING OUTCOMES—Assess Your Progress

3. List the types of normal biota presently known to occupy the genitourinary tracts of both sexes.

21.3 Urinary Tract Diseases Caused by Microorganisms

We consider two types of diseases in this section. **Urinary tract infections (UTIs)** result from invasion of the urinary system by bacteria or other microorganisms. Leptospirosis, by contrast, is a spirochete-caused disease transmitted by contact of broken skin or mucous membranes with contaminated animal urine.

Highlight Disease

Urinary Tract Infections (UTIs)

Even though the flushing action of urine helps to keep infections to a minimum in the urinary tract, urine itself is a good growth medium for many microorganisms. When urine flow is reduced, or bacteria are accidentally introduced into the bladder, an infection of that organ (known as *cystitis*) can occur. Occasionally, the infection can also affect the kidneys, in which case it is called *pyelonephritis*. If an infection is limited to the urethra, it is called *urethritis*.

▶ Signs and Symptoms

Cystitis is a disease of sudden onset. Symptoms include pain, frequent urges to urinate even when the bladder is empty, and burning pain accompanying urination (called *dysuria*). The urine can be cloudy due to the presence of bacteria and white blood cells. It may have an orange tinge from the presence of red blood cells (*hematuria*). Low-grade fever and nausea are frequently present. If back pain is present and fever is high, it is an indication that the kidneys may also be involved (pyelonephritis). Pyelonephritis is a serious infection that can result in permanent damage to the kidneys if improperly or inadequately treated. If only the bladder is involved, the condition is sometimes called acute uncomplicated UTI.

▶ Causative Agents

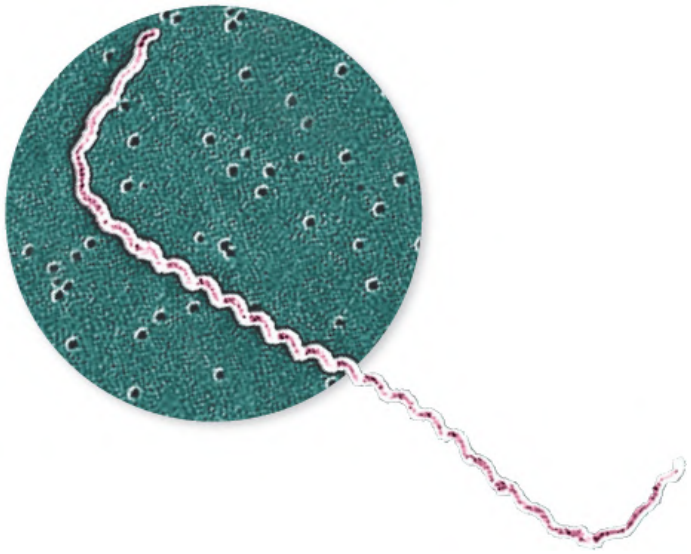
In the same way we saw in the discussion of pneumonia, it is important to distinguish between UTIs that are acquired in health care facilities and those acquired outside of the health care setting. When they occur in health care facilities, they are almost always a result of catheterization and are therefore called *catheter-associated UTIs* (*CA-UTIs*). (Be careful! The abbreviation "CA" in other infections often refers to "community-acquired"—just the opposite of what is meant here! We will spell out "community" in referring to non-healthcare-associated UTIs.)

©Photodisc/Getty Images

NCLEX® PREP

1. In providing discharge information to a client who is being treated for a urinary tract infection with phenazopyridine (Pyridium), which information should the nurse include? Select all that apply.

a. *Increase fluid intake during the course of treatment.*
b. *Urine may turn orange and cause "staining" to clothing.*
c. *Continue the medication for the full 7-day course.*
d. *Take an analgesic to treat burning and pain with urination.*



Leptospira interrogans
Source: CDC

Medical Moment

Cranberry versus UTI

It has long been claimed that cranberries help prevent, or even cure, urinary tract infections (UTIs). Cranberry supplementation is available in the form of fresh fruit, dried fruit, juice, and tablets.

Could the cranberry have a role in preventing a UTI? While results have been promising in animal studies, those in humans have been less conclusive. We do know that cranberry products can prevent the adhesion of uropathogens to the epithelium of the genitourinary tract, at least in the laboratory. However the variation in composition and dosage of cranberry products has posed challenges in extrapolating a lot of the data to humans. In some populations, using certain forms of cranberry, the data for reduction in occurrence of UTIs appear promising. For young and middle-aged women, cranberry may be effective in preventing recurrent UTIs. Data do not support effectiveness in older women, particularly those in nursing homes.

There is no evidence that cranberry products treat an active UTI. Antibiotic therapy is the recommended form of treatment. Additionally, cranberry interacts with many medications so their use should be discussed with a pharmacist or provider.

Q. Finding an effective preventive agent could be seen as a particularly pressing need for this particular infection. Why is that?

Answer in Appendix B.

In 95% of UTIs, the cause is bacteria that are normal biota in the gastrointestinal tract. *Escherichia coli* is by far the most common of these, accounting for approximately 80% of community-acquired urinary tract infections. *Staphylococcus saprophyticus* and other members of the bacterial family that contains *E. coli, Enterobacteriaceae*, especially *Klebsiella pneumoniae*, and *Proteus mirablis* are also common culprits. These last two are only referenced in **Disease Table 21.1** following the discussion of *E. coli*.

The *E. coli* species that cause UTIs are ones that exist as normal biota in the gastrointestinal tract. They are not the ones that cause diarrhea and other digestive tract diseases.

Transmission and Epidemiology

Community-acquired UTIs are nearly always "transmitted" *not* from one person to another but from one organ system to another, namely from the GI tract to the

Disease Table 21.1 Urinary Tract Infections

Causative Organism(s)	*Escherichia coli* B G−	*Staphylococcus saprophyticus* B G+	Other *Enterobacteriaceae* (*Klebsiella pneumoniae, Proteus mirablis*) B G-
Most Common Modes of Transmission	Opportunism: transfer from GI tract (community-acquired) or environment or GI tract (via catheter)		
Virulence Factors	Adhesins, motility	—	—
Culture/Diagnosis	Usually culture-based; antimicrobial susceptibilities always checked		
Prevention	Hygiene practices; in case of CA-UTIs, limit catheter usage		
Treatment	Based on susceptibility testing; members of family *Enterobacteriaceae* in **Urgent Threat** category in CDC Antibiotic Resistance Threat Report	Based on susceptibility testing	Based on susceptibility testing; members of family *Enterobacteriaceae* in **Urgent Threat** category in CDC Antibiotic Resistance Threat Report
Epidemiological Features	Causes 90% of community UTIs and 50%–70% of CA-UTIs	Causes small percentage of community UTIs and even lower percentage of CA-UTIs	Frequent cause of CA-UTIs

urinary system. They are much more common in women than in men because of the nearness of the female urethral opening to the anus (see figure 21.3). Many women experience what have been referred to as "recurrent urinary tract infections," although it is now known that some *E. coli* can invade the deeper tissue of the urinary tract and therefore avoid being destroyed by antibiotics. They can emerge later to cause symptoms again. It is not clear how many "recurrent" infections are actually infections that reactivate in this way.

The National Healthcare Safety Network is now recommending minimizing the use of urinary catheters as much as possible to limit the incidence of these infections.

▶ Treatment

Sulfa drugs such as trimethoprim-sulfamethoxazole are most often used for UTIs of various etiologies. If there is a lot of resistance to this treatment in the local area, other drugs must be used. Often another nonantibiotic drug called phenazopyridine (Pyridium) is administered simultaneously. This drug relieves the very uncomfortable symptoms of burning and urgency. However, some physicians are reluctant to administer this medication for fear that it may mask worsening symptoms. When Pyridium is used, it should be used only for a maximum of 2 days. Pyridium is an azo dye and causes the urine to turn a dark orange to red color. It may also color contact lenses. A large percentage of *E. coli* strains are resistant to penicillin derivatives, so these should be avoided. Importantly, some representatives of the family *Enterobacteriaceae* (three of which are considered here) have become resistant to carbapenem, and are designated "CRE" for carbapenem-resistant *Enterobacteriaceae*. These are in the Urgent Threat category for antibiotic resistance. They usually remain susceptible to very few antibiotics, and are most common in health care facilities. **Disease Table 21.1**.

Leptospirosis

This infection is a zoonosis associated with wild animals and domesticated animals. It can affect the kidneys, liver, brain, and eyes. It is considered in this section because it can have its major effects on

the kidneys and because its presence in animal urinary tracts causes it to be shed into the environment through animal urine.

Signs and Symptoms

Leptospirosis has two phases. During the early, or leptospiremic, phase, the pathogen appears in the blood and cerebrospinal fluid. Symptoms are sudden high fever, chills, headache, muscle aches, conjunctivitis, and vomiting. During the second phase, the blood infection is cleared by natural defenses. This period is marked by milder fever; headache due to leptospiral meningitis; and, in rare cases, *Weil's syndrome*, a cluster of symptoms characterized by kidney invasion, hepatic disease, jaundice, anemia, and neurological disturbances. Long-term disability and even death can result from damage to the kidneys and liver, but they occur primarily with the most virulent strains and in elderly persons.

Causative Agent

Leptospires are typical spirochete bacteria marked by tight, regular, individual coils with a bend or hook at one or both ends. *Leptospira interrogans* (lep″-toh-spy′-rah in-terr′-oh-ganz) is the species that causes leptospirosis in humans and animals. There are nearly 200 different serotypes of this species distributed among various animal groups, which accounts for extreme variations in the disease manifestations in humans.

Transmission and Epidemiology

Infection occurs almost entirely through contact of skin abrasions or mucous membranes with animal urine or some environmental source containing urine. In 1998, dozens of athletes competing in the swimming phase of a triathlon in Illinois contracted leptospirosis from the water. It is a common pathogen in areas of Latin America and Asia.

©Corbis

Treatment

Early treatment with doxycycline, penicillin G, or ceftriazone rapidly reduces symptoms and shortens the course of disease, but delayed therapy is less effective. Other spirochete diseases, such as syphilis (described later), exhibit this same pattern of being susceptible to antibiotics early in the infection but less so later on.

21.3 LEARNING OUTCOMES—Assess Your Progress

4. List the possible causative agents, modes of transmission, virulence factors, diagnostic techniques, and prevention/treatment for the "Highlight Disease," urinary tract infection.
5. Discuss important features of leptospirosis.

Disease Table 21.2 Leptospirosis

Causative Organism(s)	*Leptospira interrogans* B **G-**
Most Common Modes of Transmission	Vehicle: contaminated soil or water
Virulence Factors	Adhesins, invasion proteins

Culture/Diagnosis	In United States, CDC will culture specimens
Prevention	Avoiding contaminated vehicles
Treatment	Doxycycline, Penicillin G, or ceftriaxone
Epidemiological Features	United States: 100–200 cases per year, half in Hawaii; internationally: 80% of people in tropical areas are seropositive

21.4 Reproductive Tract Diseases Caused by Microorganisms

Not all reproductive tract diseases are sexually transmitted, though many are. Vaginitis/vaginosis may or may not be; prostatitis probably is not.

It was very difficult to choose a "highlight" disease for this section, though we were sure we wanted it to be a sexually transmitted disease (now often called *sexually transmitted infections*, due to the fact that so many of them are silent, while still being serious). The WHO reports that there are 1 million new sexually transmitted infections *every day* worldwide. In the United States, the CDC released a report in 2015 showing that chlamydia, gonorrhea, and syphilis were at their highest rates ever, after decades of progress. The CDC blames these increases on budget cuts to state and local STD clinics. In a recent year, 20 STD clinics closed altogether. They point out that this harms lower-income individuals disproportionately. Syphilis in particular causes devastating effects, including miscarriages, stillbirths, blindness, and strokes. At one point, syphilis was close to being eliminated in the United States; now it is increasing at an alarming rate, showing nearly a 20% increase between 2014 and 2015. The discharge diseases are responsible for unprecedented numbers of infertility cases. Herpes infections are incurable and therefore simply increase in their prevalence over time. Americans have become more familiar with the dangers of HPV infection since the introduction of the HPV vaccine, so, in a split decision, we have decided to highlight the dangers of curable—but usually undetected—discharge diseases.

Highlight Disease

Discharge Diseases with Major Manifestation in the Genitourinary Tract

Discharge diseases are those in which the infectious agent causes an increase in fluid discharge in the male and female reproductive tracts. Examples are trichomoniasis, gonorrhea, and *Chlamydia* infection. The causative agents are transferred to new hosts when the fluids in which they live contact the mucosal surfaces of the receiving partner.

Gonorrhea

Gonorrhea has been known as a sexually transmitted disease since ancient times. It was named by the Greek physician Claudius Galen, who thought that it was caused by an excess flow of semen.

A Note About STI Statistics

It is difficult to compare the incidence of different STIs to one another, for several reasons. The first is that many, many infections are "silent," and therefore infected people don't access the health care system and don't get counted. Of course, we know that many silent infections are actually causing damage that won't be noticed for years—and when it is, the original causative organism is almost never sought out. The second reason is that only some STIs are officially reportable to the CDC, and states' regulations vary. *Chlamydia* infection and gonorrhea are nationally reportable, for example, but herpes and HPV infections are not. In each section, we present estimates of the prevalence and/or incidence of the diseases wherever possible.

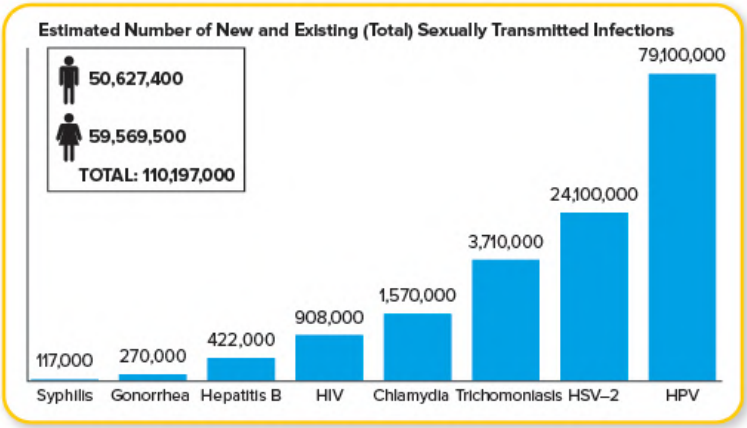
Signs and Symptoms

In the male, infection of the urethra elicits urethritis, painful urination, and a yellowish discharge, although a relatively large number of cases are asymptomatic. In most cases, infection is limited to the distal urogenital tract, but it can occasionally spread from the urethra to the prostate gland and epididymis (refer to figure 21.2). Scar tissue formed in the spermatic ducts during healing of an invasive infection can render a man infertile.

In the female, it is likely that both the urinary and genital tracts will be infected during sexual intercourse. A mucopurulent (containing mucus and pus) or bloody vaginal discharge occurs in a minority of the cases, along with painful urination if the urethra is affected. Major complications occur when the infection ascends from the vagina and cervix to higher reproductive structures such as the uterus and fallopian tubes **(figure 21.4)**. One disease resulting from this progression is **salpingitis** (sal″-pin-jy′-tis). This inflammation of the fallopian tubes may be isolated, or it may also include inflammation of other parts of the upper reproductive tract, termed *pelvic inflammatory disease (PID)*. It is not unusual for the microbe that initiates PID to become involved in mixed infections with anaerobic bacteria. The buildup of scar tissue from PID can block the fallopian tubes, causing sterility or ectopic pregnancies.

Serious consequences of gonorrhea can occur outside of the reproductive tract. In a small number of cases, the gonococcus enters the bloodstream and is disseminated to the joints and skin. Involvement of the wrist and ankle can lead to chronic arthritis and a painful, sporadic, papular rash on the limbs. Rare complications of gonococcal bacteremia are meningitis and endocarditis.

Children born to gonococcus carriers are also in danger of being infected as they pass through the birth canal. Because of the potential harm to the fetus, physicians usually screen pregnant mothers for its presence. Gonococcal eye infections are very serious and often result in keratitis, ophthalmia neonatorum, and even blindness **(figure 21.5)**. A universal precaution to prevent such complications is the use of antibiotic eyedrops or ointments (usually erythromycin) for newborn babies. The pathogen may also infect the pharynx and respiratory tract of neonates. Finding gonorrhea in children other than neonates is



Note: These numbers are from 2008 and should only be used to compare relative rates of infection.
Sex totals do not equal overall total, due to rounding.
Bars are for illustration only; not to scale, due to wide range in numbers of infections.
Source: CDC

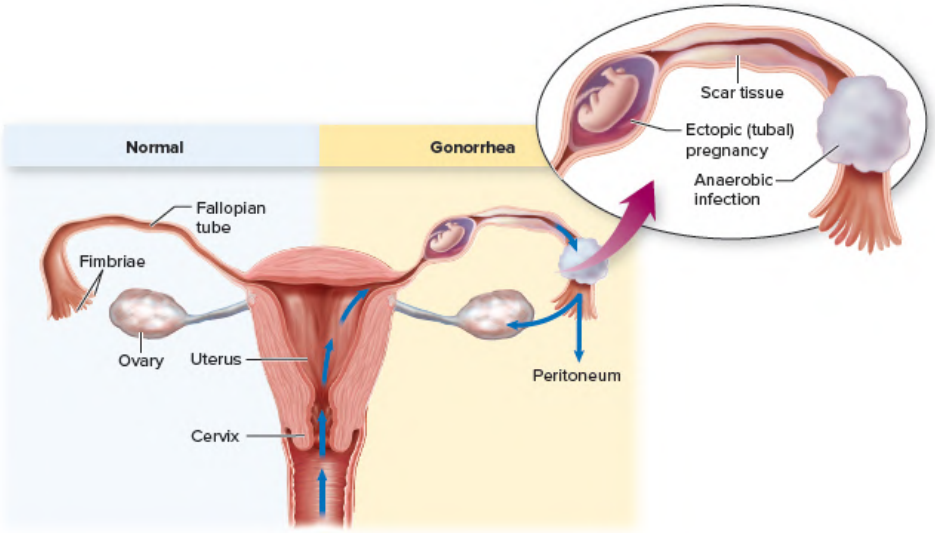


Figure 21.4 **Invasive gonorrhea in women**. *(Left)* Normal state. *(Right)* In ascending gonorrhea, the gonococcus is carried from the cervical opening up through the uterus and into the fallopian tubes. Pelvic inflammatory disease (PID) is a serious complication that can lead to scarring in the fallopian tubes, ectopic pregnancies, and mixed anaerobic infections.

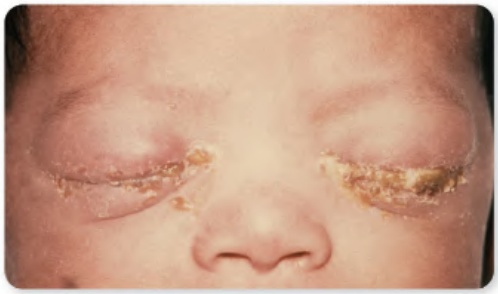


Figure 21.5 **Gonococcal ophthalmia neonatorum in a week-old infant**. The infection is marked by intense inflammation and edema; if allowed to progress, it causes damage that can lead to blindness. Fortunately, this infection is completely preventable and treatable.
Source: CDC/J. Pledger

strong evidence of sexual abuse by infected adults, and it calls for child welfare consultation along with thorough bacteriologic analysis.

Causative Agent

N. gonorrhoeae is a pyogenic (pus-forming) gram-negative diplococcus. It appears as pairs of kidney bean–shaped bacteria, with their flat sides touching **(figure 21.6)**.

Pathogenesis and Virulence Factors

Successful attachment is key to the organism's ability to cause disease. Gonococci use specific chemical groups on the tips of fimbriae to anchor themselves to mucosal epithelial cells. Once the bacterium attaches, it invades the cells and multiplies within the basement membrane.

The fimbriae may also play a role in slowing down effective immunity. The fimbrial proteins are controlled by genes that can be turned on or off, depending on the bacterium's situation. This

phenotypic change is called phase variation. In addition, the genes can rearrange themselves to put together fimbriae of different configurations. This antigenic variation confuses the body's immune system. Antibodies that previously recognized fimbrial proteins may not recognize them once they are rearranged.

The gonococcus also possesses an enzyme called IgA protease, which can cleave IgA molecules stationed for protection on mucosal surfaces. In addition, pieces of its outer membrane are shed during growth. These "blebs," containing endotoxin, probably play a role in pathogenesis because they can stimulate portions of the nonspecific defense response, resulting in localized damage.

Transmission and Epidemiology

Except for neonatal infections, the gonococcus is spread through some form of sexual contact.

Gonorrhea is a strictly human infection that occurs worldwide and ranks among the most common sexually transmitted diseases. Infections were declining for years after 2006, and were at historic lows, but have been climbing again, with approximately 400,000 new infections in 2015.

It is important to consider the reservoir of asymptomatic males and females when discussing the transmission of the infection. Because approximately 10% of infected males and 50% of infected females experience no symptoms, it is often spread unknowingly.

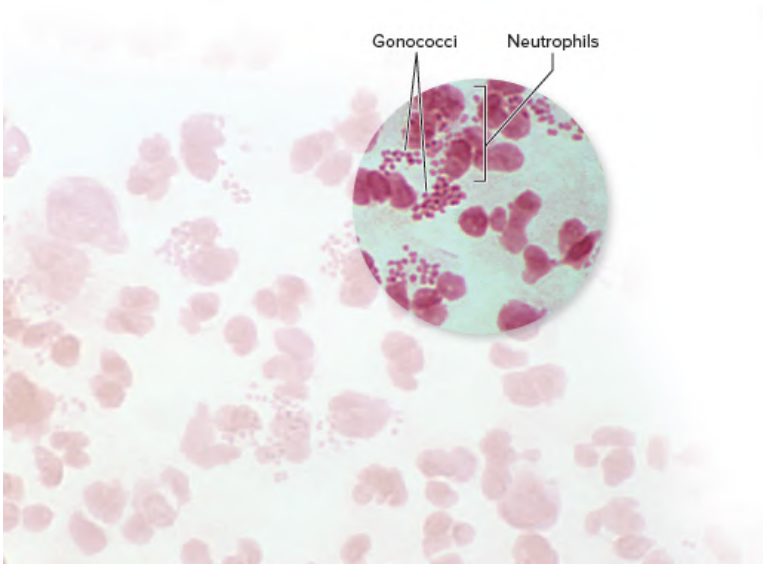


Figure 21.6 **Gram stain of urethral pus from a male patient with gonorrhea (1,000×)**. Note the intracellular (phagocytosed) gram-negative diplococci in polymorphonuclear leukocytes (neutrophils).
Source: CDC/Dr. Norman Jacobs

Culture and Diagnosis

The best method for diagnosis is a PCR test of secretions. A Gram stain of male secretions usually will yield visible gonococci inside polymorphonuclear cells, but this procedure is not considered sensitive enough to rule out infection if no bacteria are found (see figure 21.6). In females, other methods, such as ELISA or PCR tests, are called for. Alternatively, the bacterium can be cultured on Thayer-Martin agar, a rich chocolate agar base with added antibiotics that inhibit competing bacteria.

N. gonorrhoeae grows best in an atmosphere containing increased CO_2. Because *Neisseria* is so fragile, it is best to inoculate it onto media directly from the patient rather than using a transport tube. Gonococci produce catalase, enzymes for fermenting various carbohydrates, and the enzyme cytochrome oxidase, which can be used for identification as well. Gonorrhea is a reportable disease.

Prevention

No vaccine is yet available for gonorrhea. Using condoms is an effective way to avoid transmission of this and other discharge diseases.

Treatment

Because those infected with *N. gonorrhoeae* are frequently coinfected with *Chlamydia*, treatment recommendations include treating for that bacterium as well,

unless its presence has been ruled out. The CDC runs a program called the Gonococcal Isolate Surveillance Project (GISP) to monitor the growing prevalence of antibiotic-resistant strains of *N. gonorrhoeae*. Every month in 28 local STD clinics around the country, *N. gonorrhoeae* isolates from the first 25 males diagnosed with the infection are sent to regional testing labs, their antibiotic sensitivities are determined, and the data are provided to the GISP program at the CDC. The combination of increasing infection rates and increasing antibiotic resistance rates is troubling. The CDC has this bacterium in its Urgent Threat category for antibiotic resistance because it has developed resistance to nearly every drug that could be used for treatment.

Chlamydia

Genital *Chlamydia* infection is the most common reportable infectious disease in the United States. Annually, more than 1 million cases are reported, but the actual infection rate may be five to seven times that number. The overall prevalence among sexually active young women ages 14 to 19 is 6.8% according to the CDC. **Figure 21.7** demonstrates how the infection rate has been increasing steadily. The vast majority of cases are asymptomatic. When we consider the serious consequences that may follow *Chlamydia* infection, those facts are very disturbing.

©McGraw-Hill Education/Christopher Kerrigan, photographer

Signs and Symptoms

In males who experience symptoms of *Chlamydia* infection, the bacterium causes an inflammation of the urethra. The symptoms mimic gonorrhea—namely, discharge and painful urination. Untreated infections may lead to epididymitis. Females who experience symptoms have cervicitis, a discharge, and often salpingitis. Pelvic inflammatory disease is a frequent sequela of female chlamydial infection. A woman is even more likely to experience PID as a result of a *Chlamydia* infection than as a result of gonorrhea. Up to 75% of *Chlamydia* infections are asymptomatic, which puts women at risk for developing PID because they don't seek treatment for initial infections. The PID itself may be acute and painful, or it may be relatively asymptomatic, allowing damage to the upper reproductive tract to continue unchecked.

Certain strains of *C. trachomatis* can invade the lymphatic tissues, resulting in another condition called lymphogranuloma venereum. This condition is accompanied by headache, fever, and muscle aches. The lymph nodes near the lesion begin to fill with granuloma cells and become enlarged and tender. These "nodes" can cause long-term lymphatic obstruction that leads to chronic, deforming edema of the genitalia or anus. The disease is endemic to South America, Africa, and Asia, but occasionally occurs in other parts of the world. Its incidence in the United States is about 500 cases per year.

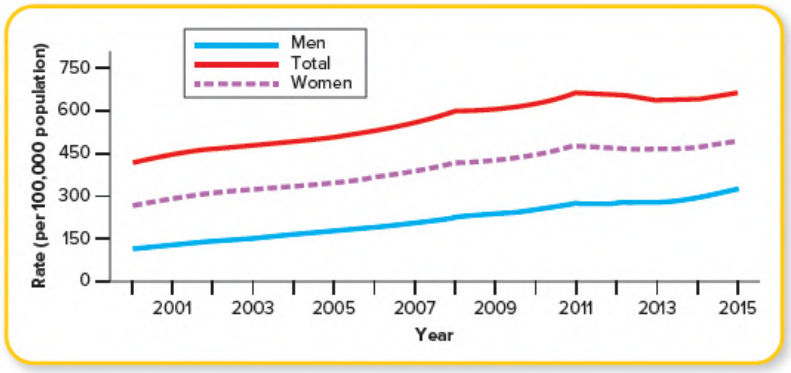
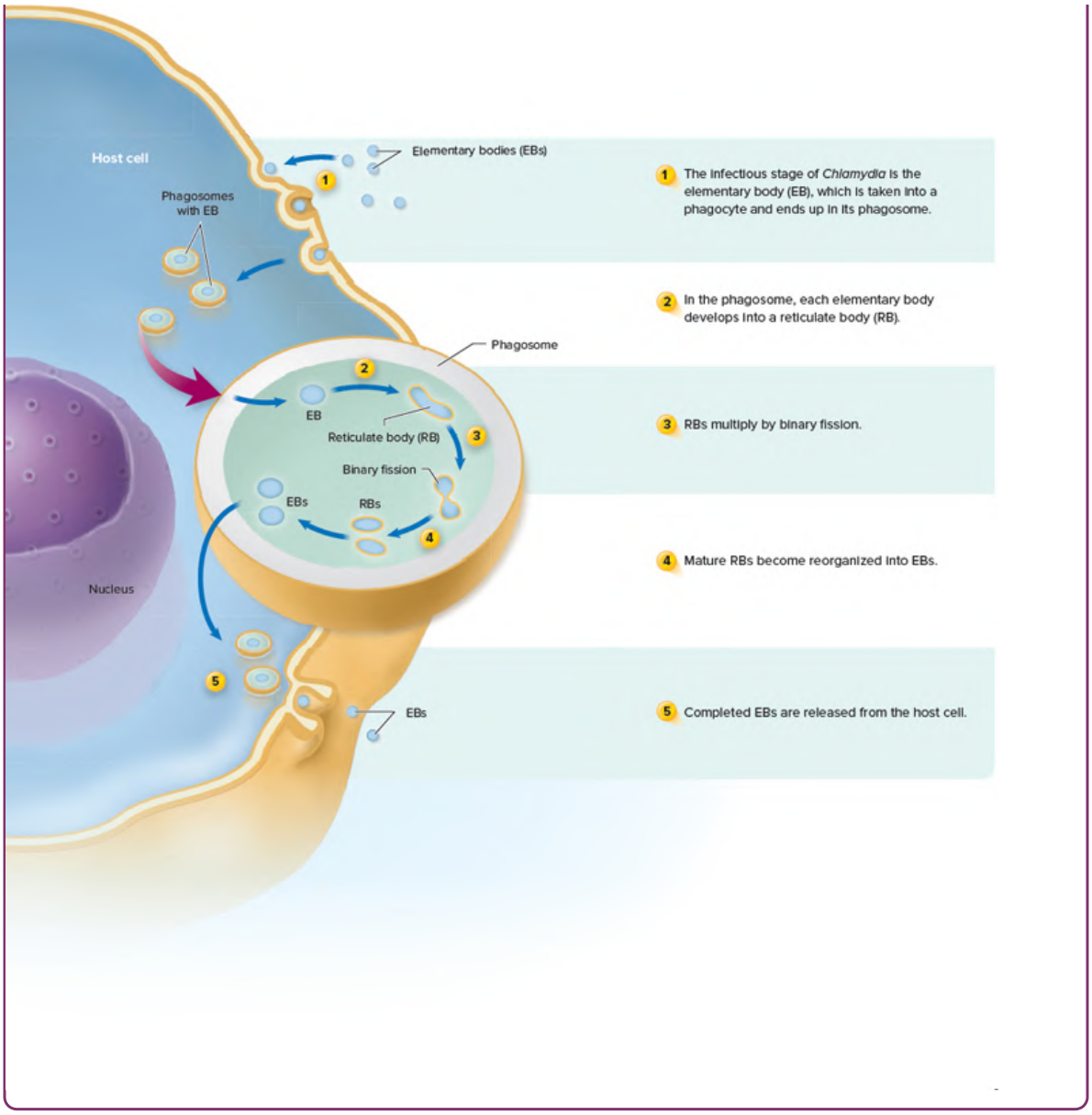


Figure 21.7 **Reported rates of *Chlamydia* in the United States from 2000–2015.**
Source: CDC

A Note About Pelvic Inflammatory Disease (PID) and Infertility

The National Center for Health Statistics estimates that more than 6 million women in the United States have impaired fertility. There are many different reasons for infertility, but the leading cause is pelvic inflammatory disease, or PID. PID is caused by infection of the upper reproductive structures in women—namely, the uterus, fallopian tubes, and ovaries. These organs have no normal biota, and when bacteria from the vagina are transported higher in the tract, they start a chain of inflammatory events that may or may not be noticeable to the patient. The inflammation can be acute, resulting in pain, abnormal vaginal discharge, fever, and nausea, or it can be chronic, with less noticeable symptoms. In acute cases, women usually seek care; in some ways, these can be considered the lucky ones. If the inflammation is curbed at an early stage by using antibiotics to kill the bacteria, chances are better that the long-term sequelae of PID can be avoided. *Chlamydia* infection is the leading cause of PID, followed closely by *N. gonorrhoeae* infection. But other bacteria, perhaps also including normal biota of the reproductive tract, can also cause PID if they are traumatically introduced into the uterus.

Table 21.1 **The Life Cycle of *Chlamydia***



Babies born to mothers with *Chlamydia* infections can develop eye infections and also pneumonia if they become infected during passage through the birth canal. Infant conjunctivitis caused by contact with maternal *Chlamydia* infection is the most prevalent form of conjunctivitis in the United States (100,000 cases per year). Antibiotic drops or ointment applied to newborns' eyes are used to eliminate both *Chlamydia* and *N. gonorrhoeae*.

Causative Agent

C. trachomatis is a very small gram-negative bacterium. It lives inside host cells as an obligate intracellular parasite. All *Chlamydia* species alternate between two distinct stages, illustrated in **Table 21.1**.

Pathogenesis and Virulence Factors

Chlamydia's ability to grow intracellularly contributes to its virulence because it escapes certain aspects of the host's immune response. Also, the bacterium has a unique cell wall that apparently prevents the phagosome from fusing with the lysosome inside phagocytes. The presence of the bacteria inside cells causes the release of cytokines that provoke intense inflammation. This defensive response leads to most of the actual tissue damage in *Chlamydia* infection. Of course, the last step

of inflammation is repair, which often results in scarring. This can have disastrous effects on a narrow-diameter structure like the fallopian tube.

Transmission and Epidemiology

The reservoir of pathogenic strains of *C. trachomatis* is the human body. The microbe shows an astoundingly broad distribution within the population, and incidence is rising. Adolescent women are more likely than older women to harbor the bacterium because it prefers to infect cells that are particularly prevalent on the adolescent cervix. It is transmitted through sexual contact and also vertically. Fifty percent of (untreated) babies born to infected mothers will acquire chlamydial conjunctivitis or pneumonia.

Culture and Diagnosis

Infection with this microorganism is usually detected initially using a rapid technique such as PCR or ELISA. Direct fluorescent antibody detection is also used. Serology is not always reliable. In addition, antibody to *Chlamydia* is very common in adults and often indicates past, not present, infection. A urine test is available, which has definite advantages for widespread screening, but it is slightly less accurate for females than males.

Prevention

Avoiding contact with infected tissues and secretions through abstinence or barrier protection (condoms) is the only means of prevention.

Treatment

The CDC recommends annual screening of young women for this often asymptomatic infection. It is also recommended that older women with some risk factor (new sexual partner, for instance) also be screened. Treatment is usually with doxycycline or azithromycin. Coinfection with gonorrhea should be assumed and treated similarly. Many patients become reinfected soon after treatment; therefore, the recommendation is that patients be rechecked for *Chlamydia* infection 3 to 4 months after treatment. Treatment of all sexual partners of the patient is also recommended to prevent reinfection. Repeated infections with *Chlamydia* increase the likelihood of PID and other serious sequelae.

Disease Table 21.3 Genital Discharge Diseases (in Addition to Vaginitis/Vaginosis)

	Gonorrhea B G−	Chlamydia B G−
Causative Organism(s)	*Neisseria gonorrhoeae*	*Chlamydia trachomatis*
Most Common Modes of Transmission	Direct contact (STI), also vertical	Direct contact (STI), vertical

	Gonorrhea (B) G−	Chlamydia (B) G−
Virulence Factors	Fimbrial adhesins, antigenic variation, IgA protease, membrane blebs/endotoxin	Intracellular growth resulting in avoiding immune system and cytokine release, unusual cell wall preventing phagolysosome fusion
Culture/Diagnosis	Gram stain in males, rapid tests (PCR, ELISA) for females, culture on Thayer-Martin agar	PCR or ELISA, can be followed by cell culture
Prevention	Avoid contact; condom use	Avoid contact; condom use
Treatment	Coinfection with gonorrhea and *C. trachomatis* should be assumed; treat with doxycycline or azithromycin. Be on alert for multidrug-resistant *N. gonorrhoeae*, which is in **Urgent Threat** category in CDC Antibiotic Resistance Report.	
Distinctive Features	Rare complications including arthritis, meningitis, endocarditis	More commonly asymptomatic than gonorrhea
Effects on Fetus	Eye infections, blindness	Eye infections, pneumonia
Epidemiological Features	United States: rates increasing since 2014; 2015 incidence = 395,000; internationally: 26 million cases	United States: increasing since 2000; 2015 incidence = 1.5 million; internationally: eye infection (trachoma) has 90% prevalence rate in developing world

Vaginitis and Vaginosis

Signs and Symptoms

Vaginitis, an inflammation of the vagina, is a condition characterized by some degree of vaginal itching, depending on the etiologic agent. Symptoms may also include burning and sometimes a discharge, which may take different forms as well. Vaginosis is similar but does not include significant inflammation.

Causative Agents

While a variety of bacteria and even protozoa can cause vaginitis, the most well-known agent is the fungus *Candida albicans*. The vaginal condition caused by this fungus is known as a *yeast infection*.

Candida albicans

C. albicans is a dimorphic fungus that is normal biota in the majority of humans, living in low numbers on many mucosal surfaces such as the mouth, gastrointestinal tract, vagina, and so on. The vaginal condition it causes is also called vulvovaginal candidiasis. The yeast is easily detectable on a wet prep or a Gram stain of material obtained during a pelvic exam (**figure 21.8**). The presence of pseudohyphae in the smear is a clear indication that the yeast is growing rapidly and causing a yeast infection.

In otherwise healthy people, the fungus is not invasive and limits itself to this surface infection. Please note, however, that *Candida* infections of the bloodstream do occur and they have high mortality rates. They do not normally stem from vaginal infections with the fungus, however, but are seen most frequently in hospitalized patients. AIDS patients are also at risk of developing systemic *Candida* infections.

Transmission and Epidemiology

Vaginal infections with this organism are nearly always opportunistic. Disruptions of the normal bacterial biota or even minor damage to the mucosal epithelium in the vagina can lead to overgrowth by this fungus. Disruptions may be mechanical, such as trauma to the vagina, or they may be chemical, as when broad-spectrum antibiotics taken for some other purpose temporarily diminish the vaginal

bacterial population. Diabetics and pregnant women are also predisposed to vaginal yeast overgrowths. Some women are prone to this condition during menstruation.

It is possible to transmit this yeast through sexual contact, especially if a woman is experiencing an overgrowth of it. The recipient's immune system may well subdue the yeast so that it acts as normal biota in them. But the yeast may be passed back to the original partner during further sexual contact after treatment. Women with HIV infection experience frequently recurring yeast infections. Also, a small percentage of women with no underlying immune disease experience chronic or recurrent vaginal infection with *Candida* for reasons that are not clear.

▶ Prevention and Treatment

No vaccine is available for *C. albicans*. Topical and oral azole drugs are used to treat vaginal candidiasis, and many of them are now available over the counter. Many women experience this condition multiple times in their lives, but if infections recur frequently or fail to resolve, it is important to see a physician.

Gardnerella Species

The bacterium *Gardnerella* is associated with a particularly common condition in women in their childbearing years. This condition is usually called *vaginosis* rather than *vaginitis* because it doesn't appear to induce inflammation in the vagina. It is also known as BV, or bacterial vaginosis. Despite the absence of an inflammatory response, a vaginal discharge is associated with the condition, which often has a fishy odor. Itching is also common, but it is also true that many women have this condition with no noticeable symptoms.

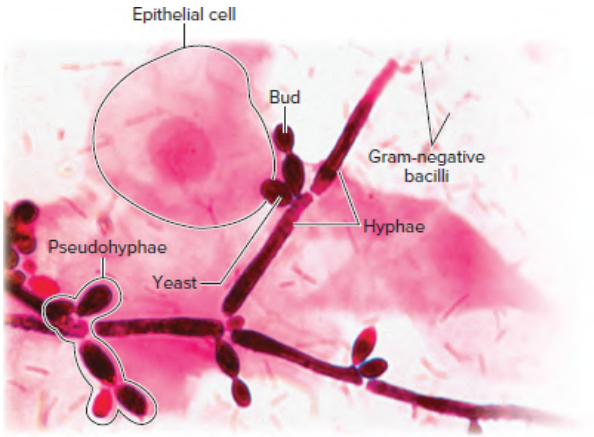


Figure 21.8 **Gram stain of *Candida albicans* in a vaginal smear.**
Courtesy Danny L. Wiedbrauk

Disease Table 21.4 Vaginitis/Vaginosis

Causative Organism(s)	*Candida albicans* (F)	Mixed infection, usually including *Gardnerella* (B)	*Trichomonas vaginalis* (P)
Most Common Modes of Transmission	Opportunism	Opportunism or STI?	Direct contact (STI)
Virulence Factors	—	—	—
Culture/Diagnosis	Wet prep or Gram stain	Visual exam of vagina, or clue cells seen in Pap smear or other smear	Protozoa seen on Pap smear or Gram stain; culture is gold standard

Prevention	—	—	Barrier use during intercourse
Treatment	Topical or oral azole drugs, some over-the-counter drugs	Metronidazole or clindamycin	Metronidazole, tinidazole
Distinctive Features	White curdlike discharge	Discharge may have fishy smell	Discharge may be greenish
Epidemiological Features	United States: 20% of all vaginitis; 75% of women report at least one case during lifetime	United States: 30% prevalence rate; internationally: prevalence rates vary from 20% to 50%	United States: 3.7 million people infected; is one of CDC's "neglected parasitic infections"

Vaginosis is most likely a result of a shift from a predominance of "good" bacteria (lactobacilli) in the vagina to a predominance of "bad" bacteria, and one of those is *Gardnerella vaginalis*. This genus of bacteria is a facultative anaerobe and gram-positive, although in a Gram stain it usually appears gram-negative. (Some texts refer to it as *gram-variable* for this reason.) Probably a mixed infection leads to the condition, however. Anaerobic streptococci and other bacteria, particularly a genus known as *Mobiluncus*, that are normally found in low numbers in a healthy vagina can also often be found in high numbers in this condition. The often-mentioned fishy odor comes from the metabolic by-products of anaerobic metabolism by these bacteria.

Pathogenesis and Virulence Factors

The mechanism of damage in this disease is not well understood, but some of the outcomes are. Besides the symptoms just mentioned, vaginosis can lead to complications such as pelvic inflammatory disease, infertility, and, more rarely, ectopic pregnancies. Babies born to some mothers with vaginosis have low birth weights.

Transmission and Epidemiology

Thirty percent of women between the ages of 15 and 44 in the United States are estimated to have bacterial vaginosis. Rates are noticeably greater in non-White women: White women have a prevalence of 23%; African-Americans, 51%; and Mexican-Americans, 32%. This mixed infection is not necessarily considered to be sexually transmitted, although women who have never had sex are less likely to develop the condition. It is very common in sexually active women. It may be that the condition is associated with sex but not transmitted by it. This situation could occur if the act of penetration or the presence of semen (or saliva) causes changes in the vaginal epithelium or in the vaginal biota. We do not know exactly what causes the increased numbers of *Gardnerella* and other normally rare biota. The low pH typical of the vagina is usually higher in vaginosis. It is not clear whether this causes or is caused by the change in bacterial biota.

Culture and Diagnosis

The condition can be diagnosed by a variety of methods. Sometimes a simple stain of vaginal secretions is used to examine sloughed vaginal epithelial cells. In vaginosis, some cells will appear to be nearly covered with adherent bacteria. In normal times, vaginal epithelial cells are sparsely covered with bacteria. These cells are called clue cells and are a helpful diagnostic indicator **(figure 21.9)**. They can also be found on Pap smears, but sometimes genomic analyses are needed.

Figure 21.9 **Clue cell in bacterial vaginosis**. These epithelial cells came from a pelvic exam. The cells in the large circle have an abundance of bacteria attached to them.
Source: CDC/M. Rein (normal); Source: CDC (abnormal)

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2. A vaginal wet prep indicates the presence of pseudohyphae. Based on this finding, the most likely differential diagnosis is
- **a.** *gonorrhea*.
- **b.** *chlamydia*.
- **c.** *vulvovaginal candidiasis*.
- **d.** *trichomoniasis*.

Prevention and Treatment

Women who find the condition uncomfortable or who are planning on becoming pregnant should be treated. Women who use intrauterine devices (IUDs) for contraception should also be treated because IUDs can provide a passageway for the bacteria to gain access to the upper reproductive tract. The usual treatment is oral or topical metronidazole or clindamycin.

Trichomonas vaginalis

Trichomonads are small, pear-shaped protozoa with four anterior flagella and an undulating membrane. *Trichomonas vaginalis* seems to cause asymptomatic infections. In approximately 50% of its victims, Trichomonads are considered asymptomatic infectious agents rather than normal biota because of evidence that some people experience long-term negative effects. Even though *Trichomonas* is a protozoan, it has no cyst form, and it does not survive long outside of the host.

Many cases are asymptomatic, and men seldom have symptoms. Women often have vaginitis symptoms, which can include a white to green frothy discharge. Chronic infection can make a person more susceptible to other infections, including HIV. Also, women who become infected during

pregnancy are predisposed to premature labor and low-birth-weight infants. Chronic infection may also lead to infertility.

Because *Trichomonas* is common biota in so many people, it is easily transmitted through sexual contact. It has been called the most common nonviral sexually transmitted infection (as shown in figure 21.4). It does not appear to undergo opportunistic shifts within its host (i.e., to become symptomatic under certain conditions), but rather, the protozoan causes symptoms when transmitted to a noncarrier. **Disease Table 21.4**.

Prostatitis

Prostatitis is an inflammation of the prostate gland (see figure 21.2). It can be acute or chronic. Acute prostatitis is virtually always caused by bacterial infection. The bacteria are usually normal biota from the intestinal tract or may have caused a previous urinary tract infection. Chronic prostatitis is also often caused by bacteria. Researchers have found that chronic prostatitis, often unresponsive to antibiotic treatment, can be caused by mixed biofilms of bacteria in the prostate. Some forms of chronic prostatitis have no known microbial cause, though many infectious disease specialists feel that one or more bacteria are involved, but they are simply not culturable with current techniques.

Symptoms may include pain in the groin and lower back, frequent urge to urinate, difficulty in urinating, blood in the urine, and painful ejaculation. Treatment is with ciprofloxacin or levofloxacin.

©Eye of Science/Science Source

Disease Table 21.5 Prostatitis

Causative Organism(s)	GI tract biota
Most Common Modes of Transmission	Endogenous transfer from GI tract; otherwise unknown
Virulence Factors	Various
Culture/Diagnosis	Digital rectal exam to examine prostate; culture of urine or semen
Prevention	None
Treatment	Antibiotics, muscle relaxers, alpha blockers
Distinctive Features	Pain in genital area and/or back, difficulty urinating
Epidemiological Features	United States: 50% of men experience during lifetime

Genital Ulcer Diseases

Three common infectious conditions can result in lesions (ulcers) on the genitals: syphilis, chancroid, and genital herpes. In this section, we consider each of these. One very important fact to remember about the ulcer diseases is that having one of them increases the chances of infection with HIV because of the open lesions.

Syphilis

Untreated syphilis is marked by distinct clinical stages designated as *primary, secondary*, and *tertiary syphilis*. The disease also has latent periods of varying duration during which it is quiescent. The spirochete appears in the lesions and blood during the primary and secondary stages and, thus, is transmissible at these times. During the early latency period between secondary and tertiary syphilis, it is also transmissible. Syphilis is largely nontransmissible during the "late latent" and tertiary stages.

Primary Syphilis

The earliest indication of syphilis infection is the appearance of a hard **chancre** (shang'-ker) at the site of entry of the pathogen. Because these ulcers tend to be painless, they may escape notice, especially when they are on internal surfaces. The chancre heals spontaneously without scarring in 3 to 6 weeks, but the healing is deceptive because the spirochete has escaped into the circulation and is entering a period of tremendous activity.

Secondary Syphilis

About 3 weeks to 6 months after the chancre heals, the secondary stage appears. By then, many systems of the body have been invaded, and the signs and symptoms are more profuse and intense. Initial symptoms are fever, headache, and sore throat, followed by lymphadenopathy and a peculiar red or brown rash that breaks out on all skin surfaces, including the palms of the hands and the soles of the feet. A person's hair often falls out. Like the chancre, the lesions contain viable spirochetes and disappear spontaneously in a few weeks. The major complications of this stage, occurring in the bones, hair follicles, joints, liver, eyes, and brain, can linger for months and years.

Latency and Tertiary Syphilis

After resolution of secondary syphilis, about 30% of infections enter a highly varied latent period that can last for 20 years or longer. During latency, although antibodies to the bacterium are readily detected, the bacterium itself is not. The final stage of the disease, tertiary syphilis, is relatively rare today because of widespread use of antibiotics. But it is so damaging that it is important to recognize. By the time a patient reaches this phase, numerous pathologic complications occur in susceptible tissues and organs. Cardiovascular syphilis results from damage to the small arteries in the aortic wall. As the fibers in the wall weaken, the aorta is subject to distension and fatal rupture. The same pathologic process can damage the aortic valves, resulting in heart failure.

In one form of tertiary syphilis, painful swollen syphilitic tumors called **gummas** (goo-mahz′) develop in tissues such as the liver, skin, bone, and cartilage **(figure 21.10)**. Gummas are usually benign and only occasionally lead to death, but they can impair function. Neurosyphilis can involve any part of the nervous system, but it shows particular affinity for the blood vessels in the brain, cranial nerves, and dorsal roots of the spinal cord. The diverse results include severe headaches, convulsions, atrophy of the optic nerve, blindness, dementia, and a sign called the Argyll-Robertson pupil—a condition caused by adhesions along the inner edge of the iris that fix the pupil's position into a small irregular circle.

Figure 21.10 **Primary, secondary, and tertiary syphilis**. **(a)** The lesion (chancre) of primary syphilis. **(b)** Secondary syphilis produces a generalized rash that appears also on the palms of the hands and soles of the feet. **(c)** Tertiary syphilis patient with gummas on his nose.
Sources: CDC/Dr. NJ Fiumara, Dr. Gavin Hart (a); CDC (b); CDC/Susan Lindsley (c)

Congenital Syphilis

The syphilis bacterium can pass from a pregnant woman's circulation into the placenta and can be carried throughout the fetal tissues. The pathogen inhibits fetal growth and disrupts critical periods of development with varied consequences, ranging from mild to the extremes of spontaneous miscarriage or stillbirth. Infants often demonstrate such signs as profuse nasal discharge **(figure 21.11*a*)**, skin eruptions, bone deformation, and nervous system abnormalities. The late form gives rise to an unusual assortment of problems in the bones, eyes, inner ear, and joints, and causes the formation of Hutchinson's teeth **(figure 21.11*b*)**.

Causative Agent

Treponema pallidum, a spirochete, is a thin, regularly coiled cell with a gram-negative cell wall. It is a strict parasite with complex growth requirements that necessitate cultivating it in living host cells.

Pathogenesis and Virulence Factors

Brought into direct contact with mucous membranes or abraded skin, *T. pallidum* binds avidly by its hooked tip to the epithelium **(figure 21.12)**. At the binding site, the spirochete multiplies and penetrates

the capillaries nearby. Within a short time, it moves into the circulation, and the body is literally transformed into a large receptacle for incubating the pathogen. Virtually any tissue is a potential target.

Transmission and Epidemiology

Humans are evidently the sole natural hosts and source of *T. pallidum*. The bacterium is extremely fastidious and sensitive and cannot survive for long outside the host, being rapidly destroyed by heat, drying, disinfectants, soap, high oxygen tension, and pH changes. It survives a few minutes to hours when protected by body secretions and about 36 hours in stored blood. The risk of infection from an infected sexual partner is 12% to 30% per encounter.

For centuries, syphilis was a common and devastating disease in the United States, so much so that major medical centers had "Departments of Syphilology." Its effect on social life was enormous. This effect diminished quickly when antibiotics were discovered. But since 2003, the rates have been increasing again in the United States, especially since 2011 **(figure 21.13)**. And syphilis continues to be a serious problem worldwide, especially in Africa and Asia. As mentioned previously, persons with syphilis often suffer concurrent infections with other STIs. Coinfection with the AIDS virus can be an especially deadly combination with a rapidly fatal course.

Culture and Diagnosis

Syphilis can be detected in patients most rapidly by using dark-field microscopy of a suspected lesion (figure 21.13). A single negative test is not enough to exclude syphilis because the patient may have removed the organisms by washing, so follow-up tests are recommended.

Very commonly, blood tests are used for this diagnosis. The best test is that which specifically reacts with treponemal antigens. There is also a test called the rapid plasmin reagin (RPR), which is a longstanding test used for syphilis that is still useful. It tests for antigens that are not from the bacterium, but that appear in the host during infection by the bacterium. Unfortunately, these antigens can appear in some other conditions. So the RPR is almost always coupled with an immunoassay specific for treponemal antigens.

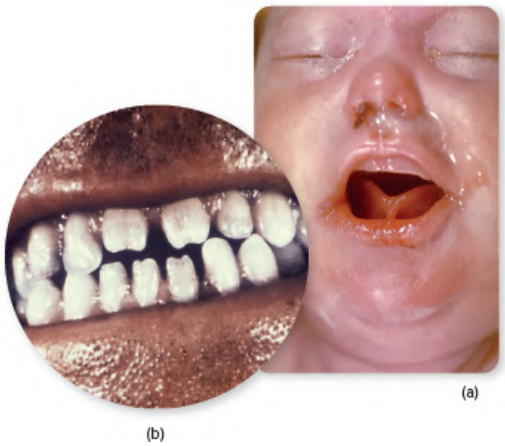

Figure 21.11 **Congenital syphilis**. **(a)** An early sign is snuffles, a profuse nasal discharge that obstructs breathing. **(b)** A common characteristic of late congenital syphilis is notched, barrel-shaped incisors (Hutchinson's teeth).
Source: CDC/Dr. Norman Cole (a); Source: CDC/Robert Sumpter (b)

Prevention

The core of an effective prevention program depends on detection and treatment of the sexual contacts of syphilitic patients. Public health departments and physicians are charged with the task of questioning patients and tracing their contacts. All individuals identified as being at risk, even if they show no signs of infection, are given immediate prophylactic penicillin in a single long-acting dose.

The barrier effect of a condom provides excellent protection during the primary phase. Protective immunity apparently does arise in humans, allowing the prospect of an effective immunization program in the future, although no vaccine exists currently.

Treatment

Throughout most of history, the treatment for syphilis was a dose of mercury or even a "mercurial rub" applied to external lesions. In 1906, Paul Ehrlich discovered that a derivative of arsenic called salvarsan could be very effective. The fact that toxic compounds like mercury and arsenic were used to treat syphilis gives some indication of how dreaded the disease was and to what lengths people would go to rid themselves of it.

Current recommendations are for ciprofloxacin or levofloxacin.

Chancroid

This ulcerative disease usually begins as a soft papule, or bump, at the point of contact. It develops into a "soft chancre" (in contrast to the hard syphilis chancre), which is very painful in men, but may be unnoticed in women **(Disease Table 21.6)**. Inguinal lymph nodes can become very swollen and tender.

Chancroid is caused by a pleomorphic gram-negative rod called *Haemophilus ducreyi*. Recent research indicates that a hemolysin (exotoxin) is important in the pathogenesis of chancroid disease. It is very common in the tropics and subtropics and is becoming more common in the United States. Chancroid is transmitted exclusively through direct contact, especially sexually. This disease is associated with sex workers and poor hygiene. Uncircumcised men seem to be more commonly infected than those who have been circumcised. People may carry this bacterium asymptomatically.

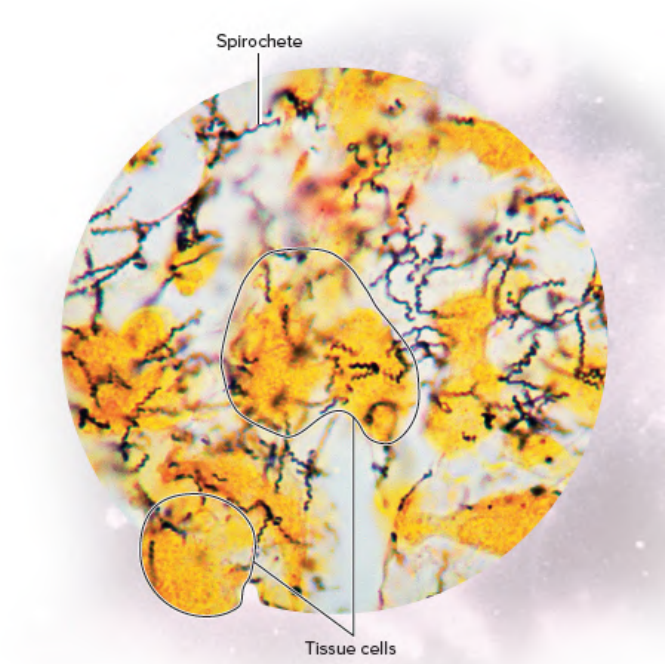

Figure 21.12 ***Treponema pallidum*** **from a syphilitic chancre, viewed with dark-field illumination**. Its tight spirals are highlighted next to human cells and tissue debris.
Source: CDC/Dr. Edwin P. Ewing, Jr.

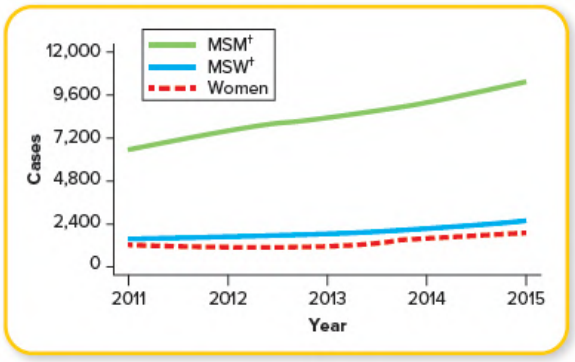

Figure 21.13 **Rates of primary and secondary syphilis from 2011–2015**. Data are represented for females (F), men who have sex with women (MSW), and men who have sex with men (MSM).
Source: CDC

Genital Herpes

Genital herpes is much more common than most people think. It is caused by herpes simplex viruses (HSVs). Two types of HSV have been identified, HSV-1 and HSV-2.

▶ Signs and Symptoms

Genital herpes infection has multiple presentations. After initial infection, a person may notice no symptoms. Alternatively, herpes could cause the appearance of single or multiple vesicles on the genitalia, perineum, thigh, and buttocks. The vesicles are small and are filled with a clear fluid (see Disease Table 21.6). They can be intensely painful to the touch. The appearance of lesions the first time you get them can be accompanied by malaise, anorexia, fever, and bilateral swelling and tenderness in the groin. Occasionally central nervous system symptoms such as meningitis or encephalitis can develop. Thus, we see that initial infection can either be completely asymptomatic or be serious enough to require hospitalization.

After recovery from initial infection, a person may have recurrent episodes of lesions. They are generally less severe than the original symptoms, although the whole gamut of possible severity is seen here as well. Some people never have recurrent lesions. Others have nearly constant outbreaks with little recovery time between them. On average, the number of recurrences is four or five a year. Their frequency tends to decrease over the course of years.

In most cases, patients remain asymptomatic or experience recurrent "surface" infections indefinitely. Very rarely, complications can occur. Every year, one or two persons per million with chronic herpes infections develop encephalitis. The virus disseminates along nerve pathways to the brain (although it can also infect the spinal cord). The effects on the central nervous system begin with headache and stiff neck and can progress to mental disturbances and coma.

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3. A neonate is brought in to the pediatrician's office by her parents for a 1-month checkup. Findings indicate inflammation and edema of the left eye. Parents deny any traumatic injury to the left eye. Which information would be most important to ascertain in order to develop a plan of care?

a. *delivery history of the neonate—vaginal or C-section*
b. *documentation of vitamin K injection postdelivery*
c. *Apgar score following delivery*
d. *maternal history of STIs*

Herpes of the Newborn

Although HSV infections in healthy adults are annoying and unpleasant, only rarely are they life-threatening. However, in the neonate and the fetus **(figure 21.14)**, HSV infections are very destructive and can be fatal. Most cases occur when infants are contaminated by the mother's reproductive tract immediately before or during birth, but they have also been traced to hand transmission from the mother's lesions to the baby.

Pregnant women with a history of recurrent infections must be monitored for any signs of viral shedding, especially in the last 4 weeks of pregnancy. If no evidence of recurrence is seen, vaginal birth is indicated, but any evidence of an outbreak at the time of delivery necessitates a cesarean section.

Causative Agent

Both HSV-1 and HSV-2 can cause genital herpes if the virus contacts the genital epithelium, although HSV-1 is thought of as a virus that infects the oral mucosa, resulting in "cold sores" or "fever blisters" **(figure 21.15)**, and HSV-2 is thought of as the genital virus. In reality, either virus can infect either region, depending on the type of contact.

Pathogenesis and Virulence Factors

Herpesviruses have a strong tendency to become latent. During latency, some type of signal causes most of the HSV genome not to be transcribed. This allows the virus to be maintained within cells of the nervous system between episodes. Recent research has found that microRNAs are in part responsible for the latency of HSV-1. It is further suggested that in some peripheral cells, viral replication takes place at a constant, slow rate, resulting in constant low-level shedding of the virus without lesion production.

Figure 21.14 **Neonatal herpes simplex**. Babies can be born with the lesions or develop them 1 to 2 weeks after birth.
Source: CDC/J.D. Millar

Reactivation of the virus can be triggered by a variety of stimuli, including stress, UV radiation (sunlight), injury, menstruation, or another microbial infection. At that point, the virus begins manufacturing large numbers of entire virions, which cause new lesions on the surface of the body served by the neuron, usually in the same site as previous lesions.

Figure 21.15 **Oral herpes infection**. Tender itchy papules erupt around the mouth and progress to vesicles that burst, drain, and scab over. These sores and fluid are highly infectious and should not be touched.
Source: CDC

Transmission and Epidemiology

It might surprise you to know that 50% to 80% of adults in the United States are seropositive for HSV-1, and 20% to 40% are positive for HSV-2. Worldwide, the WHO estimates that 4 billion people are infected; that's 4 billion of about 7.5 billion total people. Because these viruses are relatively sensitive

to the environment, transmission is primarily through direct exposure to secretions containing the virus. People with active lesions are the most significant source of infection, but studies indicate that genital herpes can be transmitted even when no lesions are present (due to the constant shedding just discussed).

Earlier in this chapter, you read that *Chlamydia* infection is the most common *reported* infectious disease in the United States. Elsewhere you might hear that gonorrhea is one of the most common reportable *STIs* in the United States. Both statements are true. It is also true that genital herpes is much more common than either of these diseases. Herpes, however, is not a nationally *reportable* disease.

Research has shown that 50% to 90% of people who are infected don't even know it, either because they have rare symptoms that they fail to recognize or because they have no symptoms at all.

Culture and Diagnosis

These two viruses are sometimes diagnosed based on the characteristic lesions alone. PCR tests are available to test for these viruses directly from lesions. Alternatively, antibody to either of the viruses can be detected from blood samples.

Prevention

No vaccine is currently licensed for HSV, but more than one is being tested in clinical trials, meaning that vaccines may become available very soon. In the meantime, avoiding contact with infected body surfaces is the only way to avoid HSV. Condoms provide good protection when they actually cover the site where the lesion is, but lesions can occur outside of the area covered by a condom.

Mothers with cold sores should be careful in handling their newborns; they should never kiss their infants on the mouth.

Treatment

Several agents are available for treatment. These agents often result in reduced viral shedding and a decrease in the frequency of lesion occurrence. They are not curative. Acyclovir and its derivatives (famciclovir or valacyclovir) are very effective. Topical formulations can be applied directly to lesions, and pills are available as well. Sometimes medicines are prescribed on an ongoing basis to decrease the frequency of recurrences, and sometimes they are prescribed to be taken at the beginning of a recurrence to shorten it. **Disease Table 21.6**.

Wart Diseases

In this section, we describe two viral STIs that cause wartlike growths. The more serious disease is caused by the *human papillomavirus* (HPV). The other condition, called *molluscum contagiosum*, apparently has no serious effects.

Medical Moment

Crabs

Pediculosis pubis, commonly known as crabs, is caused by infestation of the pubic hair by *Phthirus pubis*, a tiny (1 mm or less) insect that can multiply rapidly. The condition can be spread from person to person through sexual contact and can therefore be considered a sexually transmitted condition. The eyebrows, eyelashes, chest hair, scalp hair, and facial hair may also be affected, generally through oral sexual activities.

Although these tiny creatures do not pose much of a risk to human health, infestation with crabs may lead to a higher risk for other STIs. It is estimated that approximately 3 million people are infected yearly in the United States.

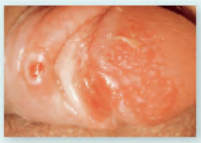
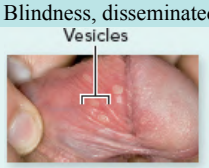
Symptoms include intense itching of the affected areas. Some people do not experience itching but while they are bathing may observe the insects, as the lice are visible to the naked eye.

Treatment is applied topically in the form of creams, shampoos, or lotions. Manual removal of adult insects and their eggs is recommended using a fine-toothed metal comb. Clothing and bedding should be laundered in hot water to prevent reinfestation.

Q. Why do you think a case of crabs can make you more susceptible to other STIs?

Answer in Appendix B

Disease Table 21.6 Genital Ulcer Diseases

	Syphilis	Chancroid	Herpes
Causative Organism(s)	*Treponema pallidum* B G-	*Haemophilus ducreyi* B G	Herpes simplex viruses 1 and 2 V
Most Common Modes of Transmission	Direct contact and vertical	Direct contact (vertical transmission not documented)	Direct contact, vertical
Virulence Factors	Lipoproteins	Rule out other ulcer diseases	Latency
Culture/Diagnosis	Direct tests (immunofluorescence, dark-field microscopy), blood tests for treponemal and nontreponemal antibodies	Culture from lesion	Clinical presentation, PCR, antibody tests, growth of virus in cell culture
Prevention	Antibiotic treatment of all possible contacts, avoiding contact	Avoiding contact	Avoiding contact, antivirals can reduce recurrences
Treatment	Ciprofloxacin, levofloxacin	Ceftriaxone or azithromycin	Acyclovir and derivatives
Distinctive Features	Three stages of disease plus latent period, possibly fatal	No systemic effects	Ranges from asymptomatic to frequent recurrences
Effects on Fetus	Congenital syphilis	None	Blindness, disseminated herpes infection
Appearance of Lesions			
Epidemiological Features	United States: estimated 24,000 new cases per year; internationally: estimated 12 million new infections per year	United States: no more than a handful per year; internationally: estimated 7 million cases annually	United States: 50%–80% prevalence of seropositivity for HSV 1; 20%–40% for HSV 2; worldwide: more than half are seropositive for one of them

©Biophoto Associates/Science Source (Syphilis); ©Dr. M.A. Ansary/Science Source (Chancroid); ©Dr. P. Marazzi/Science Source (Herpes)

Human Papillomavirus Infection

These viruses are the causative agents of genital warts. But an individual can be infected with these viruses without having any warts, while still risking serious consequences.

▶ Signs and Symptoms

Symptoms, if present, may manifest as warts—outgrowths of tissue on the genitals **(Disease Table 21.7)**. In females, these growths can occur on the vulva and in and around the vagina. In males, the warts can occur in or on the penis and the scrotum. In both sexes, the warts can appear in or on the anus and even on the skin around the groin, such as the area between the thigh and the pelvis. The warts themselves range from tiny, flat, inconspicuous bumps to extensively branching, cauliflower-like masses called **condyloma acuminata**. The warts are unsightly and can be obstructive, but they don't generally lead to more serious symptoms.

Other types of HPV can lead to more subtle symptoms. Certain types of the virus infect cells on the female cervix. This infection may be "silent," or it may lead to abnormal cell changes and malignancies

of the cervix. Approximately 4,000 women die each year in the United States from cervical cancer, and the vast majority of these are caused by HPV.

Males can also get cancer from infection with these viruses. The sites most often affected are the penis and the anus. These cases are less common than cervical cancer. Mouth and throat cancers in both sexes are also caused by HPV infection and are thought to be a consequence of oral sex.

A Note About HIV and Hepatitis B and C

This chapter is about diseases *whose major (presenting) symptoms occur in the genitourinary tract*. But some sexually transmitted diseases do not have their major symptoms in this system. HIV and hepatitis B and C can all be transmitted in several ways, one of them being through sexual contact. HIV is considered in chapter 18 because its major symptoms occur in the cardiovascular and lymphatic systems. Because the major disease manifestations of hepatitis B and C occur in the gastrointestinal tract, these diseases are discussed in chapter 20. Anyone diagnosed with any sexually transmitted disease should also be tested for HIV.

Table 21.2 Who Should Get the HPV Vaccine

Ideal	11- to 12-year-olds of both sexes should get two doses, 6 to 12 months apart
"Catch-Up"	Females who missed this through the age of 26, should get three doses Males who missed it through the age of 21, should get three doses
Others for "Catch-Up"	Men who identify as gay or bisexual through the age of 26 Transgender people through the age of 26 Immunocompromised people through the age of 26

Causative Agent

The human papillomaviruses are a group of nonenveloped DNA viruses belonging to the *Papovaviridae* family. There are more than 100 different types of HPV. Some types are specific for the mucous membranes; others invade the skin. Some of these viruses are the cause of plantar warts, which often occur on the soles of the feet. Other HPVs cause the common or "seed" warts and flat warts. In this chapter, we are concerned only with the HPVs that colonize the genital tract.

Among the HPVs that infect the genital tract, some are more likely to cause the appearance of warts. Others that have a preference for growing on the cervix can lead to cancerous changes. Five types in particular, HPV-16, -18, -31, -33, and -35, are closely associated with development of cervical and anal cancer. Other types put you at higher risk for vulvar or penile cancer.

Pathogenesis and Virulence Factors

Scientists are working hard to understand how viruses cause the growths we know as warts and also how some of them can cause cancer. The major virulence factor for cancer-causing HPVs is their **oncogenes**, which code for proteins that interfere with normal host cell function, resulting in uncontrolled growth.

Transmission and Epidemiology

The CDC states, "Nearly all sexually active adults will get HPV at some time in their lives." It is estimated that 14% of female college students become infected with this incurable condition each year.

The mode of transmission is direct contact. Autoinoculation is also possible—meaning that the virus can be spread to other parts of the body by touching warts. Indirect transmission occurs but is more common for nongenital warts caused by HPV.

Culture and Diagnosis

PCR-based screening tests can be used to test samples from a pelvic exam for the presence of dangerous HPV types. These tests are now recommended for women over the age of 30. The Pap smear is still the single best screening procedure available for cervical cancer. It will detect nearly all precancerous cells if conducted on the recommended schedule.

Prevention

Infection with these viruses is prevented the same way other sexually transmitted infections are prevented—by avoiding direct, unprotected contact, but also through vaccination. The vaccines prevent infection by up to nine types of HPV and are recommended in both girls and boys as young as age 9. There has been an unusual amount of controversy about this vaccine, which is no doubt due to the fact that it protects against a disease transmitted by sex, and parents don't like to think about their kids having sex. But the point is that it only protects you if you haven't been exposed to the virus yet. It has been repeatedly shown to be safe, and it has been shown to provide nearly 100% protection against cervical cancer as well as warts. **Table 21.2** provides guidelines for who should get the vaccine.

Preventing infection prevents the cancers that may have resulted from previous infection by an HPV. It is important to note that even though women now have access to the vaccines, cervical cancer can still result from HPV types not included in the vaccines. The good news is that cervical cancer is slow in developing, so that even if a woman is infected with a

©McGraw-Hill Education/Jill Braaten, photographer (woman); ©Science Photo Library/Getty Images (virus)

malignant HPV type, regular screening of the cervix can detect abnormal changes early. The standardized screen for cervical cell changes is the Pap smear. Precancerous changes show up very early, and the development process can be stopped by removal of the affected tissue. Women should have their first Pap smear by age 21 or within 3 years of their first sexual activity, whichever comes first. New Pap smear technologies have been developed; and depending on which one your physician uses, it is now possible that you need to be screened only once every 2 or 3 years. But you should base your screening practices on the sound advice of a physician.

Treatment

Infection with any HPV is incurable, though, unlike herpesviruses, it may resolve itself over a period of months to years. Genital warts can be removed through a variety of methods, but that does not

eliminate the virus.

Molluscum Contagiosum

An unclassified virus in the family *Poxviridae* can cause a condition called molluscum contagiosum. This disease can take the form of skin lesions, and it can also be transmitted sexually. The wartlike growths that result from this infection can be found on the mucous membranes or the skin of the genital area (see **Disease Table 21.7**). Few problems are associated with these growths beyond the warts themselves. We present them here mostly for purposes of differentiating them from HPV.

The virus causing these growths can also be transmitted through fomites such as clothing or towels and through autoinoculation.

The introduction of oral contraceptives revolutionized many aspects of society. Unfortunately, they do not protect against STIs.

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Disease Table 21.7 Wart Diseases

	HPV	Molluscum Contagiosum
Causative Organism(s)	Human papillomaviruses V	Poxvirus, sometimes called the molluscum contagiosum virus (MCV) V
Most Common Modes of Transmission	Direct contact (STI), also autoinoculation, indirect contact	Direct contact (STI), also autoinoculation, indirect contact
Virulence Factors	Oncogenes (in the case of malignant types of HPV) -	–
Culture/Diagnosis	PCR tests for certain HPV types, clinical diagnosis; Pap smear	Clinical diagnosis, also histology, PCR
Prevention	Vaccines available; avoid direct contact; prevent cancer by screening cervix	Avoid direct contact
Treatment	Warts or precancerous tissue can be removed; virus not treatable	Warts can be removed; virus not treatable
Distinguishing Features	Infection may or may not result in warts; infection may result in malignancy	Wartlike growths are only known consequence of infection
Effects on Fetus	May cause laryngeal warts	-

	HPV	Molluscum Contagiosum
Appearance of Lesions		
Epidemiological Features	United States: eventually all sexually active adults will likely have one or more types; 12,000 new cases of HPV-associated cervical cancer annually	United States: affects 2%–10% of children annually

©BSIP/age fotostock (HPV); ©Dr. Jack Jerjian/Medical Images (Molluscum Contagiosum); ©Meredun Animal Health Ltd/Science Source (Neisseria gonorrhoeae, background)

Group B *Streptococcus* "Colonization"—Neonatal Disease

Ten to forty percent of women in the United States are colonized, asymptomatically, by a beta-hemolytic *Streptococcus* in Lancefield group B. Nonpregnant women experience no ill effects from this colonization. But colonization of pregnant women with this organism is associated with preterm delivery. Additionally, about half of their infants become colonized by the bacterium during passage through the birth canal or by ascension of the bacteria through ruptured membranes. For those reasons, this colonization is considered a reproductive tract disease.

A small percentage of infected infants experience life-threatening bloodstream infections, meningitis, or pneumonia. If they recover from these acute conditions, they may have permanent disabilities, such as developmental disabilities, hearing loss, or impaired vision. In some cases, the mothers also experience disease, such as amniotic infection or subsequent stillbirths. Although group B *Streptococcus* infections have declined sharply in the United States, they remain a major threat to infant morbidity and mortality worldwide.

In 2010, the CDC reiterated recommendations that all pregnant women be screened for group B *Streptococcus* colonization at 35 to 37 weeks of pregnancy. Recommendations for earlier testing are sometimes warranted because colonization has been associated with preterm birth. Women positive for the bacterium should be treated with penicillin or ampicillin, unless the bacterium is found to be resistant to these and unless allergy to penicillin is present, in which case erythromycin may be used.

Disease Table 21.8 Group B *Streptococcus* Colonization

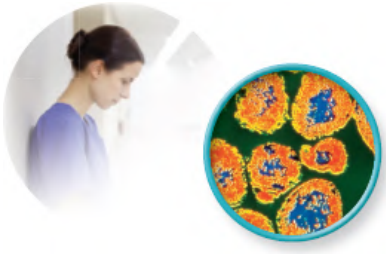
Causative Organism(s)	Group B *Streptococcus* (B) G+
Most Common Modes of Transmission	Vertical
Virulence Factors	–
Culture/Diagnosis	Culture of mother's genital tract
Prevention/Treatment	Treat mother with penicillin/ampicillin; watch for clindamycin-resistant strains as they are in **Concerning Threat** category on CDC Antibiotic Resistance Report
Epidemiological Features	United States: vaginal carriage rates 15%–45%; neonatal sepsis due to this occurs in 1.8–3.2 per 1,000 live births; internationally: vaginal carriage rates 12%–27%

21.4 LEARNING OUTCOMES—Assess Your Progress

6. List the possible causative agents, modes of transmission, virulence factors, and prevention/treatment for the "Highlight Diseases," gonorrhea and *Chlamydia* infection.
7. Distinguish between vaginitis and vaginosis.
8. Discuss prostatitis.
9. Name three diseases that result in genital ulcers, and discuss their important features.

10. Differentiate between the two diseases causing warts in the reproductive tract.
11. List at least three reasons the HPV vaccine is so strongly recommended for young adolescents.
12. Identify the most important risk group for group B *Streptococcus* infection and explain why that group is important.

CASE FILE WRAP-UP



Transmission electron micrograph of Neisseria gonorrhoeae.

©Syda Productions/Shutterstock (health care worker); ©Meredun Animal Health Ltd/Science Source (*Neisseria gonorrhoeae*)

The young patient featured in the opening case study was given a pelvic exam after consent was obtained from the patient's mother. The patient was found to have a purulent and foul-smelling vaginal discharge, in addition to fever and lower abdominal pain. Cultures of the vaginal discharge and the cervix were obtained. Blood work and an ultrasound were also ordered. The blood work revealed an elevated white blood cell count and erythrocyte sedimentation rate (ESR), while the ultrasound revealed an abscess near the left ovary. The patient was admitted with a diagnosis of pelvic inflammatory disease (PID) for IV antibiotics and possible surgical drainage of the abscess. Cultures eventually yielded the specific causative agent, *Neisseria gonorrhoeae*. The "PID shuffle" is a term used to describe the typical gait of a patient with PID, in which the feet are advanced in a shuffling manner to avoid jarring the pelvic organs, which can result in severe pain.

The patient was only 10 years of age, which should raise suspicion of child abuse. Not only should the case be reported to the appropriate state health authority, but it should also be reported to social services or the police in order to determine whether the patient was a victim of abuse.

The Microbiome

Save the World with the Vaginal Microbiome

A few years ago, a scientist named Gregor Reid made a provocative statement to a meeting of the American Society for Microbiology. He said, "To not place a huge focus on the human vaginal microbiome is like putting human survival at risk." Here is his reasoning: A healthy vaginal

microbiome is essential to successful pregnancy and delivery. Disturbances in the microbiome change the pH of the vagina, inhibit fertility, cause spontaneous abortions, and induce early-term delivery. Currently, 30% of American women have abnormal vaginal microbiomes; the rates are as high as 60% in inner-city populations. This condition is known as bacterial vaginosis. Many women who have it have no symptoms at all, and it only causes problems when women try to get pregnant or during pregnancy or delivery.

The causes of the condition are poorly understood, but douching, smoking, obesity, stress, and high numbers of sexual partners have all been associated with it. Dr. Reid and others reserve a particular disdain for the use of douche. Douching has been linked to preterm birth, an elevated risk of acquiring HIV, ectopic pregnancies, cervical cancer, and endometriosis. Some physicians feel that there is a direct link between this practice and the unusually high rates of preterm delivery and infant mortality in many urban areas.

The vaginal microbiome as the cradle of civilization? Gregor Reid thinks that if the vaginal microbiome were to suddenly shift across the human population, it would not be unreasonable to expect that the human race to go extinct. This is obviously a worst-case scenario, and an unlikely event, but it does put a spotlight on an overlooked aspect of women's and children's health.

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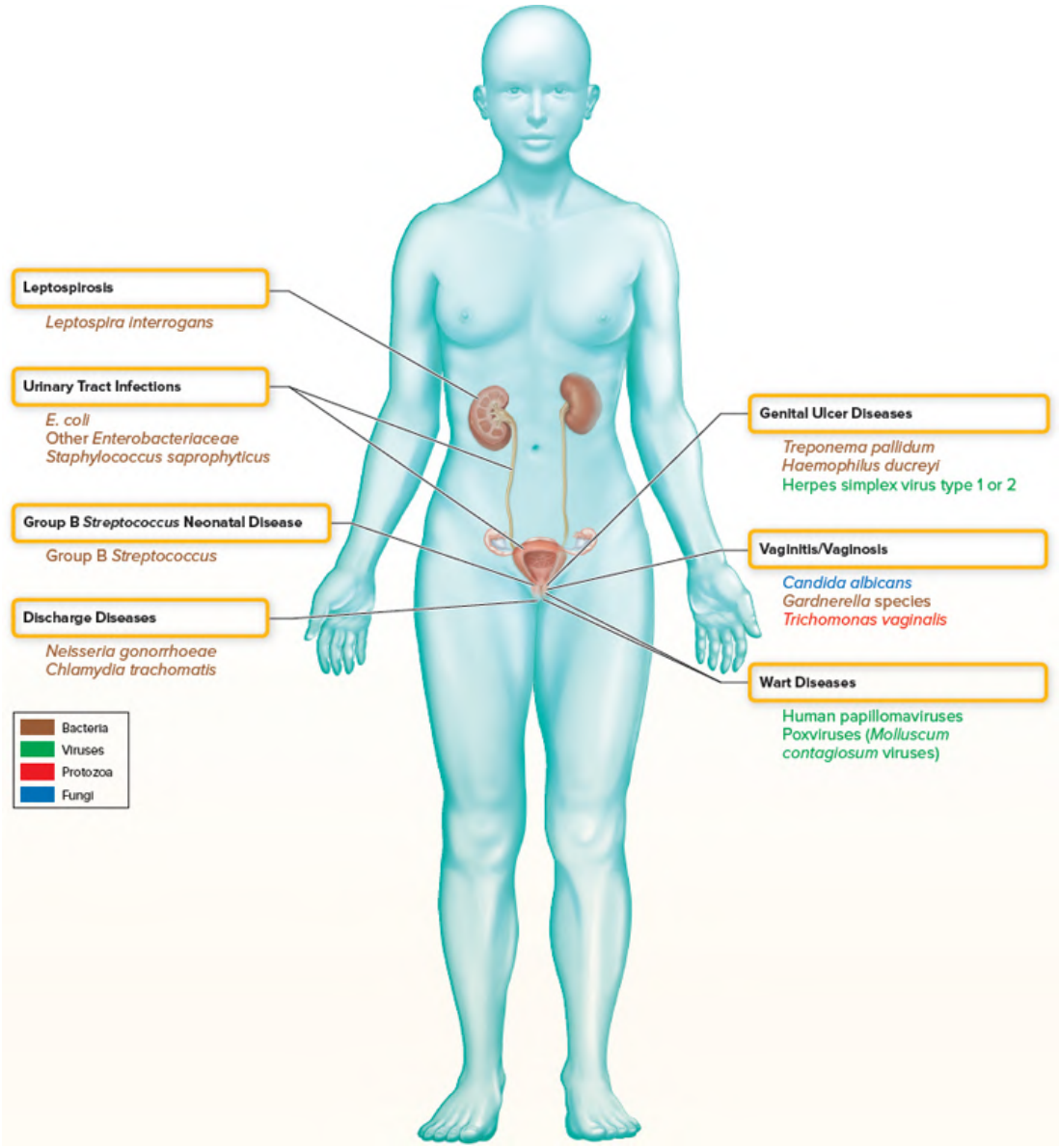
Summing Up

Taxonomic Organization Microorganisms Causing Disease in the Genitourinary Tract

Microorganism	Pronunciation	Disease Table
Gram-positive bacteria		
Staphylococcus saprophyticus	staf″-uh-lo-kok′-us sap′-pro-fit″-uh-cus	Urinary tract infection, 21.1
Gardnerella (note: stains gram-negative)	gard′-ner-el″-uh	Vaginitis or vaginosis, 21.4
Group B *Streptococcus*	groop bee′ strep′-tuh-kok″-us	Group B *Streptococcus* colonization, 21.8
Gram-negative bacteria		
Escherichia coli	esh′shur-eesh″-ee-uh col′-eye	Urinary tract infection, 21.1
Other *Enterobacteriaceae*	en″tur-oh-bak-tier′-ee-ay-cee	Urinary tract infection, 21.1
Leptospira interrogans (spirochete)	lep′-toh-spy′-ruh in-ter′-ruh-ganz	Leptospirosis, 21.2

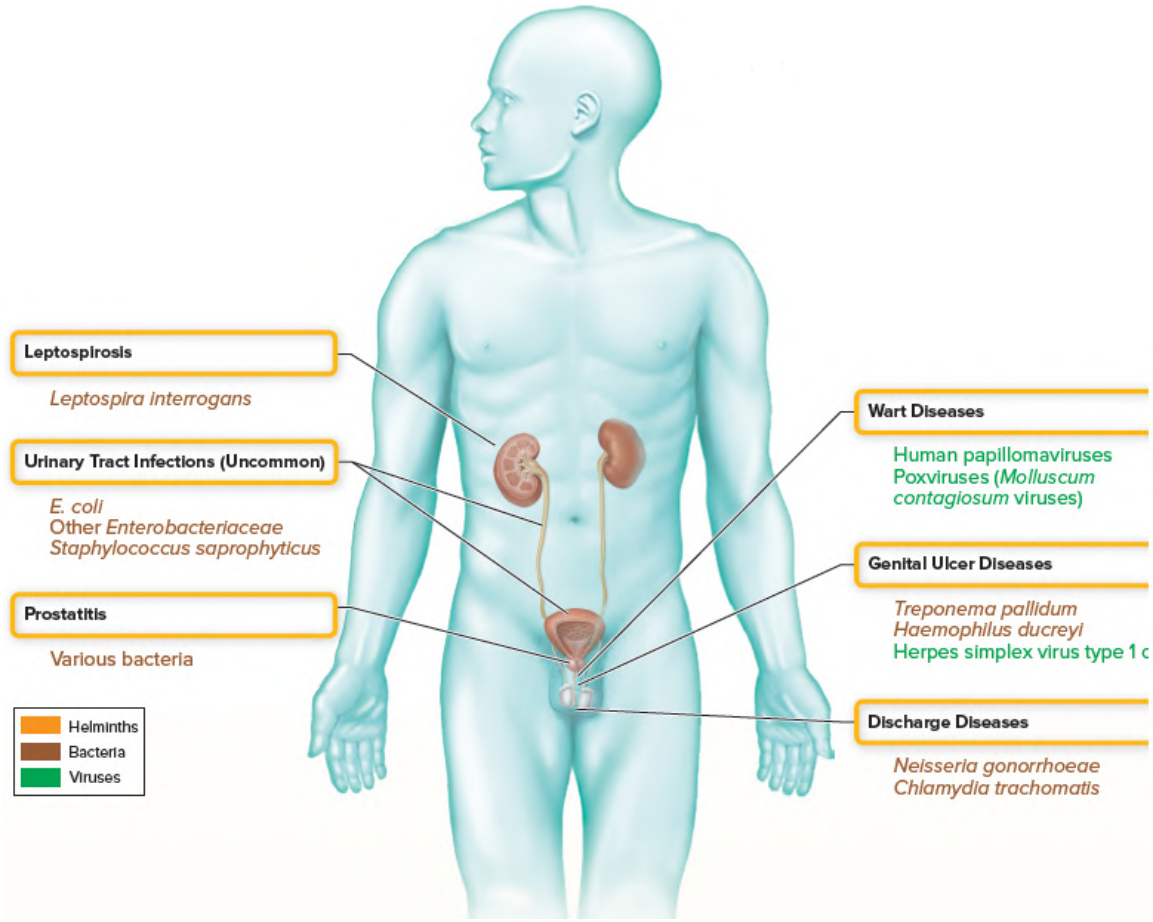
Microorganism	Pronunciation	Disease Table
Neisseria gonorrhoeae	nye-seer″-ee-uh′ gon′-uh-ree″-uh	Genital discharge disease, 21.3
Chlamydia trachomatis	kluh-mi″-dee-uh′ truh-koh′-muh-tis	Genital discharge disease, 21.3
Treponema pallidum (spirochete)	trep′-oh-nee″-ma pal′-uh-dum	Genital ulcer disease, 21.6
Haemophilus ducreyi	huh-mah′-fuh-luss doo-cray′-ee-eye	Genital ulcer disease, 21.6
DNA viruses		
Herpes simplex virus 1 and 2	hur′-peez sim′-plex vie′-russ	Genital ulcer disease, 21.6
Human papillomavirus	hew′-mun pap′-uh-loh″-muh-vie′-russ	Wart diseases, 21.7
Poxvirus	pox′-vie′-russ	Wart diseases, 21.7
Fungi		
Candida albicans	can′-duh-duh al″-buh-cans′	Vaginitis or vaginosis, 21.4
Protozoa		
Trichomonas vaginalis	trick″-uh-mon′-us vaj′-ih-nal″-us	Vaginitis or vaginosis, 21.4

Infectious Diseases Affecting
The Female Genitourinary System



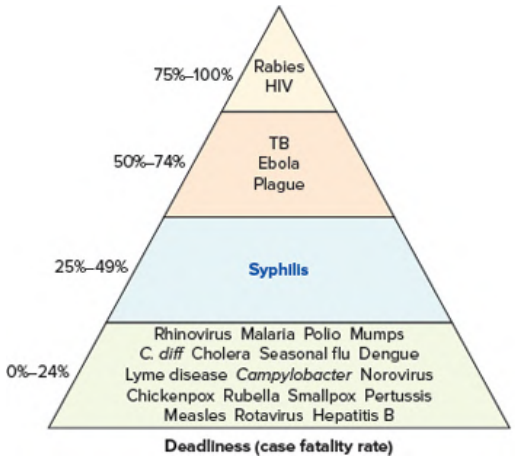
System Summary Figure 21.16*a*

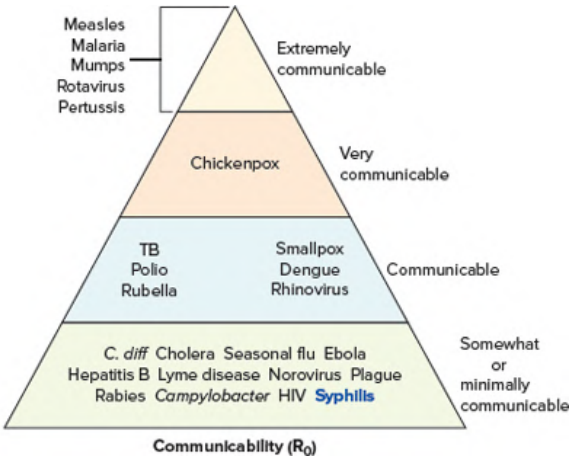
Infectious Diseases Affecting
The Male Genitourinary System



System Summary Figure 21.16*b*

Deadliness and Communicability of Selected Diseases of the Genitourinary Tract

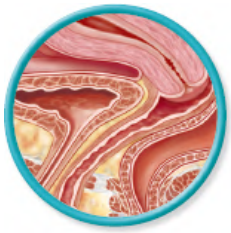




Chapter Summary

21.1 The Genitourinary Tract and Its Defenses

- The urinary system allows excretion of fluid and wastes from the body. It has mechanical and chemical defense mechanisms.
- The reproductive tract is composed of structures and substances that allow for sexual intercourse and the creation of a new fetus; protected by normal mucosal defenses and specialized features (such as low pH).



21.2 Normal Biota of the Genitourinary Tract

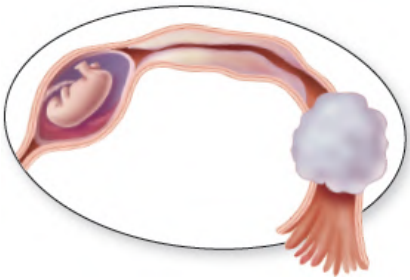
- Normal biota in the male reproductive and urinary systems are in the distal part of the urethra and resemble skin biota. The distal part of the female reproductive tract is also highly colonized, and it appears that the upper portion may have normal biota as well. Normal biota in the female reproductive tract changes over the course of her lifetime.

21.3 Urinary Tract Diseases Caused by Microorganisms

- **Urinary Tract Infections (UTIs):** Can occur in the bladder (*cystitis*), the kidneys (*pyelonephritis*), and the urethra (*urethritis*). Most common causes are *Escherichia coli*, and other members of the family *Enterobacteriaceae*, and also *Staphylococcus saprophyticus*. Community-acquired UTIs are most often transmitted from the GI tract to the urinary system. UTIs are a very common nosocomial infection.
- **Leptospirosis:** Zoonosis associated with wild animals that affects the kidneys, liver, brain, and eyes. Causative agent is spirochete *Leptospira interrogans*.

21.4 Reproductive Tract Diseases Caused by Microorganisms

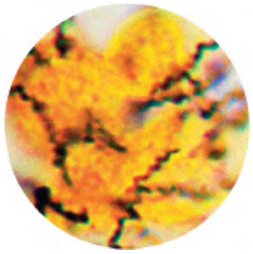
- **Discharge Diseases with Major Manifestation in the Genitourinary Tract**
  - **Gonorrhea:** Gonorrhea can elicit urethritis in males, but many cases are asymptomatic. In females, both the urinary and genital tracts may be infected during sexual intercourse. One sequela is salpingitis, which can lead to PID. Causative agent, *Neisseria gonorrhoeae*, is a gram-negative diplococcus.
  - ***Chlamydia:*** Genital *Chlamydia* infection is the most common reportable infectious disease in the United States. In males: an inflammation of the urethra. Females: cervicitis, discharge, salpingitis, and frequently PID.
    - Certain strains of *Chlamydia trachomatis* can invade lymphatic tissues, resulting in a condition called lymphogranuloma venereum.



- **Vaginitis and Vaginosis**
  - Vaginitis is most commonly caused by *Candida albicans*. Nearly always an opportunistic infection.
  - *Gardnerella* is associated with vaginosis; has a discharge but no inflammation. Could lead to complications such as pelvic inflammatory disease (PID).
  - *Trichomonas vaginalis* causes mostly asymptomatic infections in females and males. *Trichomonas*, a flagellated protozoan, is easily transmitted through sexual contact. Considered a "neglected parasitic disease" by the CDC.

Prostatitis: Inflammation of the prostate; can be acute or chronic. Not all cases established to have microbial cause, but most do.

- **Genital Ulcer Diseases**
  - **Syphilis:** Caused by spirochete *Treponema pallidum*. Three distinct clinical stages: *primary, secondary*, and *tertiary syphilis*, with a latent period between secondary and tertiary. Spirochete appears in lesions, blood during primary and secondary stages; is transmissible at these times, also during early latency period. Largely nontransmissible during "late latent" and tertiary stages. The syphilis bacterium can lead to congenital syphilis, inhibiting fetal growth and disrupting critical periods of development. This can lead to spontaneous miscarriage or stillbirth.
  - **Chancroid:** Caused by *Haemophilus ducreyi*, a pleomorphic gram-negative rod. Transmitted exclusively through direct—mainly sexual—contact.
  - **Genital Herpes:** Caused by two types of herpes simplex viruses (HSVs): HSV-1 and HSV-2. May be no symptoms, or may be fluid-filled, painful vesicles on genitalia, perineum, thigh, and buttocks. In severe cases, meningitis or encephalitis can develop. Patients remain asymptomatic or experience recurrent "surface" infections indefinitely. Infections in neonate and fetus can be fatal.



Source: CDC/Dr. Edwin P. Ewing, Jr.

- **Wart Diseases**
  - *Human papillomaviruses:* Causative agents of genital warts. Certain types infect cells on female cervix that eventually result in malignancies of the cervix. Males can also get cancer from these viral types. Infection with any human papillomavirus (HPV) is incurable. Genital warts can be removed, but virus is unaffected. Treatment of cancerous cell changes—detected through Pap smears in females—is an important part of HPV therapy. Vaccine for up to nine types of HPV is now available.
  - A virus in the family *Poxviridae* causes a condition called molluscum contagiosum. Can take the form of wartlike growths in the membranes of the genitalia, and can also be transmitted sexually.
- **Group B *Streptococcus* "Colonization"—Neonatal Disease:** Asymptomatic colonization of women by a beta-hemolytic *Streptococcus* in Lancefield group B is very common. It can cause preterm delivery and infections in newborns.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. Which of the following is on the CDC's "Urgent Threat" list because of its potential resistance to antibiotics? **a**. *Staphylococcus saprophyticus* **b**. *Trichomonas vaginalis* **c**. *Herpesvirus* **d**. *Enterobacteriaceae*	**2**. Some of the *E. coli* strains that are normal biota in the GI tract have evolved adhesive structures that can attach to the urinary tract, and cause disease there. What sequence of molecular events (in the bacteria) do you suppose led to this?	**3**. Many STIs are asymptomatic, or remain latent for long periods of time. What evolutionary advantage do you think that gives them?
Cell Structure and Function	**4**. Which of the following microbes is described as "dimorphic"? **a**. *Trichomonas vaginalis* **b**. *Gardnerella* **c**. *Candida albicans* **d**. *E. coli*	**5**. Three microorganisms in this chapter can be identified via a Gram stain. Name them and explain why this is possible for each of them.	**6**. The drug Flagyl (metronidazole) has the unusual characteristic of being effective against some bacteria as well as some protozoa. What cellular structure(s) would it be most likely to target?

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Metabolic Pathways	**7**. Which of the following bacteria is known to produce substances that raise the local pH? **a**. *Gardnerella* **b**. *E. coli* **c**. *Lactobacillus* **d**. *Leptospira*	**8**. What advantage does the particular life cycle of *Chlamydia* give to the pathogen?	**9**. Infection with *Gardnerella* seems not to induce inflammation in the vagina. Speculate on characteristics of the infection or the organism that could result in lack of inflammation.
Information Flow and Genetics	**10**. Which of the following bacteria behaves most similarly to viruses? **a**. *E. coli* **b**. *Chlamydia* **c**. *Neisseria* **d**. Haemophilus *ducreyi*	**11**. Using your knowledge of viral oncogenesis, outline the steps that the human papillomavirus might take from infection to cervical cancer.	**12**. The bacterium *Treponema palladium* is known to express an unusually low density of proteins in its outer membrane. Speculate on why that might contribute to its ability for long-term infection of the host.
Microbial Systems	**13**. Which part of the urinary tract has the most abundant and diverse microbiome? **a**. lower urethra **b**. upper urethra **c**. bladder **d**. kidneys	**14**. Why do many of the infections discussed in this chapter make the host more vulnerable to HIV infection? Provide detail in your answer.	**15**. What are some of the stimuli that can trigger reactivation of a latent herpesvirus infection? Speculate on why.

Impact of Microorganisms	**16**. Bacterial vaginosis is	**17**. A 38-year-old male living in Hawaii tried to rescue some	**18**. Use science to explain

	commonly associated with which organism? **a**. *Candida albicans* **b**. *Gardnerella* **c**. *Trichomonas* **d**. any of these	of the family's belongings as the basement flooded with water from a nearby flooded stream. Within a few days, he developed flulike symptoms, which cleared in a few days. Several weeks later he developed a painful headache and jaundice. The physician requested a urinalysis. What disease do you suspect and why?	this statement: Forensic microbiology can help identify sexual abuse of minors.
Scientific Thinking	**19**. Which of the following STIs is curable with antimicrobials? **a**. HPV **b**. herpes **c**. *Chlamydia* **d**. two of these	**20**. Why do you suppose a urine screening test for *Chlamydia* is more accurate for males than for females?	**21**. How would a laboratory technologist identify a case of vaginosis versus a case of vaginitis from a vaginal swab specimen?

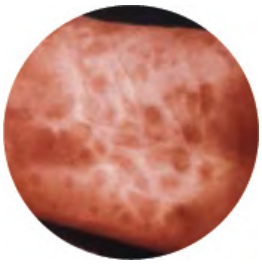
Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. **From chapters 18 and 21, figure 18.21 and figure 21.10*b***. Compare these two rashes. What kind of information would help you determine the diagnosis in both cases?

Source: CDC



Source: CDC

Resources are available through Connect, including interactive learning tools, animations, and more.

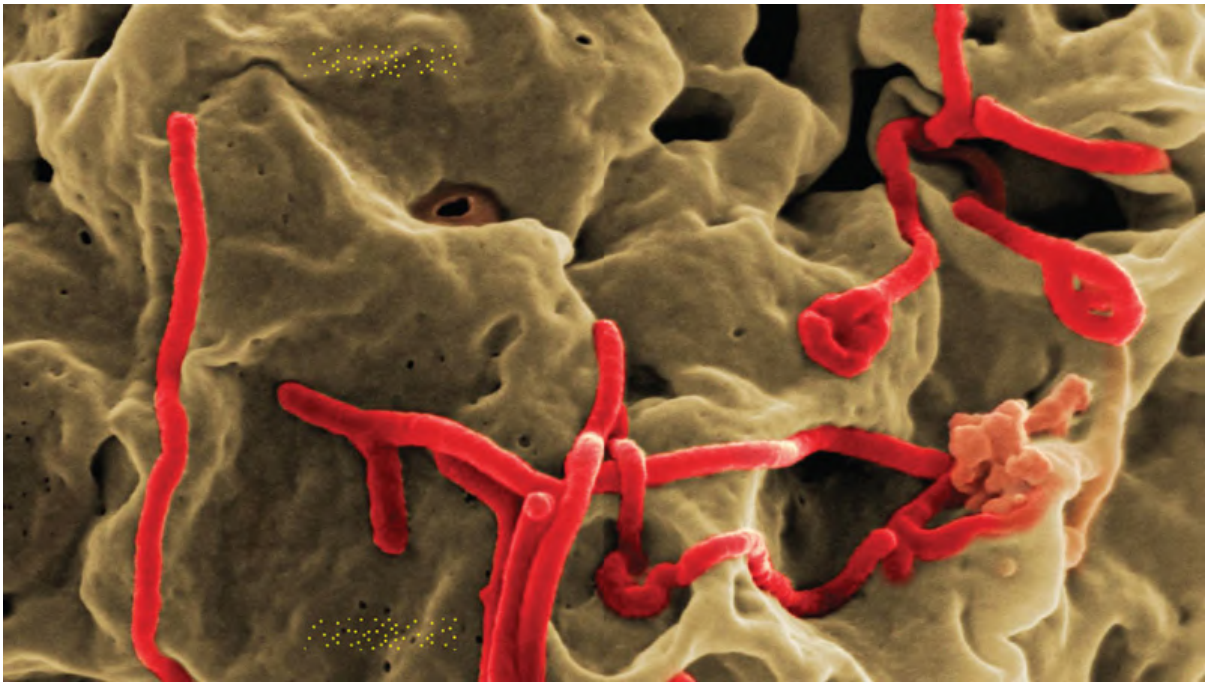
Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

22

One Health

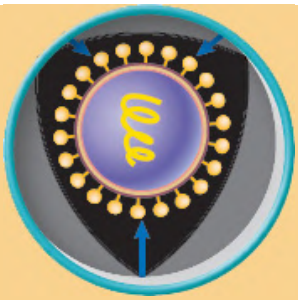
The Interconnected Health of the Environment, Humans, and Other Animals

By Ronald M. Atlas, University of Louisville, and Marjorie Kelly Cowan



Source: CDC/National Institute of Allergy and Infectious Diseases (NIAID) (virus); ©Dr. Parvinder Sethi (washing);
Source: U.S. Coast Guard/Chief Petty Officer John Kepsimelis, Atlantic Strike Team (oil spill); ©ERproductions Ltd/Blend Images LLC (health care worker)

IN THIS CHAPTER…



22.1 One Health

1. Draw your own representation or logo depicting how animal health, human health, and environmental conditions interact.

22.2 Animals and Infectious Disease: Zoonoses

2. Provide a definition for zoonosis.
3. Discuss the effects of deforestation and reforestation on the eventual emergence of Lyme disease in the northeastern United States.

22.3 The Environment and Infectious Disease

4. Differentiate between coliforms and *E. coli*.
5. Discuss the chain of environmental and biological events leading to the outbreak of hantavirus pulmonary syndrome in the southwestern United States in 1993.

22.4 Microbes to the Rescue

6. Define *biological oxygen demand*.
7. Outline the three phases in wastewater treatment.
8. Provide an example of a xenobiotic that has been made biodegradable.

CASE FILE

Ebola in the Hospital

In 2014, when the Ebola outbreak began to attract national attention, I was a nurse manager in the emergency department of a large urban medical center. My duties included developing policies and guidelines for nursing practice. I was also in charge of staff education initiatives.

When Ebola cases began to be diagnosed in the United States, it was clear as a nation that the health care system was unprepared to respond. With the help of federal funding, my institution began quickly enacting a plan for how to treat patients. We

developed protocols for patient care and infection control. We knew it would be essential to protect staff and other patients from exposure to the virus.

To identify patients who may potentially be infected, I developed a screening questionnaire for all patients who came to the emergency department. The triage team would ask about recent travel to high-risk countries. For patients with a positive travel screen, a specialized team then took over the patient assessment. All staff immediately donned personal protective equipment and the patient was escorted to a designated treatment room. The hospital's infectious disease team was immediately contacted for consultation. The patient was questioned about recent fever and other symptoms associated with Ebola.

Specific treatment rooms were designated for potential Ebola patients to keep them separated from other patients. In that room, we stored specialized isolation equipment for staff to use when caring for a potentially infected patient. Due to the complexity of donning the isolation gear and respirators, it took 15 to 20 minutes before staff could enter the treatment room—every time. They worked in pairs of two and spent 4-hour shifts in the patient room. Nurses were trained on how to manage patient care with limited access to supplies, as they could not leave the room without a lengthy doffing process to properly dispose of isolation gear.

An important part of training for nursing staff was how to manage infectious material and dispose of patient waste. Staff underwent special training for how to obtain and handle laboratory specimens safely. We developed strict protocols about the cleaning and disinfection of spilled patient waste. The trash bags leaving the patient room had to be carefully handled and the outsides sprayed with disinfectant prior to disposal in special containers.

Despite the months of preparation, our center never had to treat an Ebola patient. However, we have our protocols in place and keep staff trained with periodic drills to maintain a constant state of readiness. If a patient arrives displaying symptoms of the Ebola, we will be prepared to deliver safe care for the patient and protect our staff.

- Why did waste disposal require special attention?
- Why might keeping the staff of this emergency department trained in Ebola isolation be beneficial?

Case File Wrap-Up appears at the end of the chapter.

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22.1 One Health

We live in a complex and ever-changing world in which human health, animal health, and environmental health are tightly connected. So much today is global in nature—global travel, global economy, global movement of livestock, global climate change. It is no wonder, then, that human health, animal health, and environmental health are inextricably interrelated—and that we are beginning to appreciate that the health of all life on earth is connected.

The concept that the health of humans, animals, and the environment should be viewed in a holistic (all-inclusive) way is called *One Health*. This is not a new idea for microbiologists who have always needed to consider all the causes that lead to the spread of infectious diseases and the ability to control them. Pioneering microbiologists like Louis Pasteur and Robert Koch recognized the interconnection between animal and human health. They carried out research on diseases such as anthrax and rabies, which affected both humans and other animals. Medical and veterinary educators of that era also freely crossed the boundaries of human and animal health. In the words of Rudolf Virchow, a leading medical educator of the late 1800s, "Between animal and human medicine there are no dividing lines—nor should there be."

The contemporary revival of the *One Health* concept has expanded beyond the human—animal health interface to encompass the health and sustainability of all the world's ecosystems, including plant life. Microorganisms circulate among human hosts, animal hosts, and environmental reservoirs. Disruption of the environment can lead to

transmission of pathogens to animals and humans. Evolution of new microbial traits can occur in response to changes in the environment. In addition, reservoirs of pathogens and virulence traits can persist in the environment, poised to enter humans and other animals at an opportune time.

Global Mixing Bowl

One way to think about *One Health* is to picture three overlapping spheres representing humans, animals, and the environment **(figure 22.1)**. A change in any one of these spheres impacts the others and with that the microbes that each contains. The mixing of microbes in different animal hosts and under different environmental conditions can foster the evolution of new and potentially deadly pathogens. Human activities in particular can promote the emergence of infectious diseases, for example, through ecological disturbances and movement of animals. This occurs frequently for some microbes. Consider the example of influenza viruses, in which the mixing of different strains of influenza viruses in birds, swine, and humans results in the evolution of new recombinant strains with the potential to spread globally almost every year (see chapter 19 for the details of how this works).

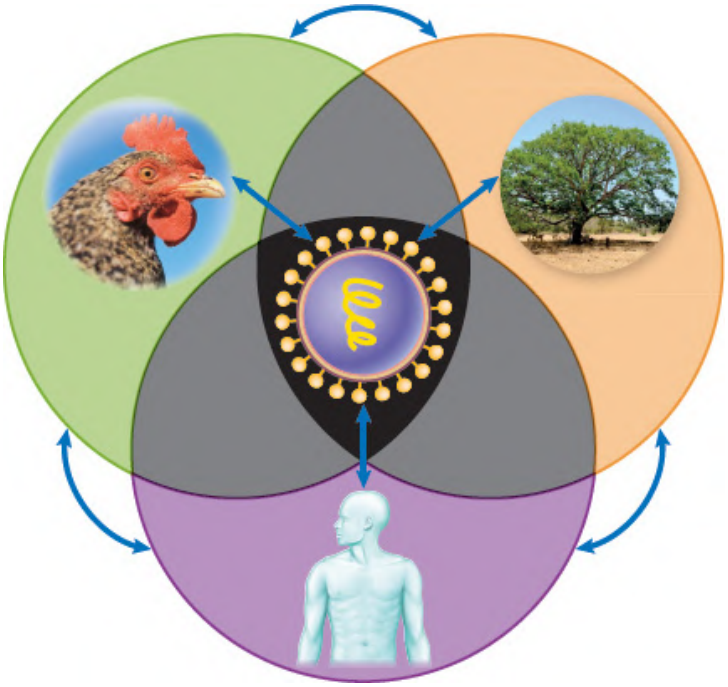


Figure 22.1 **One Health**.
©Pixtal/AGE Fotostock (chicken); ©OGphoto/Getty Images (tree)

We use the term "mixing bowl" because within the last few decades, which is no time at all in evolutionary terms, humans have created an incredible array of unique opportunities and challenges for microbes. For example, we have the ability to fly between continents in the space of a few hours (with our microbes). We have the ability to transport bacteria from one ocean to another in the bilge water of tanker ships. We use antimicrobial

drugs that put selective pressures on microbes to an unprecedented extent. The result has been the dawn of a new era of emerging and reemerging diseases **(table 22.1)**.

The patterns of newly emergent infectious diseases, such as SARS (in 2003) and West Nile fever, reflect contemporary demographic and environmental changes—more people living closer together and in greater contact with wild and domesticated animals, and habitat destruction. Another issue is the multipurpose use of resources—such as using the same water for waste disposal, irrigation, and human consumption. These changes have been driving repeated pathogen spillover from wildlife and the spread of newly evolved pathogens in dense human populations. Over the past four decades, the

Table 22.1 Emerging and Reemerging Infectious Diseases - 2017*

Babesiosis	Protozoan tick-borne disease
Bartonella henselae	Bacterial cat scratch disease
Chikungunya and Mayaro	Two arbovirus diseases that have recently moved into the Americas
Clostridium difficile	Endospore-forming bacterium causing severe GI tract infection
Crimean Congo hemorrhagic fever	Tick-borne viral disease
Ebola virus disease	Hemorrhagic fever; large epidemic in 2014–2015
Ehrlichiosis	Tick-borne bacterial disease
Elizabethkingia	Common environmental bacterium causing HAIs
Lassa fever	Viral hemorrhagic fever, confined to Africa currently
Leishmaniasis	Protozoan disease of skin and tissue
Marburg virus	Viral hemorrhagic fever, similar to Ebola
Measles	Threatens to reemerge due to lower vaccination rates
MERS	Middle East respiratory syndrome
Mumps	Threatens to reemerge, partially due to lower vaccination rates
Nipah virus	Encephalitis and respiratory illness; Asia and Africa
Rift Valley fever	Arbovirus disease; mostly in Africa
SARS	Sudden acute respiratory syndrome, emerged suddenly in 2003
Shiga-toxin-producing *E. coli*	Foodborne and water-borne infection
Zika virus	Mosquito-borne illness affecting fetuses most severely

*List compiled from CDC, WHO, and other sources

Source: U.S. Fish & Wildlife Service/Wyman Meinzer

rate of infectious disease emergence has increased in both humans and animals. Just as troubling are reemerging diseases. These are infections that have been minimized previously through vaccinations, vector controls, or sometimes through no human intervention at all, yet have resurged again—or threaten to resurge—in the human population. You will note that table 22.1 contains both measles and mumps for this reason.

22.1 LEARNING OUTCOMES—Assess Your Progress

1. Draw your own representation or logo depicting how animal health, human health, and environmental conditions interact.

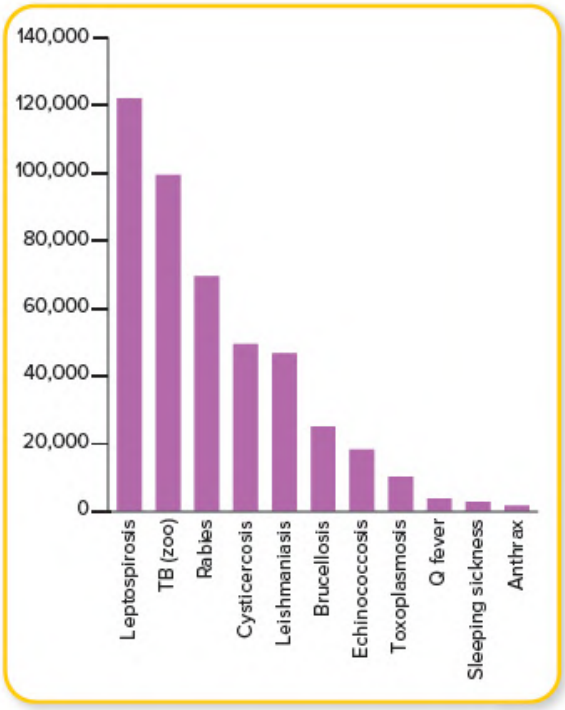


Figure 22.2 **Annual number of human deaths worldwide caused by zoonoses**.
Source: International Livestock Research Institute

22.2 Animals and Infectious Disease: Zoonoses

The term *zoonosis* was initially coined to describe the transfer of disease-causing microorganisms from vertebrate animals to humans. Worldwide, diseases that humans acquire from animals result in millions of cases of illness and perhaps a million deaths each year **(figure 22.2)**. Today, however, we also recognize that microbes can transfer back *and* forth between animals and humans. If you are trekking with gorillas in Rwanda, you may want to worry more about the danger of transmitting disease-causing microbes from yourself to the gorillas than about your own safety.

Over the last three decades, approximately 75% of newly emerging human diseases have been zoonotic and many have come from or through wildlife. Given that there are 50,000 known vertebrate species and assuming that each has 20 endemic viruses (which is likely to be an underestimate; bats alone harbor 20,000), there probably are more than

1 million vertebrate viruses. Only 2,000 or so viruses have been described, so 99.8% of vertebrate viruses remain to be discovered. These calculations begin to give you an appreciation of the enormous potential for future zoonotic diseases to emerge.

Once introduced into human populations, some zoonotic agents can then spread from human to human—in some cases causing global pandemics. For example, the HIV/AIDS epidemic, which has resulted in more than 40 million human cases worldwide, had its origin as a chimpanzee retrovirus that jumped species and then adapted itself to human-to-human transmission. Such epidemics are extremely difficult to control and can have high rates of morbidity and mortality.

©William Leaman/Alamy Stock Photo

Lyme Disease—Deer, Ticks, and Environmental Change

During the European colonization of North America, forests in New England were cleared for farming **(figure 22.3)**. At the same time, deer were hunted almost to extinction. However, during the 20th century, environmental conditions changed in the northeastern United States. People abandoned farming, and migration to cities increased. Farmland was converted back to woodland. Deer, protected from hunters and predators, proliferated. White-footed mice also flourished in the forests. With the increase in deer and mouse populations, the deer tick, which is the reservoir host for *Borrelia burgdorferi*, also thrived.

In 1976, a number of children living near Lyme, Connecticut, began developing arthritis-like symptoms. Because rheumatoid arthritis is not an infectious disease, the cluster of children with these symptoms was unusual, suggesting an underlying microbial cause. Soon this disease would be recognized as a bacterial infection caused by the spirochete *Borrelia burgdorfori* and given the name *Lyme disease*. It is now the most commonly reported arthropod-borne illness in the United States and Europe and is also found in Asia **(figure 22.4)**. The life cycle of the bacterium, depicted in figure 18.15, involves ticks, small mammals, and large mammals. The reasons for

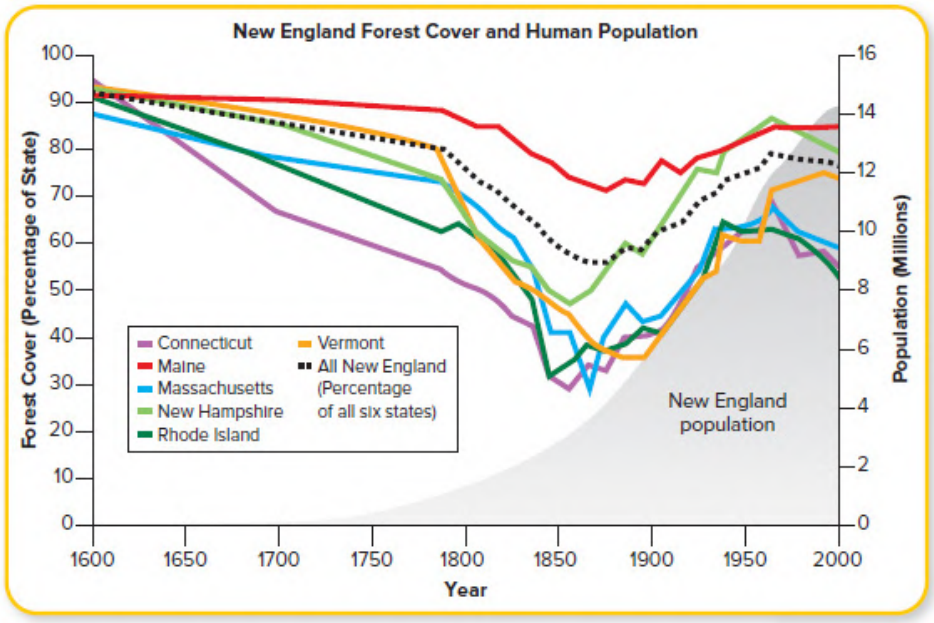


Figure 22.3 **Changes in amount of forest cover and human population over time in New England**. Note that forest cover decreased greatly in the 1700s and 1800s but started to recover around the turn of the 20th century.
Source: CDC

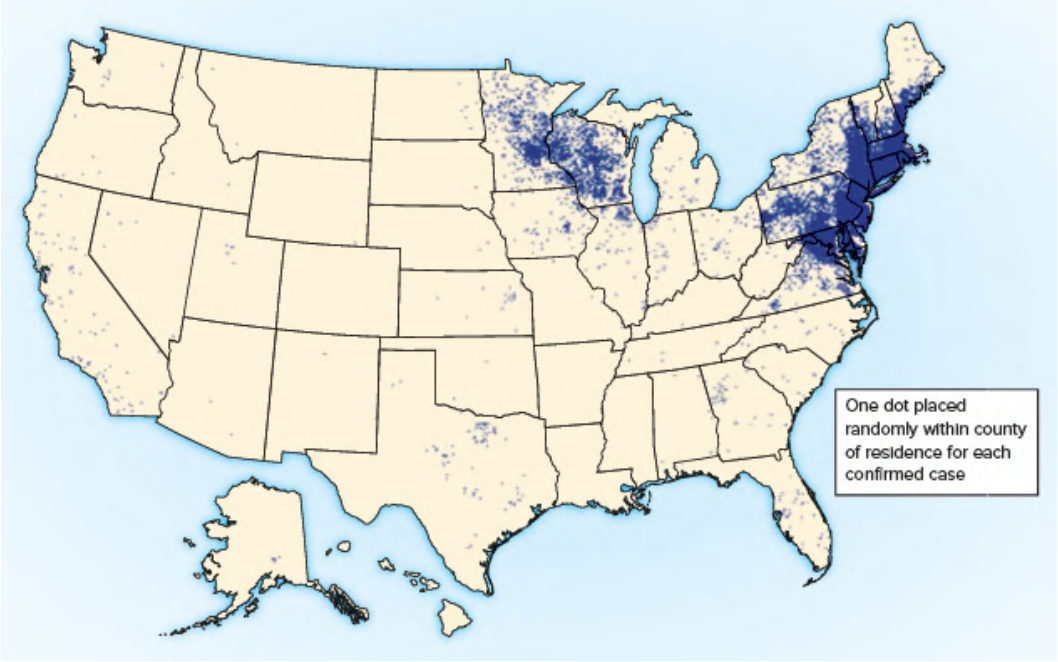


Figure 22.4 **A map of Lyme disease in the United States**.

the emergence of Lyme disease reflect changing demographics and human behavior, including efforts to restore disrupted environments.

As suburban areas of the northeastern United States became heavily populated, and growing numbers of housing developments backed up to woodland areas, conditions became ideal for *B. burgdorferi*–infected ticks to come into frequent contact with humans.

During the past 40 years, the infection has continued to spread in the northeastern United States. Climate change (covered later in this chapter) is also influencing the epidemiology of Lyme disease. Scientists mapped the places where temperatures have increased in North America between 1971 and 2010, and found a corresponding increase in the tick population in those areas. Warmer conditions mean that larval ticks are more likely to survive to maturity. This is another reason that Lyme disease—and a host of other tick-borne diseases—are on the rise in temperate climates.

We also have examples of deforestation leading to changing infection patterns. In Malaysia, humans are increasingly becoming infected with a form of malaria that previously infected mainly macaques (Old World monkeys). Researchers found that the jump from macaques to humans was correlated with deforestation activities. This brought macaques and humans closer together, close enough to "share" mosquitoes.

Macaque monkey
©kjorgen/Getty Images

22.2 LEARNING OUTCOMES—Assess Your Progress

2. Provide a definition for zoonosis.
3. Discuss the effects of deforestation and reforestation on the eventual emergence of Lyme disease in the northeastern United States.

22.3 The Environment and Infectious Disease

Clean Water and Infectious Disease

Every drop of water we drink contains thousands of microbes. This includes the nearly 30 million gallons of bottled water consumed in the United States daily. Fortunately, most

municipal and bottled water consumed in the United States is free of human pathogens—but this is not the case everywhere.

While the United States and many other nations take great care to ensure the safety of potable (drinkable) water, this is not the case worldwide—particularly in rural communities of developing countries. In fact, a large percentage of the world's population lack access to safe drinking water. According to the World Health Organization (WHO), more than 1 billion people lack access to clean water, and 2.6 billion people lack access to basic sanitation **(figure 22.5)**.

Poor water sanitation and a lack of safe drinking water take a greater human toll than war and terrorism. Waterborne diarrheal disease alone accounts for 4.1% of the daily global disease burden, and more than 3.4 million deaths each year are attributable to unsafe drinking water—primarily among children in developing countries. According to a U.N. report, 4,000 children die each day as a result of infectious diseases acquired by drinking contaminated water. The poorest and most vulnerable members of society bear the greatest burden of waterborne and diarrheal disease.

Figure 22.5 **Much of the world's population uses the same source of water for many different purposes, including bathing and drinking**.
©Dr. Parvinder Sethi

Drinking Water Quality—Microbiological Safety of Potable Water

One of the reasons potable water is generally safe in the United States and many developed nations is that great care is taken to disinfect the water and test it for bacterial content before it is consumed. Water is generally disinfected by filtration or by treatment with chlorine to ensure that it is free of potential pathogens. A typical water purification process

is shown in **figure 22.6**. When there is a breach in the water delivery system and the quality of the water cannot be ensured, a boil-water advisory is issued; that is, the public is told to boil the water to kill most potential pathogens before drinking the water.

Routine bacterial monitoring of potable waters involves testing for **indicator bacteria**, that is, microorganisms normally found in mammalian gastrointestinal tracts whose presence would indicate likely contamination with fecal matter **(figure 22.7)**. Testing of the actual pathogens of concern (protozoa like *Giardia* and *Cryptosporidium;* bacteria like *Salmonella, Shigella, Vibrio*, and *Campylobacter;* and viruses like hepatitis A and Norwalk) would be difficult and may not provide the necessary margin of safety given the large volumes of water that are distributed. The intestinal bacteria most useful in the routine monitoring of microbial pollution are gram-negative rods called **coliforms** (*E. coli* and similar bacteria), which are gram-negative bacteria that ferment lactose and produce gas. These coliform bacteria, as well as fecal streptococci, which also are useful indicator bacteria, survive in natural waters but do not multiply there. Finding them in high numbers thus indicates recent fecal contamination and the possible presence of intestinal pathogens. When significant numbers of coliform bacteria or fecal streptococci are detected, the water is not considered safe to drink.

Although total coliform counts are useful indicators for ensuring water safety, they can be misleading. In many circumstances, it is important to differentiate between coliforms that can be naturally found in soils and uncontaminated waters (*Enterobacter, Klebsiella, Citrobacter*) and **fecal coliforms** (*E. coli*) that live mainly in human and animal intestines. Although most *E. coli* strains are not pathogenic, they almost always come from a mammal's intestinal tract, so their presence in a sample is a clear indicator of fecal contamination.

Sometimes the public and the media confuse *coliform* with *E. coli*. This can cause unwarranted concerns. For example, in 1995, there was a minor panic when media outlets reported that iced tea from restaurants in Cincinnati, Ohio, contained significant numbers of "fecal coliforms." One headline read, "Iced Tea Worse Than River

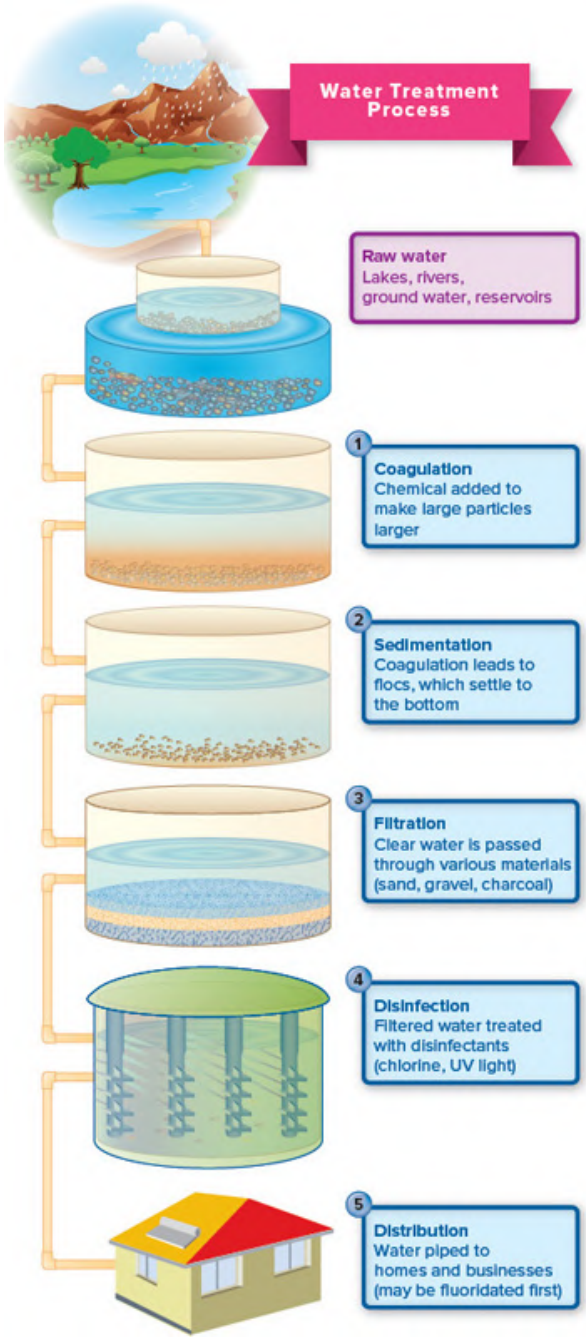


Figure 22.6 **A common pathway for water purification**.

Medical Moment

Plastic Bottles for Clean Water

Every week around the world, 30,000 people die from lack of clean water. Ninety percent of these are children under 5 years old. Clean water—taken for granted in the developed world—is a resource more precious than gold on the rest of the planet. Even though we take it for granted, the processes and infrastructure used to deliver it to us are complex and expensive. How can we export those to other

settings? Maybe we don't have to. Solar water disinfection is a method of safely disinfecting drinking water by simply placing contaminated water in a transparent plastic bottle and leaving it in the sun for 6 hours. Ultraviolet light kills bacteria and parasites and inactivates viruses, making the water safe. This technique has been used all over the world in impoverished nations where citizens have no access to clean drinking water, and it has proven to be an effective way of preventing diarrheal disease.

Q. Would you suspect that the water treated this way becomes sterile?

Answer in Appendix B.

©Khalil Senosi/AP Images

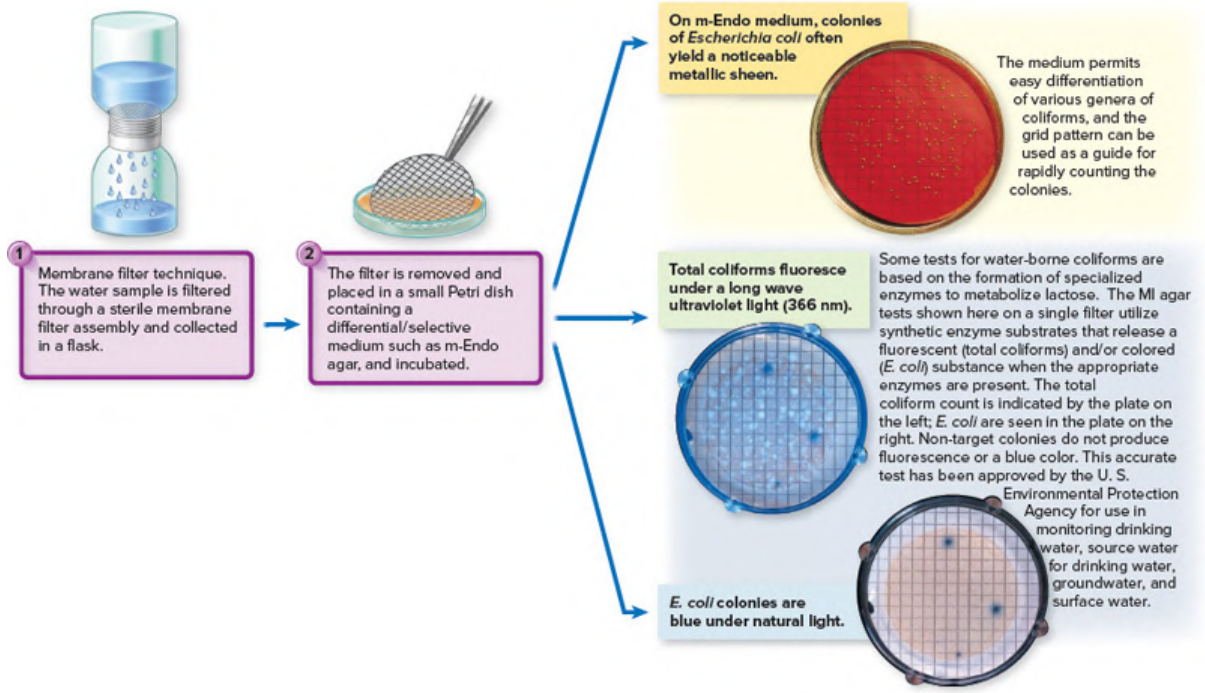


Figure 22.7 Rapid method of water analysis for coliform contamination.

©Kathleen Talaro (m-Endo medium); Reprinted from EPA Method 1604 (EPA-821-R-02-024) Courtesy Dr. Kristen Brenner from the Microbial Exposure Research Branch, Microbiological and Chemical Exposure Assessment Research Division, National Exposure Research Laboratory, Office of Research and Development, U.S Environmental Protection Agency (ultraviolet light), (natural light)

Water." Despite such alarmist reports, no one became sick from drinking the iced tea. When scientists did more detailed testing, they found that the predominant species were *Klebsiella* and *Enterobacter*, both of which are commonly found on plants, including tea leaves. Clearly, the total coliform count was misleading in this situation.

No doubt the coliform test that has been used for decades will soon be replaced with more direct testing for fecal contamination, either through specific testing for *E. coli* or through new technologies being developed such as **biosensors**, machines that detect structures on specific microbes and transmit electrical or physical signals that are easily read.

Microorganisms are just one of several substances in water sources that are closely monitored. The Safe Drinking Water Act is a federal law dating from the 1970s that guides the Environmental Protection Agency and state and local jurisdictions on water quality. In addition to microorganisms, the levels of several different chemicals is monitored. Flint, Michigan, is a city in the news because of a crisis in its water supply. Starting in 2014 officials changed the source of the city's water, leading to large-scale leaching of lead from the city's aging water pipes. City residents are experiencing major health effects from the elevated lead levels. In addition, the switch in water sources is thought to be responsible for an increased incidence of *Legionella* cases in the city.

Medical Moment

***Cryptosporidium* in Your Tap Water?**

For those who are immunocompromised, even potable water may carry risk. *Cryptosporidium*, as you recall from chapter 20, is an example of a zoonotic disease. The protozoan infects humans when they consume water that has been contaminated by infected animal feces. The protozoa represent a risk for those who are neutropenic, such as patients on immunosuppressant drugs (such as cancer patients or posttransplant patients), with disorders of immunity, or with AIDS.

Because municipalities have different standards for water sanitation, *Cryptosporidium* may be present in drinking water in some regions. For the general population, this poses a minimal risk. Immunocompromised persons should consult with their public health department as further precautions may be necessary to prevent cryptosporidiosis.

Q. What further precautions can vulnerable people take if their municipal water supply does not eliminate *Cryptosporidium?*

Answer in Appendix B.

Microbial Contamination of the Food Chain

You have only to look at the news to find the latest foodborne outbreak of disease, and many of these are described in chapter 20. *E. coli* outbreaks from beef are caused when cattle are infected, and *E. coli* outbreaks associated with vegetables are generally triggered when crops are watered with unclean water containing fecal matter

or, possibly, when infected animals or birds have access to the crops. Many helminth diseases are acquired through a contaminated environment (organisms burrowing through skin or being being swallowed) or from ingesting infected food. As has been stated already, the concept of *One Health* is an old one indeed; but in many parts of the research and regulatory communities, the last few decades have seen increasing compartmentalization so that the connections were not as well appreciated as they have now become. While pathogens can easily be transmitted from water sources, the food we eat can also carry harmful microorganisms.

Hepatitis A virus is a pathogen that can be transmitted via water or food contaminated with human fecal matter. Hepatitis A virus is widespread throughout the world. Transmission rates are very high in areas where there is no sewage treatment. Hepatitis A viral infections have an especially high incidence in developing countries and rural areas. In rural areas of South Africa, the seroprevalence is 100%. This means that all of the people in those areas have been infected with hepatitis A, although many are asymptomatic and show no signs of disease even though they are carriers of the hepatitis A virus.

Hepatitis A infections also occur in developed countries. Approximately 25,000 cases of hepatitis A viral infections are reported in the United States every year—but because many cases are asymptomatic or not reported, the actual annual incidence of hepatitis A infections is estimated to be more than 260,000. In 2003 a batch of green onions in a Pennsylvania location of a Mexican restaurant chain was the source of the largest outbreak of hepatitis A in United States history, with over 600 people sickened and 4 deaths.

©Raul Taborda/Alamy Stock Photo

Global Climate Change and Biology

Over the past 40 years, the concentrations of carbon dioxide and methane in the atmosphere have increased. They are now at their highest levels since the first humans set foot on earth **(figure 22.8)**. As a result of the increased concentrations of carbon dioxide and methane, the climate is changing. Because these compounds absorb long-wavelength energy coming from the earth's surface and reflect some of it back to earth, they cause a rise in temperatures—so things get warmer, much like in a greenhouse.

Climate change can be detected as changes in weather—more rainfall and generally higher average temperatures over the year with more variable weather patterns. Most scientists argue that human activities are the root cause and that if we do not change course there will be devastating impacts on human, animal, and environmental health.

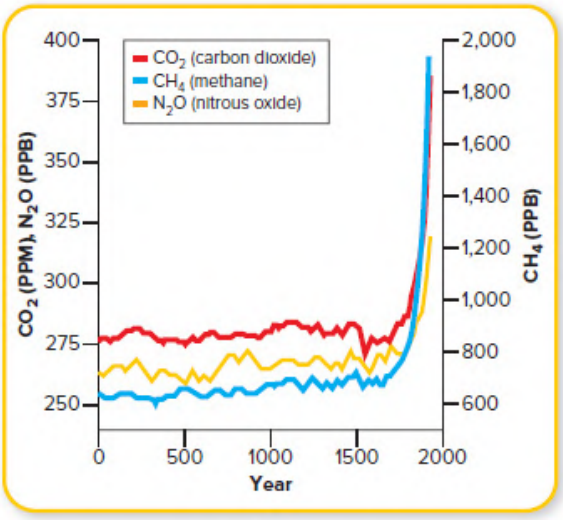
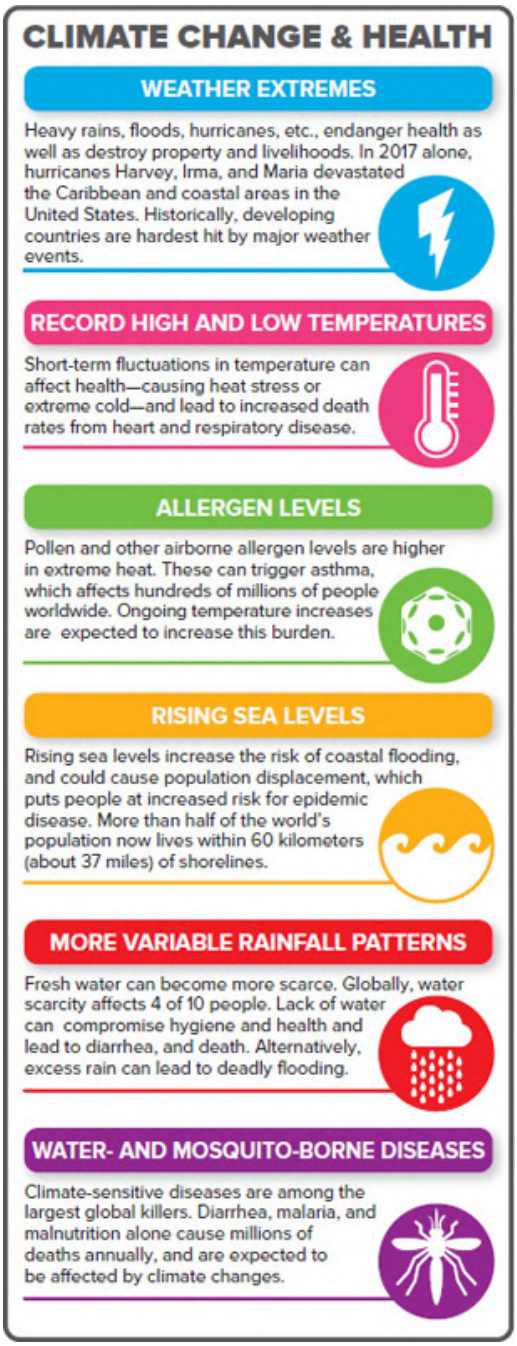


Figure 22.8 The increase in greenhouse gas concentrations over the past 2,000 years. PPM = parts per million; PPB = parts per billion.

©Shawna Lemay/Moment Open/Getty Images

We hear quite frequently about rising sea levels and the threat of devastating storms if we do not halt the atmospheric buildup of greenhouse gases **(figure 22.9)**. We should also understand that climate change may increase the global burden of disease. Drought and lack of water are likely to get worse in some places while in other areas increased rainfall likely will lead to increases in


CLIMATE CHANGE & HEALTH
WEATHER EXTREMES
Heavy rains, floods, hurricanes, etc., endanger health as well as destroy property and livelihoods. In 2017 alone, hurricanes Harvey, Irma, and Maria devastated the Caribbean and coastal areas in the United States. Historically, developing countries are hardest hit by major weather events.
RECORD HIGH AND LOW TEMPERATURES
Short-term fluctuations in temperature can affect health—causing heat stress or extreme cold—and lead to increased death rates from heart and respiratory disease.
ALLERGEN LEVELS
Pollen and other airborne allergen levels are higher in extreme heat. These can trigger asthma, which affects hundreds of millions of people worldwide. Ongoing temperature increases are expected to increase this burden.
RISING SEA LEVELS
Rising sea levels increase the risk of coastal flooding, and could cause population displacement, which puts people at increased risk for epidemic disease. More than half of the world's population now lives within 60 kilometers (about 37 miles) of shorelines.
MORE VARIABLE RAINFALL PATTERNS
Fresh water can become more scarce. Globally, water scarcity affects 4 of 10 people. Lack of water can compromise hygiene and health and lead to diarrhea, and death. Alternatively, excess rain can lead to deadly flooding.
WATER- AND MOSQUITO-BORNE DISEASES
Climate-sensitive diseases are among the largest global killers. Diarrhea, malaria, and malnutrition alone cause millions of deaths annually, and are expected to be affected by climate changes.

Source: WHO

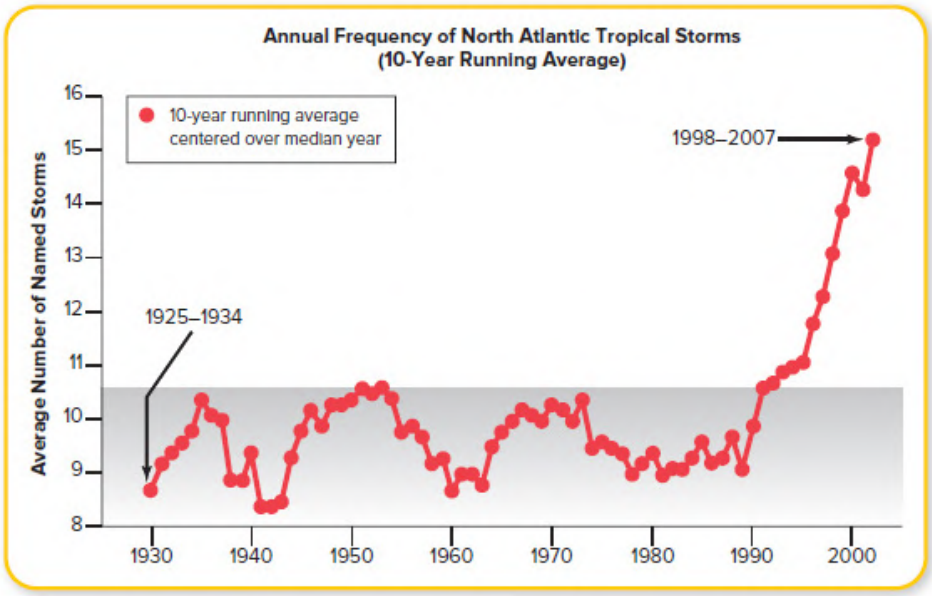


Figure 22.9 **Number of named tropical storms in the North Atlantic**.

some pathogens and vectors of infectious disease. *Vibrio cholerae*, for example, thrives in warm waters. There are predictions that the global incidence of cholera will increase as a result of global warming. Mosquito populations likely also will increase in areas of increased rainfall and with them the global incidence of infectious diseases like yellow fever and malaria. Mathematical models show that a 2- to 3-degree rise in temperature could increase the number of people affected by malaria by several hundred million.

The United States saw an elegant example of how weather patterns can lead to a sudden outbreak of disease in 1993, when hantavirus suddenly began killing healthy young adults in the Four Corners area of the American Southwest. The virus had never before been known to cause the severe, overwhelming respiratory distress and death seen in that outbreak (previously, it was known as a kidney pathogen). After the outbreak was contained and research conducted into the causes of the outbreak, the weather phenomenon El Niño, a warming trend that originates over the Pacific Ocean, looked like the ultimate culprit. Here's how: In the year preceding this outbreak, El Niño caused wetter winter conditions in the Southwest. This led to a great increase in the pine nut population, a favorite delicacy of deer mice. By the spring and summer of 1993, there was a very large deer mouse population. Deer mice are the reservoir for hantavirus, and the people who became infected all had exposure to large amounts of deer mouse feces and/or urine. When these substances dried out and were aerosolized, they delivered lethal doses of the virus to the lungs of humans. Note the interconnectedness of the environment (weather patterns), plant world (pine nuts), animal world (deer mice), and humans in this event.

Climate change will undeniably have far-reaching effects, where the change in temperature of even a few degrees can start a cascade of events that can easily lead to unusual occurrences of infectious disease. When we discussed the Zika virus earlier in this book, we pointed out that a warming climate has carried its mosquito vector to places

which were previously too cool for it. We can expect future movements of vectors and the diseases they carry to result in increased disease outbreaks if climate change continues on its current course.

22.3 LEARNING OUTCOMES—Assess Your Progress

4. Differentiate between coliforms and *E. coli*.
5. Discuss the chain of environmental and biological events leading to the outbreak of hantavirus pulmonary syndrome in the southwestern United States in 1993.

Source: NPS photo by John Good

22.4 Microbes to the Rescue

While the presence of pathogens in the environment is clearly a threat to human and animal health, non-disease-causing microbes are of extreme importance in *saving us* from the wastes and pollutants that we produce. In New York City alone, wastewater treatment plants, with the help of microbes, treat over 1 billion gallons of sewage a day, removing an average of 65% of the organic matter. Urban solid waste in the United States amounts to roughly 150 million tons per year. Microbes also help us by cleaning up our pollutants, such as industrial chemicals and oil spills, both when we specifically "ask them to" and when we do not.

©Andrew Penner/Getty Images

Liquid Waste Treatment—Sewage

Each year, trillions of gallons of human waste are released into the environment. Low levels of human wastes can be accommodated because natural waters have self-purification capacities. The naturally occurring nonpathogenic microbes in those ecosystems can degrade the wastes. However, in areas of high population densities, the wastes can be too great. When waters are overwhelmed by concentrated inputs of organic matter (including

microbes) they exhibit a high demand for oxygen. Exhaustion of the dissolved oxygen occurs and the water becomes putrid and septic. Pathogens also spread. In areas of high population densities, therefore, it is generally necessary to treat the waste to reduce risk. Several different sewage treatment processes are used, depending upon the population density and the environment into which the wastewater effluents can be released.

Many rural and suburban areas with relatively low population densities rely on septic tanks. These are containers into which the sewage flows. The solid material settles and is subject to microbial decomposition. The liquid, with its greatly reduced organic content, is allowed to overflow and is distributed through a series of perforated pipes into the surrounding soil. As long as the houses are far enough apart, the reduction of organic matter is sufficient to reduce the concentrations of organic matter being released to levels that can be accommodated without causing environmental harm. Such systems, however, are inadequate to handle the wastes from densely populated communities.

For some municipalities, the answer is to pump the sewage away—sometimes offshore into the ocean away from beaches and shellfish beds and sometimes to agricultural fields away from the city. Mexico City, for example, only treats a small percentage of the wastewater it collects, and only has a maximum capacity for treating 25% of the sewage generated by the 25 million inhabitants of the city. The remaining untreated sewage is pumped to agricultural areas—largely alfalfa but also barley, corn, and wheat fields—that collectively cover an area the size of the state of Rhode Island.

Most other major cities—from New York City to Johannesburg, South Africa—have extensive sewage treatment plants. The border cities of Tijuana in Mexico and San Diego in the United States have developed an international agreement for jointly treating their wastewater. Sewage from Tijuana is piped across the border to a treatment plant on the U.S. side of the border. This plant also treats the wastewater for San Diego.

The sewage that is piped to wastewater treatment plants is subjected to physical, biological (microbial), and sometimes chemical processes. The main aim of wastewater treatment is to reduce the organic matter content, that is, to lessen the **biological oxygen demand**, or requirement for oxygen, when the wastewater is eventually

Figure 22.10 Treatment of sewage and wastewater. **(a)** Digester tanks used in the primary phase of treatment; each tank can process several million gallons of raw sewage a day. **(b)** View inside the secondary reactor shows the large stirring paddle that mixes the sludge to aerate it to encourage microbial decomposition.

Courtesy Sanitation Districts of Los Angeles County (a, b)

released into the environment. Normally, the numbers of pathogens also decline during the treatment processes so that the water released from the plant is safer.

The processes involved in wastewater treatment are divided into (1) the primary phase **(figure 22.10*a*)**, which involves physical separation of solid materials, largely through settling; (2) a secondary phase in which the liquid portion of the waste is subjected to microbial decomposition that is largely aerobic **(figure 22.10*b*);** and (3) an optional tertiary phase, which may be chemical, physical, or biological, to remove additional inorganic substances like ammonia, nitrate, and phosphate and to eliminate pathogens.

The *trickling filter system* is a relatively simple and inexpensive secondary treatment system. Liquid waste is sprayed over a porous bed of rocks and allowed to flow downward for collection or directly into the groundwater. The rocks are covered by a biofilm of microorganisms. As the wastewater slowly percolates downward, the microorganisms in the biofilms aerobically degrade the organic compounds in the wastewater, reducing the biological oxygen demand. These trickling filters, though, have limited capacities and are not suitable for very high volumes of wastewater.

When high volumes of wastewater require treatment, the *activated sludge process* is used in the secondary stage. In this process, the liquid waste is placed into large tanks. Air is forced into the tanks and the wastewater is rigorously mixed to ensure aerobic conditions. An active microbial community develops that degrades the organic compounds in the wastewater. A sludge forms that is allowed to settle. Most of the sludge is removed for further treatment, but a portion of the sludge containing high numbers of microbes is reintroduced into the aerated treatment tank along with the next batch of wastewater to be

treated. This component is called activated sludge because the microbes are already adapted to degrading the organic compounds in the wastewater.

The activated sludge process generally reduces the biological oxygen demand by 85% to 90%. It also greatly reduces the numbers of pathogens, which are largely outcompeted by the nonpathogens and tend to settle in the sludge. The concentrations of *Salmonella, Shigella*, enteroviruses, and other pathogens are generally 90% to 99% lower in the effluent water that is discharged from the plant than in the water that

comes into the treatment facility. The water from the secondary treatment process often is released into nearby water bodies.

Sometimes, prior to release, the water from the secondary treatment tanks is subjected to tertiary treatment. Tertiary treatment removes nutrients that could support algal blooms and is important if the wastewater is going to be released into a pristine lake. The tertiary phase may also involve disinfection of the wastewater—for example, by filtration or chlorine treatment—to eliminate pathogens. Thus, cities like Cincinnati and Louisville can safely discharge the effluents from their wastewater treatment plants into the Ohio River.

The sludge from primary settling tanks and activated sludge treatment tanks can be treated in **anaerobic digestors**. Here, anaerobic bacteria and archaea further decompose the solid wastes, producing a stable solid material and methane that can be collected and used as a fuel. The gas produced from the anaerobic digestor treatment of the sludge can be used to heat the treatment facility or can be purified and sold. Thus, waste treatment and energy generation can be coupled to improve environmental health.

©PhotoAlto

Solid Waste Treatment—Composting and Landfills

Urban solid waste (what most of us know as "garbage") production in the United States amounts to roughly 150 million tons per year. Much of this material is glass, metal, and plastic that is not subject to microbial degradation. The rest is decomposable organic compounds, such as food scraps and paper.

Today, we attempt to remove the nonbiodegradable material from the solid waste and recycle it. This includes paper that is relatively difficult for microbes to decompose. In some cases, the organic wastes are dumped offshore or simply discarded on land, but excessive dumping can cause serious problems. Most municipalities in the United States, therefore, use a combination of sanitary landfills and composting to treat solid wastes.

Composting is an aerobic process in which air and inorganic nutrients support the growth of diverse aerobic microbial communities of bacteria and fungi that are able to decompose the organic wastes. The wastes are generally arranged in a heap or pile that can be managed to ensure adequate aeration and moisture retention **(figure 22.11)**. Sometimes, homeowners compost leaves and kitchen and garden wastes to get rid of them. Initially, the decomposition process is carried out by mesophilic microorganisms; but as the decomposition proceeds, heat builds up and there is a shift to thermophiles. The end result of the decomposition of the organic wastes is a material that can be added to soils as a soil conditioner or fertilizer.

The alternative to composting or decomposition in an anaerobic digestor is to send the wastes to a landfill. Generally, organic and inorganic solid wastes are deposited together on land that has minimal real estate value. Because exposed waste can cause esthetic and odor issues and also attract insects and rodents, each day's waste is covered with a layer of soil —creating a sanitary landfill **(figure 22.12)**. Eventually, the waste and soil layers build to form a hill. As the buried waste is decomposed, largely by anaerobic microorganisms, the land mass subsides. After 30 to 50 years, the land often is stable and suitable for other uses like housing development. One of the campuses of your author Kelly Cowan's university is built on top of a landfill!

Figure 22.11 **A backyard composting bin**.
©Wave Royalty Free/Alamy Stock Photo

Biodegradation and Bioremediation—Oil and Chemical Spills

Microorganisms have an extraordinary capacity to degrade and to transform chemicals that enter our environment either through natural processes or human activities. Without these microbial activities, all life would cease on earth within a few weeks. Yet, human exploitation of fossil fuels that sometimes results in major oil spills and the release into the environment of xenobiotics—novel, chemically synthesized

Figure 22.12 **A typical landfill**.
©Comstock Images/Jupiterimages

compounds that do not naturally occur—is proving challenging to microbial **biodegradation** and the sustainability of environmental quality.

Some xenobiotic compounds are totally resistant to microbial attack. When these compounds enter the environment, they accumulate and can cause environmental harm. In some cases, it has been possible to redesign these compounds so that they are biodegradable. Laundry detergents, for example, now have linear alkyl benzyl sulfonates, which are readily biodegradable, instead of the previously used branched (nonlinear) alkyl benzyl sulfonates. It was discovered that these were recalcitrant and accumulated in the environment. The story of laundry detergent design is significant because it was one of the first instances in which a xenobiotic chemical was redesigned to make it biodegradable. Today, we also have biodegradable plastics and various other substances that have been designed to be readily degraded by microorganisms.

In contrast to recalcitrant xenobiotic compounds for which microorganisms have not had sufficient time to evolve the capacity to degrade, microorganisms have long been exposed to petroleum hydrocarbons that routinely seep into the environment. Over 10 million tons of oil pollutants enter the world's oceans each year from natural see pages, accidental spillages, and the disposal of oily wastes. Microorganisms that naturally occur in the oceans have the ability to degrade most of the compounds in petroleum, which is why the oceans are not covered with a layer of oil. However, as we periodically witness, the sudden release of large amounts of oil from a supertanker accident or well blowout can overwhelm the microbial biodegradative capacity.

In the case of the 1989 *Exxon Valdez* spill, far more oil washed up on the shorelines of Prince William Sound, Alaska, than the microbes could quickly biodegrade. In particular, there was a lack of sufficient inorganic nutrients to support the microbial growth needed to consume the hydrocarbons in the oil rapidly. To overcome this limitation, inorganic nitrogen- and phosphate-containing fertilizers were added to stimulate the growth of the naturally occurring oil-degrading microbes—a process called *bioremediation*. No microbes

were added—they were already there and just needed the added fertilizer to allow them to grow faster.

©Vanessa Vick/Science Source

Likewise, within 2 to 6 days, microbes in the Gulf of Mexico were estimated to biodegrade half of the dispersed oil released from the BP *Deepwater Horizon* well explosion in 2010 **(figure 22.13)**. Even in the cold, deep waters of the Gulf, microbes consumed over 90% of the oil within a month of its release. Molecular analyses showed that diverse microbes, particularly *Oceanospirillum* and *Colwellia*, were responsible for the rapid biodegradation of this oil. Nature has an amazing self-cleaning capacity—one that is fully dependent upon microorganisms.

Figure 22.13 **The 2010 Gulf oil spill.**
Source: U.S. Coast Guard/Chief Petty Officer John Kepsimelis, Atlantic Strike Team

Counteracting Climate Change?

Some scientists think that it may be possible to manage microbial activities in soils and oceans to combat global warming. Microbes are responsible for the production and consumption of the major greenhouse gases, including carbon dioxide and methane. Some microbes produce carbon dioxide, whereas others, like cyanobacteria and algae, consume it. Some microbes, like the archaea in the guts of herbivorous animals, produce large amounts of methane. In fact, according to the U.S. Environmental Protection Agency, cows release more methane into the atmosphere than automobiles. Thus, some microorganisms contribute to the production of greenhouse gases, whereas others tend to reduce atmospheric concentrations of carbon dioxide and methane and thereby lessen the impact of human activities.

It is unclear whether scientists will be able to—and also whether they should—manipulate microbes to help counteract climate change. It is also unclear whether the balance of microbial production and consumption of carbon dioxide and methane can be controlled. What does seem certain is that if the concentrations of greenhouse gases continue to increase and climate changes continue, human, animal, and environmental health will also change—and the ultimate outcome may well depend upon the microbes.

22.4 LEARNING OUTCOMES—Assess Your Progress

6. Define *biological oxygen demand*.
7. Outline the three phases in wastewater treatment.
8. Provide an example of a xenobiotic that has been made biodegradable.

CASE FILE WRAP-UP

©ERproductions Ltd/Blend Images LLC (health care worker); Source: CDC/National Institute of Allergy and Infectious Diseases (NIAID) (virus)

Ebola is transmitted by exposure to infected body fluids or items contaminated with infected fluids. Particularly near the end of the illness, patients often bleed profusely and release urine, stool, and other body fluids—all of which is infectious, placing health care workers at great risk.

Sewage processing in the United States inactivates the Ebola virus so it was determined in our hospital that human waste could be disposed of in the toilet. All linens and cloth items in the room were disposed of as waste, instead of laundering. Solid waste (disposable plastics, for example) leaving the room were subject to federal regulations for handling and transport. Ultimately it was incinerated to destroy the contaminated material.

It is clear that American hospitals were caught off guard when the first Ebola patient appeared. Having a *One Health* perspective might have prevented that situation. As the world becomes more interconnected, infectious diseases will continue to spread between populations and even organisms. We are much better prepared for an infection like Ebola, but what will the next one be?

The Microbiome

Thanks to the Sponge, and Its Microbiome, for Letting Us Breathe

This is our last microbiome story in the book, but it is one of the first microbiome stories in the history of human evolution. As you know, early days on our planet were anaerobic; the development of complex animals and humans could not have happened before the atmosphere contained sufficient oxygen. There is evidence that a very specific microbiome—the microbiome of deep sea sponges—kick-started the accumulation of oxygen on earth.

In 2014 scientists made a discovery that explained at least partly the immense influx of oxygen onto the planet about 750 million years ago that enabled the Cambrian explosion. The Cambrian explosion refers to the rapid appearance of many types of organisms and animals that we see currently on our planet, with credit going to the sudden appearance of large amounts of oxygen, mainly in the oceans covering the earth.

But where did the oxygen come from? The research in 2014 suggested that it came from sponges—those sea creatures that seem to have great capacities for pumping and filtering water. But how did that lead to the introduction of all that oxygen? Because sponges live on very low levels of oxygen, and do all that pumping and filtering, they seem to have been producing oxygen in their habitats in the deep sea. But from what?

The oxygen was being produced by photosynthetic microorganisms in the vicinity of the sponges. The active pumping of the oxygen by the sponges created large deposits of phosphate (because phosphorus—abundant in the ocean—combines with oxygen to form the phosphate deposits). Scientists deduced that the large amounts of phosphate that started appearing in the ocean depths around this time are evidence for oxygen-producing bacteria—in the form of the microbiome associated with sponges. So it is quite possible that sea sponges, and their microbes, created the conditions that made oxygenic life on earth possible.

This chapter is titled “One Health.” The contribution of animals and environmental factors to the existence of humans is beautifully illustrated with this research.

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Chapter Summary

22.1 One Health

- The health of the environment, the health of animals, and the health of humans are intertwined. Scientists call this concept *One Health*.

22.2 Animals and Infectious Disease: Zoonoses

- Human infectious diseases that originated in animals are called zoonoses (singular, *zoonosis*). Human diseases can also be transmitted to animals.
- Lyme disease is transmitted by ticks among small and large mammals, including humans. Encroaching on the habitat of wildlife puts us in the chain of transmission.

©William Leaman/Alamy Stock Photo

22.3 The Environment and Infectious Disease

- Drinking water that does not contain microbial pathogens is fundamental to life. Most of the world's population struggles to obtain clean water.
- The presence of coliforms is used as a sign that water has been exposed to fecal material, although it is a less-than-perfect test.

- Food is another part of our environment that is easily contaminated with microbes. Even in industrialized countries, keeping pathogens out of the food chain is a constant struggle.

- Climate change results in changes in our weather patterns and the environment and plants and animals that are affected by it. This can lead to changes in the transmission of microbes to humans.

22.4 Microbes to the Rescue

- Humans have learned how to take advantage of microbial processes to help us maintain a clean environment and clean up pollution that we create.
- Sewage treatment is assisted in multiple ways by using microbes to break down toxic substances.
- Solid waste (garbage) can also be degraded by microbes via composting or anaerobic digestion.
- When humans create problems by dumping oils or chemicals into the environment, we often rely on naturally occurring microbes to degrade the oils or chemicals and clean up the spills. This is called bioremediation.
- Xenobiotics are human-made compounds that do not occur naturally on our planet. Often, they cannot be broken down in the environment. Changing the chemical structures of these chemicals can sometimes make them biodegradable.

SmartGrid: From Knowledge to Critical Thinking

This *21 Question Grid* takes the topics from this chapter and arranges them with respect to the American Society for Microbiology's Undergraduate Curriculum guidelines—all six of the important "Concepts" as well as the important "Competency" of scientific literacy. Three questions are supplied about chapter content that refer to the Concept or Competency, in increasing levels of Bloom's taxonomy for learning.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Evolution	**1**. Which of the following is/are emerging or reemerging diseases for which vaccines are widely available? **a**. Zika virus **b**. measles **c**. Ebola **d**. C. diff	**2**. In Malaysia, a form of malaria that was previously only seen in macaque monkeys is being seen in humans because deforestation activities bring the monkeys and humans closer together. Diagram the chain of events that leads to human infection, including plant life, mosquitoes, monkeys, and humans.	**3**. Develop an argument about how a sexually transmitted microbe might benefit from evolving toward lower virulence while a mosquito-borne virus would not.

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Cell Structure and Function	**4**. Which of the following emerging pathogens is a spirochete? **a**. West Nile virus **b**. *E. coli* **c**. *Borrelia burgdorferi* **d**. hantavirus	**5**. Why do you think microbes are so adept at breaking down sewage and wastewater?	**6**. Are most zoonotic viruses likely to be enveloped or non-enveloped? Provide justification for your answer.
Metabolic Pathways	**7**. Contaminated water has **a**. lower biological oxygen demand than pure water. **b**. higher biological oxygen demand than pure water. **c**. the same biological oxygen demand as pure water. no demand for oxygen. **d**.	**8**. Why do microbes exist that are capable of cleaning up oil spills in the environment?	**9**. The text states, "… cows release more methane into the atmosphere than automobiles." Would it be a good idea to dose cows with probiotics containing methane-consuming bacteria? Why or why not?

ASM Concept/Competency	A. Bloom's Level 1, 2—Remember and Understand (Choose one.)	B. Bloom's Level 3, 4—Apply and Analyze	C. Bloom's Level 5, 6—Evaluate and Create
Information Flow and Genetics	**10**. From what bacterium did the Shiga-toxin-producing *E. coli* acquire its plasmid-encoded toxin? **a**. *Salmonella* **b**. *Shigella* **c**. *Borrelia* **d**. *Shigaria*	**11**. Until the 1993 outbreak, hantaviruses were only known to cause hemorrhagic fevers accompanied by kidney damage. Genetic changes may have made it capable of causing lung disease. What aspect of its genetic makeup would suggest that it mutates easily?	**12**. With what you have learned about increasingly affordable high-throughput DNA sequencing for diagnosing infectious diseases, speculate on the pros and cons of using that technology to replace fecal coliform screening.

Microbial Systems	**13**. Which of the following refers to a process in which microbial activity is *discouraged*? **a**. composting **b**. trickling filter system **c**. bioremediation **d**. none of these	**14**. Explain how a warming climate is likely to impact the epidemiology of mosquito-borne diseases.	**15**. Conduct research to find out where the microorganisms used in wastewater treatment come from.
Impact of	**16**. In which way can	**17**. Create an	**18**. Zika virus

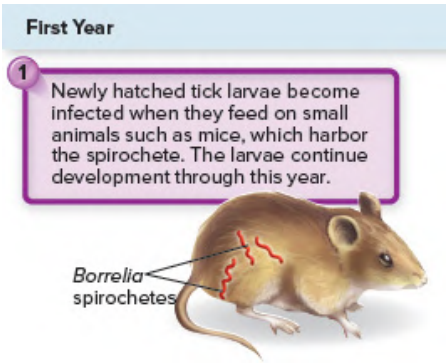
Microorganisms	infectious agents pass between humans and other animals? **a**. from animals to humans **b**. from humans to animals **c**. Both a and b are possible. **d**. Neither a nor b is possible.	infographic or visual representation that describes the similarities and differences between *E. coli* and other coliforms.	infection in adults may be asymptomatic or result in a skin rash. But it is devastating to fetuses. Suggest reasons for this and identify another virus known for the same characteristics.
Scientific Thinking	**19**. Use figure 22.2 to identify which of the following zoonoses leads to the most human deaths annually. **a**. Q fever **b**. cysticercosis **c**. leishmaniasis **d**. toxoplasmosis	**20**. Defend or refute this statement with information from this chapter: The environment would be a safer place for human health if all microbes could be eliminated from it.	**21**. Research the Coalition for Epidemic Preparedness Innovations, and write a paragraph describing the experiences that form the group's motivation for creating vaccines for emerging diseases.

Answers to the multiple-choice questions appear in Appendix A.

Visual Connections

This question connects previous images to a new concept.

1. From chapter 18, **figure 18.15**. Use this illustration to explain how the transmission of Lyme disease can be increased by changes in the weather.



connect Resources are available through Connect, including interactive learning tools, animations, and more.

Chapter design elements: Doctor in white coat holding files, close-up icon: ©David Gould/Getty Images; Note pad with pen icon: ©C Squared Studios/Getty Images

APPENDIX A

Answers to Multiple-Choice Questions in SmartGrid

Chapter 1

1. d
4. b
7. d
10. a
13. d
16. c
19. c

Chapter 2

1. d
4. a
7. d
10. a
13. b
16. d (a and b)
19. d

Chapter 3

1. d
4. b
7. c
10. b
13. d
16. d
19. b

Chapter 4

1. c
4. b
7. c
10. c

13. a
16. b
19. d

Chapter 5

1. b
4. a
7. a
10. c
13. a
16. a
19. c

Chapter 6

1. d
4. a
7. a
10. c
13. b
16. a
19. b

Chapter 7

1. b
4. a
7. b
10. d (a & c)
13. d
16. c
19. d

Chapter 8

1. a
4. d
7. b
10. b
13. a
16. d
19. c

Chapter 9

1. a
4. c
7. c
10. c
13. d
16. a
19. d

Chapter 10

1. d
4. b
7. b
10. b
13. d
16. b
19. b

Chapter 11

1. d
4. d
7. d
10. b
13. d
16. c
19. b

Chapter 12

1. b
4. d
7. d
10. b
13. b
16. a
19. a

Chapter 13

1. b
4. a
7. c
10. a
13. d
16. c
19. b

Chapter 14

1. c
4. b
7. a
10. c
13. d
16. c
19. b

Chapter 15

1. a

4. c
7. a
10. a
13. d
16. a
19. a

Chapter 16

1. b
4. a
7. d (b & c)
10. b
13. d
16. a
19. c

Chapter 17

1. d
4. a
7. c
10. a
13. b
16. a
19. d (b & c)

Chapter 18

1. b
4. a
7. c
10. d
13. d
16. b
19. b

Chapter 19

1. c
4. c
7. a
10. d
13. d
16. c
19. a

Chapter 20

1. b
4. b
7. a
10. d

13. d
16. c
19. a

Chapter 21

1. d
4. c
7. a
10. b
13. a
16. b
19. c

Chapter 22

1. b
4. c
7. b
10. b
13. d
16. c
19. b

APPENDIX B

Answers to the Medical Moments and NCLEX® Questions

Chapter 1

Medical Moments

Medications from Microbes

You might reason that it would be advantageous for one microbe to limit the number of competitors in its environment, and might therefore have evolved the ability to secrete chemicals that damage other microbes. This is, in fact, true!

Delivering Essential Nutrients

The paragraph indicates that the nutrition is delivered intravenously, which bypasses the digestive tract altogether. The gastrointestinal tract is often called the enteric tract. From the context, you could deduce that "parenteral" means, at the very least, "not via the digestive tract." In fact, it means "through direct penetration of the outer tissue (skin)."

NCLEX® Prep

1. a, b, d, e
2. a

Chapter 2

Medical Moments

The Making of the Flu Vaccine

Yes, they can. You will learn later that viruses have molecules on their surfaces that match up with molecules on the surface of only certain cells of certain species. Since influenza can infect the embryonic cells in chickens (birds), you would predict that they can also infect adult birds. This is in fact true. You have no doubt heard of "bird flu," a type of influenza virus that can infect humans, as well as birds.

Gram-Positive Versus Gram-Negative Bacteria

Since medical doctors are more interested in the effects of the molecule on a patient, they are likely to call it "endotoxin." Researchers are more likely to identify it as a structural component of the bacterial cell, and identify it as "lipopolysaccharide."

NCLEX® Prep

1. b
2. a, b, c, d
3. c

Chapter 3

Medical Moments

Healthcare-Associated Infections

Surgery compromises important barriers and can allow microbes from the environment, or from internal sites in the body, to contaminate the surgical site. Indeed, surgical-site infections are one of the main HAIs.

Collecting Sputum

Sputum would contain large quantities of normal biota from the throat and mouth. Cultures of sputum would contain these isolates as well as possible *Mycobacterium* colonies. This is one of the reasons acid-fast staining is so useful. Other bacteria will not resist the acid wash and therefore will not be stained by the main stain.

NCLEX® Prep

1. b

Chapter 4

Medical Moments

Vaginal Candidiasis

Diarrhea can have many different causes, from infection to chronic autoimmune dysfunction. Limited postantibiotic diarrhea is often caused by the temporary disruption of the normal gut biota, through the same "bystander" effects we described for vaginal yeast infections. Usually the effect is short-lived, but if the diarrhea is severe and long-lasting, other conditions should be considered.

Opportunistic Fungal Infection

Fungi are eukaryotic cells, just like human cells. It is difficult to cause harm in the invading eukaryote without causing harm to the eukaryotic human cells. Bacteria are prokaryotes.

Neglected Parasitic Infections

Malaria

NCLEX® Prep

1. c
2. c
3. a

Chapter 5

Medical Moments

Why Antibiotics Are Ineffective Against Viruses

Vaccinations were developed to prevent viral infections, since there was no effective treatment of them. Eventually vaccines against bacterial diseases were also developed.

Differentiating Between Bacterial and Viral Infections

No, a light microscope would not be useful to diagnose a viral infection since viruses are too small to be seen with light microscopes. However, light microscopy can reveal the absence of bacteria, suggesting a viral cause. It can also reveal the presence of white blood cells, which can be evidence of an infection of some type.

NCLEX® Prep

1. d, e
2. a
3. b, c, e
4. c
5. b

Chapter 6

Medical Moments

Osmosis and IV Fluids

When discussing the uses of IV fluids of varying tonicity, the semipermeable "membranes" are actually the blood vessel walls, acting to exclude larger solutes but not water.

MRSA: PCR over Culture

Patients with MRSA should preferably be housed in private rooms. Their rooms should be prioritized for daily cleaning. If possible, stethoscopes and other frequently used equipment should be dedicated to that room. The CDC has a comprehensive list of precautions and when to use them: https://www.cdc.gov/mrsa/healthcare/clinicians/precautions.html

NCLEX® Prep

1. d
2. e
3. a
4. b, c, e

Chapter 7

Medical Moments

Facultative Anaerobes

Listeria is psychrotrophic, meaning it thrives in cooler temperatures, including in refrigerated foods.

Amino Acids: Essential, Nonessential, and Conditionally Essential Amino Acids

Tyrosine is produced by mammalian enzymes from the starting material phenylalanine.

NCLEX® Prep

1. b, c
2. a
3. b

Chapter 8

Medical Moments

Gene Mutations

The redundancy of the genetic code means that fewer cases of these diseases occur than if the redundancy did not exist.

Growing Hormones

Scarcity is one of the biggest disadvantages to using growth hormone from cadavers. The supply did not nearly meet the demand among children with growth problems. Secondly, there is always a possibility of transferring infectious diseases when transplanting substances derived from human tissue. Indeed, 26 people who had received cadaveric growth hormone developed Creutzfeld-Jacob disease years after their treatment. It was determined that the prion was transmitted with the growth hormone.

NCLEX® Prep

1. a, b, c, d
2. c
3. d
4. a, b

Chapter 9

Medical Moments

The Use of Alcohol-Based Hand Cleansers

An enveloped virus is more likely to be inactivated by alcohol, since alcohol's major action is to dissolve membrane lipids.

Zap VAP: The Role of Chlorhexidine

Chlorhexidine targets bacterial cell walls and membranes, as well as proteins.

NCLEX® Prep

1. a
2. a
3. d

Chapter 10

Medical Moments

Why Do Antibiotics Cause Diarrhea?

Technically, it is neither. It is a side effect caused by the disruption of the gut microbiome and not a result of the drug harming human cells directly (toxicity), or of an inappropriate immune response to it (allergy).

NCLEX® Prep

1. b
2. c
3. d
4. c
5. a

Chapter 11

Medical Moments

When the Portal of Entry Is Compromised

Smoking damages the cilia in the upper respiratory tract, which help clear out infectious agents before they can establish themselves.

A Gene That Protects You from the Flu

The most obvious strategy to try first is to harvest or clone the IFITM3 protein to use as a treatment against the swine flu.

Eye on Careers: Infection Control Practitioner

Two of the most frequent cases of health care worker exposure to infection are accidental needlesticks and blood splashes.

NCLEX® Prep

1. b, d, e
2. d
3. d
4. b, c, e
5. a
6. b

Chapter 12

Medical Moments

Examining Lymph Nodes

This could indicate a systemic condition, infectious or noninfectious. Examples would include sepsis, a blood cancer, or an autoimmune disease.

The Tonsils

The tonsils are located on either side of the base of the tongue. The adenoids extend down the back of the nasal cavity.

NCLEX® Prep

1. c
2. a
3. a
4. a, b, c, e
5. a

Chapter 13

Medical Moments

The Thymus

Severe combined immunodeficiency is probably the most serious of these conditions, due to the fact that both the B- and T-cell systems are absent. Patients have essentially no specific immune response. They have to be completely sequestered from the environment and other humans. Bone marrow transplantation has helped some of these patients, and gene therapy has had mixed success.

Blood Exposure in Neonates

Clonal expansion after antigen encounter causes B cells and T cells to proliferate.

NCLEX® Prep

1. a
2. b
3. c

Chapter 14

Medical Moments

Hand Washing

There may be several reasons for that. Hand sanitizers contain either alcohol or povidone-iodine or some other antimicrobial chemical, while soaps may only contain the surfactant molecule. Probably most important is the fact that hand sanitizers are left on the hands to evaporate, instead of being rinsed off, allowing more time for the ingredients to act.

Patch Testing

The dendritic cells, just under the surface of the skin, will process and present the antigen to other cells of the immune system.

NCLEX® Prep

1. a

2. a, b, d
3. b
4. d

Chapter 15

Medical Moments

Qualitative Versus Quantitative Diagnosis

The lateral-flow test, which is an immunochromatographic method.

Understanding Lab Results

It was not necessary for you to know which antibiotic should be used for which bacterium; this information is likely to change over time anyway. What is important is that you read the lab results carefully and noticed that the antibiotic he was being treated with did not inhibit the bacterium he is infected with in lab tests. Your thoroughness got him on the correct drug as much as half a day earlier than would have otherwise happened.

Detecting and Treating TB

The infected person can continue to spread TB in the time it takes to definitively diagnose the disease. Also, the infected person will experience more pathology from the disease before being treated with an effective antibiotic.

NCLEX® Prep

1. d
2. e
3. e

Chapter 16

Medical Moments

Scabies

It suggests that its need for a living host is not absolute. In fact, it can live on inanimate objects for 24 to 48 hours.

Scrum Pox: Herpes Gladiatorum

The modes described in the Medical Moment are *direct contact* and *indirect contact* (fomites).

NCLEX® Prep

1. c
2. b
3. d
4. d
5. c

Chapter 17

Medical Moments

Acute Flaccid Myelitis

Botulism is characterized by flaccid paralysis.

NCLEX® Prep

1. a
2. b
3. a
4. c, d, e

Chapter 18

Medical Moments

Postexposure Prophylaxis

Hepatitis B

Where Does the Fluid Go?

Septic shock

Ebola Epidemic

Since this is the largest number of Ebola patients seen to date, the date could indeed inform a new calculation of the case fatality rate. In this

epidemic the CFR was closer to 39%. Other factors are also considered when designating a CFR, and they are rarely calculated using only a single epidemic.

NCLEX® Prep

1. b
2. d
3. a, b, e
4. c
5. a

Chapter 19

Medical Moments

Epiglottitis

Meningitis, otitis media, and pneumonia can be caused by *Haemophilus influenzae* b (Hib) and the incidence of these diseases caused by Hib has fallen drastically.

Tuberculosis—A Lesson in Inadequate Treatment

In poor countries sometimes the correct antibiotics are not available. Even when they are, supplies can be unreliable, and treatment may be interrupted for even the most compliant patients. Also, especially before there was much awareness that resistant strains were being created, physicians incorrectly treated tuberculosis sequentially with single antibiotics, encouraging the development of resistance.

NCLEX® Prep

1. c
2. d
3. b
4. a, b, d, e

Chapter 20

Medical Moments

Dehydration

Dehydration harms bodies by depriving vital organs of the correct tonicity of fluids. Smaller bodies have smaller margins for loss of water (increasing hypertonicity disproportionately). Organ damage, and death, occur more quickly.

Assessing Jaundice

Since jaundice is the visible effect of elevated bilirubin levels in the bloodstream, and the blood infiltrates every organ, including the skin and eyes, every organ will have symptoms of the bilirubin elevation.

NCLEX® Prep

1. e
2. a, b, c, d, e
3. b
4. d
5. f

Chapter 21

Medical Moments

Cranberry Versus UTI

Because the main causes of UTIs can be resistant to many antibiotics, and may eventually become resistant to most if not all antibiotics.

Crabs

Crabs lay their eggs at the base of pubic hairs, which can cause itching. Scratching the affected areas can damage the skin and break down the barriers to other microbes entering.

NCLEX® Prep

1. a, b, c
2. c
3. a, d

Chapter 22

Medical Moments

Plastic Bottles for Clean Water

No. UV light has to come into direct contact with microbes in order to kill them. The water is contained in plastic, which reduces the UV's effectiveness. But the sunlight treatment does reduce the microbial load to levels that make the water safer.

Cryptosporidium in Your Tap Water?

Water should be boiled or filtered after it comes out of the tap. Ice should be made from water treated this way, as well. Alternatively, bottled water can be substituted for tap water. Raw fruits and vegetables should only be washed with treated water before eating.

Glossary

A

abiogenesis The belief in spontaneous generation as a source of life.

abscess An inflamed, fibrous lesion enclosing a core of pus.

acid-fast stain A solution containing carbol fuchsin, which, when bound to lipids in the envelopes of *Mycobacterium* species, cannot be removed with an acid wash.

acidophilic Thriving in low-pH environments.

active immunity Immunity acquired through direct stimulation of the immune system by antigen.

active transport Nutrient transport method that requires carrier proteins in the membranes of the living cells and the expenditure of energy.

acute Characterized by rapid onset and short duration.

adenine (A) One of the nitrogen bases found in DNA and RNA with a purine form.

adenosine deaminase (ADA) deficiency An immunodeficiency disorder and one type of SCID that is caused by an inborn error in the metabolism of adenine. The accumulation of adenine destroys both B and T lymphocytes.

adenosine triphosphate (ATP) A nucleotide that is the primary source of energy to cells.

adhesion The process by which microbes gain a more stable foothold at the portal of entry; often involves a specific interaction between the molecules on the microbial surface and the receptors on the host cell.

adjuvant In immunology, a chemical vehicle that enhances antigenicity, presumably by prolonging antigen retention at the injection site.

adsorption A process of adhering one molecule onto the surface of another molecule.

aerobic respiration Respiration in which the final electron acceptor in the electron transport chain is oxygen (O_2).

agammaglobulinemia Also called *hypogammaglobulinemia*. The absence of or severely reduced levels of antibodies in serum.

agar A polysaccharide found in seaweed and commonly used to prepare solid culture media.

agglutination The aggregation by antibodies of suspended cells or similar-size particles (agglutinogens) into clumps that settle.

agranulocyte One form of leukocyte (white blood cell) having globular, nonlobed nuclei and lacking prominent cytoplasmic granules.

AIDS Acquired immunodeficiency syndrome. The complex of signs and symptoms characteristic of the late phase of human immunodeficiency virus (HIV) infection.

algae Photosynthetic, plantlike organisms that generally lack the complex structure of plants; they may be single-celled or multicellular and inhabit diverse habitats such as marine and freshwater environments, glaciers, and hot springs.

allergen A substance that provokes an allergic response.

allergy The altered, usually exaggerated, immune response to an allergen. Also called *hypersensitivity*.

alloantigen An antigen that is present in some but not all members of the same species.

allograft Relatively compatible tissue exchange between nonidentical members of the same species. Also called *homograft*.

alternative complement pathway Complement cascade initiated by spontaneous breakdown of a blood protein called C3 in the presence of microbes.

amino acids The building blocks of protein. Amino acids exist in 20 naturally occurring forms that impart different characteristics to the various proteins they compose.

amphibolism Pertaining to the metabolic pathways that serve multiple functions in the breakdown, synthesis, and conversion of metabolites.

amphitrichous Having a single flagellum or a tuft of flagella at opposite poles of a microbial cell.

anabolism The energy-consuming process of incorporating nutrients into protoplasm through biosynthesis.

anaerobic digesters Closed chambers used in a microbial process that converts organic sludge from waste treatment plants into useful fuels such as methane and hydrogen gases. Also called *bioreactors*.

anaerobic respiration Respiration in which the final electron acceptor in the electron transport chain is an inorganic molecule containing sulfate, nitrate, nitrite, carbonate, and so on.

anamnestic response In immunology, an augmented response or memory related to a prior stimulation of the immune system by antigen. It boosts the levels of immune substances.

anaphylaxis The unusual or exaggerated allergic reaction to antigen that leads to severe respiratory and cardiac complications.

antagonism Relationship in which microorganisms compete for survival in a common environment by taking actions that inhibit or destroy another organism.

antibody A large protein molecule evoked in response to an antigen that interacts specifically with that antigen.

anticodon The trinucleotide sequence of transfer RNA that is complementary to the trinucleotide sequence of messenger RNA (the codon).

antigen Any cell, particle, or chemical that induces a specific immune response by B cells or T cells and can stimulate resistance to an infection or a toxin. See **immunogen**.

antigen binding site Specific region at the ends of the antibody molecule that recognize specific antigens. These sites have numerous shapes to fit a wide variety of antigens.

antigenic drift Minor antigenic changes in the influenza A virus due to mutations in the spikes' genes.

antigenic shift Major changes in the influenza A virus due to recombination of viral strains from two different host species.

antigen-presenting cells (APCs) Cells of the immune system that digest foreign cells and particles and place pieces of them on their own surfaces in such a way that other cells of the immune system recognize them.

antihistamine A drug that counters the action of histamine and is useful in allergy treatment.

antimicrobial peptides Short protein molecules found in epithelial cells; have the ability to kill bacteria.

antiparallel A description of the two strands of DNA, which are parallel to each other, but the orientation of the deoxyribose and phosphate groups run in the opposite directions, with the 5′

carbon at the top of the leading strand and the 3′ carbon at the top of the lagging strand.

antiseptic A growth-inhibiting agent used on tissues to prevent infection.

antitoxin Globulin fraction of serum that neutralizes a specific toxin. Also refers to the specific antitoxin antibody itself.

apoenzyme The protein part of an enzyme, as opposed to the nonprotein or inorganic cofactors.

G-2

appendages Accessory structures that sprout from the surface of bacteria. They can be divided into two major groups: those that provide motility and those that enable adhesion.

aqueous Referring to solutions in which water is used as the solvent.

archaea Prokaryotic single-celled organisms of primitive origin that have unusual anatomy, physiology, and genetics and live in harsh habitats; when capitalized **(Archaea)**, the term refers to one of the three domains of living organisms as proposed by Woese.

arthroconidia Reproductive body of *Coccidioides immitis;* also *arthrospore*.

Arthus reaction An immune complex phenomenon that develops after repeat injection. This localized inflammation results from aggregates of antigen and antibody that bind, complement, and attract neutrophils.

artificial immunity Immunity that is induced as a medical intervention, either by exposing an individual to an antigen or administering immune substances to him or her.

asepsis A condition free of viable pathogenic microorganisms.

aseptic technique Method of handling microbial cultures, patient specimens, and other sources of microbes in a way that prevents infection of the handler and others who may be exposed.

assay medium Microbiological medium used to test the effects of specific treatments to bacteria, such as antibiotic or disinfectant treatment.

assembly (viral) The step in viral multiplication in which capsids and genetic material are packaged into virions.

asthma A type of chronic allergy in which the airways become constricted and produce excess mucus in reaction to allergens, exercise, stress, or cold temperatures.

asymptomatic An infection that produces no noticeable symptoms even though the microbe is active in the host tissue.

atopy Allergic reaction classified as type I, with a strong familial relationship; caused by allergens such as pollen, insect venom, food, and dander; involves IgE antibody; includes symptoms of hay fever, asthma, and skin rash.

ATP synthase A unique enzyme located in the mitochondrial cristae and chloroplast grana that harnesses the flux of hydrogen ions to the synthesis of ATP.

autoantibody An “anti-self” antibody having an affinity for tissue antigens of the subject in which it is formed.

autoclave A sterilization chamber that allows the use of steam under pressure to sterilize materials. The most common temperature/pressure combination for an autoclave is 121°C and 15 psi.

autograft Tissue or organ surgically transplanted to another site on the same subject.

autoimmune disease The pathologic condition arising from the production of antibodies against autoantigens. Example: rheumatoid arthritis. Also called *autoimmunity*.

autotroph A microorganism that requires only inorganic nutrients and whose sole source of carbon is carbon dioxide.

B

bacillus Bacterial cell shape that is cylindrical (longer than it is wide).

back-mutation A mutation that counteracts an earlier mutation, resulting in the restoration of the original DNA sequence.

bacteremia The presence of viable bacteria in circulating blood.

Bacteria When capitalized, can refer to one of the three domains of living organisms proposed by Woese containing all nonarchaea prokaryotes.

bacteria (singular, ***bacterium***) Category of prokaryotes with peptidoglycan in their cell walls and circular chromosome(s). This group of small cells is widely distributed in the earth's habitats.

bacterial chromosome A circular body in bacteria that contains the primary genetic material. Also called *nucleoid*.

bactericide An agent that kills bacteria.

bacteriophage A virus that specifically infects bacteria.

bacteristatic Any process or agent that inhibits bacterial growth.

barophile A microorganism that thrives under high (usually hydrostatic) pressure.

basement membrane A thin layer (1–6 μm) of protein and polysaccharide found at the base of epithelial tissues.

beta oxidation The degradation of long-chain fatty acids. Two-carbon fragments are formed as a result of enzymatic attack directed against the second or beta carbon of the hydrocarbon chain. Aided by coenzyme A, the fragments enter the Krebs cycle and are processed for ATP synthesis.

binary fission The formation of two new cells of approximately equal size as the result of parent cell division.

binomial system Scientific method of assigning names to organisms that employs two names to identify every organism—genus name plus species name.

biodegradation The breaking down of materials through the action of microbes or insects.

biofilm A complex association that arises from a mixture of microorganisms growing together on the surface of a habitat.

biogenesis Belief that living things can only arise from others of the same kind.

biological oxygen demand Requirement for oxygen by microbes in a niche; used to measure drinking water quality.

bioremediation Decomposition of harmful chemicals by microbes or consortia of microbes.

biosensor A device used to detect microbes or trace amounts of compounds through PCR, genome techniques, or electrochemical signaling.

biota Beneficial or harmless resident bacteria commonly found on and/or in the human body.

biotechnology The intentional use by humans of living organisms or their products to accomplish a goal related to health or the environment.

blocking antibody The IgG class of immunoglobulins that competes with IgE antibody for allergens, thus blocking the degranulation of basophils and mast cells.

blood cells Cellular components of the blood consisting of red blood cells, primarily responsible for the transport of oxygen and carbon dioxide, and white blood cells, primarily responsible for host defense and immune reactions.

blood-brain barrier Decreased permeability of the walls of blood vessels in the brain, restricting access to that compartment.

botulinum toxin *Clostridium botulinum* toxin. Ingestion of this potent exotoxin leads to flaccid paralysis.

bradykinin An active polypeptide that is a potent vasodilator released from IgE-coated mast cells during anaphylaxis.

broad-spectrum Denotes drugs that have an effect on a wide variety of microorganisms.

bubo The swelling of one or more lymph nodes due to inflammation.

bubonic plague The form of plague in which bacterial growth is primarily restricted to the lymph and is characterized by the appearance of a swollen lymph node referred to as a *bubo*.

bulbar poliomyelitis Complication of polio infection in which the brain stem, medulla, or cranial nerves are affected. Leads to loss of respiratory control and paralysis of the trunk and limbs.

bullous Consisting of fluid-filled blisters.

C

calculus Dental deposit formed when plaque becomes mineralized with calcium and phosphate crystals. Also called *tartar*.

capsid The protein covering of a virus's nucleic acid core. Capsids exhibit symmetry due to the regular arrangement of subunits called *capsomers*. See **icosahedron**.

capsomere A subunit of the virus capsid shaped as a triangle or disc.

capsular staining Any staining method that highlights the outermost polysaccharide and/or protein structure on a bacterial, fungal, or protozoal cell.

capsule In bacteria, the loose, gel-like covering or slime made chiefly of polysaccharides. This layer is protective and can be associated with virulence.

carbohydrate A compound containing primarily carbon, hydrogen, and oxygen in a 1:2:1 ratio.

carbohydrate fermentation medium A growth medium that contains sugars that are converted to acids through fermentation. Usually contains a pH indicator to detect acid protection.

carrier A person who harbors infections and inconspicuously spreads them to others. Also, a chemical agent that can accept an atom, chemical radical, or subatomic particle from one compound and pass it on to another.

caseous lesion Necrotic area of lung tubercle superficially resembling cheese. Typical of tuberculosis.

catabolism The chemical breakdown of complex compounds into simpler units to be used in cell metabolism.

catalyst A substance that alters the rate of a reaction without being consumed or permanently changed by it. In cells, enzymes are catalysts.

catarrhal A term referring to the secretion of mucus or fluids; term for the first stage of pertussis.

cell An individual membrane-bound living entity; the smallest unit capable of an independent existence.

cell wall In bacteria, a rigid structure made of peptidoglycan that lies just outside the cytoplasmic membrane; eukaryotes also have a cell wall but may be composed of a variety of materials.

cellulose A long, fibrous polymer composed of β-glucose; one of the most common substances on earth.

cestode The common name for tapeworms that parasitize humans and domestic animals.

chancre The primary sore of syphilis that forms at the site of penetration by *Treponema pallidum*. It begins as a hard, dull red, painless papule that erodes from the center.

chemical mediators Small molecules that are released during inflammation and specific immune reactions that allow communication between the cells of the immune system and facilitate surveillance, recognition, and attack.

chemoautotroph An organism that relies upon inorganic chemicals for its energy and carbon dioxide for its carbon. Also called a *chemolithotroph*.

chemoheterotroph Microorganisms that derive their nutritional needs from organic compounds.

chemokine Chemical mediators (cytokines) that stimulate the movement and migration of white blood cells.

chemostat A growth chamber with an outflow that is equal to the continuous inflow of nutrient media. This steady-state growth device is used to study such events as cell division, mutation rates, and enzyme regulation.

chemotactic factors Chemical mediators that stimulate the movement of white blood cells. See **chemokine**.

chemotaxis The tendency of organisms to move in response to a chemical gradient (toward an attractant or to avoid adverse stimuli).

chemotroph Organism that oxidizes compounds to feed on nutrients.

chitin A polysaccharide similar to cellulose in chemical structure. This polymer makes up the horny substance of the exoskeletons of arthropods and certain fungi.

chloroplast An organelle containing chlorophyll that is found in photosynthetic eukaryotes.

cholesterol Best-known member of a group of lipids called *steroids*. Cholesterol is commonly found in cell membranes and animal hormones.

chromatin The genetic material of the nucleus. Chromatin is made up of nucleic acid and stains readily with certain dyes.

chromosome The tightly coiled bodies in cells that are the primary sites of genes.

class In the levels of classification, the division of organisms that follows phylum.

classical complement pathway Pathway of complement activation initiated by a specific antigen-antibody interaction.

clonal deletion The selective elimination of lymphocytes that would recognize self markers.

clonal selection A conceptual explanation for the development of lymphocyte specificity and variety during immune maturation.

clone A colony of cells (or group of organisms) derived from a single cell (or single organism) by asexual reproduction. All units share identical characteristics. Also used as a verb to refer to the process of producing a genetically identical population of cells or genes.

cloning host An organism such as a bacterium or a yeast that receives and replicates a foreign piece of DNA inserted during a genetic engineering experiment.

coccobacillus An elongated coccus; a short, thick, oval-shaped bacterial rod.

coccus A spherical-shaped bacterial cell.

codon A specific sequence of three nucleotides in mRNA (or the sense strand of DNA) that constitutes the genetic code for a particular amino acid.

coenzyme A complex organic molecule, several of which are derived from vitamins (e.g., nicotinamide, riboflavin). A coenzyme operates in conjunction with an enzyme. Coenzymes serve as transient carriers of specific atoms or functional groups during metabolic reactions.

cofactor An enzyme accessory. It can be organic, such as coenzymes, or inorganic, such as Fe^{2+}, Mn^{2+}, or Zn^{2+} ions.

coliform A collective term that includes normal enteric bacteria that are gram-negative and lactose-fermenting.

colonize The act of taking up long-term residence; as in microbes establishing a steady relationship with a host.

colony A macroscopic cluster of cells appearing on a solid medium, each arising from the multiplication of a single cell.

colostrum The clear yellow early product of breast milk that is very high in secretory antibodies. Provides passive intestinal protection.

commensalism An unequal relationship in which one species derives benefit without harming the other.

common-source epidemic An outbreak of disease in which all affected individuals were exposed to a single source of the pathogen, even if they were exposed at different times.

communicable infection Capable of being transmitted from one individual to another.

competent Referring to bacterial cells that are capable of absorbing free DNA in their environment either naturally or through induction by exposure to chemicals or electrical currents.

competitive inhibition Control process that relies on the ability of metabolic analogs to control microbial growth by successfully competing with a necessary enzyme to halt the growth of bacterial cells.

complement In immunology, serum protein components that act in a definite sequence when set in motion either by an antigen-antibody complex or by factors of the alternative (properdin) pathway.

complementary DNA (cDNA) DNA created by using reverse transcriptase to synthesize DNA from RNA templates.

condyloma acuminata Extensive, branched masses of genital warts caused by infection with human papillomavirus.

congenital Transmission of an infection from mother to fetus.

congenital rubella Transmission of the rubella virus to a fetus *in utero*. Injury to the fetus is generally much more serious than it is to the mother.

conidia Asexual fungal spores shed as free units from the tips of fertile hyphae.

conidiospore A type of asexual spore in fungi; not enclosed in a sac.

conjugated vaccines Subunit vaccines combined with carrier proteins, often from other microbes, to make them more immunogenic.

conjugation In bacteria, the contact between donor and recipient cells associated with the transfer of genetic material such as plasmids.

Can involve special (sex) pili. Also a form of sexual recombination in ciliated protozoa.

conjunctiva The thin fluid-secreting tissue that covers the eye and lines the eyelid.

constitutive enzyme An enzyme present in bacterial cells in constant amounts, regardless of the presence of substrate. Enzymes of the central catabolic pathways are typical examples.

contagious Communicable; transmissible by direct contact with infected people and their fresh secretions or excretions.

contaminant An impurity; any undesirable material or organism.

convalescence Recovery; the period between the end of a disease and the complete restoration of health in a patient.

cornea The transparent, dome-shaped tissue covering the iris, pupil, and anterior chamber of the eye composed of five to six layers of quickly regenerating epithelial cells.

cortex The outer rim of a lymph node.

Creutzfeldt-Jakob disease (CJD) A spongiform encephalopathy caused by infection with a prion. The disease is marked by dementia, impaired senses, and uncontrollable muscle contractions.

CRISPR Clustered regularly interspaced short palindromic repeats found in bacteria and archaea used to combat invasion by foreign DNA. Used by scientists to direct DNA manipulation.

crista The infolded inner membrane of a mitochondrion that is the site of the respiratory chain and oxidative phosphorylation.

cross-presentation The simultaneous stimulation of T-helper cells and T-cytotoxic cells by antigen.

culture The visible accumulation of microorganisms in or on a nutrient medium. Also, the propagation of microorganisms with various media.

cyst The resistant, dormant but infectious form of protozoa. Can be important in spread of infectious agents such as *Entamoeba histolytica* and *Giardia lamblia*.

cysticercosis Condition caused by *Taenia solium* tapeworm. Larval cysts embed in brain, muscle, and other tissue, causing seizures and other symptoms.

cystine An amino acid, $HOOC—CH(NH_2)—CH_2—S—S—CH_2—CH(NH_2)COOH$. An oxidation product of two cysteine molecules in which the OSH (sulfhydryl) groups form a disulfide union. Also called *dicysteine*.

cytochrome A group of heme protein compounds whose chief role is in electron and/or hydrogen transport occurring in the last phase of aerobic respiration.

cytokine Regulatory chemical released by cells of the immune system that serves as signal between different cells.

cytopathic effect The degenerative changes in cells associated with virus infection. Examples: the formation of multinucleate giant cells (Negri bodies), the prominent cytoplasmic inclusions of nerve cells infected by rabies virus.

cytoplasm Dense fluid encased by the cytoplasmic membrane; the site of many of the cell's biochemical and synthetic activities.

cytosine (C) One of the nitrogen bases found in DNA and RNA, with a pyrimidine form.

D

deamination The removal of an amino group from an amino acid.

death phase End of the cell growth due to lack of nutrition, depletion of environment, and accumulation of wastes. Population of cells begins to die.

definitive host The organism in which a parasite develops into its adult or sexually mature stage. Also called the *final host*.

denaturation The loss of normal characteristics resulting from some molecular alteration. Usually in reference to the action of heat or chemicals on proteins whose function depends upon an unaltered tertiary structure.

dendritic cell A large, antigen-processing cell characterized by long, branchlike extensions of the cell membrane.

denitrification The end of the nitrogen cycle when nitrogen compounds are returned to the reservoir in the air.

deoxyribose A 5-carbon sugar that is an important component of DNA.

dermatophytes A group of fungi that cause infections of the skin and other integument components. They survive by metabolizing keratin.

dermolytic Capable of damaging the skin.

desiccation To dry thoroughly. To preserve by drying.

desquamate To shed the cuticle in scales; to peel off the outer layer of a surface.

diapedesis The migration of intact blood cells between endothelial cells of a blood vessel such as a venule.

differential medium A single substrate that discriminates between groups of microorganisms on the basis of differences in their appearance due to different chemical reactions.

differential stain A technique that utilizes two dyes to distinguish between different microbial groups or cell parts by color reaction.

differentiation Making one object distinguishable from another; also, the process of biological cells changing from one state to a more mature state.

diffusion The dispersal of molecules, ions, or microscopic particles propelled down a concentration gradient by spontaneous random motion to achieve a uniform distribution.

DiGeorge syndrome A birth defect usually caused by a missing or incomplete thymus that results in abnormally low or absent T cells and other developmental abnormalities.

dimorphic In mycology, the tendency of some pathogens to alter their growth form from mold to yeast in response to rising temperature.

direct or total cell count 1. Counting total numbers of individual cells being viewed with magnification. 2. Counting isolated colonies of organisms growing on a plate of media as a way to determine population size.

disaccharide A sugar containing two monosaccharides. Example: sucrose (fructose + glucose).

disease Any deviation from health, as when the effects of microbial infection damage or disrupt tissues and organs.

division In the levels of classification, an alternate term for *phylum*.

DNA polymerase Enzyme responsible for the replication of DNA. Several versions of the enzyme exist, each completing a unique portion of the replication process.

DNA profiling A pattern of restriction enzyme fragments that is unique for an individual organism.

DNA sequencing Determining the exact order of nucleotides in a fragment of DNA. Most commonly done using the Sanger dideoxy sequencing method.

DNA vaccine A newer vaccine preparation based on inserting DNA from pathogens into host cells to encourage them to express the foreign protein and stimulate immunity.

domain In the levels of classification, the broadest general category to which an organism is assigned. Members of a domain share only one or a few general characteristics.

doubling time Time required for a complete fission cycle—from parent cell to two new daughter cells. Also called *generation time*.

drug resistance An adaptive response in which microorganisms begin to tolerate an amount of drug that would ordinarily be inhibitory.

dysentery Diarrheal illness in which stools contain blood and/or mucus.

E

eczema An acute or chronic allergy of the skin associated with itching and burning sensations. Typically, red, edematous, vesicular lesions erupt, leaving the skin scaly and sometimes hyperpigmented.

edema The accumulation of excess fluid in cells, tissues, or serous cavities. Also called *swelling*.

ELISA Abbreviation for **e**nzyme-**l**inked **i**mmuno**s**orbent **a**ssay, a very sensitive serological test used to detect antibodies in diseases such as AIDS.

emetic Inducing to vomit.

endemic disease A native disease that prevails continuously in a geographic region.

endergonic reaction A chemical reaction that occurs with the absorption and storage of surrounding energy. See **exergonic reaction**.

endocytosis The process whereby solid and liquid materials are taken into the cell through membrane invagination and engulfment into a vesicle.

endogenous Originating or produced within an organism or one of its parts.

endoplasmic reticulum (ER) An intracellular network of flattened sacs or tubules with or without ribosomes on their surfaces.

endospore A small, dormant, resistant derivative of a bacterial cell that germinates under favorable growth conditions into a vegetative cell. The bacterial genera *Bacillus* and *Clostridium* are typical endospore formers.

endosymbiosis The theory that eukaryotic cells came to be when primordial cell types engulfed bacteria or other primordial cell types that later became organelles inside the more complex cells.

endotoxic shock A massive drop in blood pressure caused by the release of endotoxin from gram-negative bacteria multiplying in the bloodstream.

enriched medium A nutrient medium supplemented with blood, serum, or some growth factor to promote the multiplication of fastidious microorganisms.

enteroaggregative The term used to describe certain types of intestinal bacteria that tend to stick to each other in large clumps.

enteroinvasive Predisposed to invade the intestinal tissues.

enteropathogenic Pathogenic to the alimentary canal.

enterotoxigenic Having the capacity to produce toxins that act on the intestinal tract.

enterotoxin A bacterial toxin that specifically targets intestinal mucous membrane cells. Enterotoxigenic strains of *Escherichia coli* and *Staphylococcus aureus* are typical sources.

enumeration medium Microbiological medium that does not encourage growth and allows for the counting of microbes in food, water, or environmental samples.

enveloped virus A virus whose nucleocapsid is enclosed by a membrane derived in part from the host cell. It usually contains exposed glycoprotein spikes specific for the virus.

enzyme A protein biocatalyst that facilitates metabolic reactions.

enzyme induction One of the controls on enzyme synthesis. This occurs when enzymes appear only when suitable substrates are present.

enzyme repression The inhibition of enzyme synthesis by the end product of a catabolic pathway.

eosinophilia An increase in eosinophil concentration in the bloodstream, often in response to helminthic infection.

epidemic A sudden and simultaneous outbreak or increase in the number of cases of disease in a community.

epidemiology The study of the factors affecting the prevalence and spread of disease within a community.

epigenetic Refers to "above the gene," specifically additions to the DNA which do not change the gene but can change its expression.

epimutation Heritable change in gene expression that does not involve change in the gene itself but chemical additions to it.

epitope The precise molecular group of an antigen that defines its specificity and triggers the immune response.

Epstein-Barr virus (EBV) Herpesvirus linked to infectious mononucleosis, Burkitt's lymphoma, and nasopharyngeal carcinoma.

erythrogenic toxin An exotoxin produced by lysogenized group A strains of β-hemolytic streptococci that is responsible for the severe fever and rash of scarlet fever in the nonimmune individual. Also called a *pyrogenic toxin*.

eschar A dark, sloughing scab that is the lesion of anthrax and certain rickettsioses.

essential nutrient Any ingredient such as a certain amino acid, fatty acid, vitamin, or mineral that cannot be formed by an organism and must be supplied in the diet. A growth factor.

etiologic agent The microbial cause of disease; the pathogen.

Eukarya One of the three domains (sometimes called superkingdoms) of living organisms, as proposed by Woese; contains all eukaryotes.

eukaryotic cell A cell that differs from a prokaryotic cell chiefly by having a nuclear membrane (a well-defined nucleus), membrane-bound subcellular organelles, and mitotic cell division.

evolution Scientific principle that states that living things change gradually through hundreds of millions of years, and these changes are expressed in structural and functional adaptations in each organism. Evolution presumes that those traits that favor survival are preserved and passed on to following generations, and those traits that do not favor survival are lost.

exanthem An eruption or rash of the skin.

exergonic reaction A chemical reaction associated with the release of energy to the surroundings. See **endergonic reaction**.

exoenzyme An extracellular enzyme chiefly for hydrolysis of nutrient macromolecules that are otherwise impervious to the cell membrane. It functions in saprobic decomposition of organic debris and can be a factor in invasiveness of pathogens.

exogenous Originating outside the body.

exon A stretch of eukaryotic DNA coding for a corresponding portion of mRNA that is translated into peptides. Intervening stretches of DNA that are not expressed are called *introns*. During transcription, exons are separated from introns and are spliced together into a continuous mRNA transcript.

exotoxin A toxin (usually protein) that is secreted and acts upon a specific cellular target. Examples: botulin, tetanospasmin, diphtheria toxin, and erythrogenic toxin.

exponential Pertaining to the use of exponents, numbers that are typically written as a superscript to indicate how many times a factor is to be multiplied. Exponents are used in scientific notation to render large, cumbersome numbers into small workable quantities.

exponential growth phase The period of maximum growth rate in a growth curve. Cell population increases logarithmically.

extrapulmonary tuberculosis A condition in which tuberculosis bacilli have spread to organs other than the lungs.

exudate Fluid that escapes cells into the extracellular spaces during the inflammatory response.

F

facultative Pertaining to the capacity of microbes to adapt or adjust to variations; not obligate. Example: The presence of oxygen is not obligatory for a facultative anaerobe to grow. See **obligate**.

family In the levels of classification, a mid-level division of organisms that groups more closely related organisms than previous levels. An order is divided into families.

fastidious Requiring special nutritional or environmental conditions for growth; said of bacteria.

fecal coliforms Any species of gram-negative lactose-positive bacteria (primarily *Escherichia coli*) that live primarily in the intestinal tract and not the environment. Finding evidence of these bacteria in a water or food sample is substantial evidence of fecal contamination and potential for infection. See **coliform**.

fermentation The extraction of energy through anaerobic degradation of substrates into simpler, reduced metabolites. In large industrial processes, fermentation can mean any use of microbial metabolism to manufacture organic chemicals or other products.

fertility (F) factor Donor plasmid that allows synthesis of a pilus in bacterial conjugation. Presence of the factor is indicated by F^+, and lack of the factor is indicated by F^-.

filament A helical structure composed of proteins that is part of bacterial flagella.

fimbria A short, numerous-surface appendage on some bacteria that provides adhesion but not locomotion.

Firmicutes Taxonomic category of bacteria that have gram-positive cell envelopes.

flagellar staining A staining method that highlights the flagellum of a bacterium.

flagellum A structure that is used to propel the organism through a fluid environment.

fluorescence The property possessed by certain minerals and dyes to emit visible light when excited by ultraviolet radiation. A fluorescent dye combined with specific antibody provides a sensitive test for the presence of antigen.

food poisoning Symptoms in the intestines (which may include vomiting) induced by preformed exotoxin from bacteria.

frameshift mutation An insertion or deletion mutation that changes the codon reading frame from the point of the mutation to the final codon. Almost always leads to a nonfunctional protein.

fructose One of the carbohydrates commonly referred to as sugars. Fructose is commonly fruit sugars.

fungemia The condition of fungi multiplying in the bloodstream.

fungi Macroscopic and microscopic heterotrophic eukaryotic organisms that can be uni- or multicellular.

G

gel electrophoresis A laboratory technique for separating DNA fragments according to length by employing electricity to force the DNA through a gel-like matrix typically made of agarose. Smaller DNA fragments move more quickly through the gel, thereby moving farther than larger fragments during the same period of time.

gene A site on a chromosome that provides information for a certain cell function. A specific segment of DNA that contains the necessary code to make a protein or RNA molecule.

gene drive A genetic engineering technique that increases the density of a particular gene in a population.

gene probe Short strand of single-stranded nucleic acid that hybridizes specifically with complementary stretches of nucleotides on test samples and thereby serves as a tagging and identification device.

general-purpose media Laboratory agar or broth that allow most microorganisms to grow, without differentiating among them.

gene therapy The introduction of normal functional genes into people with genetic diseases such as sickle-cell anemia and cystic fibrosis. This is usually accomplished by a virus vector.

generation time Time required for a complete fission cycle—from parent cell to two new daughter cells. Also called *doubling time*.

genetic engineering A field involving deliberate alterations (recombinations) of the genomes of microbes, plants, and animals through special technological processes.

genetics The science of heredity.

genome The complete set of chromosomes and genes in an organism.

genomic libraries Collections of DNA fragments representing the entire genome of an organism inserted into plasmids and stored in vectors such as bacteria or yeasts.

genomics The systematic study of an organism's genes and their functions.

genotype The genetic makeup of an organism. The genotype is ultimately responsible for an organism's phenotype, or expressed characteristics.

genus In the levels of classification, the second most specific level. A family is divided into several genera.

germ theory of disease A theory first originating in the 1800s that proposed that microorganisms can be the cause of diseases. The concept is actually so well established in the present time that it is considered a fact.

germicide An agent lethal to non-endospore-forming pathogens.

gingivitis Inflammation of the gum tissue in contact with the roots of the teeth.

glucose One of the carbohydrates commonly referred to as sugars. Glucose is characterized by its 6-carbon structure.

glycerol A 3-carbon alcohol, with three OH groups that serve as binding sites.

glycocalyx A filamentous network of carbohydrate-rich molecules that coats cells.

glycolysis The energy-yielding breakdown (fermentation) of glucose to pyruvic or lactic acid. It is often called *anaerobic glycolysis* because no molecular oxygen is consumed in the degradation.

Golgi apparatus An organelle of eukaryotes that participates in packaging and secretion of molecules.

Gracilicutes Taxonomic category of bacteria that have gram-negative envelopes.

graft versus host disease (GVHD) A condition associated with a bone marrow transplant in which T cells in the transplanted tissue mount an immune response against the recipient's (host) normal tissues.

Gram stain A differential stain for bacteria useful in identification and taxonomy. Gram-positive organisms appear purple from crystal violet mordant retention, whereas gram-negative organisms appear red after loss of crystal violet and absorbance of the safranin counterstain.

gram-negative A category of bacterial cells that describes bacteria with an outer membrane, a cytoplasmic membrane, and a thin cell wall.

gram-positive A category of bacterial cells that describes bacteria with a thick cell wall and no outer membrane.

granulocyte A mature leukocyte that contains noticeable granules in a Wright stain. Examples: neutrophils, eosinophils, and basophils.

granuloma A solid mass or nodule of inflammatory tissue containing modified macrophages and lymphocytes. Usually a chronic pathologic process of diseases such as tuberculosis or syphilis.

granzymes Enzymes secreted by cytotoxic T cells that damage proteins of target cells.

Graves' disease A malfunction of the thyroid gland in which autoantibodies directed at thyroid cells stimulate an overproduction of thyroid hormone (hyperthyroidism).

growth curve A graphical representation of the change in population size over time. This graph has four periods known as lag phase, exponential or log phase, stationary phase, and death phase.

growth factor An organic compound such as a vitamin or amino acid that must be provided in the diet to facilitate growth. An essential nutrient.

guanine (G) One of the nitrogen bases found in DNA and RNA in the purine form.

guide RNA A sequence of RNA used to target specific gene sequences during use of the CRISPR system.

Guillain-Barré syndrome A neurological complication of infection or vaccination.

gumma A nodular, infectious granuloma characteristic of tertiary syphilis.

gut-associated lymphoid tissue (GALT) A collection of lymphoid tissue in the gastrointestinal tract that includes the appendix, the lacteals, and Peyer's patches.

H

halophile A microbe whose growth is either stimulated by salt or requires a high concentration of salt for growth.

hapten An incomplete or partial antigen. Although it constitutes the determinative group and can bind antigen, hapten cannot stimulate a full immune response without being carried by a larger protein molecule.

hay fever A form of atopic allergy marked by seasonal acute inflammation of the conjunctiva and mucous membranes of the respiratory passages. Symptoms are irritative itching and rhinitis.

healthcare-associated infection (HAI) Formerly referred to as *nosocomial infection*, any infection acquired as a direct result of a patient's presence in a hospital or health care setting.

helical Having a spiral or coiled shape.

helical capsid Protein covering of viruses made of rod-shaped capsomeres that form a coiled nucleic acid/capsid structure.

helminth A term that designates all parasitic worms.

hematopoiesis The process by which the various types of blood cells are formed, such as in the bone marrow.

hemolysin Any biological agent that is capable of destroying red blood cells and causing the release of hemoglobin. Many bacterial pathogens produce exotoxins that act as hemolysins.

hemolytic disease of the newborn (HDN) Incompatible Rh factor between mother and fetus causes maternal antibodies to attack the fetus and trigger complement-mediated lysis in the fetus.

hemolytic uremic syndrome (HUS) Severe hemolytic anemia leading to kidney damage or failure; can accompany *E. coli* O157:H7 intestinal infection.

hemolyze When red blood cells burst and release hemoglobin pigment.

hepatitis Inflammation and necrosis of the liver, often the result of viral infection.

hepatitis D The delta agent; a defective RNA virus that cannot reproduce on its own unless a cell is also infected with the hepatitis B virus.

heredity Genetic inheritance.

hermaphroditic Containing the sex organs for both male and female in one individual.

herpes zoster A recurrent infection caused by latent chickenpox virus. Its manifestation on the skin tends to correspond to dermatomes and to occur in patches that "girdle" the trunk. Also called *shingles*.

heterotroph An organism that relies upon organic compounds for its carbon and energy needs.

hexose A 6-carbon sugar such as glucose and fructose.

histamine A cytokine released when mast cells and basophils release their granules. An important mediator of allergy, its effects include smooth muscle contraction, increased vascular permeability, and increased mucus secretion.

histiocyte Another term for *macrophage*.

histone Proteins associated with eukaryotic DNA. These simple proteins serve as winding spools to compact and condense the chromosomes.

holoenzyme An enzyme complete with its apoenzyme and cofactors.

horizontal gene transfer Transmission of genetic material from one cell to another through nonreproductive mechanisms, that is, from one organism to another living in the same habitat.

host range The limitation imposed by the characteristics of the host cell on the type of virus that can successfully invade it.

human immunodeficiency virus (HIV) A retrovirus that causes acquired immunodeficiency syndrome (AIDS).

human microbiome The complete complement of microorganisms that live in or on humans.

Human Microbiome Project (HMP) A project of the National Institutes of Health to identify microbial inhabitants of the human body and their role in health and disease; uses metagenomic techniques instead of culturing.

hybridization A process that matches complementary strands of nucleic acid (DNA-DNA, RNA-DNA, RNA-RNA). Used for locating specific sites or types of nucleic acids.

hypertonic Having a greater osmotic pressure than a reference solution.

hyphae The tubular threads that make up filamentous fungi (molds). This web of branched and intertwining fibers is called a *mycelium*.

hypogammaglobulinemia An inborn disease in which the gamma globulin (antibody) fraction of serum is greatly reduced. The condition is associated with a high susceptibility to pyogenic infections.

hyposensitivity diseases Diseases in which there is a diminished or lack of immune reaction to pathogens due to incomplete immune system development, immune suppression, or destruction of the immune system.

hypotonic Having a lower osmotic pressure than a reference solution.

I

icosahedron A regular geometric figure having 20 surfaces that meet to form 12 corners. Some virions have capsids that resemble icosahedral crystals.

immune complex reaction Type III hypersensitivity of the immune system. It is characterized by the reaction of soluble antigen with antibody and the deposition of the resulting complexes in basement membranes of epithelial tissue.

immune privilege The restriction or reduction of immune response in certain areas of the body that reduces the potential damage to tissues that a normal inflammatory response could cause.

immune tolerance Tolerance to self; the inability of one's immune system to react to self proteins or antigens.

immunochromatography Use of devices with antigens or antibodies embedded in matrices that detect the presence of antigens or antibodies in patient samples.

immunocompetence The ability of the body to recognize and react with multiple foreign substances.

immunodeficiency Immune function is incompletely developed, suppressed, or destroyed.

immunogen Any substance that induces a state of sensitivity or resistance after processing by the immune system of the body.

immunoglobulin (Ig) The chemical class of proteins to which antibodies belong.

immunology The study of the system of body defenses that protect against infection.

immunopathology The study of disease states associated with overreactivity or underreactivity of the immune response.

incidence In epidemiology, the number of new cases of a disease occurring during a period.

incubate To isolate a sample culture in a temperature-controlled environment to encourage growth.

incubation period The period from the initial contact with an infectious agent to the appearance of the first symptoms.

index case The first case of a disease identified in an outbreak or epidemic.

indicator bacteria In water analysis, any easily cultured bacteria that may be found in the intestine and can be used as an index of fecal contamination. The category includes coliforms and enterococci. Discovery of these bacteria in a sample means that pathogens may also be present.

induced mutation Any alteration in DNA that occurs as a consequence of exposure to chemical or physical mutagens.

induction The process whereby a bacteriophage in the prophage state is activated and begins replication and enters the lytic cycle.

induration Area of hardened, reddened tissue associated with the tuberculin test.

infection The entry, establishment, and multiplication of pathogenic organisms within a host.

infectious disease The state of damage or toxicity in the body caused by an infectious agent.

inflammasome A large protein in phagocytic cells that contains pattern recognition receptors (PRRs) to help these cells initiate the inflammatory response.

inflammation A natural, nonspecific response to tissue injury that protects the host from further damage. It stimulates immune reactivity and blocks the spread of an infectious agent.

inoculation The implantation of microorganisms into or upon culture media.

integument The outer surfaces of the body: skin, hair, nails, sweat glands, and oil glands.

interferon (IFN) Natural human chemical that inhibits viral replication; used therapeutically to combat viral infections and cancer.

interleukins A class of chemicals released from host cells that have potent effects on immunity.

intermediate filament Proteinaceous fibers in eukaryotic cells that help provide support to the cells and their organelles.

intoxication Poisoning that results from the introduction of a toxin into body tissues through ingestion or injection.

intron The segments on split genes of eukaryotes that do not code for polypeptide. They can have regulatory functions. See **exon**.

irradiation The application of radiant energy for diagnosis, therapy, disinfection, or sterilization.

isograft Transplanted tissue from one monozygotic twin to the other; transplants between highly inbred animals that are genetically identical.

isolation The separation of microbial cells by serial dilution or mechanical dispersion on solid media to create discrete colonies.

isotonic Two solutions having the same osmotic pressure such that, when separated by a semipermeable membrane, there is no net movement of solvent in either direction.

J

jaundice The yellowish pigmentation of skin, mucous membranes, sclera, deeper tissues, and excretions due to abnormal deposition of bile pigments. Jaundice is associated with liver infection, as with hepatitis B virus and leptospirosis.

JC virus (JCV) Causes a form of encephalitis (progressive multifocal leukoencephalopathy), especially in AIDS patients.

K

keratin Protein produced by outermost skin cells provides protection from trauma and moisture loss.

killed or inactivated vaccine A whole cell or intact virus preparation in which the microbes are dead or preserved and cannot multiply but are still capable of conferring immunity.

killer T cells A T lymphocyte programmed to directly affix cells and kill them. See **cytotoxicity**.

kingdom In the levels of classification, the second division from more general to more specific. Each domain is divided into kingdoms.

Koch's postulates A procedure to establish the specific cause of disease. In all cases of infection: (1) The agent must be found; (2) inoculations of a pure culture must reproduce the same disease in animals; (3) the agent must again be present in the experimental animal; and (4) a pure culture must again be obtained.

Krebs cycle or **tricarboxylic acid (TCA) cycle** The second pathway of the three pathways that complete the process of primary catabolism. Also called the *citric acid cycle*.

L

L form A stage in the lives of some bacteria in which they have no peptidoglycan.

labile In chemistry, molecules or compounds that are chemically unstable in the presence of environmental changes.

lactose One of the carbohydrates commonly referred to as sugars. Lactose is commonly found in milk.

lactose (*lac*) operon Control system that manages the regulation of lactose metabolism. It is composed of three DNA segments, including a regulator, a control locus, and a structural locus.

lag phase The early phase of population growth during which no signs of growth occur.

lagging strand The newly forming 5′ DNA strand that is discontinuously replicated in segments (Okazaki fragments).

latency The state of being inactive. Example: a latent virus or latent infection.

leading strand The newly forming 3′ DNA strand that is replicated in a continuous fashion without segments.

lesion A wound, injury, or some other pathologic change in tissues.

leukocidin A heat-labile substance formed by some pyogenic cocci that impairs and sometimes lyses leukocytes.

leukocytes White blood cells. The primary infection-fighting blood cells.

leukocytosis An abnormally large number of leukocytes in the blood, which can be indicative of acute infection.

leukopenia A lower-than-normal leukocyte count in the blood that can be indicative of blood infection or disease.

leukotriene An unsaturated fatty acid derivative of arachidonic acid. Leukotriene functions in chemotactic activity, smooth muscle contractility, mucus secretion, and capillary permeability.

ligase An enzyme required to seal the sticky ends of DNA pieces after splicing.

lipase A fat-splitting enzyme. Example: Triacylglycerol lipase separates the fatty acid chains from the glycerol backbone of triglycerides.

lipid A term used to describe a variety of substances that are not soluble in polar solvents, such as water, but will dissolve in nonpolar solvents such as benzene and chloroform. Lipids include triglycerides, phospholipids, steroids, and waxes.

lipopolysaccharide A molecular complex of lipid and carbohydrate found in the bacterial cell wall. The lipopolysaccharide (LPS) of gram-negative bacteria is an endotoxin with generalized pathologic effects such as fever.

lipoteichoic acid Anionic polymers containing glycerol that are anchored in the cytoplasmic membranes of gram-positive bacteria.

liquid [media] Growth-supporting substance in fluid form.

lithoautotroph Bacteria that rely on inorganic minerals to supply their nutritional needs. Sometimes referred to as *chemoautotrophs*.

live attenuated vaccines Vaccines composed of living organisms that have been weakened and cannot cause disease.

logarithmic or log phase Maximum rate of cell division during which growth is geometric in its rate of increase. Also called *exponential growth phase*.

lophotrichous Describing bacteria having a tuft of flagella at one or both poles.

lymphadenitis Inflammation of one or more lymph nodes. Also called *lymphadenopathy*.

lymphatic system A system of vessels and organs that serve as sites for development of immune cells and immune reactions. It includes the spleen, thymus, lymph nodes, and gut-associated lymphoid tissue (GALT).

lyophilization A method for preserving microorganisms (and other substances) by freezing and then drying them directly from the frozen state.

lyse To burst.

lysis The physical rupture or deterioration of a cell.

lysogenic conversion A bacterium acquires a new genetic trait due to the presence of genetic material from an infecting phage.

lysogeny The indefinite persistence of bacteriophage DNA in a host without bringing about the production of virions.

lysosome A cytoplasmic organelle containing lysozyme and other hydrolytic enzymes.

lysozyme An enzyme found in sweat, tears, and saliva that breaks down bacterial peptidoglycan.

M

macromolecules Large, molecular compounds assembled from smaller subunits, most notably biochemicals.

macronutrient A chemical substance required in large quantities (phosphate, for example).

macrophage A white blood cell derived from a monocyte that leaves the circulation and enters tissues. These cells are important in nonspecific phagocytosis and in regulating, stimulating, and cleaning up after immune responses.

major histocompatibility complex (MHC) A set of genes in mammals that produces molecules on surfaces of cells that differentiate among different individuals in the species. See **HLA**.

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maltose One of the carbohydrates referred to as sugars. A fermentable sugar formed from starch.

Mantoux test An intradermal screening test for tuberculin hypersensitivity. A red, firm patch of skin at the injection site greater than 10 mm in diameter after 48 hours is a positive result that indicates current or prior exposure to the TB bacillus.

marker Any trait or factor of a cell, virus, or molecule that makes it distinct and recognizable; example: a genetic marker.

maximum temperature The highest temperature at which an organism will grow.

MDR-TB Multidrug-resistant tuberculosis.

medium (plural, *media*) A nutrient used to grow organisms outside of their natural habitats.

membrane attack complex A group of proteins that insert themselves into bacterial or infected cell membranes as a result of complement activation.

memory (immunologic memory) The capacity of the immune system to recognize and act against an antigen upon second and subsequent encounters.

Mendosicutes Taxonomic category of bacteria that have unusual cell walls; archaea.

meninges The tough tri-layer membrane covering the brain and spinal cord. Consists of the dura mater, arachnoid mater, and pia mater.

meningitis An inflammation of the membranes (meninges) that surround and protect the brain. It is often caused by bacteria such as *Neisseria meningitidis* (the meningococcus) and *Haemophilus influenzae*.

mesophile Microorganisms that grow at intermediate temperatures.

messenger RNA (mRNA) A single-stranded transcript that is a copy of the DNA template that corresponds to a gene.

metabolism A general term for the totality of chemical and physical processes occurring in a cell.

metabolomics The study of the complete complement of small chemicals present in a cell at any given time.

metagenomics Analysis of total DNA from an environmental or patient sample, without culturing any microorganisms.

microbial antagonism Relationship in which microorganisms compete for survival in a common environment by taking actions that inhibit or destroy another organism.

microbicides Chemicals that kill microorganisms.

microbiology A specialized area of biology that deals with living things ordinarily too small to be seen without magnification, including bacteria, archaea, fungi, protozoa, and viruses.

microbistatic The quality of inhibiting the growth of microbes.

micronutrient A chemical substance required in small quantities (trace metals, for example).

microorganism A living thing ordinarily too small to be seen without magnification; an organism of microscopic size.

microscopic Invisible to the naked eye.

microtubules Long hollow tubes in eukaryotic cells; maintain the shape of the cell and transport substances from one part of cell to another; involved in separating chromosomes in mitosis.

minimum inhibitory concentration (MIC) The smallest concentration of drug needed to visibly control microbial growth.

minimum temperature The lowest temperature at which an organism will grow.

missense mutation A mutation in which a change in the DNA sequence results in a different amino acid being incorporated into a protein, with varying results.

mitochondrion A double-membrane organelle of eukaryotes that is the main site for aerobic respiration.

monocyte A large mononuclear leukocyte normally found in the lymph nodes, spleen, bone marrow, and loose connective tissue. This type of cell makes up 3% to 7% of circulating leukocytes.

monomer A simple molecule that can be linked by chemical bonds to form larger molecules.

mononuclear phagocyte system (MPS) A collection of monocytes and macrophages scattered throughout the extracellular spaces that function to engulf and degrade foreign molecules.

monosaccharide A simple sugar such as glucose that is a basic building block for more complex carbohydrates.

monotrichous Describing a microorganism that bears a single flagellum.

morbidity rate The number of persons afflicted with an illness under question or with illness in general, expressed as a numerator, with the denominator being some unit of population (as in *x*/100,000).

mortality rate The number of persons who have died as the result of a particular cause or due to all causes, expressed as a numerator, with the denominator being some unit of population (as in *x*/100,000).

motility Self-propulsion.

mucosa-associated lymphoid tissue (MALT) Patches of lymphatic tissue containing B and T cells that underlie the surface of most mucosal surfaces in the body.

mutagen Any agent that induces genetic mutation; examples: certain chemical substances, ultraviolet light, radioactivity.

mutant strain A subspecies of microorganism that has undergone a mutation, causing expression of a trait that differs from other members of that species.

mutualism Organisms living in an obligatory but mutually beneficial relationship.

mycelium The filamentous mass that makes up a mold. Composed of hyphae.

N

nanotubes Extensions of bacterial membranes that are channels for nutrient or energy exchange.

narrow-spectrum Denotes drugs that are selective and limited in their effects. For example, they inhibit either gram-negative or gram-positive bacteria, but not both.

natural immunity Any immunity that arises naturally in an organism via previous experience with the antigen.

negative stain A staining technique that renders the background opaque or colored and leaves the object unstained so that it is outlined as a colorless area.

nematode A common name for helminths called roundworms.

neurons Cells that make up the tissues of the brain and spinal cord that receive and transmit signals to and from the peripheral nervous system and central nervous system.

neurotropic Having an affinity for the nervous system. Most likely to affect the spinal cord.

neutralization The process of combining an acid and a base until they reach a balanced proportion, with a pH value close to 7.

neutrophil A mature granulocyte present in peripheral circulation, exhibiting a multilobular nucleus and numerous cytoplasmic granules that retain a neutral stain. The neutrophil is an active phagocytic cell in bacterial infection.

nitrogen base A ringed compound of which pyrimidines and purines are types.

nitrogenous base A nitrogen-containing molecule found in DNA and RNA that provides the basis for the genetic code. Adenine, guanine, and cytosine are found in both DNA and RNA, while thymine is found exclusively in DNA and uracil is found exclusively in RNA.

noncommunicable An infectious disease that does not arrive through transmission of an infectious agent from host to host.

noncompetitive inhibition Form of enzyme inhibition that involves binding of a regulatory molecule to a site other than the active site.

nonself Molecules recognized by the immune system as containing foreign markers, indicating a need for immune response.

nonsense mutation A mutation that changes an amino-acid-producing codon into a stop codon, leading to premature termination of a protein.

nosocomial infection An infection not present upon admission to a hospital but incurred while being treated there.

nucleic acid A polymeric strand of nucleotides; exists in two forms: ribonucleic acid (RNA) and deoxyribonucleic acid (DNA).

nucleocapsid In viruses, the close physical combination of the nucleic acid with its protective covering.

nucleoid The basophilic nuclear region or nuclear body that contains the bacterial chromosome.

nucleolus A granular mass containing RNA that is contained within the nucleus of a eukaryotic cell.

nucleotide The basic structural unit of DNA and RNA; each nucleotide consists of a phosphate, a sugar (ribose in RNA, deoxyribose in DNA), and a nitrogenous base such as adenine, guanine, cytosine, thymine (DNA only), or uracil (RNA only).

O

Okazaki fragment In replication of DNA, a segment formed on the lagging strand in which biosynthesis is conducted in a discontinuous manner dictated by the 5′ → 3′ DNA polymerase orientation.

oncogene A naturally occurring type of gene that when activated can transform a normal cell into a cancer cell.

oncovirus Mammalian virus capable of causing malignant tumors.

operator In an operon sequence, the DNA segment where transcription of structural genes is initiated.

operon A genetic operational unit that regulates metabolism by controlling mRNA production. In sequence, the unit consists of a regulatory gene, inducer or repressor control sites, and structural genes.

opportunistic In infection, ordinarily nonpathogenic or weakly pathogenic microbes that cause disease primarily in an immunologically compromised host.

opsonization The process of stimulating phagocytosis by affixing molecules (opsonins such as antibodies and complement) to the surfaces of foreign cells or particles.

optimum temperature The temperature at which a species shows the most rapid growth rate.

order In the levels of classification, the division of organisms that follows class. Increasing similarity may be noticed among organisms assigned to the same order.

organelle A small component of eukaryotic cells that is bounded by a membrane and specialized in function.

osmophile A microorganism that thrives in a medium having high osmotic pressure.

osmosis The diffusion of water across a selectively permeable membrane in the direction of lower water concentration.

outer membrane (OM) An additional membrane possessed by gram-negative bacteria; a lipid bilayer containing specialized proteins and polysaccharides. It lies outside of the cell wall.

oxidation In chemical reactions, the loss of electrons by one reactant.

oxidation-reduction Redox reactions in which paired sets of molecules participate in electron transfers.

oxidative phosphorylation The synthesis of ATP using energy given off during the electron transport phase of respiration.

P

palindrome A word, verse, number, or sentence that reads the same forward or backward. Palindromes of nitrogen bases in DNA have genetic significance as transposable elements, as regulatory protein targets, and in DNA splicing.

palisades The characteristic arrangement of *Corynebacterium* cells resembling a row of fence posts and created by snapping.

pandemic A disease afflicting an increased proportion of the population over a wide geographic area (often worldwide).

paracortical area A layer of the lymph node internal to the cortex; houses T cells.

parasite An organism that lives on or within another organism (the host) from which it obtains nutrients and enjoys protection. The parasite produces some degree of harm in the host.

parasitism A relationship between two organisms in which the host is harmed in some way while the colonizer benefits.

paroxysmal Events characterized by sharp spasms or convulsions; sudden onset of a symptom such as fever and chills.

passive immunity Specific resistance that is acquired indirectly by donation of preformed immune substances (antibodies) produced in the body of another individual.

pasteurization Heat treatment of perishable fluids such as milk, fruit juices, or wine to destroy heat-sensitive vegetative cells, followed by rapid chilling to inhibit growth of survivors and germination of spores. It prevents infection and spoilage.

pathogen Any agent (usually a virus, bacterium, fungus, protozoan, or helminth) that causes disease.

pathogen-associated molecular patterns (PAMPs) Molecules on the surfaces of many types of microbes that are not present on host cells that mark the microbes as foreign.

pathogenicity The capacity of microbes to cause disease.

pattern recognition receptors (PRRs) Molecules on the surface of host defense cells that recognize pathogen-associated molecular patterns on microbes.

pellicle A membranous cover; a thin skin, film, or scum on a liquid surface; a thin film of salivary glycoproteins that forms over newly cleaned tooth enamel when exposed to saliva.

penetration (viral) The step in viral multiplication in which virus enters the host cell.

pentose A monosaccharide with five carbon atoms per molecule; examples: arabinose, ribose, xylose.

peptide Molecule composed of short chains of amino acids, such as a dipeptide (two amino acids), a tripeptide (three), and a tetrapeptide (four).

peptide bond The covalent union between two amino acids that forms between the amine group of one and the carboxyl group of the other. The basic bond of proteins.

peptidoglycan (PG) A network of polysaccharide chains cross-linked by short peptides that forms the rigid part of bacterial cell walls. Gram-negative bacteria have a smaller amount of this rigid structure than do gram-positive bacteria.

perforin Proteins released by cytotoxic T cells that produce pores in target cells.

peritrichous In bacterial morphology, having flagella distributed over the entire cell.

petechiae Minute hemorrhagic spots in the skin that range from pinpoint- to pinhead-size.

Peyer's patches Oblong lymphoid aggregates of the gut located chiefly in the wall of the terminal and small intestine. Along with the tonsils and appendix, Peyer's patches make up the gut-associated lymphoid tissue that responds to local invasion by infectious agents.

pH The symbol for the negative logarithm of the H ion concentration; p (power) or $[H^+]_{10}$. A system for rating acidity and alkalinity.

phagocyte A class of white blood cells capable of engulfing other cells and particles.

phagocytosis A type of endocytosis in which the cell membrane actively engulfs large particles or cells into vesicles.

phase variation The process of bacteria turning on or off a group of genes that changes its phenotype in a heritable manner.

phenotype The observable characteristics of an organism produced by the interaction between its genetic potential (genotype) and the environment.

phosphate An acidic salt containing phosphorus and oxygen that is an essential inorganic component of DNA, RNA, and ATP.

phosphorylation Process in which inorganic phosphate is added to a compound.

photoautotroph An organism that utilizes light for its energy and carbon dioxide chiefly for its carbon needs.

photosynthesis A process occurring in plants, algae, and some bacteria that traps the sun's energy and converts it to ATP in the cell. This energy is used to fix CO_2 into organic compounds.

phototrophs Microbes that use photosynthesis to feed.

phylum In the levels of classification, the third level of classification from general to more specific. Each kingdom is divided into numerous phyla. Sometimes referred to as a *division*.

pili (singular, ***pilus***) Long, tubular structures made of pilin protein produced by gram-negative bacteria and used for conjugation.

pinocytosis The engulfment, or endocytosis, of liquids by extensions of the cell membrane.

plaque In virus propagation methods, the clear zone of lysed cells in tissue culture or chick embryo membrane that corresponds to the area containing viruses. In dental application, the filamentous mass of microbes that adheres tenaciously to the tooth and predisposes to caries, calculus, or inflammation.

plasma The carrier fluid element of blood.

plasmids Extra chromosomal genetic units characterized by several features. A plasmid is a double-stranded DNA that is smaller than and replicates independently of the cell chromosome; it bears genes that are not essential for cell growth; it can bear genes that code for adaptive traits; and it is transmissible to other bacteria.

pleomorphism Normal variability of cell shapes in a single species.

pneumonia An inflammation of the lung leading to accumulation of fluid and respiratory compromise.

pneumonic plague The acute, frequently fatal form of pneumonia caused by *Yersinia pestis*.

point mutation A change that involves the loss, substitution, or addition of one or a few nucleotides.

point-source epidemic An outbreak of disease in which all affected individuals were exposed to a single source of the pathogen at a single point in time.

polymer A macromolecule made up of a chain of repeating units; examples: starch, protein, DNA.

polymerase chain reaction (PCR) A technique that amplifies segments of DNA for testing. Using denaturation, primers, and heat-resistant DNA polymerase, the number can be increased several-million-fold.

polymicrobial Involving multiple distinct microorganisms.

polypeptide A relatively large chain of amino acids linked by peptide bonds.

polyribosomal complex An assembly line for mass production of proteins composed of a chain of ribosomes involved in mRNA transcription.

polysaccharide A carbohydrate that can be hydrolyzed into a number of monosaccharides; examples: cellulose, starch, glycogen.

portal of entry Route of entry for an infectious agent; typically a cutaneous or membranous route.

portal of exit Route through which a pathogen departs from the host organism.

positive stain A method for coloring microbial specimens that involves a chemical that sticks to the specimen to give it color.

posttranslational Referring to modifications to the protein structure that occur after protein synthesis is complete, including removal of formyl methionine, further folding of the protein, addition of functional groups, or addition of the protein to a quaternary structure.

prebiotics Nutrients used to stimulate the growth of favorable biota in the intestine.

prevalence The total number of cases of a disease in a certain area and time period.

primary lymphatic organ Sites where B and T lymphocytes are generated and become mature. In the human, the red bone marrow and the thymus are primary lymphatic organs.

primary response The first response of the immune system when exposed to an antigen.

primary structure Initial protein organization described by type, number, and order of amino acids in the chain. The primary structure varies extensively from protein to protein.

primers Synthetic oligonucleotides of known sequence that serve as landmarks to indicate where DNA amplification will begin.

prion A concocted word to denote "proteinaceous infectious agent"; a cytopathic protein associated with the slow-virus spongiform encephalopathies of humans and animals.

probes Small fragments of single-stranded DNA (RNA) that are known to be complementary to the specific sequence of DNA being studied.

probiotics Preparations of live microbes used as a preventive or therapeutic measure to displace or compete with potential pathogens.

prodromal stage A short period of mild symptoms occurring at the end of the period of incubation. It indicates the onset of disease.

progressive multifocal leukoencephalopathy (PML) An uncommon, fatal complication of infection with JC virus (polyoma virus).

prokaryotic cells Small cells, lacking special structures such as a nucleus and organelles. All **prokaryotes** are microorganisms.

promoter Part of an operon sequence. The DNA segment that is recognized by RNA polymerase as the starting site for transcription.

promoter region The site composed of a short signaling DNA sequence that RNA polymerase recognizes and binds to to commence transcription.

propagated epidemic An outbreak of disease in which the causative agent is passed from affected persons to new persons over the course of time.

prophage A lysogenized bacteriophage; a phage that is latently incorporated into the host chromosome instead of undergoing viral replication and lysis.

prostaglandin A hormonelike substance that regulates many body functions. Prostaglandin comes from a family of organic acids containing 5-carbon rings that are essential to the human diet.

protease Enzymes that act on proteins, breaking them down into component parts.

protein Predominant organic molecule in cells, formed by long chains of amino acids.

proteomics The study of an organism's complement of proteins (its *proteome*) and functions mediated by the proteins.

protozoa A group of single-celled, eukaryotic organisms.

provirus The genome of a virus when it is integrated into a host cell's DNA.

pseudohypha A chain of easily separated, spherical to sausage-shaped yeast cells partitioned by constrictions rather than by septa.

psychrophile A microorganism that thrives at low temperature (0°C–20°C), with a temperature optimum of 0°C–15°C.

pure culture A container growing a single species of microbe whose identity is known.

purine A nitrogen base that is an important encoding component of DNA and RNA. The two most common purines are adenine and guanine.

purpura Purple-colored spots or blotches on the skin.

pus The viscous, opaque, usually yellowish matter formed by an inflammatory infection. It consists of serum exudate, tissue debris, leukocytes, and microorganisms.

pyogenic Pertains to pus formers, especially the pyogenic cocci: pneumococci, streptococci, staphylococci, and neisseriae.

pyrimidine Nitrogen bases that help form the genetic code on DNA and RNA. Uracil, thymine, and cytosine are the most important pyrimidines.

pyrogen A substance that causes a rise in body temperature. It can come from pyrogenic microorganisms or from polymorphonuclear leukocytes (endogenous pyrogens).

Q

quaternary structure Most complex protein structure characterized by the formation of large, multiunit proteins by more than one of the polypeptides. This structure is typical of antibodies and some enzymes that act in cell synthesis.

quinine A substance derived from cinchona trees that was used as an antimalarial treatment; has been replaced by synthetic derivatives.

quorum sensing The ability of bacteria to regulate their gene expression in response to sensing bacterial density.

R

rabies The only rhabdovirus that infects humans. Zoonotic disease characterized by fatal meningoencephalitis.

radiation Electromagnetic waves or rays, such as those of light given off from an energy source.

rales Sounds in the lung, ranging from clicking to rattling; indicate respiratory illness.

real image An image formed at the focal plane of a convex lens. In the compound light microscope, it is the image created by the objective lens.

recombinant An organism that contains genes that originated in another organism, whether through deliberate laboratory manipulation or natural processes.

recombinant DNA technology A technology, also known as *genetic engineering*, that deliberately modifies the genetic structure of an organism to create novel products, microbes, animals, plants, and viruses.

recombination A type of genetic transfer in which DNA from one organism is donated to another.

reducing agent An atom or a compound that can donate electrons in a chemical reaction.

reducing medium A growth medium that absorbs oxygen and allows anaerobic bacteria to grow.

redundancy The property of the genetic code that allows an amino acid to be specified by several different codons.

refractive index The measurement of the degree of light that is bent, or refracted, as it passes between two substances such as air, water, or glass.

regulated enzymes Enzymes whose extent of transcription or translation is influenced by changes in the environment.

regulator DNA segment that codes for a protein capable of repressing an operon.

release The final step in the multiplication cycle of viruses in which the assembled virus particle exits the host cell and moves on to infect another cell.

reportable disease Those diseases that must be reported to health authorities by law.

repressor The protein product of a repressor gene that combines with the operator and arrests the transcription and translation of structural genes.

reproduction rate The average number of susceptible persons one infected host will spread an infection to; describes communicability of an infectious disease.

reservoir In disease communication, the natural host or habitat of a pathogen.

resistance (R) factor Plasmids, typically shared among bacteria by conjugation, that provide resistance to the effects of antibiotics.

resolving power The capacity of a microscope lens system to accurately distinguish between two separate entities that lie close to each other. Also called *resolution*.

respiratory syncytial virus (RSV) An RNA virus that infects the respiratory tract. RSV is the most prevalent cause of respiratory infection in newborns.

restriction endonuclease An enzyme present naturally in cells that cleaves specific locations on DNA. It is an important means of inactivating viral genomes, and it is also used to splice genes in genetic engineering.

restriction factors Host cell molecules that inhibit viral replication.

restriction fragment length polymorphisms (RFLPs) Variations in the lengths of DNA fragments produced when a specific restriction endonuclease acts on different DNA sequences.

restriction fragments Short pieces of DNA produced when DNA is exposed to restriction endonucleases.

reverse transcriptase (RT) The enzyme possessed by retroviruses that carries out the reversion of RNA to DNA—a form of reverse transcription.

Rh factor An isoantigen that can trigger hemolytic disease in newborns due to incompatibility between maternal and infant blood factors.

ribose A 5-carbon monosaccharide found in RNA.

ribosomal RNA (rRNA) A single-stranded transcript that is a copy of part of the DNA template.

ribosome A bilobed macromolecular complex of ribonucleoprotein that coordinates the codons of mRNA with tRNA anticodons and, in so doing, constitutes the peptide assembly site.

RNA polymerase Enzyme process that translates the code of DNA to RNA.

rolling circle An intermediate stage in viral replication of circular DNA into linear DNA.

rough endoplasmic reticulum (RER) Microscopic series of tunnels that originates in the outer membrane of the nuclear envelope and is used in transport and storage. Large numbers of ribosomes, partly attached to the membrane, give the rough appearance.

rubeola (red measles) Acute disease caused by infection with *Morbillivirus*.

S

S layer Single layer of thousands of copies of a single type of protein linked together on the surface of a bacterial cell that is produced when the cell is in a hostile environment.

saccharide Scientific term for sugar. Refers to a simple carbohydrate with a sweet taste.

salpingitis Inflammation of the fallopian tubes.

saprobe A microbe that decomposes organic remains from dead organisms. Also known as a *saprophyte* or *saprotroph*.

sarcina A cubical packet of 8, 16, or more cells; the cellular arrangement of the genus *Sarcina* in the family *Micrococcaceae*.

schistosomiasis Infection by blood fluke, often as a result of contact with contaminated water in rivers and streams. Symptoms appear in liver, spleen, or urinary system depending on species of *Schistosoma*. Infection may be chronic.

sebaceous glands The sebum- (oily, fatty) secreting glands of the skin.

sebum Low pH, oil-based secretion of the sebaceous glands.

secondary lymphatic organs Tissue locations where T and B lymphocytes perform their actions. Examples are lymph nodes, MALT, SALT.

secondary response The rapid rise in antibody titer following a repeat exposure to an antigen that has been recognized from a previous exposure. This response is brought about by memory cells produced as a result of the primary exposure.

secondary structure Protein structure that occurs when the functional groups on the outer surface of the molecule interact by forming hydrogen bonds. These bonds cause the amino acid chain to either twist, forming a helix, or to pleat into an accordion pattern called a *β-pleated sheet*.

selective media Nutrient media designed to favor the growth of certain microbes and to inhibit undesirable competitors.

selectively toxic Property of an antimicrobial agent to be highly toxic against its target microbe while being far less toxic to other cells, particularly those of the host organism.

self Natural markers of the body that are recognized by the immune system.

semiconservative replication In DNA replication, the synthesis of paired daughter strands, each retaining a parent strand template.

semisolid [media] Nutrient media with a firmness midway between that of a broth (a liquid medium) and an ordinary solid medium; motility media.

sepsis The state of putrefaction; the presence of pathogenic organisms or their toxins in tissue or blood.

septic shock Blood infection resulting in a pathological state of low blood pressure accompanied by a reduced amount of blood circulating to vital organs. Endotoxins of all gram-negative bacteria can cause shock, but most clinical cases are due to gram-negative enteric rods.

septicemia Systemic infection associated with microorganisms multiplying in circulating blood.

septicemic plague A form of infection with *Yersinia pestis* occurring mainly in the bloodstream and leading to high mortality rates.

septum (plural, ***septa***) A partition or cellular cross wall, as in certain fungal hyphae.

sequela A morbid complication that follows a disease.

serology The branch of immunology that deals with *in vitro* diagnostic testing of serum.

serotonin A vasoconstrictor that inhibits gastric secretion and stimulates smooth muscle.

serotyping The subdivision of a species or subspecies into an immunologic type, based upon antigenic characteristics.

serous Referring to serum, the clear fluid that escapes cells during the inflammatory response.

serum sickness A type of immune complex disease in which immune complexes enter circulation, are carried throughout the body, and are deposited in the blood vessels of the kidney, heart, skin, and joints. The condition may become chronic.

severe combined immunodeficiency (SCID) A collection of syndromes occurring in newborns caused by a genetic defect that knocks out both B- and T-cell types of immunity. There are several versions of this disease.

Shiga toxin Heat-labile exotoxin released by some *Shigella* species and by *E. coli* O157:H7; responsible for worst symptoms of these infections.

shingles Lesions produced by reactivated human herpesvirus 3 (chickenpox) infection; also known as herpes zoster.

sign Any abnormality uncovered upon physical diagnosis that indicates the presence of disease. A *sign* is an objective assessment of disease, as opposed to a *symptom*, which is the subjective assessment perceived by the patient.

silent mutation A mutation that, because of the degeneracy of the genetic code, results in a nucleotide change in both the DNA and mRNA but not the resultant amino acid and, thus, not the protein.

simple stain Type of positive staining technique that uses a single dye to add color to cells so that they are easier to see. This technique tends to color all cells the same color.

single nucleotide polymorphism (SNP) A mutation of a single nucleotide in DNA causing differences among individuals carrying that mutation from those not carrying it.

skin-associated lymphoid tissue (SALT) Patches of lymphatic tissue containing B and T cells that underlie the surface of many skin surfaces in the body.

slime layer A diffuse, unorganized layer of polysaccharides and/or proteins on the outside of some bacteria.

smooth endoplasmic reticulum (SER) A microscopic series of tunnels lacking ribosomes that functions in the nutrient processing function of a cell.

species In the levels of classification, the most specific level of organization.

specificity In immunity, the concept that some parts of the immune system only react with antigens that originally activated them.

spirillum A type of bacterial cell with a rigid spiral shape and external flagella.

spirochete A coiled, spiral-shaped bacterium that has endoflagella and flexes as it moves.

spliceosome A molecule composed of RNA and protein that removes introns from eukaryotic mRNA before it is translated by forming a loop in the intron, cutting it from the mRNA, and joining exons together.

spontaneous generation Early belief that living things arose from vital forces present in nonliving, or decomposing, matter.

spontaneous mutation A mutation in DNA caused by random mistakes in replication and not known to be influenced by any mutagenic agent. These mutations give rise to an organism's natural, or background, rate of mutation.

sporadic Description of a disease that exhibits new cases at irregular intervals in unpredictable geographic locales.

sporangiospore A form of asexual spore in fungi; enclosed in a sac.

sporangium A fungal cell in which asexual spores are formed by multiple cell cleavage.

spore A differentiated, specialized cell form that can be used for dissemination, for survival in times of adverse conditions, and/or for reproduction. Spores are usually unicellular and may develop into gametes or vegetative organisms.

sporozoite One of many minute elongated bodies generated by multiple division of the oocyst. It is the infectious form of the malarial parasite that is harbored in the salivary gland of the mosquito and inoculated into the victim during feeding.

sporulation The process of spore formation.

stationary growth phase Survival mode in which cells either stop growing or grow very slowly.

stem cells Pluripotent, undifferentiated cells.

sterile Completely free of all life forms, including spores and viruses.

streptolysin A hemolysin produced by streptococci.

structural gene A gene that codes for the amino acid sequence (peptide structure) of a protein.

subacute Indicates an intermediate status between acute and chronic disease.

subacute sclerosing panencephalitis (SSPE) A complication of measles infection in which progressive neurological degeneration of the cerebral cortex invariably leads to coma and death.

subclinical A period of inapparent manifestations that occurs before symptoms and signs of disease appear.

substrate The specific molecule upon which an enzyme acts.

subunit vaccine A vaccine preparation that contains only antigenic fragments such as surface receptors from the microbe. Usually in reference to virus vaccines.

sucrose One of the carbohydrates commonly referred to as sugars. Common table or cane sugar.

superantigens Bacterial toxins that are potent stimuli for T cells and can be a factor in diseases such as toxic shock.

superficial mycosis A fungal infection located in hair, nails, and the epidermis of the skin.

superinfection An infection occurring during antimicrobial therapy that is caused by an overgrowth of drug-resistant microorganisms.

symbiosis An intimate association between individuals from two species; used as a synonym for *mutualism*.

symptom The subjective evidence of infection and disease as perceived by the patient.

syncytium (plural, ***syncytia***) A multinucleated protoplasmic mass formed by consolidation of individual cells.

syndrome The collection of signs and symptoms that, taken together, paint a portrait of the disease.

synergism The coordinated or correlated action by two or more drugs or microbes that results in a heightened response or greater activity.

synthesis (viral) The step in viral multiplication in which viral genetic material and proteins are made through replication and transcription/translation.

systemic Occurring throughout the body; said of infections that invade many compartments and organs via the circulation.

T

taxa Taxonomic categories.

taxonomy The formal system for organizing, classifying, and naming living things.

teichoic acid Anionic polymers containing glycerol that appear in the walls of gram-positive bacteria.

temperate phage A bacteriophage that enters into a less virulent state by becoming incorporated into the host genome as a prophage instead of in the vegetative or lytic form that eventually destroys the cell.

template strand The strand in a double-stranded DNA molecule that is used as a model to synthesize a complementary strand of DNA or RNA during replication or transcription.

Tenericutes Taxonomic category of bacteria that lack cell walls.

teratogenic Causing abnormal fetal development.

tertiary structure Protein structure that results from additional bonds forming between functional groups in a secondary structure, creating a three-dimensional mass.

tetanospasmin The neurotoxin of *Clostridium tetani*, the agent of tetanus. Its chief action is directed upon the inhibitory synapses of the anterior horn motor neurons.

tetrads Groups of four.

theory of evolution The evidence cited to explain how evolution occurs.

therapeutic index The ratio of the toxic dose to the effective therapeutic dose that is used to assess the safety and reliability of the drug.

thermal death point (TDP) The lowest temperature that achieves sterilization in a given quantity of broth culture upon a 10-minute exposure. Examples: 55°C for *Escherichia coli*, 60°C for *Mycobacterium tuberculosis*, and 120°C for endospores.

thermal death time (TDT) The least time required to kill all cells of a culture at a specified temperature.

thermophile A microorganism that thrives at a temperature of 50°C or higher.

thymine (T) One of the nitrogen bases found in DNA but not in RNA. Thymine is in a pyrimidine form.

thymus Butterfly-shaped organ near the tip of the sternum that is the site of T-cell maturation.

tincture A medicinal substance dissolved in an alcoholic solvent.

tinea Ringworm; a fungal infection of the hair, skin, or nails.

titer In immunochemistry, a measure of antibody level in a patient, determined by agglutination methods.

tonsils A ring of lymphoid tissue in the pharynx that acts as a repository for lymphocytes.

topoisomerases Enzymes that can add or remove DNA twists and thus regulate the degree of supercoiling.

toxin A specific chemical product of microbes, plants, and some animals that is poisonous to other organisms.

toxoid A toxin that has been rendered nontoxic but is still capable of eliciting the formation of protective antitoxin antibodies; used in vaccines.

trace elements Micronutrients (zinc, nickel, and manganese) that occur in small amounts and are involved in enzyme function and maintenance of protein structure.

transcription Messenger RNA (mRNA) synthesis; the process by which a strand of RNA is produced against a DNA template.

transduction The transfer of genetic material from one bacterium to another by means of a bacteriophage vector.

transfection The introduction of DNA into eukaryotic cells from the environment by exposing cells to chemicals or electrical currents; similar to **transformation**.

transformation In microbial genetics, the transfer of genetic material contained in “naked” DNA fragments from a donor cell to a competent recipient cell.

translation Protein synthesis; the process of decoding the messenger RNA code into a polypeptide.

transmissible spongiform encephalopathies (TSEs) Diseases caused by proteinaceous infectious particles (also known as *prions*).

transport medium Microbiological medium that is used to transport specimens.

transposon A DNA segment with an insertion sequence at each end, enabling it to migrate to another plasmid, to the bacterial chromosome, or to a bacteriophage.

trematode A category of helminth; also known as *flatworm* or *fluke*.

triglyceride A type of lipid composed of a glycerol molecule bound to three fatty acids.

trophozoite A vegetative protozoan (feeding form) as opposed to a resting (cyst) form.

true pathogen A microbe capable of causing infection and disease in healthy persons with normal immune defenses.

tubercle In tuberculosis, the granulomatous well-defined lung lesion that can serve as a focus for latent infection.

tuberculin reaction A diagnostic test in which PPD, or purified protein derivative (of *M. tuberculosis*), is injected superficially under the skin and the area of reaction measured; also called the *Mantoux test*.

turbid Cloudy appearance of nutrient solution in a test tube due to growth of microbe population.

U

ubiquitous Present everywhere at the same time.

uncoating The process of removal of the viral coat and release of the viral genome by its newly invaded host cell.

uracil (U) One of the nitrogen bases in RNA but not in DNA. Uracil is in a pyrimidine form.

urinary tract infection (UTI) Invasion and infection of the urethra and bladder by bacterial residents, most often *E. coli*.

V

vaccine Originally used in reference to inoculation with the cowpox or vaccinia virus to protect against smallpox. In general, the term now pertains to injection of whole microbes (killed or attenuated), toxoids, or parts of microbes as a prevention or cure for disease.

vacuoles In the cell, membrane-bounded sacs containing fluids or solid particles to be digested, excreted, or stored.

variable (V) region The antigen binding fragment of an immunoglobulin molecule, consisting of a combination of heavy and light chains whose molecular conformation is specific for the antigen.

varicella Informal name for virus responsible for chickenpox as well as shingles; also known as *human herpesvirus 3* (HHV-3).

variolation A hazardous, outmoded process of deliberately introducing smallpox material scraped from a victim into the nonimmune subject in the hope of inducing resistance.

vasoactive Referring to chemical mediators involved in the immune response that act on endothelial cells or the smooth muscle of blood vessels causing them to either restrict or relax.

vector An animal that transmits infectious agents from one host to another, usually a biting or piercing arthropod like the tick, mosquito, or fly. Infectious agents can be conveyed mechanically by simple contact or biologically whereby the parasite develops in the vector. A genetic element such as a plasmid or a bacteriophage used to introduce genetic material into a cloning host during recombinant DNA experiments.

vegetative In describing microbial developmental stages, a metabolically active feeding and dividing form, as opposed to a dormant, seemingly inert, nondividing form. Examples: a bacterial cell versus its spore; a protozoal trophozoite versus its cyst.

viable nonculturable (VNC) A description of a state in which bacteria are alive but are not metabolizing at an appreciable rate and will not grow when inoculated onto laboratory medium.

vibrio A curved, rod-shaped bacterial cell.

viremia The presence of viruses in the bloodstream.

virion An elementary virus particle in its complete morphological and thus infectious form. A virion consists of the nucleic acid core surrounded by a capsid, which can be enclosed in an envelope.

viroid An infectious agent that, unlike a virion, lacks a capsid and consists of a closed circular RNA molecule. Although known viroids are all plant pathogens, it is conceivable that animal versions exist.

virome The complete array of viruses in a given ecosystem, such as the human body.

virtual image In optics, an image formed by diverging light rays; in the compound light microscope, the second, magnified visual impression formed by the ocular from the real image formed by the objective.

virulence In infection, the relative capacity of a pathogen to invade and harm host cells.

virulence factors A microbe's structures or capabilities that allow it to establish itself in a host and cause damage.

virus Microscopic, acellular agent composed of nucleic acid surrounded by a protein coat.

vitamins A component of coenzymes critical to nutrition and the metabolic function of coenzyme complexes.

W

Western blot test A procedure for separating and identifying antigen or antibody mixtures by two-dimensional electrophoresis in polyacrylamide gel, followed by immune labeling.

whole blood A liquid connective tissue consisting of blood cells suspended in plasma.

wild type The natural, nonmutated form of a genetic trait.

wobble A characteristic of amino acid codons in which the third base of a codon can be altered without changing the code for the amino acid.

X

XDR-TB Extensively drug-resistant tuberculosis (worse than multidrug-resistant tuberculosis).

xenograft The transfer of a tissue or an organ from an animal of one species to a recipient of another species.

Z

zoonosis An infectious disease indigenous to animals that humans can acquire through direct or indirect contact with infected animals.

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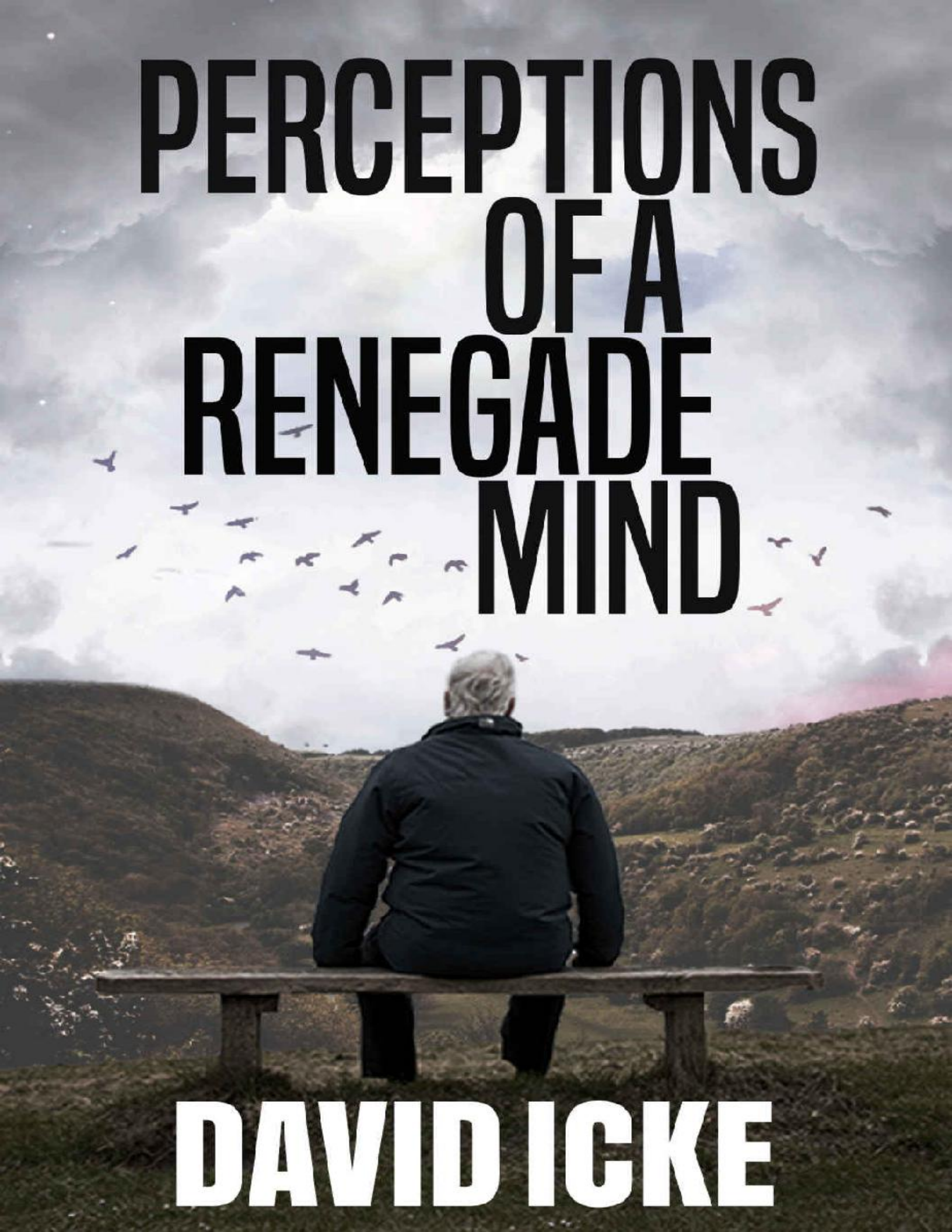

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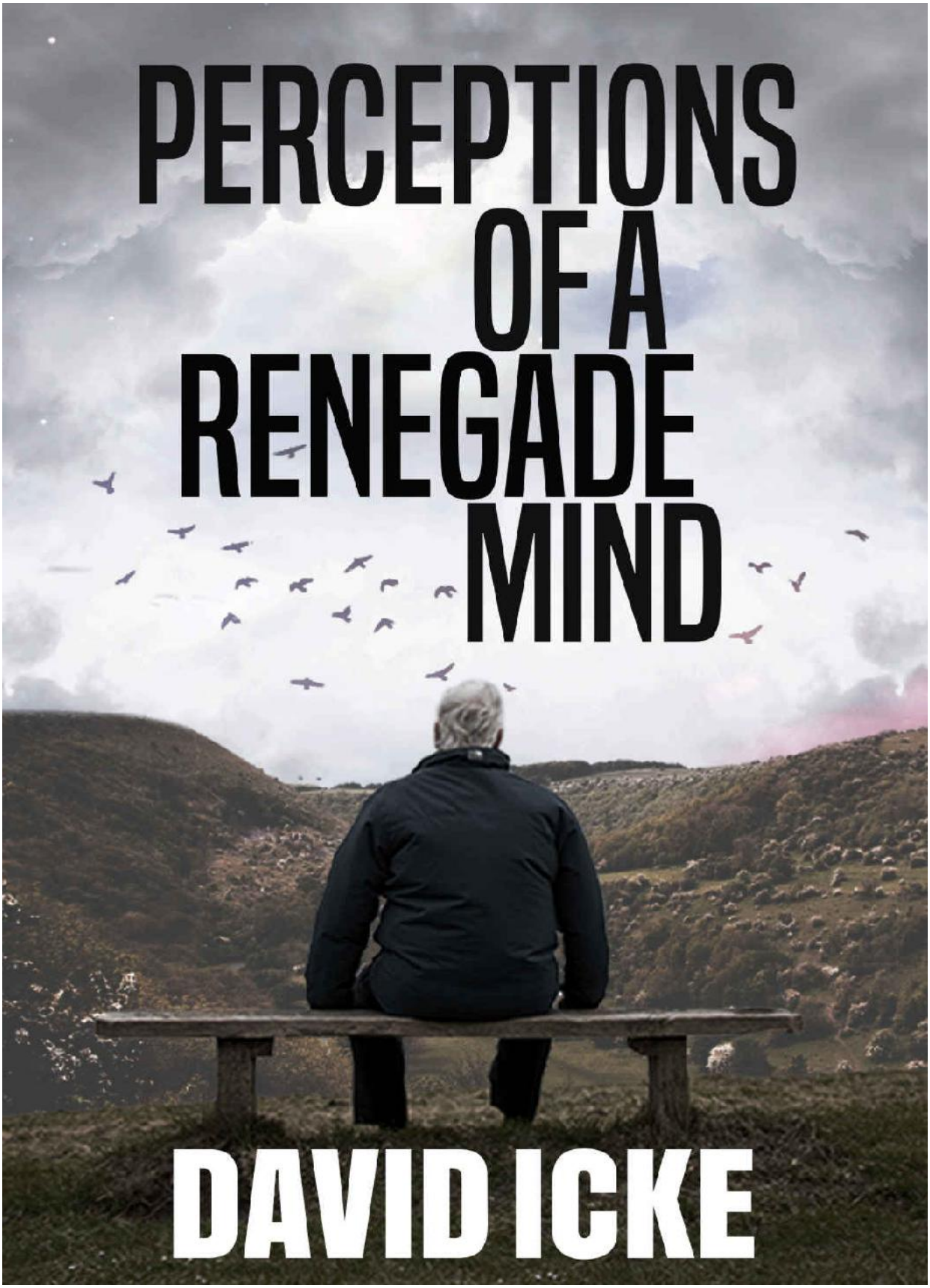
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PERCEPTIONS OF A RENEGADE MIND

DAVID ICKE

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Renegade:
Adjective
‘Having rejected tradition: Unconventional.’
Merriam-Webster Dictionary

Acquiescence to tyranny is the death of the spirit

You may be 38 years old, as I happen to be. And one day, some great opportunity stands before you and calls you to stand up for some great principle, some great issue, some great cause. And you refuse to do it because you are afraid … You refuse to do it because you want to live longer … You're afraid that you will lose your job, or you are afraid that you will be criticised or that you will lose your popularity, or you're afraid that somebody will stab you, or shoot at you or bomb your house; so you refuse to take the stand.

Well, you may go on and live until you are 90, but you're just as dead at 38 as you would be at 90. And the cessation of breathing in your life is but the belated announcement of an earlier death of the spirit.

Martin Luther King

How the few control the many and always have – the many do whatever they're told

'Forward, the Light Brigade!'
Was there a man dismayed?
Not though the soldier knew
Someone had blundered.
Theirs not to make reply,
Theirs not to reason why,
Theirs but to do and die.
Into the valley of Death
Rode the six hundred.

Cannon to right of them,
Cannon to left of them,
Cannon in front of them
Volleyed and thundered;
Stormed at with shot and shell,
Boldly they rode and well,
Into the jaws of Death,
Into the mouth of hell
Rode the six hundred

Alfred Lord Tennyson (1809-1892)

The mist is lifting slowly
I can see the way ahead
And I've left behind the empty streets
That once inspired my life
And the strength of the emotion
Is like thunder in the air
'Cos the promise that we made each other
Haunts me to the end

The secret of your beauty
And the mystery of your soul
I've been searching for in everyone I meet
And the times I've been mistaken
It's impossible to say
And the grass is growing
Underneath our feet

The words that I remember
From my childhood still are true
That there's none so blind
As those who will not see
And to those who lack the courage
And say it's dangerous to try
Well they just don't know
That love eternal will not be denied

I know you're out there somewhere
Somewhere, somewhere
I know you're out there somewhere

Somewhere you can hear my voice
I know I'll find you somehow
Somehow, somehow
I know I'll find you somehow
And somehow I'll return again to you

The Moody Blues

Are you a gutless wonder - or a Renegade Mind?

Monuments put from pen to paper,
Turns me into a gutless wonder,
And if you tolerate this,
Then your children will be next.
Gravity keeps my head down,
Or is it maybe shame ...

Manic Street Preachers

Rise like lions after slumber
In unvanquishable number.
Shake your chains to earth like dew
Which in sleep have fallen on you.
Ye are many – they are few.

Percy Shelley

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CHAPTER ONE

I'm thinking' – Oh, but *are* you?

Think for yourself and let others enjoy the privilege of doing so too
Voltaire

French-born philosopher, mathematician and scientist René Descartes became famous for his statement in Latin in the 17th century which translates into English as: 'I think, therefore I am.'

On the face of it that is true. Thought reflects perception and perception leads to both behaviour and self-identity. In that sense 'we' are what we think. But who or what is doing the thinking and is thinking the only route to perception? Clearly, as we shall see, 'we' are not always the source of 'our' perception, indeed with regard to humanity as a whole this is rarely the case; and thinking is far from the only means of perception. Thought is the village idiot compared with other expressions of consciousness that we all have the potential to access and tap into. This has to be true when we *are* those other expressions of consciousness which are infinite in nature. We have forgotten this, or, more to the point, been manipulated to forget.

These are not just the esoteric musings of the navel. The whole foundation of human control and oppression is control of perception. Once perception is hijacked then so is behaviour which is dictated by perception. Collective perception becomes collective behaviour and collective behaviour is what we call human society. Perception is all and those behind human control know that which is

why perception is the target 24/7 of the psychopathic manipulators that I call the Global Cult. They know that if they dictate perception they will dictate behaviour and collectively dictate the nature of human society. They are further aware that perception is formed from information received and if they control the circulation of information they will to a vast extent direct human behaviour. Censorship of information and opinion has become globally Nazi-like in recent years and never more blatantly than since the illusory 'virus pandemic' was triggered out of China in 2019 and across the world in 2020. Why have billions submitted to house arrest and accepted fascistic societies in a way they would have never believed possible? Those controlling the information spewing from government, mainstream media and Silicon Valley (all controlled by the same Global Cult networks) told them they were in danger from a 'deadly virus' and only by submitting to house arrest and conceding their most basic of freedoms could they and their families be protected. This monumental and provable lie became the *perception* of the billions and therefore the *behaviour* of the billions. In those few words you have the whole structure and modus operandi of human control. Fear is a perception – **F**alse **E**motion **A**ppearing **R**eal – and fear is the currency of control. In short … get them by the balls (or give them the impression that you have) and their hearts and minds will follow. Nothing grips the dangly bits and freezes the rear-end more comprehensively than fear.

World number 1

There are two 'worlds' in what appears to be one 'world' and the prime difference between them is knowledge. First we have the mass of human society in which the population is maintained in coldly-calculated ignorance through control of information and the 'education' (indoctrination) system. That's all you really need to control to enslave billions in a perceptual delusion in which what are perceived to be *their* thoughts and opinions are ever-repeated mantras that the system has been downloading all their lives through 'education', media, science, medicine, politics and academia

in which the personnel and advocates are themselves overwhelmingly the perceptual products of the same repetition. Teachers and academics in general are processed by the same programming machine as everyone else, but unlike the great majority they never leave the 'education' program. It gripped them as students and continues to grip them as programmers of subsequent generations of students. The programmed become the programmers – the programmed programmers. The same can largely be said for scientists, doctors and politicians and not least because as the American writer Upton Sinclair said: 'It is difficult to get a man to understand something when his salary depends upon his not understanding it.' If your career and income depend on thinking the way the system demands then you will – bar a few free-minded exceptions – concede your mind to the Perceptual Mainframe that I call the Postage Stamp Consensus. This is a tiny band of perceived knowledge and possibility 'taught' (downloaded) in the schools and universities, pounded out by the mainstream media and on which all government policy is founded. Try thinking, and especially speaking and acting, outside of the 'box' of consensus and see what that does for your career in the Mainstream Everything which bullies, harasses, intimidates and ridicules the population into compliance. Here we have the simple structure which enslaves most of humanity in a perceptual prison cell for an entire lifetime and I'll go deeper into this process shortly. Most of what humanity is taught as fact is nothing more than programmed belief. American science fiction author Frank Herbert was right when he said: 'Belief can be manipulated. Only knowledge is dangerous.' In the 'Covid' age belief is promoted and knowledge is censored. It was always so, but never to the extreme of today.

World number 2

A 'number 2' is slang for 'doing a poo' and how appropriate that is when this other 'world' is doing just that on humanity every minute of every day. World number 2 is a global network of secret societies and semi-secret groups dictating the direction of society via

governments, corporations and authorities of every kind. I have spent more than 30 years uncovering and exposing this network that I call the Global Cult and knowing its agenda is what has made my books so accurate in predicting current and past events. Secret societies are secret for a reason. They want to keep their hoarded knowledge to themselves and their chosen initiates and to hide it from the population which they seek through ignorance to control and subdue. The whole foundation of the division between World 1 and World 2 is *knowledge*. What number 1 knows number 2 must not. Knowledge they have worked so hard to keep secret includes (a) the agenda to enslave humanity in a centrally-controlled global dictatorship, and (b) the nature of reality and life itself. The latter (b) must be suppressed to allow the former (a) to prevail as I shall be explaining. The way the Cult manipulates and interacts with the population can be likened to a spider's web. The 'spider' sits at the centre in the shadows and imposes its will through the web with each strand represented in World number 2 by a secret society, satanic or semi-secret group, and in World number 1 – the world of the seen – by governments, agencies of government, law enforcement, corporations, the banking system, media conglomerates and Silicon Valley (Fig 1 overleaf). The spider and the web connect and coordinate all these organisations to pursue the same global outcome while the population sees them as individual entities working randomly and independently. At the level of the web governments *are* the banking system *are* the corporations *are* the media *are* Silicon Valley *are* the World Health Organization working from their inner cores as one unit. Apparently unconnected countries, corporations, institutions, organisations and people are on the *same team* pursuing the same global outcome. Strands in the web immediately around the spider are the most secretive and exclusive secret societies and their membership is emphatically restricted to the Cult inner-circle emerging through the generations from particular bloodlines for reasons I will come to. At the core of the core you would get them in a single room. That's how many people are dictating the direction of human society and its transformation

through the ‘Covid’ hoax and other means. As the web expands out from the spider we meet the secret societies that many people will be aware of – the Freemasons, Knights Templar, Knights of Malta, Opus Dei, the inner sanctum of the Jesuit Order, and such like. Note how many are connected to the Church of Rome and there is a reason for that. The Roman Church was established as a revamp, a rebranding, of the relocated ‘Church’ of Babylon and the Cult imposing global tyranny today can be tracked back to Babylon and Sumer in what is now Iraq.

Figure 1: The global web through which the few control the many. (Image Neil Hague.)

Inner levels of the web operate in the unseen away from the public eye and then we have what I call the cusp organisations located at the point where the hidden meets the seen. They include a series of satellite organisations answering to a secret society founded in London in the late 19th century called the Round Table and among them are the Royal Institute of International Affairs (UK, founded in 1920); Council on Foreign Relations (US, 1921); Bilderberg Group (worldwide, 1954); Trilateral Commission (US/worldwide, 1972); and the Club of Rome (worldwide, 1968) which was created to exploit environmental concerns to justify the centralisation of global power to ‘save the planet’. The Club of Rome instigated with others the human-caused climate change hoax which has led to all the ‘green

new deals' demanding that very centralisation of control. Cusp organisations, which include endless 'think tanks' all over the world, are designed to coordinate a single global policy between political and business leaders, intelligence personnel, media organisations and anyone who can influence the direction of policy in their own sphere of operation. Major players and regular attenders will know what is happening – or some of it – while others come and go and are kept overwhelmingly in the dark about the big picture. I refer to these cusp groupings as semi-secret in that they can be publicly identified, but what goes on at the inner-core is kept very much 'in house' even from most of their members and participants through a fiercely-imposed system of compartmentalisation. Only let them know what they need to know to serve your interests and no more. The structure of secret societies serves as a perfect example of this principle. Most Freemasons never get higher than the bottom three levels of 'degree' (degree of knowledge) when there are 33 official degrees of the Scottish Rite. Initiates only qualify for the next higher 'compartment' or degree if those at that level choose to allow them. Knowledge can be carefully assigned only to those considered 'safe'. I went to my local Freemason's lodge a few years ago when they were having an 'open day' to show how cuddly they were and when I chatted to some of them I was astonished at how little the rank and file knew even about the most ubiquitous symbols they use. The mushroom technique – keep them in the dark and feed them bullshit – applies to most people in the web as well as the population as a whole. Sub-divisions of the web mirror in theme and structure transnational corporations which have a headquarters somewhere in the world dictating to all their subsidiaries in different countries. Subsidiaries operate in their methodology and branding to the same centrally-dictated plan and policy in pursuit of particular ends. The Cult web functions in the same way. Each country has its own web as a subsidiary of the global one. They consist of networks of secret societies, semi-secret groups and bloodline families and their job is to impose the will of the spider and the global web in their particular country. Subsidiary networks control and manipulate the national political system, finance, corporations, media, medicine, etc. to

ensure that they follow the globally-dictated Cult agenda. These networks were the means through which the 'Covid' hoax could be played out with almost every country responding in the same way.

The 'Yessir' pyramid

Compartmentalisation is the key to understanding how a tiny few can dictate the lives of billions when combined with a top-down sequence of imposition and acquiescence. The inner core of the Cult sits at the peak of the pyramidal hierarchy of human society (Fig 2 overleaf). It imposes its will – its agenda for the world – on the level immediately below which acquiesces to that imposition. This level then imposes the Cult will on the level below them which acquiesces and imposes on the next level. Very quickly we meet levels in the hierarchy that have no idea there even is a Cult, but the sequence of imposition and acquiescence continues down the pyramid in just the same way. 'I don't know why we are doing this but the order came from "on-high" and so we better just do it.' Alfred Lord Tennyson said of the cannon fodder levels in his poem *The Charge of the Light Brigade*: 'Theirs not to reason why; theirs but to do and die.' The next line says that 'into the valley of death rode the six hundred' and they died because they obeyed without question what their perceived 'superiors' told them to do. In the same way the population capitulated to 'Covid'. The whole hierarchical pyramid functions like this to allow the very few to direct the enormous many. Eventually imposition-acquiescence-imposition-acquiescence comes down to the mass of the population at the foot of the pyramid. If they acquiesce to those levels of the hierarchy imposing on them (governments/law enforcement/doctors/media) a circuit is completed between the population and the handful of super-psychopaths in the Cult inner core at the top of the pyramid. Without a circuit-breaking refusal to obey, the sequence of imposition and acquiescence allows a staggeringly few people to impose their will upon the entirety of humankind. We are looking at the very sequence that has subjugated billions since the start of 2020. Our freedom has not been taken from us. Humanity has given it

away. Fascists do not impose fascism because there are not enough of them. Fascism is imposed by the population acquiescing to fascism. Put another way allowing their perceptions to be programmed to the extent that leads to the population giving their freedom away by giving their perceptions – their mind – away. If this circuit is not broken by humanity ceasing to cooperate with their own enslavement then nothing can change. For that to happen people have to critically think and see through the lies and window dressing and then summon the backbone to act upon what they see. The Cult spends its days working to stop either happening and its methodology is systematic and highly detailed, but it can be overcome and that is what this book is all about.

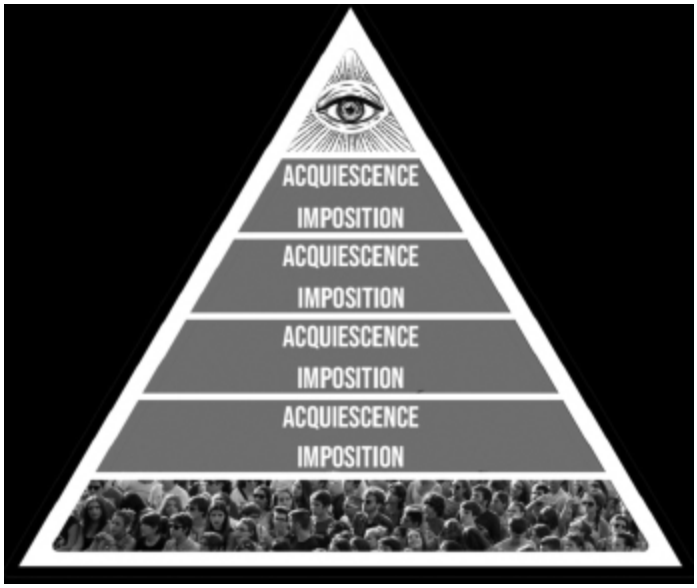

Figure 2: The simple sequence of imposition and compliance that allows a handful of people at the peak of the pyramid to dictate the lives of billions.

The Life Program

Okay, back to world number 1 or the world of the 'masses'. Observe the process of what we call 'life' and it is a perceptual download from cradle to grave. The Cult has created a global structure in which perception can be programmed and the program continually topped-up with what appears to be constant confirmation that the program is indeed true reality. The important word here is 'appears'.

This is the structure, the fly-trap, the Postage Stamp Consensus or Perceptual Mainframe, which represents that incredibly narrow band of perceived possibility delivered by the 'education' system, mainstream media, science and medicine. From the earliest age the download begins with parents who have themselves succumbed to the very programming their children are about to go through. Most parents don't do this out of malevolence and mostly it is quite the opposite. They do what they believe is best for their children and that is what the program has told them is best. Within three or four years comes the major transition from parental programming to full-blown state (Cult) programming in school, college and university where perceptually-programmed teachers and academics pass on their programming to the next generations. Teachers who resist are soon marginalised and their careers ended while children who resist are called a problem child for whom Ritalin may need to be prescribed. A few years after entering the 'world' children are under the control of authority figures representing the state telling them when they have to be there, when they can leave and when they can speak, eat, even go to the toilet. This is calculated preparation for a lifetime of obeying authority in all its forms. Reflex-action fear of authority is instilled by authority from the start. Children soon learn the carrot and stick consequences of obeying or defying authority which is underpinned daily for the rest of their life. Fortunately I daydreamed through this crap and never obeyed authority simply because it told me to. This approach to my alleged 'betters' continues to this day. There can be consequences of pursuing open-minded freedom in a world of closed-minded conformity. I spent a lot of time in school corridors after being ejected from the classroom for not taking some of it seriously and now I spend a lot of time being ejected from Facebook, YouTube and Twitter. But I can tell you that being true to yourself and not compromising your self-respect is far more exhilarating than bowing to authority for authority's sake. You don't have to be a sheep to the shepherd (authority) and the sheep dog (fear of not obeying authority).

The perceptual download continues throughout the formative years in school, college and university while script-reading 'teachers', 'academics' 'scientists', 'doctors' and 'journalists' insist that ongoing generations must be as programmed as they are. Accept the program or you will not pass your 'exams' which confirm your 'degree' of programming. It is tragic to think that many parents pressure their offspring to work hard at school to download the program and qualify for the next stage at college and university. The late, great, American comedian George Carlin said: 'Here's a bumper sticker I'd like to see: We are proud parents of a child who has resisted his teachers' attempts to break his spirit and bend him to the will of his corporate masters.' Well, the best of luck finding many of those, George. Then comes the moment to leave the formal programming years in academia and enter the 'adult' world of work. There you meet others in your chosen or prescribed arena who went through the same Postage Stamp Consensus program before you did. There is therefore overwhelming agreement between almost everyone on the basic foundations of Postage Stamp reality and the rejection, even contempt, of the few who have a mind of their own and are prepared to use it. This has two major effects. Firstly, the consensus confirms to the programmed that their download is really how things are. I mean, everyone knows that, right? Secondly, the arrogance and ignorance of Postage Stamp adherents ensure that anyone questioning the program will have unpleasant consequences for seeking their own truth and not picking their perceptions from the shelf marked: 'Things you must believe without question and if you don't you're a dangerous lunatic conspiracy theorist and a harebrained nutter'.

Every government, agency and corporation is founded on the same Postage Stamp prison cell and you can see why so many people believe the same thing while calling it their own 'opinion'. Fusion of governments and corporations in pursuit of the same agenda was the definition of fascism described by Italian dictator Benito Mussolini. The pressure to conform to perceptual norms downloaded for a lifetime is incessant and infiltrates society right

down to family groups that become censors and condemners of their own 'black sheep' for not, ironically, being sheep. We have seen an explosion of that in the 'Covid' era. Cult-owned global media unleashes its propaganda all day every day in support of the Postage Stamp and targets with abuse and ridicule anyone in the public eye who won't bend their mind to the will of the tyranny. Any response to this is denied (certainly in my case). They don't want to give a platform to expose official lies. Cult-owned-and-created Internet giants like Facebook, Google, YouTube and Twitter delete you for having an unapproved opinion. Facebook boasts that its AI censors delete 97-percent of 'hate speech' before anyone even reports it. Much of that 'hate speech' will simply be an opinion that Facebook and its masters don't want people to see. Such perceptual oppression is widely known as fascism. Even Facebook executive Benny Thomas, a 'CEO Global Planning Lead', said in comments secretly recorded by investigative journalism operation Project Veritas that Facebook is 'too powerful' and should be broken up:

> I mean, no king in history has been the ruler of two billion people, but Mark Zuckerberg is … And he's 36. That's too much for a 36-year-old ... You should not have power over two billion people. I just think that's wrong.

Thomas said Facebook-owned platforms like Instagram, Oculus, and WhatsApp needed to be separate companies. 'It's too much power when they're all one together'. That's the way the Cult likes it, however. We have an executive of a Cult organisation in Benny Thomas that doesn't know there is a Cult such is the compartmentalisation. Thomas said that Facebook and Google 'are no longer companies, they're countries'. Actually they are more powerful than countries on the basis that if you control information you control perception and control human society.

I love my oppressor

Another expression of this psychological trickery is for those who realise they are being pressured into compliance to eventually

convince themselves to believe the official narratives to protect their self-respect from accepting the truth that they have succumbed to meek and subservient compliance. Such people become some of the most vehement defenders of the system. You can see them everywhere screaming abuse at those who prefer to think for themselves and by doing so reminding the compliers of their own capitulation to conformity. 'You are talking dangerous nonsense you Covidiot!!' Are you trying to convince me or yourself? It is a potent form of Stockholm syndrome which is defined as: 'A psychological condition that occurs when a victim of abuse identifies and attaches, or bonds, positively with their abuser.' An example is hostages bonding and even 'falling in love' with their kidnappers. The syndrome has been observed in domestic violence, abused children, concentration camp inmates, prisoners of war and many and various Satanic cults. These are some traits of Stockholm syndrome listed at goodtherapy.org:

- Positive regard towards perpetrators of abuse or captor [see 'Covid'].
- Failure to cooperate with police and other government authorities when it comes to holding perpetrators of abuse or kidnapping accountable [or in the case of 'Covid' cooperating with the police to enforce and defend their captors' demands].
- Little or no effort to escape [see 'Covid'].
- Belief in the goodness of the perpetrators or kidnappers [see 'Covid'].
- Appeasement of captors. This is a manipulative strategy for maintaining one's safety. As victims get rewarded – perhaps with less abuse or even with life itself – their appeasing behaviours are reinforced [see 'Covid'].
- Learned helplessness. This can be akin to 'if you can't beat 'em, join 'em'. As the victims fail to escape the abuse or captivity, they may start giving up and soon realize it's just easier for everyone if they acquiesce all their power to their captors [see 'Covid'].

- Feelings of pity toward the abusers, believing they are actually victims themselves. Because of this, victims may go on a crusade or mission to 'save' [protect] their abuser [see the venom unleashed on those challenging the official 'Covid' narrative].
- Unwillingness to learn to detach from their perpetrators and heal. In essence, victims may tend to be less loyal to themselves than to their abuser [*definitely* see 'Covid'].

Ponder on those traits and compare them with the behaviour of great swathes of the global population who have defended governments and authorities which have spent every minute destroying their lives and livelihoods and those of their children and grandchildren since early 2020 with fascistic lockdowns, house arrest and employment deletion to 'protect' them from a 'deadly virus' that their abusers' perceptually created to bring about this very outcome. We are looking at mass Stockholm syndrome. All those that agree to concede their freedom will believe those perceptions are originating in their own independent 'mind' when in fact by conceding their reality to Stockholm syndrome they have by definition conceded any independence of mind. Listen to the 'opinions' of the acquiescing masses in this 'Covid' era and what gushes forth is the repetition of the official version of everything delivered unprocessed, unfiltered and unquestioned. The whole programming dynamic works this way. I must be free because I'm told that I am and so I think that I am.

You can see what I mean with the chapter theme of 'I'm thinking – Oh, but *are* you?' The great majority are not thinking, let alone for themselves. They are repeating what authority has told them to believe which allows them to be controlled. Weaving through this mentality is the fear that the 'conspiracy theorists' are right and this again explains the often hysterical abuse that ensues when you dare to contest the official narrative of anything. Denial is the mechanism of hiding from yourself what you don't want to be true. Telling people what they want to hear is easy, but it's an infinitely greater challenge to tell them what they would rather not be happening.

One is akin to pushing against an open door while the other is met with vehement resistance no matter what the scale of evidence. I don't want it to be true so I'll convince myself that it's not. Examples are everywhere from the denial that a partner is cheating despite all the signs to the reflex-action rejection of any idea that world events in which country after country act in exactly the same way are centrally coordinated. To accept the latter is to accept that a force of unspeakable evil is working to destroy your life and the lives of your children with nothing too horrific to achieve that end. Who the heck wants that to be true? But if we don't face reality the end is duly achieved and the consequences are far worse and ongoing than breaking through the walls of denial today with the courage to make a stand against tyranny.

Connect the dots – but how?

A crucial aspect of perceptual programming is to portray a world in which everything is random and almost nothing is connected to anything else. Randomness cannot be coordinated by its very nature and once you perceive events as random the idea they could be connected is waved away as the rantings of the tinfoil-hat brigade. You can't plan and coordinate random you idiot! No, you can't, but you can hide the coldly-calculated and long-planned behind the *illusion* of randomness. A foundation manifestation of the Renegade Mind is to scan reality for patterns that connect the apparently random and turn pixels and dots into pictures. This is the way I work and have done so for more than 30 years. You look for similarities in people, modus operandi and desired outcomes and slowly, then ever quicker, the picture forms. For instance: There would seem to be no connection between the 'Covid pandemic' hoax and the human-caused global-warming hoax and yet they are masks (appropriately) on the same face seeking the same outcome. Those pushing the global warming myth through the Club of Rome and other Cult agencies are driving the lies about 'Covid' – Bill Gates is an obvious one, but they are endless. Why would the same people be involved in both when they are clearly not connected? Oh, but they

are. Common themes with personnel are matched by common goals. The 'solutions' to both 'problems' are centralisation of global power to impose the will of the few on the many to 'save' humanity from 'Covid' and save the planet from an 'existential threat' (we need 'zero Covid' and 'zero carbon emissions'). These, in turn, connect with the 'dot' of globalisation which was coined to describe the centralisation of global power in every area of life through incessant political and corporate expansion, trading blocks and superstates like the European Union. If you are the few and you want to control the many you have to centralise power and decision-making. The more you centralise power the more power the few at the centre will have over the many; and the more that power is centralised the more power those at the centre have to centralise even quicker. The momentum of centralisation gets faster and faster which is exactly the process we have witnessed. In this way the hoaxed 'pandemic' and the fakery of human-caused global warming serve the interests of globalisation and the seizure of global power in the hands of the Cult inner-circle which is behind 'Covid', 'climate change' *and* globalisation. At this point random 'dots' become a clear and obvious picture or pattern.

Klaus Schwab, the classic Bond villain who founded the Cult's Gates-funded World Economic Forum, published a book in 2020, *The Great Reset*, in which he used the 'problem' of 'Covid' to justify a total transformation of human society to 'save' humanity from 'climate change'. Schwab said: 'The pandemic represents a rare but narrow window of opportunity to reflect, reimagine, and reset our world.' What he didn't mention is that the Cult he serves is behind both hoaxes as I show in my book *The Answer*. He and the Cult don't have to reimagine the world. They know precisely what they want and that's why they destroyed human society with 'Covid' to 'build back better' in their grand design. Their job is not to imagine, but to get humanity to imagine and agree with their plans while believing it's all random. It must be pure coincidence that 'The Great Reset' has long been the Cult's code name for the global imposition of fascism and replaced previous code-names of the 'New World

Order' used by Cult frontmen like Father George Bush and the 'New Order of the Ages' which emerged from Freemasonry and much older secret societies. New Order of the Ages appears on the reverse of the Great Seal of the United States as 'Novus ordo seclorum' underneath the Cult symbol used since way back of the pyramid and all seeing-eye (Fig 3). The pyramid is the hierarchy of human control headed by the illuminated eye that symbolises the force behind the Cult which I will expose in later chapters. The term 'Annuit Coeptis' translates as 'He favours our undertaking'. We are told the 'He' is the Christian god, but 'He' is not as I will be explaining.

Figure 3: The all-seeing eye of the Cult 'god' on the Freemason-designed Great Seal of the United States and also on the dollar bill.

Having you on

Two major Cult techniques of perceptual manipulation that relate to all this are what I have called since the 1990s Problem-Reaction-Solution (PRS) and the Totalitarian Tiptoe (TT). They can be uncovered by the inquiring mind with a simple question: Who benefits? The answer usually identifies the perpetrators of a given action or happening through the concept of 'he who most benefits from a crime is the one most likely to have committed it'. The Latin 'Cue bono?' – Who benefits? – is widely attributed to the Roman orator and statesman Marcus Tullius Cicero. No wonder it goes back so far when the concept has been relevant to human behaviour since

history was recorded. Problem-Reaction-Solution is the technique used to manipulate us every day by covertly creating a problem (or the illusion of one) and offering the solution to the problem (or the illusion of one). In the first phase you create the problem and blame someone or something else for why it has happened. This may relate to a financial collapse, terrorist attack, war, global warming or pandemic, anything in fact that will allow you to impose the 'solution' to change society in the way you desire at that time. The 'problem' doesn't have to be real. PRS is manipulation of perception and all you need is the population to believe the problem is real. Human-caused global warming and the 'Covid pandemic' only have to be *perceived* to be real for the population to accept the 'solutions' of authority. I refer to this technique as NO-Problem-Reaction-Solution. Billions did not meekly accept house arrest from early 2020 because there was a real deadly 'Covid pandemic' but because they perceived – believed – that to be the case. The antidote to Problem-Reaction-Solution is to ask who benefits from the proposed solution. Invariably it will be anyone who wants to justify more control through deletion of freedom and centralisation of power and decision-making.

The two world wars were Problem-Reaction-Solutions that transformed and realigned global society. Both were manipulated into being by the Cult as I have detailed in books since the mid-1990s. They dramatically centralised global power, especially World War Two, which led to the United Nations and other global bodies thanks to the overt and covert manipulations of the Rockefeller family and other Cult bloodlines like the Rothschilds. The UN is a stalking horse for full-blown world government that I will come to shortly. The land on which the UN building stands in New York was donated by the Rockefellers and the same Cult family was behind Big Pharma scalpel and drug 'medicine' and the creation of the World Health Organization as part of the UN. They have been stalwarts of the eugenics movement and funded Hitler's race-purity expert' Ernst Rudin. The human-caused global warming hoax has been orchestrated by the Club of Rome through the UN which is

manufacturing both the 'problem' through its Intergovernmental Panel on Climate Change and imposing the 'solution' through its Agenda 21 and Agenda 2030 which demand the total centralisation of global power to 'save the world' from a climate hoax the United Nations is itself perpetrating. What a small world the Cult can be seen to be particularly among the inner circles. The bedfellow of Problem-Reaction-Solution is the Totalitarian Tiptoe which became the Totalitarian Sprint in 2020. The technique is fashioned to hide the carefully-coordinated behind the cover of apparently random events. You start the sequence at 'A' and you know you are heading for 'Z'. You don't want people to know that and each step on the journey is presented as a random happening while all the steps strung together lead in the same direction. The speed may have quickened dramatically in recent times, but you can still see the incremental approach of the Tiptoe in the case of 'Covid' as each new imposition takes us deeper into fascism. Tell people they have to do this or that to get back to 'normal', then this and this and this. With each new demand adding to the ones that went before the population's freedom is deleted until it disappears. The spider wraps its web around the flies more comprehensively with each new diktat. I'll highlight this in more detail when I get to the 'Covid' hoax and how it has been pulled off. Another prime example of the Totalitarian Tiptoe is how the Cult-created European Union went from a 'free-trade zone' to a centralised bureaucratic dictatorship through the Tiptoe of incremental centralisation of power until nations became mere administrative units for Cult-owned dark suits in Brussels.

The antidote to ignorance is knowledge which the Cult seeks vehemently to deny us, but despite the systematic censorship to that end the Renegade Mind can overcome this by vociferously seeking out the facts no matter the impediments put in the way. There is also a method of thinking and perceiving – *knowing* – that doesn't even need names, dates, place-type facts to identify the patterns that reveal the story. I'll get to that in the final chapter. All you need to know about the manipulation of human society and to what end is still out there – *at the time of writing* – in the form of books, videos

and websites for those that really want to breach the walls of programmed perception. To access this knowledge requires the abandonment of the mainstream media as a source of information in the awareness that this is owned and controlled by the Cult and therefore promotes mass perceptions that suit the Cult. Mainstream media lies all day, every day. That is its function and very reason for being. Where it does tell the truth, here and there, is only because the truth and the Cult agenda very occasionally coincide. If you look for fact and insight to the BBC, CNN and virtually all the rest of them you are asking to be conned and perceptually programmed.

Know the outcome and you'll see the journey

Events seem random when you have no idea where the world is being taken. Once you do the random becomes the carefully planned. Know the outcome and you'll see the journey is a phrase I have been using for a long time to give context to daily happenings that appear unconnected. Does a problem, or illusion of a problem, trigger a proposed 'solution' that further drives society in the direction of the outcome? Invariably the answer will be yes and the random – *abracadabra* – becomes the clearly coordinated. So what is this outcome that unlocks the door to a massively expanded understanding of daily events? I will summarise its major aspects – the fine detail is in my other books – and those new to this information will see that the world they thought they were living in is a very different place. The foundation of the Cult agenda is the incessant centralisation of power and all such centralisation is ultimately in pursuit of Cult control on a global level. I have described for a long time the planned world structure of top-down dictatorship as the Hunger Games Society. The term obviously comes from the movie series which portrayed a world in which a few living in military-protected hi-tech luxury were the overlords of a population condemned to abject poverty in isolated 'sectors' that were not allowed to interact. 'Covid' lockdowns and travel bans anyone? The 'Hunger Games' pyramid of structural control has the inner circle of the Cult at the top with pretty much the entire

population at the bottom under their control through dependency for survival on the Cult. The whole structure is planned to be protected and enforced by a military-police state (Fig 4).

Here you have the reason for the global lockdowns of the fake pandemic to coldly destroy independent incomes and livelihoods and make everyone dependent on the 'state' (the Cult that controls the 'states'). I have warned in my books for many years about the plan to introduce a 'guaranteed income' – a barely survivable pittance – designed to impose dependency when employment was destroyed by AI technology and now even more comprehensively at great speed by the 'Covid' scam. Once the pandemic was played and lockdown consequences began to delete independent income the authorities began to talk right on cue about the need for a guaranteed income and a 'Great Reset'. Guaranteed income will be presented as benevolent governments seeking to help a desperate people – desperate as a direct result of actions of the same governments. The truth is that such payments are a trap. You will only get them if you do exactly what the authorities demand including mass vaccination (genetic manipulation). We have seen this theme already in Australia where those dependent on government benefits have them reduced if parents don't agree to have their children vaccinated according to an insane health-destroying government-dictated schedule. Calculated economic collapse applies to governments as well as people. The Cult wants rid of countries through the creation of a world state with countries broken up into regions ruled by a world government and super states like the European Union. Countries must be bankrupted, too, to this end and it's being achieved by the trillions in 'rescue packages' and furlough payments, trillions in lost taxation, and money-no-object spending on 'Covid' including constant all-medium advertising (programming) which has made the media dependent on government for much of its income. The day of reckoning is coming – as planned – for government spending and given that it has been made possible by printing money and not by production/taxation there is inflation on the way that has the

potential to wipe out monetary value. In that case there will be no need for the Cult to steal your money. It just won't be worth anything (see the German Weimar Republic before the Nazis took over). Many have been okay with lockdowns while getting a percentage of their income from so-called furlough payments without having to work. Those payments are dependent, however, on people having at least a theoretical job with a business considered non-essential and ordered to close. As these business go under because they are closed by lockdown after lockdown the furlough stops and it will for everyone eventually. Then what? The 'then what?' is precisely the idea.

Figure 4: The Hunger Games Society structure I have long warned was planned and now the 'Covid' hoax has made it possible. This is the real reason for lockdowns.

Hired hands

Between the Hunger Games Cult elite and the dependent population is planned to be a vicious military-police state (a fusion of the two into one force). This has been in the making for a long time with police looking ever more like the military and carrying weapons to match. The pandemic scam has seen this process accelerate so fast as

lockdown house arrest is brutally enforced by carefully recruited fascist minds and gormless system-servers. The police and military are planned to merge into a centrally-directed world army in a global structure headed by a world government which wouldn't be elected even by the election fixes now in place. The world army is not planned even to be human and instead wars would be fought, primarily against the population, using robot technology controlled by artificial intelligence. I have been warning about this for decades and now militaries around the world are being transformed by this very AI technology. The global regime that I describe is a particular form of fascism known as a technocracy in which decisions are not made by clueless and co-opted politicians but by unelected technocrats – scientists, engineers, technologists and bureaucrats. Cult-owned-and-controlled Silicon Valley giants are examples of technocracy and they already have far more power to direct world events than governments. They are with their censorship *selecting* governments. I know that some are calling the 'Great Reset' a Marxist communist takeover, but fascism and Marxism are different labels for the same tyranny. Tell those who lived in fascist Germany and Stalinist Russia that there was a difference in the way their freedom was deleted and their lives controlled. I could call it a fascist technocracy or a Marxist technocracy and they would be equally accurate. The Hunger Games society with its world government structure would oversee a world army, world central bank and single world cashless currency imposing its will on a microchipped population (Fig 5). Scan its different elements and see how the illusory pandemic is forcing society in this very direction at great speed. Leaders of 23 countries and the World Health Organization (WHO) backed the idea in March, 2021, of a global treaty for 'international cooperation' in 'health emergencies' and nations should 'come together as a global community for peaceful cooperation that extends beyond this crisis'. Cut the Orwellian bullshit and this means another step towards global government. The plan includes a cashless digital money system that I first warned about in 1993. Right at the start of 'Covid' the deeply corrupt Tedros

Adhanom Ghebreyesus, the crooked and merely gofer 'head' of the World Health Organization, said it was possible to catch the 'virus' by touching cash and it was better to use cashless means. The claim was ridiculous nonsense and like the whole 'Covid' mind-trick it was nothing to do with 'health' and everything to do with pushing every aspect of the Cult agenda. As a result of the Tedros lie the use of cash has plummeted. The Cult script involves a single world digital currency that would eventually be technologically embedded in the body. China is a massive global centre for the Cult and if you watch what is happening there you will know what is planned for everywhere. The Chinese government is developing a digital currency which would allow fines to be deducted immediately via AI for anyone caught on camera breaking its fantastic list of laws and the money is going to be programmable with an expiry date to ensure that no one can accrue wealth except the Cult and its operatives.

Figure 5: The structure of global control the Cult has been working towards for so long and this has been enormously advanced by the 'Covid' illusion.

Serfdom is so smart

The Cult plan is far wider, extreme, and more comprehensive than even most conspiracy researchers appreciate and I will come to the true depths of deceit and control in the chapters 'Who controls the

Cult?' and 'Escaping Wetiko'. Even the world that we know is crazy enough. We are being deluged with ever more sophisticated and controlling technology under the heading of 'smart'. We have smart televisions, smart meters, smart cards, smart cars, smart driving, smart roads, smart pills, smart patches, smart watches, smart skin, smart borders, smart pavements, smart streets, smart cities, smart communities, smart environments, smart growth, smart planet ... smart *everything* around us. Smart technologies and methods of operation are designed to interlock to create a global Smart Grid connecting the entirety of human society including human minds to create a centrally-dictated 'hive' mind. 'Smart cities' is code for densely-occupied megacities of total surveillance and control through AI. Ever more destructive frequency communication systems like 5G have been rolled out without any official testing for health and psychological effects (colossal). 5G/6G/7G systems are needed to run the Smart Grid and each one becomes more destructive of body and mind. Deleting independent income is crucial to forcing people into these AI-policed prisons by ending private property ownership (except for the Cult elite). The Cult's Great Reset now openly foresees a global society in which no one will own any possessions and everything will be rented while the Cult would own literally everything under the guise of government and corporations. The aim has been to use the lockdowns to destroy sources of income on a mass scale and when the people are destitute and in unrepayable amounts of debt (problem) Cult assets come forward with the pledge to write-off debt in return for handing over all property and possessions (solution). Everything – literally everything including people – would be connected to the Internet via AI. I was warning years ago about the coming Internet of Things (IoT) in which all devices and technology from your car to your fridge would be plugged into the Internet and controlled by AI. Now we are already there with much more to come. The next stage is the Internet of Everything (IoE) which is planned to include the connection of AI to the human brain and body to replace the human mind with a centrally-controlled AI mind. Instead of perceptions

being manipulated through control of information and censorship those perceptions would come direct from the Cult through AI. What do you think? You think whatever AI decides that you think. In human terms there would be no individual 'think' any longer. Too incredible? The ravings of a lunatic? Not at all. Cult-owned crazies in Silicon Valley have been telling us the plan for years without explaining the real motivation and calculated implications. These include Google executive and 'futurist' Ray Kurzweil who highlights the year 2030 for when this would be underway. He said:

Our thinking ... will be a hybrid of biological and non-biological thinking ... humans will be able to extend their limitations and 'think in the cloud' ... We're going to put gateways to the cloud in our brains ... We're going to gradually merge and enhance ourselves ... In my view, that's the nature of being human – we transcend our limitations.

As the technology becomes vastly superior to what we are then the small proportion that is still human gets smaller and smaller and smaller until it's just utterly negligible.

The sales-pitch of Kurzweil and Cult-owned Silicon Valley is that this would make us 'super-human' when the real aim is to make us post-human and no longer 'human' in the sense that we have come to know. The entire global population would be connected to AI and become the centrally-controlled 'hive-mind' of externally-delivered perceptions. The Smart Grid being installed to impose the Cult's will on the world is being constructed to allow particular locations – even one location – to control the whole global system. From these prime control centres, which absolutely include China and Israel, anything connected to the Internet would be switched on or off and manipulated at will. Energy systems could be cut, communication via the Internet taken down, computer-controlled driverless autonomous vehicles driven off the road, medical devices switched off, the potential is limitless given how much AI and Internet connections now run human society. We have seen nothing yet if we allow this to continue. Autonomous vehicle makers are working with law enforcement to produce cars designed to automatically pull over if they detect a police or emergency vehicle flashing from up to 100 feet away. At a police stop the car would be unlocked and the

window rolled down automatically. Vehicles would only take you where the computer (the state) allowed. The end of petrol vehicles and speed limiters on all new cars in the UK and EU from 2022 are steps leading to electric computerised transport over which ultimately you have no control. The picture is far bigger even than the Cult global network or web and that will become clear when I get to the nature of the 'spider'. There is a connection between all these happenings and the instigation of DNA-manipulating 'vaccines' (which aren't 'vaccines') justified by the 'Covid' hoax. That connection is the unfolding plan to transform the human body from a biological to a synthetic biological state and this is why synthetic biology is such a fast-emerging discipline of mainstream science. 'Covid vaccines' are infusing self-replicating synthetic genetic material into the cells to cumulatively take us on the Totalitarian Tiptoe from Human 1.0 to the synthetic biological Human 2.0 which will be physically and perceptually attached to the Smart Grid to one hundred percent control every thought, perception and deed. Humanity needs to wake up and *fast*.

This is the barest explanation of where the 'outcome' is planned to go but it's enough to see the journey happening all around us. Those new to this information will already see 'Covid' in a whole new context. I will add much more detail as we go along, but for the minutiae evidence see my mega-works, *The Answer, The Trigger* and *Everything You Need to Know But Have Never Been Told*.

Now – how does a Renegade Mind see the 'world'?

CHAPTER TWO

Renegade Perception

It is one thing to be clever and another to be wise
George R.R. Martin

A simple definition of the difference between a programmed mind and a Renegade Mind would be that one sees only dots while the other connects them to see the picture. Reading reality with accuracy requires the observer to (a) know the planned outcome and (b) realise that everything, but *everything*, is connected.

The entirety of infinite reality is connected – that's its very nature – and with human society an expression of infinite reality the same must apply. Simple cause and effect is a connection. The effect is triggered by the cause and the effect then becomes the cause of another effect. Nothing happens in isolation because it *can't*. Life in whatever reality is simple choice and consequence. We make choices and these lead to consequences. If we don't like the consequences we can make different choices and get different consequences which lead to other choices and consequences. The choice and the consequence are not only connected they are indivisible. You can't have one without the other as an old song goes. A few cannot control the world unless those being controlled allow that to happen – cause and effect, choice and consequence. Control – who has it and who doesn't – is a two-way process, a symbiotic relationship, involving the controller and controlled. 'They took my freedom away!!' Well, yes, but you also gave it to them. Humanity is

subjected to mass control because humanity has acquiesced to that control. This is all cause and effect and literally a case of give and take. In the same way world events of every kind are connected and the Cult works incessantly to sell the illusion of the random and coincidental to maintain the essential (to them) perception of dots that hide the picture. Renegade Minds know this and constantly scan the world for patterns of connection. This is absolutely pivotal in understanding the happenings in the world and without that perspective clarity is impossible. First you know the planned outcome and then you identify the steps on the journey – the day-by-day apparently random which, when connected in relation to the outcome, no longer appear as individual events, but as the proverbial *chain* of events leading in the same direction. I'll give you some examples:

Political puppet show

We are told to believe that politics is 'adversarial' in that different parties with different beliefs engage in an endless tussle for power. There may have been some truth in that up to a point – and only a point – but today divisions between 'different' parties are rhetorical not ideological. Even the rhetorical is fusing into one-speak as the parties eject any remaining free thinkers while others succumb to the ever-gathering intimidation of anyone with the 'wrong' opinion. The Cult is not a new phenomenon and can be traced back thousands of years as my books have documented. Its intergenerational initiates have been manipulating events with increasing effect the more that global power has been centralised. In ancient times the Cult secured control through the system of monarchy in which 'special' bloodlines (of which more later) demanded the right to rule as kings and queens simply by birthright and by vanquishing others who claimed the same birthright. There came a time, however, when people had matured enough to see the unfairness of such tyranny and demanded a say in who governed them. Note the word – *governed* them. Not served them – *governed* them, hence government defined as 'the political direction and control exercised over the

actions of the members, citizens, or inhabitants of communities, societies, and states; direction of the affairs of a state, community, etc.' Governments exercise control over rather than serve just like the monarchies before them. Bizarrely there are still countries like the United Kingdom which are ruled by a monarch *and* a government that officially answers to the monarch. The UK head of state and that of Commonwealth countries such as Canada, Australia and New Zealand is 'selected' by who in a *single family* had unprotected sex with whom and in what order. Pinch me it can't be true. Ouch! Shit, it is. The demise of monarchies in most countries offered a potential vacuum in which some form of free and fair society could arise and the Cult had that base covered. Monarchies had served its interests but they couldn't continue in the face of such widespread opposition and, anyway, replacing a 'royal' dictatorship that people could see with a dictatorship 'of the people' hiding behind the concept of 'democracy' presented far greater manipulative possibilities and ways of hiding coordinated tyranny behind the illusion of 'freedom'.

Democracy is quite wrongly defined as government selected by the population. This is not the case at all. It is government selected by *some* of the population (and then only in theory). This 'some' doesn't even have to be the majority as we have seen so often in first-past-the-post elections in which the so-called majority party wins fewer votes than the 'losing' parties combined. Democracy can give total power to a party in government from a minority of the votes cast. It's a sleight of hand to sell tyranny as freedom. Seventy-four million Trump-supporting Americans didn't vote for the 'Democratic' Party of Joe Biden in the distinctly dodgy election in 2020 and yet far from acknowledging the wishes and feelings of that great percentage of American society the Cult-owned Biden government set out from day one to destroy them and their right to a voice and opinion. Empty shell Biden and his Cult handlers said they were doing this to 'protect democracy'. Such is the level of lunacy and sickness to which politics has descended. Connect the dots and relate them to the desired outcome – a world government run by self-appointed technocrats and no longer even elected

politicians. While operating through its political agents in government the Cult is at the same time encouraging public distain for politicians by putting idiots and incompetents in theoretical power on the road to deleting them. The idea is to instil a public reaction that says of the technocrats: 'Well, they couldn't do any worse than the pathetic politicians.' It's all about controlling perception and Renegade Minds can see through that while programmed minds cannot when they are ignorant of both the planned outcome and the manipulation techniques employed to secure that end. This knowledge can be learned, however, and fast if people choose to get informed.

Politics may at first sight appear very difficult to control from a central point. I mean look at the 'different' parties and how would you be able to oversee them all and their constituent parts? In truth, it's very straightforward because of their structure. We are back to the pyramid of imposition and acquiescence. Organisations are structured in the same way as the system as a whole. Political parties are not open forums of free expression. They are hierarchies. I was a national spokesman for the British Green Party which claimed to be a different kind of politics in which influence and power was devolved; but I can tell you from direct experience – and it's far worse now – that Green parties are run as hierarchies like all the others however much they may try to hide that fact or kid themselves that it's not true. A very few at the top of all political parties are directing policy and personnel. They decide if you are elevated in the party or serve as a government minister and to do that you have to be a yes man or woman. Look at all the maverick political thinkers who never ascended the greasy pole. If you want to progress within the party or reach 'high-office' you need to fall into line and conform. Exceptions to this are rare indeed. Should you want to run for parliament or Congress you have to persuade the local or state level of the party to select you and for that you need to play the game as dictated by the hierarchy. If you secure election and wish to progress within the greater structure you need to go on conforming to what is acceptable to those running the hierarchy

from the peak of the pyramid. Political parties are perceptual gulags and the very fact that there are party 'Whips' appointed to 'whip' politicians into voting the way the hierarchy demands exposes the ridiculous idea that politicians are elected to serve the people they are supposed to represent. Cult operatives and manipulation has long seized control of major parties that have any chance of forming a government and at least most of those that haven't. A new party forms and the Cult goes to work to infiltrate and direct. This has reached such a level today that you see video compilations of 'leaders' of all parties whether Democrats, Republicans, Conservative, Labour and Green parroting the same Cult mantra of 'Build Back Better' and the 'Great Reset' which are straight off the Cult song-sheet to describe the transformation of global society in response to the Cult-instigated hoaxes of the 'Covid pandemic' and human-caused 'climate change'. To see Caroline Lucas, the Green Party MP that I knew when I was in the party in the 1980s, speaking in support of plans proposed by Cult operative Klaus Schwab representing the billionaire global elite is a real head-shaker.

Many parties – one master

The party system is another mind-trick and was instigated to change the nature of the dictatorship by swapping 'royalty' for dark suits that people believed – though now ever less so – represented their interests. Understanding this trick is to realise that a single force (the Cult) controls all parties either directly in terms of the major ones or through manipulation of perception and ideology with others. You don't need to manipulate Green parties to demand your transformation of society in the name of 'climate change' when they are obsessed with the lie that this is essential to 'save the planet'. You just give them a platform and away they go serving your interests while believing they are being environmentally virtuous. America's political structure is a perfect blueprint for how the two or multi-party system is really a one-party state. The Republican Party is controlled from one step back in the shadows by a group made up of billionaires and their gofers known as neoconservatives or Neocons.

I have exposed them in fine detail in my books and they were the driving force behind the policies of the imbecilic presidency of Boy George Bush which included 9/11 (see *The Trigger* for a comprehensive demolition of the official story), the subsequent 'war on terror' (war *of* terror) and the invasions of Afghanistan and Iraq. The latter was a No-Problem-Reaction-Solution based on claims by Cult operatives, including Bush and British Prime Minister Tony Blair, about Saddam Hussein's 'weapons of mass destruction' which did not exist as war criminals Bush and Blair well knew.

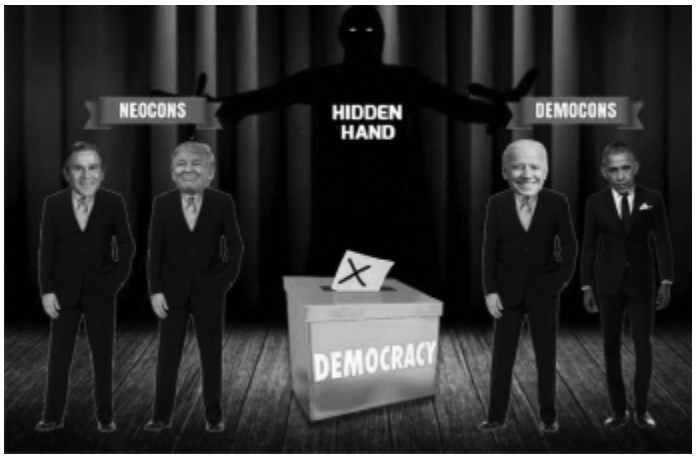

Figure 6: Different front people, different parties – same control system.

The Democratic Party has its own 'Neocon' group controlling from the background which I call the 'Democons' and here's the penny-drop – the Neocons and Democons answer to the same masters one step further back into the shadows (Fig 6). At that level of the Cult the Republican and Democrat parties are controlled by the same people and no matter which is in power the Cult is in power. This is how it works in almost every country and certainly in Britain with Conservative, Labour, Liberal Democrat and Green parties now all on the same page whatever the rhetoric may be in their feeble attempts to appear different. Neocons operated at the time of Bush through a think tank called The Project for the New American Century which in September, 2000, published a document entitled *Rebuilding America's Defenses: Strategies, Forces, and Resources*

For a New Century demanding that America fight 'multiple, simultaneous major theatre wars' as a 'core mission' to force regime-change in countries including Iraq, Libya and Syria. Neocons arranged for Bush ('Republican') and Blair ('Labour Party') to front-up the invasion of Iraq and when they departed the Democons orchestrated the targeting of Libya and Syria through Barack Obama ('Democrat') and British Prime Minister David Cameron ('Conservative Party'). We have 'different' parties and 'different' people, but the same unfolding script. The more the Cult has seized the reigns of parties and personnel the more their policies have transparently pursued the same agenda to the point where the fascist 'Covid' impositions of the Conservative junta of Jackboot Johnson in Britain were opposed by the Labour Party because they were not fascist enough. The Labour Party is likened to the US Democrats while the Conservative Party is akin to a British version of the Republicans and on both sides of the Atlantic they all speak the same language and support the direction demanded by the Cult although some more enthusiastically than others. It's a similar story in country after country because it's all centrally controlled. Oh, but what about Trump? I'll come to him shortly. Political 'choice' in the 'party' system goes like this: You vote for Party A and they get into government. You don't like what they do so next time you vote for Party B and they get into government. You don't like what they do when it's pretty much the same as Party A and why wouldn't that be with both controlled by the same force? Given that only two, sometimes three, parties have any chance of forming a government to get rid of Party B that you don't like you have to vote again for Party A which … you don't like. This, ladies and gentlemen, is what they call 'democracy' which we are told – wrongly – is a term interchangeable with 'freedom'.

The cult of cults

At this point I need to introduce a major expression of the Global Cult known as Sabbatian-Frankism. Sabbatian is also spelt as Sabbatean. I will summarise here. I have published major exposés

and detailed background in other works. Sabbatian-Frankism combines the names of two frauds posing as 'Jewish' men, Sabbatai Zevi (1626-1676), a rabbi, black magician and occultist who proclaimed he was the Jewish messiah; and Jacob Frank (1726-1791), the Polish 'Jew', black magician and occultist who said he was the reincarnation of 'messiah' Zevi and biblical patriarch Jacob. They worked across two centuries to establish the Sabbatian-Frankist cult that plays a major, indeed central, role in the manipulation of human society by the Global Cult which has its origins much further back in history than Sabbatai Zevi. I should emphasise two points here in response to the shrill voices that will scream 'anti-Semitism': (1) Sabbatian-Frankists are NOT Jewish and only pose as such to hide their cult behind a Jewish façade; and (2) my information about this cult has come from Jewish sources who have long realised that their society and community has been infiltrated and taken over by interloper Sabbatian-Frankists. Infiltration has been the foundation technique of Sabbatian-Frankism from its official origin in the 17th century. Zevi's Sabbatian sect attracted a massive following described as the biggest messianic movement in Jewish history, spreading as far as Africa and Asia, and he promised a return for the Jews to the 'Promised Land' of Israel. Sabbatianism was not Judaism but an inversion of everything that mainstream Judaism stood for. So much so that this sinister cult would have a feast day when Judaism had a fast day and whatever was forbidden in Judaism the Sabbatians were encouraged and even commanded to do. This included incest and what would be today called Satanism. Members were forbidden to marry outside the sect and there was a system of keeping their children ignorant of what they were part of until they were old enough to be trusted not to unknowingly reveal anything to outsiders. The same system is employed to this day by the Global Cult in general which Sabbatian-Frankism has enormously influenced and now largely controls.

Zevi and his Sabbatians suffered a setback with the intervention by the Sultan of the Islamic Ottoman Empire in the Middle East and what is now the Republic of Turkey where Zevi was located. The

Sultan gave him the choice of proving his 'divinity', converting to Islam or facing torture and death. Funnily enough Zevi chose to convert or at least appear to. Some of his supporters were disillusioned and drifted away, but many did not with 300 families also converting – only in theory – to Islam. They continued behind this Islamic smokescreen to follow the goals, rules and rituals of Sabbatianism and became known as 'crypto-Jews' or the 'Dönmeh' which means 'to turn'. This is rather ironic because they didn't 'turn' and instead hid behind a fake Islamic persona. The process of appearing to be one thing while being very much another would become the calling card of Sabbatianism especially after Zevi's death and the arrival of the Satanist Jacob Frank in the 18th century when the cult became Sabbatian-Frankism and plumbed still new depths of depravity and infiltration which included – still includes – human sacrifice and sex with children. Wherever Sabbatians go paedophilia and Satanism follow and is it really a surprise that Hollywood is so infested with child abuse and Satanism when it was established by Sabbatian-Frankists and is still controlled by them? Hollywood has been one of the prime vehicles for global perceptual programming and manipulation. How many believe the version of 'history' portrayed in movies when it is a travesty and inversion (again) of the truth? Rabbi Marvin Antelman describes Frankism in his book, *To Eliminate the Opiate*, as 'a movement of complete evil' while Jewish professor Gershom Scholem said of Frank in *The Messianic Idea in Judaism*: 'In all his actions [he was] a truly corrupt and degenerate individual ... one of the most frightening phenomena in the whole of Jewish history.' Frank was excommunicated by traditional rabbis, as was Zevi, but Frank was undeterred and enjoyed vital support from the House of Rothschild, the infamous banking dynasty whose inner-core are Sabbatian-Frankists and not Jews. Infiltration of the Roman Church and Vatican was instigated by Frank with many Dönmeh 'turning' again to convert to Roman Catholicism with a view to hijacking the reins of power. This was the ever-repeating modus operandi and continues to be so. Pose as an advocate of the religion, culture or country that you want to control and then

manipulate your people into the positions of authority and influence largely as advisers, administrators and Svengalis for those that appear to be in power. They did this with Judaism, Christianity (Christian Zionism is part of this), Islam and other religions and nations until Sabbatian-Frankism spanned the world as it does today.

Sabbatian Saudis and the terror network

One expression of the Sabbatian-Frankist Dönmeh within Islam is the ruling family of Saudi Arabia, the House of Saud, through which came the vile distortion of Islam known as Wahhabism. This is the violent creed followed by terrorist groups like Al-Qaeda and ISIS or Islamic State. Wahhabism is the hand-chopping, head-chopping 'religion' of Saudi Arabia which is used to keep the people in a constant state of fear so the interloper House of Saud can continue to rule. Al-Qaeda and Islamic State were lavishly funded by the House of Saud while being created and directed by the Sabbatian-Frankist network in the United States that operates through the Pentagon, CIA and the government in general of whichever 'party'. The front man for the establishment of Wahhabism in the middle of the 18th century was a Sabbatian-Frankist 'crypto-Jew' posing as Islamic called Muhammad ibn Abd al-Wahhab. His daughter would marry the son of Muhammad bin Saud who established the first Saudi state before his death in 1765 with support from the British Empire. Bin Saud's successors would establish modern Saudi Arabia in league with the British and Americans in 1932 which allowed them to seize control of Islam's major shrines in Mecca and Medina. They have dictated the direction of Sunni Islam ever since while Iran is the major centre of the Shiite version and here we have the source of at least the public conflict between them. The Sabbatian network has used its Wahhabi extremists to carry out Problem-Reaction-Solution terrorist attacks in the name of 'Al-Qaeda' and 'Islamic State' to justify a devastating 'war on terror', ever-increasing surveillance of the population and to terrify people into compliance. Another insight of the Renegade Mind is the streetwise understanding that

just because a country, location or people are attacked doesn't mean that those apparently representing that country, location or people are not behind the attackers. Often they are *orchestrating* the attacks because of the societal changes that can be then justified in the name of 'saving the population from terrorists'.

I show in great detail in *The Trigger* how Sabbatian-Frankists were the real perpetrators of 9/11 and not '19 Arab hijackers' who were blamed for what happened. Observe what was justified in the name of 9/11 alone in terms of Middle East invasions, mass surveillance and control that fulfilled the demands of the Project for the New American Century document published by the Sabbatian Neocons. What appear to be enemies are on the deep inside players on the same Sabbatian team. Israel and Arab 'royal' dictatorships are all ruled by Sabbatians and the recent peace agreements between Israel and Saudi Arabia, the United Arab Emirates (UAE) and others are only making formal what has always been the case behind the scenes. Palestinians who have been subjected to grotesque tyranny since Israel was bombed and terrorised into existence in 1948 have never stood a chance. Sabbatian-Frankists have controlled Israel (so the constant theme of violence and war which Sabbatians love) and they have controlled the Arab countries that Palestinians have looked to for real support that never comes. 'Royal families' of the Arab world in Saudi Arabia, Bahrain, UAE, etc., are all Sabbatians with allegiance to the aims of the cult and not what is best for their Arabic populations. They have stolen the oil and financial resources from their people by false claims to be 'royal dynasties' with a genetic right to rule and by employing vicious militaries to impose their will.

Satanic 'illumination'

The Satanist Jacob Frank formed an alliance in 1773 with two other Sabbatians, Mayer Amschel Rothschild (1744-1812), founder of the Rothschild banking dynasty, and Jesuit-educated fraudulent Jew, Adam Weishaupt, and this led to the formation of the Bavarian Illuminati, firstly under another name, in 1776. The Illuminati would

be the manipulating force behind the French Revolution (1789-1799) and was also involved in the American Revolution (1775-1783) before and after the Illuminati's official creation. Weishaupt would later become (in public) a Protestant Christian in archetypal Sabbatian style. I read that his name can be decoded as Adam-Weishaupt or 'the first man to lead those who know'. He wasn't a leader in the sense that he was a subordinate, but he did lead those below him in a crusade of transforming human society that still continues today. The theme was confirmed as early as 1785 when a horseman courier called Lanz was reported to be struck by lighting and extensive Illuminati documents were found in his saddlebags. They made the link to Weishaupt and detailed the plan for world takeover. Current events with 'Covid' fascism have been in the making for a very long time. Jacob Frank was jailed for 13 years by the Catholic Inquisition after his arrest in 1760 and on his release he headed for Frankfurt, Germany, home city and headquarters of the House of Rothschild where the alliance was struck with Mayer Amschel Rothschild and Weishaupt. Rothschild arranged for Frank to be given the title of Baron and he became a wealthy nobleman with a big following of Jews in Germany, the Austro-Hungarian Empire and other European countries. Most of them would have believed he was on their side.

The name 'Illuminati' came from the Zohar which is a body of works in the Jewish mystical 'bible' called the Kabbalah. 'Zohar' is the foundation of Sabbatian-Frankist belief and in Hebrew 'Zohar' means 'splendour', 'radiance', 'illuminated', and so we have 'Illuminati'. They claim to be the 'Illuminated Ones' from their knowledge systematically hidden from the human population and passed on through generations of carefully-chosen initiates in the global secret society network or Cult. Hidden knowledge includes an awareness of the Cult agenda for the world and the nature of our collective reality that I will explore later. Cult 'illumination' is symbolised by the torch held by the Statue of Liberty which was gifted to New York by French Freemasons in Paris who knew exactly what it represents. 'Liberty' symbolises the goddess worshipped in

Babylon as Queen Semiramis or Ishtar. The significance of this will become clear. Notice again the ubiquitous theme of inversion with the Statue of 'Liberty' really symbolising mass control (Fig 7). A mirror-image statute stands on an island in the River Seine in Paris from where New York Liberty originated (Fig 8). A large replica of the Liberty flame stands on top of the Pont de l'Alma tunnel in Paris where Princess Diana died in a Cult ritual described in *The Biggest Secret*. Lucifer 'the light bringer' is related to all this (and much more as we'll see) and 'Lucifer' is a central figure in Sabbatian-Frankism and its associated Satanism. Sabbatians reject the Jewish Torah, or Pentateuch, the 'five books of Moses' in the Old Testament known as Genesis, Exodus, Leviticus, Numbers, and Deuteronomy which are claimed by Judaism and Christianity to have been dictated by 'God' to Moses on Mount Sinai. Sabbatians say these do not apply to them and they seek to replace them with the Zohar to absorb Judaism and its followers into their inversion which is an expression of a much greater global inversion. They want to delete all religions and force humanity to worship a one-world religion – Sabbatian Satanism that also includes worship of the Earth goddess. Satanic themes are being more and more introduced into mainstream society and while Christianity is currently the foremost target for destruction the others are planned to follow.

Figure 7: The Cult goddess of Babylon disguised as the Statue of Liberty holding the flame of Lucifer the 'light bringer'.

Figure 8: Liberty's mirror image in Paris where the New York version originated.

Marx brothers

Rabbi Marvin Antelman connects the Illuminati to the Jacobins in *To Eliminate the Opiate* and Jacobins were the force behind the French Revolution. He links both to the Bund der Gerechten, or League of the Just, which was the network that inflicted communism/Marxism on the world. Antelman wrote:

> The original inner circle of the Bund der Gerechten consisted of born Catholics, Protestants and Jews [Sabbatian-Frankist infiltrators], and those representatives of respective subdivisions formulated schemes for the ultimate destruction of their faiths. The heretical Catholics laid plans which they felt would take a century or more for the ultimate destruction of the church; the apostate Jews for the ultimate destruction of the Jewish religion.

Sabbatian-created communism connects into this anti-religion agenda in that communism does not allow for the free practice of religion. The Sabbatian 'Bund' became the International Communist Party and Communist League and in 1848 'Marxism' was born with the Communist Manifesto of Sabbatian assets Karl Marx and Friedrich Engels. It is absolutely no coincidence that Marxism, just a different name for fascist and other centrally-controlled tyrannies, is being imposed worldwide as a result of the 'Covid' hoax and nor that Marxist/fascist China was the place where the hoax originated. The reason for this will become very clear in the chapter 'Covid: The calculated catastrophe'. The so-called 'Woke' mentality has hijacked

traditional beliefs of the political left and replaced them with far-right make-believe 'social justice' better known as Marxism. Woke will, however, be swallowed by its own perceived 'revolution' which is really the work of billionaires and billionaire corporations feigning being 'Woke'. Marxism is being touted by Wokers as a replacement for 'capitalism' when we don't have 'capitalism'. We have cartelism in which the market is stitched up by the very Cult billionaires and corporations bankrolling Woke. Billionaires love Marxism which keeps the people in servitude while they control from the top. Terminally naïve Wokers think they are 'changing the world' when it's the Cult that is doing the changing and when they have played their vital part and become surplus to requirements they, too, will be targeted. The Illuminati-Jacobins were behind the period known as 'The Terror' in the French Revolution in 1793 and 1794 when Jacobin Maximillian de Robespierre and his Orwellian 'Committee of Public Safety' killed 17,000 'enemies of the Revolution' who had once been 'friends of the Revolution'. Karl Marx (1818-1883), whose Sabbatian creed of Marxism has cost the lives of at least 100 million people, is a hero once again to Wokers who have been systematically kept ignorant of real history by their 'education' programming. As a result they now promote a Sabbatian 'Marxist' abomination destined at some point to consume them. Rabbi Antelman, who spent decades researching the Sabbatian plot, said of the League of the Just and Karl Marx:

> Contrary to popular opinion Karl Marx did not originate the Communist Manifesto. He was paid for his services by the League of the Just, which was known in its country of origin, Germany, as the Bund der Geaechteten.

Antelman said the text attributed to Marx was the work of other people and Marx 'was only repeating what others already said'. Marx was 'a hired hack – lackey of the wealthy Illuminists'. Marx famously said that religion was the 'opium of the people' (part of the Sabbatian plan to demonise religion) and Antelman called his books, *To Eliminate the Opiate*. Marx was born Jewish, but his family converted to Christianity (Sabbatian modus operandi) and he

attacked Jews, not least in his book, *A World Without Jews*. In doing so he supported the Sabbatian plan to destroy traditional Jewishness and Judaism which we are clearly seeing today with the vindictive targeting of orthodox Jews by the Sabbatian government of Israel over 'Covid' laws. I don't follow any religion and it has done much damage to the world over centuries and acted as a perceptual straightjacket. Renegade Minds, however, are always asking *why* something is being done. It doesn't matter if they agree or disagree with what is happening – *why* is it happening is the question. The 'why?' can be answered with regard to religion in that religions create interacting communities of believers when the Cult wants to dismantle all discourse, unity and interaction (see 'Covid' lockdowns) and the ultimate goal is to delete all religions for a one-world religion of Cult Satanism worshipping their 'god' of which more later. We see the same 'why?' with gun control in America. I don't have guns and don't want them, but why is the Cult seeking to disarm the population at the same time that law enforcement agencies are armed to their molars and why has every tyrant in history sought to disarm people before launching the final takeover? They include Hitler, Stalin, Pol Pot and Mao who followed confiscation with violent seizing of power. You know it's a Cult agenda by the people who immediately race to the microphones to exploit dead people in multiple shootings. Ultra-Zionist Cult lackey Senator Chuck Schumer was straight on the case after ten people were killed in Boulder, Colorado in March, 2121. Simple rule … if Schumer wants it the Cult wants it and the same with his ultra-Zionist mate the wild-eyed Senator Adam Schiff. At the same time they were calling for the disarmament of Americans, many of whom live a long way from a police response, Schumer, Schiff and the rest of these pampered clowns were sitting on Capitol Hill behind a razor-wired security fence protected by thousands of armed troops in addition to their own armed bodyguards. Mom and pop in an isolated home? They're just potential mass shooters.

Zion Mainframe

Sabbatian-Frankists and most importantly the Rothschilds were behind the creation of 'Zionism', a political movement that demanded a Jewish homeland in Israel as promised by Sabbatai Zevi. The very symbol of Israel comes from the German meaning of the name Rothschild. Dynasty founder Mayer Amschel Rothschild changed the family name from Bauer to Rothschild, or 'Red-Shield' in German, in deference to the six-pointed 'Star of David' hexagram displayed on the family's home in Frankfurt. The symbol later appeared on the flag of Israel after the Rothschilds were centrally involved in its creation. Hexagrams are not a uniquely Jewish symbol and are widely used in occult ('hidden') networks often as a symbol for Saturn (see my other books for why). Neither are Zionism and Jewishness interchangeable. Zionism is a political movement and philosophy and not a 'race' or a people. Many Jews oppose Zionism and many non-Jews, including US President Joe Biden, call themselves Zionists as does Israel-centric Donald Trump. America's support for the Israel government is pretty much a gimme with ultra-Zionist billionaires and corporations providing fantastic and dominant funding for both political parties. Former Congresswoman Cynthia McKinney has told how she was approached immediately she ran for office to 'sign the pledge' to Israel and confirm that she would always vote in that country's best interests. All American politicians are approached in this way. Anyone who refuses will get no support or funding from the enormous and all-powerful Zionist lobby that includes organisations like mega-lobby group AIPAC, the American Israel Public Affairs Committee. Trump's biggest funder was ultra-Zionist casino and media billionaire Sheldon Adelson while major funders of the Democratic Party include ultra-Zionist George Soros and ultra-Zionist financial and media mogul, Haim Saban. Some may reel back at the suggestion that Soros is an Israel-firster (Sabbatian-controlled Israel-firster), but Renegade Minds watch the actions not the words and everywhere Soros donates his billions the Sabbatian agenda benefits. In the spirit of Sabbatian inversion Soros pledged $1 billion for a new university network to promote 'liberal values and tackle intolerance'. He made the announcement during his annual speech

at the Cult-owned World Economic Forum in Davos, Switzerland, in January, 2020, after his 'harsh criticism' of 'authoritarian rulers' around the world. You can only laugh at such brazen mendacity. How *he* doesn't laugh is the mystery. Translated from the Orwellian 'liberal values and tackle intolerance' means teaching non-white people to hate white people and for white people to loathe themselves for being born white. The reason for that will become clear.

The 'Anti-Semitism' fraud

Zionists support the Jewish homeland in the land of Palestine which has been the Sabbatian-Rothschild goal for so long, but not for the benefit of Jews. Sabbatians and their global Anti-Semitism Industry have skewed public and political opinion to equate opposing the violent extremes of Zionism to be a blanket attack and condemnation of all Jewish people. Sabbatians and their global Anti-Semitism Industry have skewed public and political opinion to equate opposing the violent extremes of Zionism to be a blanket attack and condemnation of all Jewish people. This is nothing more than a Sabbatian protection racket to stop legitimate investigation and exposure of their agendas and activities. The official definition of 'anti-Semitism' has more recently been expanded to include criticism of Zionism – a *political movement* – and this was done to further stop exposure of Sabbatian infiltrators who created Zionism as we know it today in the 19th century. Renegade Minds will talk about these subjects when they know the shit that will come their way. People must decide if they want to know the truth or just cower in the corner in fear of what others will say. Sabbatians have been trying to label me as 'anti-Semitic' since the 1990s as I have uncovered more and more about their background and agendas. Useless, gutless, fraudulent 'journalists' then just repeat the smears without question and on the day I was writing this section a pair of unquestioning repeaters called Ben Quinn and Archie Bland (how appropriate) outright called me an 'anti-Semite' in the establishment propaganda sheet, the London *Guardian*, with no supporting evidence. The

Sabbatian Anti-Semitism Industry said so and who are they to question that? They wouldn't dare. Ironically 'Semitic' refers to a group of languages in the Middle East that are almost entirely Arabic. 'Anti-Semitism' becomes 'anti-Arab' which if the consequences of this misunderstanding were not so grave would be hilarious. Don't bother telling Quinn and Bland. I don't want to confuse them, bless 'em. One reason I am dubbed 'anti-Semitic' is that I wrote in the 1990s that Jewish operatives (Sabbatians) were heavily involved in the Russian Revolution when Sabbatians overthrew the Romanov dynasty. This apparently made me 'anti-Semitic'. Oh, really? Here is a section from *The Trigger*:

British journalist Robert Wilton confirmed these themes in his 1920 book *The Last Days of the Romanovs* when he studied official documents from the Russian government to identify the members of the Bolshevik ruling elite between 1917 and 1919. The Central Committee included 41 Jews among 62 members; the Council of the People's Commissars had 17 Jews out of 22 members; and 458 of the 556 most important Bolshevik positions between 1918 and 1919 were occupied by Jewish people. Only 17 were Russian. Then there were the 23 Jews among the 36 members of the vicious Cheka Soviet secret police established in 1917 who would soon appear all across the country.

Professor Robert Service of Oxford University, an expert on 20th century Russian history, found evidence that ['Jewish'] Leon Trotsky had sought to make sure that Jews were enrolled in the Red Army and were disproportionately represented in the Soviet civil bureaucracy that included the Cheka which performed mass arrests, imprisonment and executions of 'enemies of the people'. A US State Department Decimal File (861.00/5339) dated November 13th, 1918, names [Rothschild banking agent in America] Jacob Schiff and a list of ultra-Zionists as funders of the Russian Revolution leading to claims of a 'Jewish plot', but the key point missed by all is they were not 'Jews' – they were Sabbatian-Frankists.

Britain's Winston Churchill made the same error by mistake or otherwise. He wrote in a 1920 edition of the *Illustrated Sunday Herald* that those behind the Russian revolution were part of a 'worldwide conspiracy for the overthrow of civilisation and for the reconstitution of society on the basis of arrested development, of envious malevolence, and impossible equality' (see 'Woke' today because that has been created by the same network). Churchill said there was no need to exaggerate the part played in the creation of Bolshevism and in the actual bringing about of the Russian

Revolution 'by these international and for the most part atheistical Jews' ['atheistical Jews' = Sabbatians]. Churchill said it is certainly a very great one and probably outweighs all others: 'With the notable exception of Lenin, the majority of the leading figures are Jews.' He went on to describe, knowingly or not, the Sabbatian modus operandi of placing puppet leaders nominally in power while they control from the background:

> Moreover, the principal inspiration and driving power comes from the Jewish leaders. Thus Tchitcherin, a pure Russian, is eclipsed by his nominal subordinate, Litvinoff, and the influence of Russians like Bukharin or Lunacharski cannot be compared with the power of Trotsky, or of Zinovieff, the Dictator of the Red Citadel (Petrograd), or of Krassin or Radek – all Jews. In the Soviet institutions the predominance of Jews is even more astonishing. And the prominent, if not indeed the principal, part in the system of terrorism applied by the Extraordinary Commissions for Combatting Counter-Revolution has been taken by Jews, and in some notable cases by Jewesses.

What I said about seriously disproportionate involvement in the Russian Revolution by Jewish 'revolutionaries' (Sabbatians) is provable fact, but truth is no defence against the Sabbatian Anti-Semitism Industry, its repeater parrots like Quinn and Bland, and the now breathtaking network of so-called 'Woke' 'anti-hate' groups with interlocking leaderships and funding which have the role of discrediting and silencing anyone who gets too close to exposing the Sabbatians. We have seen 'truth is no defence' confirmed in legal judgements with the Saskatchewan Human Rights Commission in Canada decreeing this: 'Truthful statements can be presented in a manner that would meet the definition of hate speech, and not all truthful statements must be free from restriction.' Most 'anti-hate' activists, who are themselves consumed by hatred, are too stupid and ignorant of the world to know how they are being used. They are far too far up their own virtue-signalling arses and it's far too dark for them to see anything.

The 'revolution' game

The background and methods of the 'Russian' Revolution are straight from the Sabbatian playbook seen in the French Revolution

and endless others around the world that appear to start as a revolution of the people against tyrannical rule and end up with a regime change to more tyrannical rule overtly or covertly. Wars, terror attacks and regime overthrows follow the Sabbatian cult through history with its agents creating them as Problem-Reaction-Solutions to remove opposition on the road to world domination. Sabbatian dots connect the Rothschilds with the Illuminati, Jacobins of the French Revolution, the 'Bund' or League of the Just, the International Communist Party, Communist League and the Communist Manifesto of Karl Marx and Friedrich Engels that would lead to the Rothschild-funded Russian Revolution. The sequence comes under the heading of 'creative destruction' when you advance to your global goal by continually destroying the status quo to install a new status quo which you then also destroy. The two world wars come to mind. With each new status quo you move closer to your planned outcome. Wars and mass murder are to Sabbatians a collective blood sacrifice ritual. They are obsessed with death for many reasons and one is that death is an inversion of life. Satanists and Sabbatians are obsessed with death and often target churches and churchyards for their rituals. Inversion-obsessed Sabbatians explain the use of inverted symbolism including the *inverted* pentagram and *inverted* cross. The inversion of the cross has been related to targeting Christianity, but the cross was a religious symbol long before Christianity and its inversion is a statement about the Sabbatian mentality and goals more than any single religion.

Sabbatians operating in Germany were behind the rise of the occult-obsessed Nazis and the subsequent Jewish exodus from Germany and Europe to Palestine and the United States after World War Two. The Rothschild dynasty was at the forefront of this both as political manipulators and by funding the operation. Why would Sabbatians help to orchestrate the horrors inflicted on Jews by the Nazis and by Stalin after they organised the Russian Revolution? Sabbatians hate Jews and their religion, that's why. They pose as Jews and secure positions of control within Jewish society and play the 'anti-Semitism' card to protect themselves from exposure

through a global network of organisations answering to the Sabbatian-created-and-controlled globe-spanning intelligence network that involves a stunning web of military-intelligence operatives and operations for a tiny country of just nine million. Among them are Jewish assets who are not Sabbatians but have been convinced by them that what they are doing is for the good of Israel and the Jewish community to protect them from what they have been programmed since childhood to believe is a Jew-hating hostile world. The Jewish community is just a highly convenient cover to hide the true nature of Sabbatians. Anyone getting close to exposing their game is accused by Sabbatian place-people and gofers of 'anti-Semitism' and claiming that all Jews are part of a plot to take over the world. I am not saying that. I am saying that Sabbatians – the *real* Jew-haters – have infiltrated the Jewish community to use them both as a cover and an 'anti-Semitic' defence against exposure. Thus we have the Anti-Semitism Industry targeted researchers in this way and most Jewish people think this is justified and genuine. They don't know that their 'Jewish' leaders and institutions of state, intelligence and military are not controlled by Jews at all, but cultists and stooges of Sabbatian-Frankism. I once added my name to a pro-Jewish freedom petition online and the next time I looked my name was gone and text had been added to the petition blurb to attack me as an 'anti-Semite' such is the scale of perceptual programming.

Moving on America

I tell the story in *The Trigger* and a chapter called 'Atlantic Crossing' how particularly after Israel was established the Sabbatians moved in on the United States and eventually grasped control of government administration, the political system via both Democrats and Republicans, the intelligence community like the CIA and National Security Agency (NSA), the Pentagon and mass media. Through this seriously compartmentalised network Sabbatians and their operatives in Mossad, Israeli Defense Forces (IDF) and US agencies pulled off 9/11 and blamed it on 19 'Al-Qaeda hijackers' dominated by men from, or connected to, Sabbatian-ruled Saudi

Arabia. The '19' were not even on the planes let alone flew those big passenger jets into buildings while being largely incompetent at piloting one-engine light aircraft. 'Hijacker' Hani Hanjour who is said to have flown American Airlines Flight 77 into the Pentagon with a turn and manoeuvre most professional pilots said they would have struggled to do was banned from renting a small plane by instructors at the Freeway Airport in Bowie, Maryland, just *six weeks* earlier on the grounds that he was an incompetent pilot. The Jewish population of the world is just 0.2 percent with even that almost entirely concentrated in Israel (75 percent Jewish) and the United States (around two percent). This two percent and globally 0.2 percent refers to *Jewish* people and not Sabbatian interlopers who are a fraction of that fraction. What a sobering thought when you think of the fantastic influence on world affairs of tiny Israel and that the Project for the New America Century (PNAC) which laid out the blueprint in September, 2000, for America's war on terror and regime change wars in Iraq, Libya and Syria was founded and dominated by Sabbatians known as 'Neocons'. The document conceded that this plan would not be supported politically or publicly without a major attack on American soil and a Problem-Reaction-Solution excuse to send troops to war across the Middle East. Sabbatian Neocons said:

> ... [The] process of transformation ... [war and regime change] ... is likely to be a long one, absent some catastrophic and catalysing event – like a new Pearl Harbor.

Four months later many of those who produced that document came to power with their inane puppet George Bush from the long-time Sabbatian Bush family. They included Sabbatian Dick Cheney who was officially vice-president, but really de-facto president for the entirety of the 'Bush' government. Nine months after the 'Bush' inauguration came what Bush called at the time 'the Pearl Harbor of the 21st century' and with typical Sabbatian timing and symbolism 2001 was the 60th anniversary of the attack in 1941 by the Japanese Air Force on Pearl Harbor, Hawaii, which allowed President Franklin Delano Roosevelt to take the United States into a Sabbatian-

instigated Second World War that he said in his election campaign that he never would. The evidence is overwhelming that Roosevelt and his military and intelligence networks knew the attack was coming and did nothing to stop it, but they did make sure that America's most essential naval ships were not in Hawaii at the time. Three thousand Americans died in the Pearl Harbor attacks as they did on September 11th. By the 9/11 year of 2001 Sabbatians had widely infiltrated the US government, military and intelligence operations and used their compartmentalised assets to pull off the 'Al-Qaeda' attacks. If you read *The Trigger* it will blow your mind to see the utterly staggering concentration of 'Jewish' operatives (Sabbatian infiltrators) in essential positions of political, security, legal, law enforcement, financial and business power before, during, and after the attacks to make them happen, carry them out, and then cover their tracks – and I do mean *staggering* when you think of that 0.2 percent of the world population and two percent of Americans which are Jewish while Sabbatian infiltrators are a fraction of that. A central foundation of the 9/11 conspiracy was the hijacking of government, military, Air Force and intelligence computer systems in real time through 'back-door' access made possible by Israeli (Sabbatian) 'cyber security' software. Sabbatian-controlled Israel is on the way to rivalling Silicon Valley for domination of cyberspace and is becoming the dominant force in cyber-security which gives them access to entire computer systems and their passcodes across the world. Then add to this that Zionists head (officially) Silicon Valley giants like Google (Larry Page and Sergey Brin), Google-owned YouTube (Susan Wojcicki), Facebook (Mark Zuckerberg and Sheryl Sandberg), and Apple (Chairman Arthur D. Levinson), and that ultra-Zionist hedge fund billionaire Paul Singer has a $1 billion stake in Twitter which is only nominally headed by 'CEO' pothead Jack Dorsey. As cable news host Tucker Carlson said of Dorsey: 'There used to be debate in the medical community whether dropping a ton of acid had permanent effects and I think that debate has now ended.' Carlson made the comment after Dorsey told a hearing on Capitol Hill (if you cut through his bullshit) that he

believed in free speech so long as he got to decide what you can hear and see. These 'big names' of Silicon Valley are only front men and women for the Global Cult, not least the Sabbatians, who are the true controllers of these corporations. Does anyone still wonder why these same people and companies have been ferociously censoring and banning people (like me) for exposing any aspect of the Cult agenda and especially the truth about the 'Covid' hoax which Sabbatians have orchestrated?

The Jeffrey Epstein paedophile ring was a Sabbatian operation. He was officially 'Jewish' but he was a Sabbatian and women abused by the ring have told me about the high number of 'Jewish' people involved. The Epstein horror has Sabbatian written all over it and matches perfectly their modus operandi and obsession with sex and ritual. Epstein was running a Sabbatian blackmail ring in which famous people with political and other influence were provided with young girls for sex while everything was being filmed and recorded on hidden cameras and microphones at his New York house, Caribbean island and other properties. Epstein survivors have described this surveillance system to me and some have gone public. Once the famous politician or other figure knew he or she was on video they tended to do whatever they were told. Here we go again …when you've got them by the balls their hearts and minds will follow. Sabbatians use this blackmail technique on a wide scale across the world to entrap politicians and others they need to act as demanded. Epstein's private plane, the infamous 'Lolita Express', had many well-known passengers including Bill Clinton while Bill Gates has flown on an Epstein plane and met with him four years after Epstein had been jailed for paedophilia. They subsequently met many times at Epstein's home in New York according to a witness who was there. Epstein's infamous side-kick was Ghislaine Maxwell, daughter of Mossad agent and ultra-Zionist mega-crooked British businessman, Bob Maxwell, who at one time owned the *Daily Mirror* newspaper. Maxwell was murdered at sea on his boat in 1991 by Sabbatian-controlled Mossad when he became a liability with his

business empire collapsing as a former Mossad operative has confirmed (see *The Trigger*).

Money, money, money, funny money …

Before I come to the Sabbatian connection with the last three US presidents I will lay out the crucial importance to Sabbatians of controlling banking and finance. Sabbatian Mayer Amschel Rothschild set out to dominate this arena in his family's quest for total global control. What is freedom? It is, in effect, choice. The more choices you have the freer you are and the fewer your choices the more you are enslaved. In the global structure created over centuries by Sabbatians the biggest decider and restrictor of choice is … money. Across the world if you ask people what they would like to do with their lives and why they are not doing that they will reply 'I don't have the money'. This is the idea. A global elite of multi-billionaires are described as 'greedy' and that is true on one level; but control of money – who has it and who doesn't – is not primarily about greed. It's about control. Sabbatians have seized ever more control of finance and sucked the wealth of the world out of the hands of the population. We talk now, after all, about the 'One-percent' and even then the wealthiest are a lot fewer even than that. This has been made possible by a money scam so outrageous and so vast it could rightly be called the scam of scams founded on creating 'money' out of nothing and 'loaning' that with interest to the population. Money out of nothing is called 'credit'. Sabbatians have asserted control over governments and banking ever more completely through the centuries and secured financial laws that allow banks to lend hugely more than they have on deposit in a confidence trick known as fractional reserve lending. Imagine if you could lend money that doesn't exist and charge the recipient interest for doing so. You would end up in jail. Bankers by contrast end up in mansions, private jets, Malibu and Monaco.

Banks are only required to keep a fraction of their deposits and wealth in their vaults and they are allowed to lend 'money' they don't have called 'credit. Go into a bank for a loan and if you succeed

the banker will not move any real wealth into your account. They will type into your account the amount of the agreed 'loan' – say £100,000. This is not wealth that really exists; it is non-existent, fresh-air, created-out-of-nothing 'credit' which has never, does not, and will never exist except in theory. Credit is backed by nothing except wind and only has buying power because people think that it has buying power and accept it in return for property, goods and services. I have described this situation as like those cartoon characters you see chasing each other and when they run over the edge of a cliff they keep running forward on fresh air until one of them looks down, realises what's happened, and they all crash into the ravine. The whole foundation of the Sabbatian financial system is to stop people looking down except for periodic moments when they want to crash the system (as in 2008 and 2020 ongoing) and reap the rewards from all the property, businesses and wealth their borrowers had signed over as 'collateral' in return for a 'loan' of fresh air. Most people think that money is somehow created by governments when it comes into existence from the start as a debt through banks 'lending' illusory money called credit. Yes, the very currency of exchange is a *debt* from day one issued as an interest-bearing loan. Why don't governments create money interest-free and lend it to their people interest-free? Governments are controlled by Sabbatians and the financial system is controlled by Sabbatians for whom interest-free money would be a nightmare come true. Sabbatians underpin their financial domination through their global network of central banks, including the privately-owned US Federal Reserve and Britain's Bank of England, and this is orchestrated by a privately-owned central bank coordination body called the Bank for International Settlements in Basle, Switzerland, created by the usual suspects including the Rockefellers and Rothschilds. Central bank chiefs don't answer to governments or the people. They answer to the Bank for International Settlements or, in other words, the Global Cult which is dominated today by Sabbatians.

Built-in disaster

There are so many constituent scams within the overall banking scam. When you take out a loan of thin-air credit only the amount of that loan is theoretically brought into circulation to add to the amount in circulation; but you are paying back the principle plus interest. The additional interest is not created and this means that with every 'loan' there is a shortfall in the money in circulation between what is borrowed and what has to be paid back. There is never even close to enough money in circulation to repay all outstanding public and private debt including interest. Coldly weaved in the very fabric of the system is the certainty that some will lose their homes, businesses and possessions to the banking 'lender'. This is less obvious in times of 'boom' when the amount of money in circulation (and the debt) is expanding through more people wanting and getting loans. When a downturn comes and the money supply contracts it becomes painfully obvious that there is not enough money to service all debt and interest. This is less obvious in times of 'boom' when the amount of money in circulation (and the debt) is expanding through more people wanting and getting loans. When a downturn comes and the money supply contracts and it becomes painfully obvious – as in 2008 and currently – that there is not enough money to service all debt and interest. Sabbatian banksters have been leading the human population through a calculated series of booms (more debt incurred) and busts (when the debt can't be repaid and the banks get the debtor's tangible wealth in exchange for non-existent 'credit'). With each 'bust' Sabbatian bankers have absorbed more of the world's tangible wealth and we end up with the One-percent. Governments are in bankruptcy levels of debt to the same system and are therefore owned by a system they do not control. The Federal Reserve, 'America's central bank', is privately-owned and American presidents only nominally appoint its chairman or woman to maintain the illusion that it's an arm of government. It's not. The 'Fed' is a cartel of private banks which handed billions to its associates and friends after the crash of 2008 and has been Sabbatian-controlled since it was manipulated into being in 1913 through the covert trickery of Rothschild banking agents Jacob Schiff and Paul

Warburg, and the Sabbatian Rockefeller family. Somehow from a Jewish population of two-percent and globally 0.2 percent (Sabbatian interlopers remember are far smaller) ultra-Zionists headed the Federal Reserve for 31 years between 1987 and 2018 in the form of Alan Greenspan, Bernard Bernanke and Janet Yellen (now Biden's Treasury Secretary) with Yellen's deputy chairman a Israeli-American duel citizen and ultra-Zionist Stanley Fischer, a former governor of the Bank of Israel. Ultra-Zionist Fed chiefs spanned the presidencies of Ronald Reagan ('Republican'), Father George Bush ('Republican'), Bill Clinton ('Democrat'), Boy George Bush ('Republican') and Barack Obama ('Democrat'). We should really add the pre-Greenspan chairman, Paul Adolph Volcker, 'appointed' by Jimmy Carter ('Democrat') who ran the Fed between 1979 and 1987 during the Carter and Reagan administrations before Greenspan took over. Volcker was a long-time associate and business partner of the Rothschilds. No matter what the 'party' officially in power the United States economy was directed by the same force. Here are members of the Obama, Trump and Biden administrations and see if you can make out a common theme.

Barack Obama ('Democrat')

Ultra-Zionists Robert Rubin, Larry Summers, and Timothy Geithner ran the US Treasury in the Clinton administration and two of them reappeared with Obama. Ultra-Zionist Fed chairman Alan Greenspan had manipulated the crash of 2008 through deregulation and jumped ship just before the disaster to make way for ultra-Zionist Bernard Bernanke to hand out trillions to Sabbatian 'too big to fail' banks and businesses, including the ubiquitous ultra-Zionist Goldman Sachs which has an ongoing staff revolving door operation between itself and major financial positions in government worldwide. Obama inherited the fallout of the crash when he took office in January, 2009, and fortunately he had the support of his ultra-Zionist White House Chief of Staff Rahm Emmanuel, son of a terrorist who helped to bomb Israel into being in 1948, and his ultra-Zionist senior adviser David Axelrod, chief strategist in Obama's two

successful presidential campaigns. Emmanuel, later mayor of Chicago and former senior fundraiser and strategist for Bill Clinton, is an example of the Sabbatian policy after Israel was established of migrating insider families to America so their children would be born American citizens. 'Obama' chose this financial team throughout his administration to respond to the Sabbatian-instigated crisis:

Timothy Geithner (ultra-Zionist) Treasury Secretary; Jacob J. Lew, Treasury Secretary; Larry Summers (ultra-Zionist), director of the White House National Economic Council; Paul Adolph Volcker (Rothschild business partner), chairman of the Economic Recovery Advisory Board; Peter Orszag (ultra-Zionist), director of the Office of Management and Budget overseeing all government spending; Penny Pritzker (ultra-Zionist), Commerce Secretary; Jared Bernstein (ultra-Zionist), chief economist and economic policy adviser to Vice President Joe Biden; Mary Schapiro (ultra-Zionist), chair of the Securities and Exchange Commission (SEC); Gary Gensler (ultra-Zionist), chairman of the Commodity Futures Trading Commission (CFTC); Sheila Bair (ultra-Zionist), chair of the Federal Deposit Insurance Corporation (FDIC); Karen Mills (ultra-Zionist), head of the Small Business Administration (SBA); Kenneth Feinberg (ultra-Zionist), Special Master for Executive [bail-out] Compensation. Feinberg would be appointed to oversee compensation (with strings) to 9/11 victims and families in a campaign to stop them having their day in court to question the official story. At the same time ultra-Zionist Bernard Bernanke was chairman of the Federal Reserve and these are only some of the ultra-Zionists with allegiance to Sabbatian-controlled Israel in the Obama government. Obama's biggest corporate donor was ultra-Zionist Goldman Sachs which had employed many in his administration.

Donald Trump ('Republican')

Trump claimed to be an outsider (he wasn't) who had come to 'drain the swamp'. He embarked on this goal by immediately appointing ultra-Zionist Steve Mnuchin, a Goldman Sachs employee for 17

years, as his Treasury Secretary. Others included Gary Cohn (ultra-Zionist), chief operating officer of Goldman Sachs, his first Director of the National Economic Council and chief economic adviser, who was later replaced by Larry Kudlow (ultra-Zionist). Trump's senior adviser throughout his four years in the White House was his sinister son-in-law Jared Kushner, a life-long friend of Israel Prime Minister Benjamin Netanyahu. Kushner is the son of a convicted crook who was pardoned by Trump in his last days in office. Other ultra-Zionists in the Trump administration included: Stephen Miller, Senior Policy Adviser; Avrahm Berkowitz, Deputy Adviser to Trump and his Senior Adviser Jared Kushner; Ivanka Trump, Adviser to the President, who converted to Judaism when she married Jared Kushner; David Friedman, Trump lawyer and Ambassador to Israel; Jason Greenblatt, Trump Organization executive vice president and chief legal officer, who was made Special Representative for International Negotiations and the Israeli-Palestinian Conflict; Rod Rosenstein, Deputy Attorney General; Elliot Abrams, Special Representative for Venezuela, then Iran; John Eisenberg, National Security Council Legal Adviser and Deputy Council to the President for National Security Affairs; Anne Neuberger, Deputy National Manager, National Security Agency; Ezra Cohen-Watnick, Acting Under Secretary of Defense for Intelligence; Elan Carr, Special Envoy to monitor and combat anti-Semitism; Len Khodorkovsky, Deputy Special Envoy to monitor and combat anti-Semitism; Reed Cordish, Assistant to the President, Intragovernmental and Technology Initiatives. Trump Vice President Mike Pence and Secretary of State Mike Pompeo, both Christian Zionists, were also vehement supporters of Israel and its goals and ambitions.

Donald 'free-speech believer' Trump pardoned a number of financial and violent criminals while ignoring calls to pardon Julian Assange and Edward Snowden whose crimes are revealing highly relevant information about government manipulation and corruption and the widespread illegal surveillance of the American people by US 'security' agencies. It's so good to know that Trump is on the side of freedom and justice and not mega-criminals with

allegiance to Sabbatian-controlled Israel. These included a pardon for Israeli spy Jonathan Pollard who was jailed for life in 1987 under the Espionage Act. Aviem Sella, the Mossad agent who recruited Pollard, was also pardoned by Trump while Assange sat in jail and Snowden remained in exile in Russia. Sella had 'fled' (was helped to escape) to Israel in 1987 and was never extradited despite being charged under the Espionage Act. A Trump White House statement said that Sella's clemency had been 'supported by Benjamin Netanyahu, Ron Dermer, Israel's US Ambassador, David Friedman, US Ambassador to Israel and Miriam Adelson, wife of leading Trump donor Sheldon Adelson who died shortly before. Other friends of Jared Kushner were pardoned along with Sholom Weiss who was believed to be serving the longest-ever white-collar prison sentence of more than 800 years in 2000. The sentence was commuted of Ponzi-schemer Eliyahu Weinstein who defrauded Jews and others out of $200 million. I did mention that Assange and Snowden were ignored, right? Trump gave Sabbatians almost everything they asked for in military and political support, moving the US Embassy from Tel Aviv to Jerusalem with its critical symbolic and literal implications for Palestinian statehood, and the 'deal of the Century' designed by Jared Kushner and David Friedman which gave the Sabbatian Israeli government the green light to substantially expand its already widespread program of building illegal Jewish-only settlements in the occupied land of the West Bank. This made a two-state 'solution' impossible by seizing all the land of a potential Palestinian homeland and that had been the plan since 1948 and then 1967 when the Arab-controlled Gaza Strip, West Bank, Sinai Peninsula and Syrian Golan Heights were occupied by Israel. All the talks about talks and road maps and delays have been buying time until the West Bank was physically occupied by Israeli real estate. Trump would have to be a monumentally ill-informed idiot not to see that this was the plan he was helping to complete. The Trump administration was in so many ways the Kushner administration which means the Netanyahu administration which means the Sabbatian administration. I understand why many opposing Cult fascism in all its forms gravitated to Trump, but he

was a crucial part of the Sabbatian plan and I will deal with this in the next chapter.

Joe Biden ('Democrat')

A barely cognitive Joe Biden took over the presidency in January, 2021, along with his fellow empty shell, Vice-President Kamala Harris, as the latest Sabbatian gofers to enter the White House. Names on the door may have changed and the 'party' – the force behind them remained the same as Zionists were appointed to a stream of pivotal areas relating to Sabbatian plans and policy. They included: Janet Yellen, Treasury Secretary, former head of the Federal Reserve, and still another ultra-Zionist running the US Treasury after Mnuchin (Trump), Lew and Geithner (Obama), and Summers and Rubin (Clinton); Anthony Blinken, Secretary of State; Wendy Sherman, Deputy Secretary of State (so that's 'Biden's' Sabbatian foreign policy sorted); Jeff Zients, White House coronavirus coordinator; Rochelle Walensky, head of the Centers for Disease Control; Rachel Levine, transgender deputy health secretary (that's 'Covid' hoax policy under control); Merrick Garland, Attorney General; Alejandro Mayorkas, Secretary of Homeland Security; Cass Sunstein, Homeland Security with responsibility for new immigration laws; Avril Haines, Director of National Intelligence; Anne Neuberger, National Security Agency cybersecurity director (note, cybersecurity); David Cohen, CIA Deputy Director; Ronald Klain, Biden's Chief of Staff (see Rahm Emanuel); Eric Lander, a 'leading geneticist', Office of Science and Technology Policy director (see Smart Grid, synthetic biology agenda); Jessica Rosenworcel, acting head of the Federal Communications Commission (FCC) which controls Smart Grid technology policy and electromagnetic communication systems including 5G. How can it be that so many pivotal positions are held by two-percent of the American population and 0.2 percent of the world population administration after administration no matter who is the president and what is the party? It's a coincidence? Of course it's not and this is why Sabbatians have built their colossal global web of interlocking 'anti-

hate' hate groups to condemn anyone who asks these glaring questions as an 'anti-Semite'. The way that Jewish people horrifically abused in Sabbatian-backed Nazi Germany are exploited to this end is stomach-turning and disgusting beyond words.

Political fusion

Sabbatian manipulation has reversed the roles of Republicans and Democrats and the same has happened in Britain with the Conservative and Labour Parties. Republicans and Conservatives were always labelled the 'right' and Democrats and Labour the 'left', but look at the policy positions now and the Democrat-Labour 'left' has moved further to the 'right' than Republicans and Conservatives under the banner of 'Woke', the Cult-created far-right tyranny. Where once the Democrat-Labour 'left' defended free speech and human rights they now seek to delete them and as I said earlier despite the 'Covid' fascism of the Jackboot Johnson Conservative government in the UK the Labour Party of leader Keir Starmer demanded even more extreme measures. The Labour Party has been very publicly absorbed by Sabbatians after a political and media onslaught against the previous leader, the weak and inept Jeremy Corbyn, over made-up allegations of 'anti-Semitism' both by him and his party. The plan was clear with this 'anti-Semite' propaganda and what was required in response was a swift and decisive 'fuck off' from Corbyn and a statement to expose the Anti-Semitism Industry (Sabbatian) attempt to silence Labour criticism of the Israeli government (Sabbatians) and purge the party of all dissent against the extremes of ultra-Zionism (Sabbatians). Instead Corbyn and his party fell to their knees and appeased the abusers which, by definition, is impossible. Appeasing one demand leads only to a new demand to be appeased until takeover is complete. Like I say – 'fuck off' would have been a much more effective policy and I have used it myself with great effect over the years when Sabbatians are on my case which is most of the time. I consider that fact a great compliment, by the way. The outcome of the Labour Party capitulation is that we now have a Sabbatian-controlled

Conservative Party 'opposed' by a Sabbatian-controlled Labour Party in a one-party Sabbatian state that hurtles towards the extremes of tyranny (the Sabbatian cult agenda). In America the situation is the same. Labour's Keir Starmer spends his days on his knees with his tongue out pointing to Tel Aviv, or I guess now Jerusalem, while Boris Johnson has an 'anti-Semitism czar' in the form of former Labour MP John Mann who keeps Starmer company on his prayer mat.

Sabbatian influence can be seen in Jewish members of the Labour Party who have been ejected for criticism of Israel including those from families that suffered in Nazi Germany. Sabbatians despise real Jewish people and target them even more harshly because it is so much more difficult to dub them 'anti-Semitic' although in their desperation they do try.

CHAPTER THREE

The Pushbacker sting

Until you realize how easy it is for your mind to be manipulated, you remain the puppet of someone else's game
Evita Ochel

I will use the presidencies of Trump and Biden to show how the manipulation of the one-party state plays out behind the illusion of political choice across the world. No two presidencies could – on the face of it – be more different and apparently at odds in terms of direction and policy.

A Renegade Mind sees beyond the obvious and focuses on outcomes and consequences and not image, words and waffle. The Cult embarked on a campaign to divide America between those who blindly support its agenda (the mentality known as 'Woke') and those who are pushing back on where the Cult and its Sabbatians want to go. This presents infinite possibilities for dividing and ruling the population by setting them at war with each other and allows a perceptual ring fence of demonisation to encircle the Pushbackers in a modern version of the Little Big Horn in 1876 when American cavalry led by Lieutenant Colonel George Custer were drawn into a trap, surrounded and killed by Native American tribes defending their land of thousands of years from being seized by the government. In this modern version the roles are reversed and it's those defending themselves from the Sabbatian government who are surrounded and the government that's seeking to destroy them. This trap was set years ago and to explain how we must return to 2016

and the emergence of Donald Trump as a candidate to be President of the United States. He set out to overcome the best part of 20 other candidates in the Republican Party before and during the primaries and was not considered by many in those early stages to have a prayer of living in the White House. The Republican Party was said to have great reservations about Trump and yet somehow he won the nomination. When you know how American politics works – politics in general – there is no way that Trump could have become the party's candidate unless the Sabbatian-controlled 'Neocons' that run the Republican Party wanted that to happen. We saw the proof in emails and documents made public by WikiLeaks that the Democratic Party hierarchy, or Democons, systematically undermined the campaign of Bernie Sanders to make sure that Sabbatian gofer Hillary Clinton won the nomination to be their presidential candidate. If the Democons could do that then the Neocons in the Republican Party could have derailed Trump in the same way. But they didn't and at that stage I began to conclude that Trump could well be the one chosen to be president. If that was the case the 'why' was pretty clear to see – the goal of dividing America between Cult agenda-supporting Wokers and Pushbackers who gravitated to Trump because he was telling them what they wanted to hear. His constituency of support had been increasingly ignored and voiceless for decades and profoundly through the eight years of Sabbatian puppet Barack Obama. Now here was someone speaking their language of pulling back from the incessant globalisation of political and economic power, the exporting of American jobs to China and elsewhere by 'American' (Sabbatian) corporations, the deletion of free speech, and the mass immigration policies that had further devastated job opportunities for the urban working class of all races and the once American heartlands of the Midwest.

Beware the forked tongue

Those people collectively sighed with relief that at last a political leader was apparently on their side, but another trait of the Renegade Mind is that you look even harder at people telling you

what you want to hear than those who are telling you otherwise. Obviously as I said earlier people wish what they want to hear to be true and genuine and they are much more likely to believe that than someone saying what they don't want to here and don't want to be true. Sales people are taught to be skilled in eliciting by calculated questioning what their customers want to hear and repeating that back to them as their own opinion to get their targets to like and trust them. Assets of the Cult are also sales people in the sense of selling perception. To read Cult manipulation you have to play the long and expanded game and not fall for the Vaudeville show of party politics. Both American parties are vehicles for the Cult and they exploit them in different ways depending on what the agenda requires at that moment. Trump and the Republicans were used to be the focus of dividing America and isolating Pushbackers to open the way for a Biden presidency to become the most extreme in American history by advancing the full-blown Woke (Cult) agenda with the aim of destroying and silencing Pushbackers now labelled Nazi Trump supporters and white supremacists.

Sabbatians wanted Trump in office for the reasons described by ultra-Zionist Saul Alinsky (1909-1972) who was promoting the Woke philosophy through 'community organising' long before anyone had heard of it. In those days it still went by its traditional name of Marxism. The reason for the manipulated Trump phenomenon was laid out in Alinsky's 1971 book, *Rules for Radicals*, which was his blueprint for overthrowing democratic and other regimes and replacing them with Sabbatian Marxism. Not surprisingly his to-do list was evident in the Sabbatian French and Russian 'Revolutions' and that in China which will become very relevant in the next chapter about the 'Covid' hoax. Among Alinsky's followers have been the deeply corrupt Barack Obama, House Speaker Nancy Pelosi and Hillary Clinton who described him as a 'hero'. All three are Sabbatian stooges with Pelosi personifying the arrogant corrupt idiocy that so widely fronts up for the Cult inner core. Predictably as a Sabbatian advocate of the 'light-bringer' Alinsky features Lucifer on the dedication page of his book as the original radical who gained

his own kingdom ('Earth' as we shall see). One of Alinsky's golden radical rules was to pick an individual and focus all attention, hatred and blame on them and not to target faceless bureaucracies and corporations. *Rules for Radicals* is really a Sabbatian handbook with its contents repeatedly employed all over the world for centuries and why wouldn't Sabbatians bring to power their designer-villain to be used as the individual on which all attention, hatred and blame was bestowed? This is what they did and the only question for me is how much Trump knew that and how much he was manipulated. A bit of both, I suspect. This was Alinsky's Trump technique from a man who died in 1972. The technique has spanned history:

Pick the target, freeze it, personalize it, polarize it. Don't try to attack abstract corporations or bureaucracies. Identify a responsible individual. Ignore attempts to shift or spread the blame.

From the moment Trump came to illusory power everything was about him. It wasn't about Republican policy or opinion, but all about Trump. Everything he did was presented in negative, derogatory and abusive terms by the Sabbatian-dominated media led by Cult operations such as CNN, MSNBC, *The New York Times* and the Jeff Bezos-owned *Washington Post* – 'Pick the target, freeze it, personalize it, polarize it.' Trump was turned into a demon to be vilified by those who hated him and a demi-god loved by those who worshipped him. This, in turn, had his supporters, too, presented as equally demonic in preparation for the punchline later down the line when Biden was about to take office. It was here's a Trump, there's a Trump, everywhere a Trump, Trump. Virtually every news story or happening was filtered through the lens of 'The Donald'. You loved him or hated him and which one you chose was said to define you as Satan's spawn or a paragon of virtue. Even supporting some Trump policies or statements and not others was enough for an assault on your character. No shades of grey were or are allowed. Everything is black and white (literally and figuratively). A Californian I knew had her head utterly scrambled by her hatred for Trump while telling people they should love each other. She was so totally consumed by

Trump Derangement Syndrome as it became to be known that this glaring contradiction would never have occurred to her. By definition anyone who criticised Trump or praised his opponents was a hero and this lady described Joe Biden as 'a kind, honest gentleman' when he's a provable liar, mega-crook and vicious piece of work to boot. Sabbatians had indeed divided America using Trump as the fall-guy and all along the clock was ticking on the consequences for his supporters.

In hock to his masters

Trump gave Sabbatians via Israel almost everything they wanted in his four years. Ask and you shall receive was the dynamic between himself and Benjamin Netanyahu orchestrated by Trump's ultra-Zionist son-in-law Jared Kushner, his ultra-Zionist Ambassador to Israel, David Friedman, and ultra-Zionist 'Israel adviser', Jason Greenblatt. The last two were central to the running and protecting from collapse of his business empire, the Trump Organisation, and colossal business failures made him forever beholding to Sabbatian networks that bailed him out. By the start of the 1990s Trump owed $4 billion to banks that he couldn't pay and almost $1billion of that was down to him personally and not his companies. This mega-disaster was the result of building two new casinos in Atlantic City and buying the enormous Taj Mahal operation which led to crippling debt payments. He had borrowed fantastic sums from 72 banks with major Sabbatian connections and although the scale of debt should have had him living in a tent alongside the highway they never foreclosed. A plan was devised to lift Trump from the mire by BT Securities Corporation and Rothschild Inc. and the case was handled by Wilber Ross who had worked for the Rothschilds for 27 years. Ross would be named US Commerce Secretary after Trump's election. Another crucial figure in saving Trump was ultra-Zionist 'investor' Carl Icahn who bought the Taj Mahal casino. Icahn was made special economic adviser on financial regulation in the Trump administration. He didn't stay long but still managed to find time to make a tidy sum of a reported $31.3 million when he sold his

holdings affected by the price of steel three days before Trump imposed a 235 percent tariff on steel imports. What amazing bits of luck these people have. Trump and Sabbatian operatives have long had a close association and his mentor and legal adviser from the early 1970s until 1986 was the dark and genetically corrupt ultra-Zionist Roy Cohn who was chief counsel to Senator Joseph McCarthy's 'communist' witch-hunt in the 1950s. *Esquire* magazine published an article about Cohn with the headline 'Don't mess with Roy Cohn'. He was described as the most feared lawyer in New York and 'a ruthless master of dirty tricks ... [with] ... more than one Mafia Don on speed dial'. Cohn's influence, contacts, support and protection made Trump a front man for Sabbatians in New York with their connections to one of Cohn's many criminal employers, the 'Russian' Sabbatian Mafia. Israel-centric media mogul Rupert Murdoch was introduced to Trump by Cohn and they started a long friendship. Cohn died in 1986 weeks after being disbarred for unethical conduct by the Appellate Division of the New York State Supreme Court. The wheels of justice do indeed run slow given the length of Cohn's crooked career.

QAnon-sense

We are asked to believe that Donald Trump with his fundamental connections to Sabbatian networks and operatives has been leading the fight to stop the Sabbatian agenda for the fascistic control of America and the world. Sure he has. A man entrapped during his years in the White House by Sabbatian operatives and whose biggest financial donor was casino billionaire Sheldon Adelson who was Sabbatian to his DNA?? Oh, do come on. Trump has been used to divide America and isolate Pushbackers on the Cult agenda under the heading of 'Trump supporters', 'insurrectionists' and 'white supremacists'. The US Intelligence/Mossad Psyop or psychological operation known as QAnon emerged during the Trump years as a central pillar in the Sabbatian campaign to lead Pushbackers into the trap set by those that wished to destroy them. I knew from the start that QAnon was a scam because I had seen the same scenario many

times before over 30 years under different names and I had written about one in particular in the books. 'Not again' was my reaction when QAnon came to the fore. The same script is pulled out every few years and a new name added to the letterhead. The story always takes the same form: 'Insiders' or 'the good guys' in the government-intelligence-military 'Deep State' apparatus were going to instigate mass arrests of the 'bad guys' which would include the Rockefellers, Rothschilds, Barack Obama, Hillary Clinton, George Soros, etc., etc. Dates are given for when the 'good guys' are going to move in, but the dates pass without incident and new dates are given which pass without incident. The central message to Pushbackers in each case is that they don't have to do anything because there is 'a plan' and it is all going to be sorted by the 'good guys' on the inside. 'Trust the plan' was a QAnon mantra when the only plan was to misdirect Pushbackers into putting their trust in a Psyop they believed to be real. Beware, beware, those who tell you what you want to hear and always check it out. Right up to Biden's inauguration QAnon was still claiming that 'the Storm' was coming and Trump would stay on as president when Biden and his cronies were arrested and jailed. It was never going to happen and of course it didn't, but what did happen as a result provided that punchline to the Sabbatian Trump/QAnon Psyop.

On January 6th, 2021, a very big crowd of Trump supporters gathered in the National Mall in Washington DC down from the Capitol Building to protest at what they believed to be widespread corruption and vote fraud that stopped Trump being re-elected for a second term as president in November, 2020. I say as someone that does not support Trump or Biden that the evidence is clear that major vote-fixing went on to favour Biden, a man with cognitive problems so advanced he can often hardly string a sentence together without reading the words written for him on the Teleprompter. Glaring ballot discrepancies included serious questions about electronic voting machines that make vote rigging a comparative cinch and hundreds of thousands of paper votes that suddenly appeared during already advanced vote counts and virtually all of

them for Biden. Early Trump leads in crucial swing states suddenly began to close and disappear. The pandemic hoax was used as the excuse to issue almost limitless numbers of mail-in ballots with no checks to establish that the recipients were still alive or lived at that address. They were sent to streams of people who had not even asked for them. Private organisations were employed to gather these ballots and who knows what they did with them before they turned up at the counts. The American election system has been manipulated over decades to become a sick joke with more holes than a Swiss cheese for the express purpose of dictating the results. Then there was the criminal manipulation of information by Sabbatian tech giants like Facebook, Twitter and Google-owned YouTube which deleted pro-Trump, anti-Biden accounts and posts while everything in support of Biden was left alone. Sabbatians wanted Biden to win because after the dividing of America it was time for full-on Woke and every aspect of the Cult agenda to be unleashed.

Hunter gatherer

Extreme Silicon Valley bias included blocking information by the *New York Post* exposing a Biden scandal that should have ended his bid for president in the final weeks of the campaign. Hunter Biden, his monumentally corrupt son, is reported to have sent a laptop to be repaired at a local store and failed to return for it. Time passed until the laptop became the property of the store for non-payment of the bill. When the owner saw what was on the hard drive he gave a copy to the FBI who did nothing even though it confirmed widespread corruption in which the Joe Biden family were using his political position, especially when he was vice president to Obama, to make multiple millions in countries around the world and most notably Ukraine and China. Hunter Biden's one-time business partner Tony Bobulinski went public when the story broke in the *New York Post* to confirm the corruption he saw and that Joe Biden not only knew what was going on he also profited from the spoils. Millions were handed over by a Chinese company with close

connections – like all major businesses in China – to the Chinese communist party of President Xi Jinping. Joe Biden even boasted at a meeting of the Cult's World Economic Forum that as vice president he had ordered the government of Ukraine to fire a prosecutor. What he didn't mention was that the same man just happened to be investigating an energy company which was part of Hunter Biden's corrupt portfolio. The company was paying him big bucks for no other reason than the influence his father had. Overnight Biden's presidential campaign should have been over given that he had lied publicly about not knowing what his son was doing. Instead almost the entire Sabbatian-owned mainstream media and Sabbatian-owned Silicon Valley suppressed circulation of the story. This alone went a mighty way to rigging the election of 2020. Cult assets like Mark Zuckerberg at Facebook also spent hundreds of millions to be used in support of Biden and vote 'administration'.

The Cult had used Trump as the focus to divide America and was now desperate to bring in moronic, pliable, corrupt Biden to complete the double-whammy. No way were they going to let little things like the will of the people thwart their plan. Silicon Valley widely censored claims that the election was rigged because it *was* rigged. For the same reason anyone claiming it was rigged was denounced as a 'white supremacist' including the pathetically few Republican politicians willing to say so. Right across the media where the claim was mentioned it was described as a 'false claim' even though these excuses for 'journalists' would have done no research into the subject whatsoever. Trump won seven million more votes than any sitting president had ever achieved while somehow a cognitively-challenged soon to be 78-year-old who was hidden away from the public for most of the campaign managed to win more votes than any presidential candidate in history. It makes no sense. You only had to see election rallies for both candidates to witness the enthusiasm for Trump and the apathy for Biden. Tens of thousands would attend Trump events while Biden was speaking in empty car parks with often only television crews attending and framing their shots to hide the fact that no one was there. It was pathetic to see

footage come to light of Biden standing at a podium making speeches only to TV crews and party fixers while reading the words written for him on massive Teleprompter screens. So, yes, those protestors on January 6th had a point about election rigging, but some were about to walk into a trap laid for them in Washington by the Cult Deep State and its QAnon Psyop. This was the Capitol Hill riot ludicrously dubbed an 'insurrection'.

The spider and the fly

Renegade Minds know there are not two 'sides' in politics, only one side, the Cult, working through all 'sides'. It's a stage show, a puppet show, to direct the perceptions of the population into focusing on diversions like parties and candidates while missing the puppeteers with their hands holding all the strings. The Capitol Hill 'insurrection' brings us back to the Little Big Horn. Having created two distinct opposing groupings – Woke and Pushbackers – the trap was about to be sprung. Pushbackers were to be encircled and isolated by associating them all in the public mind with Trump and then labelling Trump as some sort of Confederate leader. I knew immediately that the Capitol riot was a set-up because of two things. One was how easy the rioters got into the building with virtually no credible resistance and secondly I could see – as with the 'Covid' hoax in the West at the start of 2020 – how the Cult could exploit the situation to move its agenda forward with great speed. My experience of Cult techniques and activities over more than 30 years has showed me that while they do exploit situations they haven't themselves created this never happens with events of fundamental agenda significance. Every time major events giving cultists the excuse to rapidly advance their plan you find they are manipulated into being for the specific reason of providing that excuse – Problem-Reaction-Solution. Only a tiny minority of the huge crowd of Washington protestors sought to gain entry to the Capitol by smashing windows and breaching doors. That didn't matter. The whole crowd and all Pushbackers, even if they did not support Trump, were going to be lumped together as dangerous

insurrectionists and conspiracy theorists. The latter term came into widespread use through a CIA memo in the 1960s aimed at discrediting those questioning the nonsensical official story of the Kennedy assassination and it subsequently became widely employed by the media. It's still being used by inept 'journalists' with no idea of its origin to discredit anyone questioning anything that authority claims to be true. When you are perpetrating a conspiracy you need to discredit the very word itself even though the dictionary definition of conspiracy is merely 'the activity of secretly planning with other people to do something bad or illegal' and 'a general agreement to keep silent about a subject for the purpose of keeping it secret'. On that basis there are conspiracies almost wherever you look. For obvious reasons the Cult and its lapdog media have to claim there are no conspiracies even though the word appears in state laws as with conspiracy to defraud, to murder, and to corrupt public morals.

Agent provocateurs are widely used by the Cult Deep State to manipulate genuine people into acting in ways that suit the desired outcome. By genuine in this case I mean protestors genuinely supporting Trump and claims that the election was stolen. In among them, however, were agents of the state wearing the garb of Trump supporters and QAnon to pump-prime the Capital riot which some genuine Trump supporters naively fell for. I described the situation as 'Come into my parlour said the spider to the fly'. Leaflets appeared through the Woke paramilitary arm Antifa, the anti-fascist fascists, calling on supporters to turn up in Washington looking like Trump supporters even though they hated him. Some of those arrested for breaching the Capitol Building were sourced to Antifa and its stable mate Black Lives Matter. Both organisations are funded by Cult billionaires and corporations. One man charged for the riot was according to his lawyer a former FBI agent who had held top secret security clearance for 40 years. Attorney Thomas Plofchan said of his client, 66-year-old Thomas Edward Caldwell:

He has held a Top Secret Security Clearance since 1979 and has undergone multiple Special Background Investigations in support of his clearances. After retiring from the Navy, he

worked as a section chief for the Federal Bureau of Investigation from 2009-2010 as a GS-12 [mid-level employee].

He also formed and operated a consulting firm performing work, often classified, for U.S government customers including the US. Drug Enforcement Agency, Department of Housing and Urban Development, the US Coast Guard, and the US Army Personnel Command.

A judge later released Caldwell pending trial in the absence of evidence about a conspiracy or that he tried to force his way into the building. *The New York Post* reported a 'law enforcement source' as saying that 'at least two known Antifa members were spotted' on camera among Trump supporters during the riot while one of the rioters arrested was John Earle Sullivan, a seriously extreme Black Lives Matter Trump-hater from Utah who was previously arrested and charged in July, 2020, over a BLM-Antifa riot in which drivers were threatened and one was shot. Sullivan is the founder of Utah-based Insurgence USA which is an affiliate of the Cult-created-and-funded Black Lives Matter movement. Footage appeared and was then deleted by Twitter of Trump supporters calling out Antifa infiltrators and a group was filmed changing into pro-Trump clothing before the riot. Security at the building was *pathetic* – as planned. Colonel Leroy Fletcher Prouty, a man with long experience in covert operations working with the US security apparatus, once described the tell-tale sign to identify who is involved in an assassination. He said:

No one has to direct an assassination – it happens. The active role is played secretly by permitting it to happen. This is the greatest single clue. Who has the power to call off or reduce the usual security precautions?

This principle applies to many other situations and certainly to the Capitol riot of January 6th, 2021.

The sting

With such a big and potentially angry crowd known to be gathering near the Capitol the security apparatus would have had a major police detail to defend the building with National Guard troops on

standby given the strength of feeling among people arriving from all over America encouraged by the QAnon Psyop and statements by Donald Trump. Instead Capitol Police 'security' was flimsy, weak, and easily breached. The same number of officers was deployed as on a regular day and that is a blatant red flag. They were not staffed or equipped for a possible riot that had been an obvious possibility in the circumstances. No protective and effective fencing worth the name was put in place and there were no contingency plans. The whole thing was basically a case of standing aside and waving people in. Once inside police mostly backed off apart from one Capitol police officer who ridiculously shot dead unarmed Air Force veteran protestor Ashli Babbitt without a warning as she climbed through a broken window. The 'investigation' refused to name or charge the officer after what must surely be considered a murder in the circumstances. They just lifted a carpet and swept. The story was endlessly repeated about five people dying in the 'armed insurrection' when there was no report of rioters using weapons. Apart from Babbitt the other four died from a heart attack, strokes and apparently a drug overdose. Capitol police officer Brian Sicknick was reported to have died after being bludgeoned with a fire extinguisher when he was alive after the riot was over and died later of what the Washington Medical Examiner's Office said was a stroke. Sicknick had no external injuries. The lies were delivered like rapid fire. There was a narrative to build with incessant repetition of the lie until the lie became the accepted 'everybody knows that' truth. The 'Big Lie' technique of Nazi Propaganda Minister Joseph Goebbels is constantly used by the Cult which was behind the Nazis and is today behind the 'Covid' and 'climate change' hoaxes. Goebbels said:

If you tell a lie big enough and keep repeating it, people will eventually come to believe it. The lie can be maintained only for such time as the State can shield the people from the political, economic and/or military consequences of the lie. It thus becomes vitally important for the State to use all of its powers to repress dissent, for the truth is the mortal enemy of the lie, and thus by extension, the truth is the greatest enemy of the State.

Most protestors had a free run of the Capitol Building. This allowed pictures to be taken of rioters in iconic parts of the building including the Senate chamber which could be used as propaganda images against all Pushbackers. One Congresswoman described the scene as 'the worst kind of non-security anybody could ever imagine'. Well, the first part was true, but someone obviously did imagine it and made sure it happened. Some photographs most widely circulated featured people wearing QAnon symbols and now the Psyop would be used to dub all QAnon followers with the ubiquitous fit-all label of 'white supremacist' and 'insurrectionists'. When a Muslim extremist called Noah Green drove his car at two police officers at the Capitol Building killing one in April, 2021, there was no such political and media hysteria. They were just disappointed he wasn't white.

The witch-hunt

Government prosecutor Michael Sherwin, an aggressive, dark-eyed, professional Rottweiler led the 'investigation' and to call it over the top would be to understate reality a thousand fold. Hundreds were tracked down and arrested for the crime of having the wrong political views and people were jailed who had done nothing more than walk in the building, committed no violence or damage to property, took a few pictures and left. They were labelled a 'threat to the Republic' while Biden sat in the White House signing executive orders written for him that were dismantling 'the Republic'. Even when judges ruled that a mother and son should not be in jail the government kept them there. Some of those arrested have been badly beaten by prison guards in Washington and lawyers for one man said he suffered a fractured skull and was made blind in one eye. Meanwhile a woman is shot dead for no reason by a Capitol Police officer and we are not allowed to know who he is never mind what has happened to him although that will be *nothing*. The Cult's QAnon/Trump sting to identify and isolate Pushbackers and then target them on the road to crushing and deleting them was a resounding success. You would have thought the Russians had

invaded the building at gunpoint and lined up senators for a firing squad to see the political and media reaction. Congresswoman Alexandria Ocasio-Cortez is a child in a woman's body, a terrible-twos, me, me, me, Woker narcissist of such proportions that words have no meaning. She said she thought she was going to die when 'insurrectionists' banged on her office door. It turned out she wasn't even in the Capitol Building when the riot was happening and the 'banging' was a Capitol Police officer. She referred to herself as a 'survivor' which is an insult to all those true survivors of violent and sexual abuse while she lives her pampered and privileged life talking drivel for a living. Her Woke colleague and fellow mega-narcissist Rashida Tlaib broke down describing the devastating effect on her, too, of *not being* in the building when the rioters were there. Ocasio-Cortez and Tlaib are members of a fully-Woke group of Congresswomen known as 'The Squad' along with Ilhan Omar and Ayanna Pressley. The Squad from what I can see can be identified by its vehement anti-white racism, anti-white men agenda, and, as always in these cases, the absence of brain cells on active duty.

The usual suspects were on the riot case immediately in the form of Democrat ultra-Zionist senators and operatives Chuck Schumer and Adam Schiff demanding that Trump be impeached for 'his part in the insurrection'. The same pair of prats had led the failed impeachment of Trump over the invented 'Russia collusion' nonsense which claimed Russia had helped Trump win the 2016 election. I didn't realise that Tel Aviv had been relocated just outside Moscow. I must find an up-to-date map. The Russia hoax was a Sabbatian operation to keep Trump occupied and impotent and to stop any rapport with Russia which the Cult wants to retain as a perceptual enemy to be pulled out at will. Puppet Biden began attacking Russia when he came to office as the Cult seeks more upheaval, division and war across the world. A two-year stage show 'Russia collusion inquiry' headed by the not-very-bright former 9/11 FBI chief Robert Mueller, with support from 19 lawyers, 40 FBI agents plus intelligence analysts, forensic accountants and other

staff, devoured tens of millions of dollars and found no evidence of Russia collusion which a ten-year-old could have told them on day one. Now the same moronic Schumer and Schiff wanted a second impeachment of Trump over the Capitol 'insurrection' (riot) which the arrested development of Schumer called another 'Pearl Harbor' while others compared it with 9/11 in which 3,000 died and, in the case of CNN, with the Rwandan genocide in the 1990s in which an estimated 500,000 to 600,000 were murdered, between 250, 000 and 500,000 women were raped, and populations of whole towns were hacked to death with machetes. To make those comparisons purely for Cult political reasons is beyond insulting to those that suffered and lost their lives and confirms yet again the callous inhumanity that we are dealing with. Schumer is a monumental idiot and so is Schiff, but they serve the Cult agenda and do whatever they're told so they get looked after. Talking of idiots – another inane man who spanned the Russia and Capitol impeachment attempts was Senator Eric Swalwell who had the nerve to accuse Trump of collusion with the Russians while sleeping with a Chinese spy called Christine Fang or 'Fang Fang' which is straight out of a Bond film no doubt starring Klaus Schwab as the bloke living on a secret island and controlling laser weapons positioned in space and pointing at world capitals. Fang Fang plays the part of Bond's infiltrator girlfriend which I'm sure she would enjoy rather more than sharing a bed with the brainless Swalwell, lying back and thinking of China. The FBI eventually warned Swalwell about Fang Fang which gave her time to escape back to the Chinese dictatorship. How very thoughtful of them. The second Trump impeachment also failed and hardly surprising when an impeachment is supposed to remove a sitting president and by the time it happened Trump was no longer president. These people are running your country America, well, officially anyway. Terrifying isn't it?

Outcomes tell the story - always

The outcome of all this – and it's the *outcome* on which Renegade Minds focus, not the words – was that a vicious, hysterical and

obviously pre-planned assault was launched on Pushbackers to censor, silence and discredit them and even targeted their right to earn a living. They have since been condemned as 'domestic terrorists' that need to be treated like Al-Qaeda and Islamic State. 'Domestic terrorists' is a label the Cult has been trying to make stick since the period of the Oklahoma bombing in 1995 which was blamed on 'far-right domestic terrorists'. If you read *The Trigger* you will see that the bombing was clearly a Problem-Reaction-Solution carried out by the Deep State during a Bill Clinton administration so corrupt that no dictionary definition of the term would even nearly suffice. Nearly 30, 000 troops were deployed from all over America to the empty streets of Washington for Biden's inauguration. Ten thousand of them stayed on with the pretext of protecting the capital from insurrectionists when it was more psychological programming to normalise the use of the military in domestic law enforcement in support of the Cult plan for a police-military state. Biden's fascist administration began a purge of 'wrong-thinkers' in the military which means anyone that is not on board with Woke. The Capitol Building was surrounded by a fence with razor wire and the Land of the Free was further symbolically and literally dismantled. The circle was completed with the installation of Biden and the exploitation of the QAnon Psyop.

America had never been so divided since the civil war of the 19th century, Pushbackers were isolated and dubbed terrorists and now, as was always going to happen, the Cult immediately set about deleting what little was left of freedom and transforming American society through a swish of the hand of the most controlled 'president' in American history leading (officially at least) the most extreme regime since the country was declared an independent state on July 4th, 1776. Biden issued undebated, dictatorial executive orders almost by the hour in his opening days in office across the whole spectrum of the Cult wish-list including diluting controls on the border with Mexico allowing thousands of migrants to illegally enter the United States to transform the demographics of America and import an election-changing number of perceived Democrat

voters. Then there were Biden deportation amnesties for the already illegally resident (estimated to be as high as 20 or even 30 million). A bill before Congress awarded American citizenship to anyone who could prove they had worked in agriculture for just 180 days in the previous two years as 'Big Ag' secured its slave labour long-term. There were the plans to add new states to the union such as Puerto Rico and making Washington DC a state. They are all parts of a plan to ensure that the Cult-owned Woke Democrats would be permanently in power.

Border – what border?

I have exposed in detail in other books how mass immigration into the United States and Europe is the work of Cult networks fuelled by the tens of billions spent to this and other ends by George Soros and his global Open Society (open borders) Foundations. The impact can be seen in America alone where the population has increased by *100 million* in little more than 30 years mostly through immigration. I wrote in *The Answer* that the plan was to have so many people crossing the southern border that the numbers become unstoppable and we are now there under Cult-owned Biden. El Salvador in Central America puts the scale of what is happening into context. A third of the population now lives in the United States, much of it illegally, and many more are on the way. The methodology is to crush Central and South American countries economically and spread violence through machete-wielding psychopathic gangs like MS-13 based in El Salvador and now operating in many American cities. Biden-imposed lax security at the southern border means that it is all but open. He said before his 'election' that he wanted to see a surge towards the border if he became president and that was the green light for people to do just that after election day to create the human disaster that followed for both America and the migrants. When that surge came the imbecilic Alexandria Ocasio-Cortez said it wasn't a 'surge' because they are 'children, not insurgents' and the term 'surge' (used by Biden) was a claim of 'white supremacists'.

This disingenuous lady may one day enter the realm of the most basic intelligence, but it won't be any time soon.

Sabbatians and the Cult are in the process of destroying America by importing violent people and gangs in among the genuine to terrorise American cities and by overwhelming services that cannot cope with the sheer volume of new arrivals. Something similar is happening in Europe as Western society in general is targeted for demographic and cultural transformation and upheaval. The plan demands violence and crime to create an environment of intimidation, fear and division and Soros has been funding the election of district attorneys across America who then stop prosecuting many crimes, reduce sentences for violent crimes and free as many violent criminals as they can. Sabbatians are creating the chaos from which order – their order – can respond in a classic Problem-Reaction-Solution. A Freemasonic moto says 'Ordo Ab Chao' (Order out of Chaos) and this is why the Cult is constantly creating chaos to impose a new 'order'. Here you have the reason the Cult is constantly creating chaos. The 'Covid' hoax can be seen with those entering the United States by plane being forced to take a 'Covid' test while migrants flooding through southern border processing facilities do not. Nothing is put in the way of mass migration and if that means ignoring the government's own 'Covid' rules then so be it. They know it's all bullshit anyway. Any pushback on this is denounced as 'racist' by Wokers and Sabbatian fronts like the ultra-Zionist Anti-Defamation League headed by the appalling Jonathan Greenblatt which at the same time argues that Israel should not give citizenship and voting rights to more Palestinian Arabs or the 'Jewish population' (in truth the Sabbatian network) will lose control of the country.

Society-changing numbers

Biden's masters have declared that countries like El Salvador are so dangerous that their people must be allowed into the United States for humanitarian reasons when there are fewer murders in large parts of many Central American countries than in US cities like

Baltimore. That is not to say Central America cannot be a dangerous place and Cult-controlled American governments have been making it so since way back, along with the dismantling of economies, in a long-term plan to drive people north into the United States. Parts of Central America are very dangerous, but in other areas the story is being greatly exaggerated to justify relaxing immigration criteria. Migrants are being offered free healthcare and education in the United States as another incentive to head for the border and there is no requirement to be financially independent before you can enter to prevent the resources of America being drained. You can't blame migrants for seeking what they believe will be a better life, but they are being played by the Cult for dark and nefarious ends. The numbers since Biden took office are huge. In February, 2021, more than 100,000 people were known to have tried to enter the US illegally through the southern border (it was 34,000 in the same month in 2020) and in March it was 170,000 – a 418 percent increase on March, 2020. These numbers are only known people, not the ones who get in unseen. The true figure for migrants illegally crossing the border in a single month was estimated by one congressman at 250,000 and that number will only rise under Biden's current policy. Gangs of murdering drug-running thugs that control the Mexican side of the border demand money – thousands of dollars – to let migrants cross the Rio Grande into America. At the same time gun battles are breaking out on the border several times a week between rival Mexican drug gangs (which now operate globally) who are equipped with sophisticated military-grade weapons, grenades and armoured vehicles. While the Capitol Building was being 'protected' from a non-existent 'threat' by thousands of troops, and others were still deployed at the time in the Cult Neocon war in Afghanistan, the southern border of America was left to its fate. This is not incompetence, it is cold calculation.

By March, 2021, there were 17,000 unaccompanied children held at border facilities and many of them are ensnared by people traffickers for paedophile rings and raped on their journey north to America. This is not conjecture – this is fact. Many of those designated

children are in reality teenage boys or older. Meanwhile Wokers posture their self-purity for encouraging poor and tragic people to come to America and face this nightmare both on the journey and at the border with the disgusting figure of House Speaker Nancy Pelosi giving disingenuous speeches about caring for migrants. The woman's evil. Wokers condemned Trump for having children in cages at the border (so did Obama, *Shhhh*), but now they are sleeping on the floor without access to a shower with one border facility 729 percent over capacity. The Biden insanity even proposed flying migrants from the southern border to the northern border with Canada for 'processing'. The whole shambles is being overseen by ultra-Zionist Secretary of Homeland Security, the moronic liar Alejandro Mayorkas, who banned news cameras at border facilities to stop Americans seeing what was happening. Mayorkas said there was not a ban on news crews; it was just that they were not allowed to film. Alongside him at Homeland Security is another ultra-Zionist Cass Sunstein appointed by Biden to oversee new immigration laws. Sunstein despises conspiracy researchers to the point where he suggests they should be banned or *taxed* for having such views. The man is not bonkers or anything. He's perfectly well-adjusted, but adjusted to what is the question. Criticise what is happening and you are a 'white supremacist' when earlier non-white immigrants also oppose the numbers which effect their lives and opportunities. Black people in poor areas are particularly damaged by uncontrolled immigration and the increased competition for work opportunities with those who will work for less. They are also losing voting power as Hispanics become more dominant in former black areas. It's a downward spiral for them while the billionaires behind the policy drone on about how much they care about black people and 'racism'. None of this is about compassion for migrants or black people – that's just wind and air. Migrants are instead being mercilessly exploited to transform America while the countries they leave are losing their future and the same is true in Europe. Mass immigration may now be the work of Woke Democrats, but it can be traced back to the 1986 Immigration Reform and Control Act (it

wasn't) signed into law by Republican hero President Ronald Reagan which gave amnesty to millions living in the United States illegally and other incentives for people to head for the southern border. Here we have the one-party state at work again.

Save me syndrome

Almost every aspect of what I have been exposing as the Cult agenda was on display in even the first days of 'Biden' with silencing of Pushbackers at the forefront of everything. A Renegade Mind will view the Trump years and QAnon in a very different light to their supporters and advocates as the dots are connected. The QAnon/Trump Psyop has given the Cult all it was looking for. We may not know how much, or little, that Trump realised he was being used, but that's a side issue. This pincer movement produced the desired outcome of dividing America and having Pushbackers isolated. To turn this around we have to look at new routes to empowerment which do not include handing our power to other people and groups through what I will call the 'Save Me Syndrome' – 'I want someone else to do it so that I don't have to'. We have seen this at work throughout human history and the QAnon/Trump Psyop is only the latest incarnation alongside all the others. Religion is an obvious expression of this when people look to a 'god' or priest to save them or tell them how to be saved and then there are 'save me' politicians like Trump. Politics is a diversion and not a 'saviour'. It is a means to block positive change, not make it possible.

Save Me Syndrome always comes with the same repeating theme of handing your power to whom or what you believe will save you while your real 'saviour' stares back from the mirror every morning. Renegade Minds are constantly vigilant in this regard and always asking the question 'What can I do?' rather than 'What can someone else do for me?' Gandhi was right when he said: 'You must be the change you want to see in the world.' We are indeed the people we have been waiting for. We are presented with a constant raft of reasons to concede that power to others and forget where the real power is. Humanity has the numbers and the Cult does not. It has to

use diversion and division to target the unstoppable power that comes from unity. Religions, governments, politicians, corporations, media, QAnon, are all different manifestations of this power-diversion and dilution. Refusing to give your power to governments and instead handing it to Trump and QAnon is not to take a new direction, but merely to recycle the old one with new names on the posters. I will explore this phenomenon as we proceed and how to break the cycles and recycles that got us here through the mists of repeating perception and so repeating history.

For now we shall turn to the most potent example in the entire human story of the consequences that follow when you give your power away. I am talking, of course, of the 'Covid' hoax.

CHAPTER FOUR

'Covid': Calculated catastrophe

Facts are threatening to those invested in fraud
DaShanne Stokes

We can easily unravel the real reason for the 'Covid pandemic' hoax by employing the Renegade Mind methodology that I have outlined this far. We'll start by comparing the long-planned Cult outcome with the 'Covid pandemic' outcome. Know the outcome and you'll see the journey.

I have highlighted the plan for the Hunger Games Society which has been in my books for so many years with the very few controlling the very many through ongoing dependency. To create this dependency it is essential to destroy independent livelihoods, businesses and employment to make the population reliant on the state (the Cult) for even the basics of life through a guaranteed pittance income. While independence of income remained these Cult ambitions would be thwarted. With this knowledge it was easy to see where the 'pandemic' hoax was going once talk of 'lockdowns' began and the closing of all but perceived 'essential' businesses to 'save' us from an alleged 'deadly virus'. Cult corporations like Amazon and Walmart were naturally considered 'essential' while mom and pop shops and stores had their doors closed by fascist decree. As a result with every new lockdown and new regulation more small and medium, even large businesses not owned by the Cult, went to the wall while Cult giants and their frontmen and women grew financially fatter by the second. Mom and pop were

denied an income and the right to earn a living and the wealth of people like Jeff Bezos (Amazon), Mark Zuckerberg (Facebook) and Sergei Brin and Larry Page (Google/Alphabet) have reached record levels. The Cult was increasing its own power through further dramatic concentrations of wealth while the competition was being destroyed and brought into a state of dependency. Lockdowns have been instigated to secure that very end and were never anything to do with health. My brother Paul spent 45 years building up a bus repair business, but lockdowns meant buses were running at a fraction of normal levels for months on end. Similar stories can told in their hundreds of millions worldwide. Efforts of a lifetime coldly destroyed by Cult multi-billionaires and their lackeys in government and law enforcement who continued to earn their living from the taxation of the people while denying the right of the same people to earn theirs. How different it would have been if those making and enforcing these decisions had to face the same financial hardships of those they affected, but they never do.

Gates of Hell

Behind it all in the full knowledge of what he is doing and why is the psychopathic figure of Cult operative Bill Gates. His puppet Tedros at the World Health Organization declared 'Covid' a pandemic in March, 2020. The WHO had changed the definition of a 'pandemic' in 2009 just a month before declaring the 'swine flu pandemic' which would not have been so under the previous definition. The same applies to 'Covid'. The definition had included… 'an infection by an infectious agent, occurring simultaneously in different countries, with a significant mortality rate relative to the proportion of the population infected'. The new definition removed the need for 'significant mortality'. The 'pandemic' has been fraudulent even down to the definition, but Gates demanded economy-destroying lockdowns, school closures, social distancing, mandatory masks, a 'vaccination' for every man, woman and child on the planet and severe consequences and restrictions for those that refused. Who gave him this power? The

Cult did which he serves like a little boy in short trousers doing what his daddy tells him. He and his psychopathic missus even smiled when they said that much worse was to come (what they knew was planned to come). Gates responded in the matter-of-fact way of all psychopaths to a question about the effect on the world economy of what he was doing:

> Well, it won't go to zero but it will shrink. Global GDP is probably going to take the biggest hit ever [Gates was smiling as he said this] … in my lifetime this will be the greatest economic hit. But you don't have a choice. People act as if you have a choice. People don't feel like going to the stadium when they might get infected … People are deeply affected by seeing these stats, by knowing they could be part of the transmission chain, old people, their parents and grandparents, could be affected by this, and so you don't get to say ignore what is going on here.
>
> There will be the ability to open up, particularly in rich countries, if things are done well over the next few months, but for the world at large normalcy only returns when we have largely vaccinated the entire population.

The man has no compassion or empathy. How could he when he's a psychopath like all Cult players? My own view is that even beyond that he is very seriously mentally ill. Look in his eyes and you can see this along with his crazy flailing arms. You don't do what he has done to the world population since the start of 2020 unless you are mentally ill and at the most extreme end of psychopathic. You especially don't do it when to you know, as we shall see, that cases and deaths from 'Covid' are fakery and a product of monumental figure massaging. 'These stats' that Gates referred to are based on a 'test' that's not testing for the 'virus' as he has known all along. He made his fortune with big Cult support as an infamously ruthless software salesman and now buys global control of 'health' (death) policy without the population he affects having any say. It's a breathtaking outrage. Gates talked about people being deeply affected by fear of 'Covid' when that was because of *him* and his global network lying to them minute-by-minute supported by a lying media that he seriously influences and funds to the tune of hundreds of millions. He's handed big sums to media operations including the BBC, NBC, Al Jazeera, Univision, *PBS NewsHour*,

ProPublica, National Journal, The Guardian, The Financial Times, The Atlantic, Texas Tribune, USA Today publisher Gannett, *Washington Monthly, Le Monde,* Center for Investigative Reporting, Pulitzer Center on Crisis Reporting, National Press Foundation, International Center for Journalists, Solutions Journalism Network, the Poynter Institute for Media Studies, and many more. Gates is everywhere in the 'Covid' hoax and the man must go to prison – or a mental facility – for the rest of his life and his money distributed to those he has taken such enormous psychopathic pleasure in crushing.

The Muscle

The Hunger Games global structure demands a police-military state – a fusion of the two into one force – which viciously imposes the will of the Cult on the population and protects the Cult from public rebellion. In that regard, too, the 'Covid' hoax just keeps on giving. Often unlawful, ridiculous and contradictory 'Covid' rules and regulations have been policed across the world by moronic automatons and psychopaths made faceless by face-nappy masks and acting like the Nazi SS and fascist blackshirts and brownshirts of Hitler and Mussolini. The smallest departure from the rules decreed by the psychos in government and their clueless gofers were jumped upon by the face-nappy fascists. Brutality against public protestors soon became commonplace even on girls, women and old people as the brave men with the batons – the Face-Nappies as I call them – broke up peaceful protests and handed out fines like confetti to people who couldn't earn a living let alone pay hundreds of pounds for what was once an accepted human right. Robot Face-Nappies of Nottingham police in the English East Midlands fined one group £11,000 for attending a child's birthday party. For decades I charted the transformation of law enforcement as genuine, decent officers were replaced with psychopaths and the brain dead who would happily and brutally do whatever their masters told them. Now they were let loose on the public and I would emphasise the point that none of this just happened. The step-by-step change in the dynamic between police and public was orchestrated from the shadows by

those who knew where this was all going and the same with the perceptual reframing of those in all levels of authority and official administration through 'training courses' by organisations such as Common Purpose which was created in the late 1980s and given a massive boost in Blair era Britain until it became a global phenomenon. Supposed public 'servants' began to view the population as the enemy and the same was true of the police. This was the start of the explosion of behaviour manipulation organisations and networks preparing for the all-war on the human psyche unleashed with the dawn of 2020. I will go into more detail about this later in the book because it is a core part of what is happening.

Police desecrated beauty spots to deter people gathering and arrested women for walking in the countryside alone 'too far' from their homes. We had arrogant, clueless sergeants in the Isle of Wight police where I live posting on Facebook what they insisted the population must do or else. A schoolmaster sergeant called Radford looked young enough for me to ask if his mother knew he was out, but he was posting what he *expected* people to do while a Sergeant Wilkinson boasted about fining lads for meeting in a McDonald's car park where they went to get a lockdown takeaway. Wilkinson added that he had even cancelled their order. What a pair of prats these people are and yet they have increasingly become the norm among Jackboot Johnson's Yellowshirts once known as the British police. This was the theme all over the world with police savagery common during lockdown protests in the United States, the Netherlands, and the fascist state of Victoria in Australia under its tyrannical and again moronic premier Daniel Andrews. Amazing how tyrannical and moronic tend to work as a team and the same combination could be seen across America as arrogant, narcissistic Woke governors and mayors such as Gavin Newsom (California), Andrew Cuomo (New York), Gretchen Whitmer (Michigan), Lori Lightfoot (Chicago) and Eric Garcetti (Los Angeles) did their Nazi and Stalin impressions with the full support of the compliant brutality of their enforcers in uniform as they arrested small business owners defying

fascist shutdown orders and took them to jail in ankle shackles and handcuffs. This happened to bistro owner Marlena Pavlos-Hackney in Gretchen Whitmer's fascist state of Michigan when police arrived to enforce an order by a state-owned judge for 'putting the community at risk' at a time when other states like Texas were dropping restrictions and migrants were pouring across the southern border without any 'Covid' questions at all. I'm sure there are many officers appalled by what they are ordered to do, but not nearly enough of them. If they were truly appalled they would not do it. As the months passed every opportunity was taken to have the military involved to make their presence on the streets ever more familiar and 'normal' for the longer-term goal of police-military fusion.

Another crucial element to the Hunger Games enforcement network has been encouraging the public to report neighbours and others for 'breaking the lockdown rules'. The group faced with £11,000 in fines at the child's birthday party would have been dobbed-in by a neighbour with a brain the size of a pea. The technique was most famously employed by the Stasi secret police in communist East Germany who had public informants placed throughout the population. A police chief in the UK says his force doesn't need to carry out 'Covid' patrols when they are flooded with so many calls from the public reporting other people for visiting the beach. Dorset police chief James Vaughan said people were so enthusiastic about snitching on their fellow humans they were now operating as an auxiliary arm of the police: 'We are still getting around 400 reports a week from the public, so we will respond to reports …We won't need to be doing hotspot patrols because people are very quick to pick the phone up and tell us.' Vaughan didn't say that this is a pillar of all tyrannies of whatever complexion and the means to hugely extend the reach of enforcement while spreading distrust among the people and making them wary of doing anything that might get them reported. Those narcissistic Isle of Wight sergeants Radford and Wilkinson never fail to add a link to their Facebook posts where the public can inform on their fellow slaves.

Neither would be self-aware enough to realise they were imitating the Stasi which they might well never have heard of. Government psychologists that I will expose later laid out a policy to turn communities against each other in the same way.

A coincidence? Yep, and I can knit fog

I knew from the start of the alleged pandemic that this was a Cult operation. It presented limitless potential to rapidly advance the Cult agenda and exploit manipulated fear to demand that every man, woman and child on the planet was 'vaccinated' in a process never used on humans before which infuses self-replicating *synthetic* material into human cells. Remember the plan to transform the human body from a biological to a synthetic biological state. I'll deal with the 'vaccine' (that's not actually a vaccine) when I focus on the genetic agenda. Enough to say here that mass global 'vaccination' justified by this 'new virus' set alarms ringing after 30 years of tracking these people and their methods. The 'Covid' hoax officially beginning in China was also a big red flag for reasons I will be explaining. The agenda potential was so enormous that I could dismiss any idea that the 'virus' appeared naturally. Major happenings with major agenda implications never occur without Cult involvement in making them happen. My questions were twofold in early 2020 as the media began its campaign to induce global fear and hysteria: Was this alleged infectious agent released on purpose by the Cult or did it even exist at all? I then did what I always do in these situations. I sat, observed and waited to see where the evidence and information would take me. By March and early April synchronicity was strongly – and ever more so since then – pointing me in the direction of *there is no 'virus'*. I went public on that with derision even from swathes of the alternative media that voiced a scenario that the Chinese government released the 'virus' in league with Deep State elements in the United States from a top-level bio-lab in Wuhan where the 'virus' is said to have first appeared. I looked at that possibility, but I didn't buy it for several reasons. Deaths from the 'virus' did not in any way match what they

would have been with a 'deadly bioweapon' and it is much more effective if you sell the *illusion* of an infectious agent rather than having a real one unless you can control through injection who has it and who doesn't. Otherwise you lose control of events. A made-up 'virus' gives you a blank sheet of paper on which you can make it do whatever you like and have any symptoms or mutant 'variants' you choose to add while a real infectious agent would limit you to what it actually does. A phantom disease allows you to have endless ludicrous 'studies' on the 'Covid' dollar to widen the perceived impact by inventing ever more 'at risk' groups including one study which said those who walk slowly may be almost four times more likely to die from the 'virus'. People are in psychiatric wards for less.

A real 'deadly bioweapon' can take out people in the hierarchy that are not part of the Cult, but essential to its operation. Obviously they don't want that. Releasing a real disease means you immediately lose control of it. Releasing an illusory one means you don't. Again it's vital that people are extra careful when dealing with what they want to hear. A bioweapon unleashed from a Chinese laboratory in collusion with the American Deep State may fit a conspiracy narrative, but is it true? Would it not be far more effective to use the excuse of a 'virus' to justify the real bioweapon – the 'vaccine'? That way your disease agent does not have to be transmitted and arrives directly through a syringe. I saw a French virologist Luc Montagnier quoted in the alternative media as saying he had discovered that the alleged 'new' severe acute respiratory syndrome coronavirus , or SARS-CoV-2, was made artificially and included elements of the human immunodeficiency 'virus' (HIV) and a parasite that causes malaria. SARS-CoV-2 is alleged to trigger an alleged illness called Covid-19. I remembered Montagnier's name from my research years before into claims that an HIV 'retrovirus' causes AIDs – claims that were demolished by Berkeley virologist Peter Duesberg who showed that no one had ever proved that HIV causes acquired immunodeficiency syndrome or AIDS. Claims that become accepted as fact, publicly and medically, with no proof whatsoever are an ever-recurring story that profoundly applies to

'Covid'. Nevertheless, despite the lack of proof, Montagnier's team at the Pasteur Institute in Paris had a long dispute with American researcher Robert Gallo over which of them discovered and isolated the HIV 'virus' and with *no evidence* found it to cause AIDS. You will see later that there is also no evidence that any 'virus' causes any disease or that there is even such a thing as a 'virus' in the way it is said to exist. The claim to have 'isolated' the HIV 'virus' will be presented in its real context as we come to the shocking story – and it is a story – of SARS-CoV-2 and so will Montagnier's assertion that he identified the full SARS-CoV-2 genome.

Hoax in the making

We can pick up the 'Covid' story in 2010 and the publication by the Rockefeller Foundation of a document called 'Scenarios for the Future of Technology and International Development'. The inner circle of the Rockefeller family has been serving the Cult since John D. Rockefeller (1839-1937) made his fortune with Standard Oil. It is less well known that the same Rockefeller – the Bill Gates of his day – was responsible for establishing what is now referred to as 'Big Pharma', the global network of pharmaceutical companies that make outrageous profits dispensing scalpel and drug 'medicine' and are obsessed with pumping vaccines in ever-increasing number into as many human arms and backsides as possible. John D. Rockefeller was the driving force behind the creation of the 'education' system in the United States and elsewhere specifically designed to program the perceptions of generations thereafter. The Rockefeller family donated exceptionally valuable land in New York for the United Nations building and were central in establishing the World Health Organization in 1948 as an agency of the UN which was created from the start as a Trojan horse and stalking horse for world government. Now enter Bill Gates. His family and the Rockefellers have long been extremely close and I have seen genealogy which claims that if you go back far enough the two families fuse into the same bloodline. Gates has said that the Bill and Melinda Gates Foundation was inspired by the Rockefeller Foundation and why not

when both are serving the same Cult? Major tax-exempt foundations are overwhelmingly criminal enterprises in which Cult assets fund the Cult agenda in the guise of 'philanthropy' while avoiding tax in the process. Cult operatives can become mega-rich in their role of front men and women for the psychopaths at the inner core and they, too, have to be psychopaths to knowingly serve such evil. Part of the deal is that a big percentage of the wealth gleaned from representing the Cult has to be spent advancing the ambitions of the Cult and hence you have the Rockefeller Foundation, Bill and Melinda Gates Foundation (and *so* many more) and people like George Soros with his global Open Society Foundations spending their billions in pursuit of global Cult control. Gates is a global public face of the Cult with his interventions in world affairs including Big Tech influence; a central role in the 'Covid' and 'vaccine' scam; promotion of the climate change shakedown; manipulation of education; geoengineering of the skies; and his food-control agenda as the biggest owner of farmland in America, his GMO promotion and through other means. As one writer said: 'Gates monopolizes or wields disproportionate influence over the tech industry, global health and vaccines, agriculture and food policy (including biopiracy and fake food), weather modification and other climate technologies, surveillance, education and media.' The almost limitless wealth secured through Microsoft and other not-allowed-to-fail ventures (including vaccines) has been ploughed into a long, long list of Cult projects designed to enslave the entire human race. Gates and the Rockefellers have been working as one unit with the Rockefeller-established World Health Organization leading global 'Covid' policy controlled by Gates through his mouth-piece Tedros. Gates became the WHO's biggest funder when Trump announced that the American government would cease its donations, but Biden immediately said he would restore the money when he took office in January, 2021. The Gates Foundation (the Cult) owns through limitless funding the world health system and the major players across the globe in the 'Covid' hoax.

Okay, with that background we return to that Rockefeller Foundation document of 2010 headed 'Scenarios for the Future of Technology and International Development' and its 'imaginary' epidemic of a virulent and deadly influenza strain which infected 20 percent of the global population and killed eight million in seven months. The Rockefeller scenario was that the epidemic destroyed economies, closed shops, offices and other businesses and led to governments imposing fierce rules and restrictions that included mandatory wearing of face masks and body-temperature checks to enter communal spaces like railway stations and supermarkets. The document predicted that even after the height of the Rockefeller-envisaged epidemic the authoritarian rule would continue to deal with further pandemics, transnational terrorism, environmental crises and rising poverty. Now you may think that the Rockefellers are our modern-day seers or alternatively, and rather more likely, that they well knew what was planned a few years further on. Fascism had to be imposed, you see, to 'protect citizens from risk and exposure'. The Rockefeller scenario document said:

During the pandemic, national leaders around the world flexed their authority and imposed airtight rules and restrictions, from the mandatory wearing of face masks to body-temperature checks at the entries to communal spaces like train stations and supermarkets. Even after the pandemic faded, this more authoritarian control and oversight of citizens and their activities stuck and even intensified. In order to protect themselves from the spread of increasingly global problems – from pandemics and transnational terrorism to environmental crises and rising poverty – leaders around the world took a firmer grip on power.

At first, the notion of a more controlled world gained wide acceptance and approval. Citizens willingly gave up some of their sovereignty – and their privacy – to more paternalistic states in exchange for greater safety and stability. Citizens were more tolerant, and even eager, for top-down direction and oversight, and national leaders had more latitude to impose order in the ways they saw fit.

In developed countries, this heightened oversight took many forms: biometric IDs for all citizens, for example, and tighter regulation of key industries whose stability was deemed vital to national interests. In many developed countries, enforced cooperation with a suite of new regulations and agreements slowly but steadily restored both order and, importantly, economic growth.

There we have the prophetic Rockefellers in 2010 and three years later came their paper for the Global Health Summit in Beijing, China, when government representatives, the private sector, international organisations and groups met to discuss the next 100 years of 'global health'. The Rockefeller Foundation-funded paper was called 'Dreaming the Future of Health for the Next 100 Years and more prophecy ensued as it described a dystopian future: 'The abundance of data, digitally tracking and linking people may mean the 'death of privacy' and may replace physical interaction with transient, virtual connection, generating isolation and raising questions of how values are shaped in virtual networks.' Next in the 'Covid' hoax preparation sequence came a 'table top' simulation in 2018 for another 'imaginary' pandemic of a disease called Clade X which was said to kill 900 million people. The exercise was organised by the Gates-funded Johns Hopkins University's Center for Health Security in the United States and this is the very same university that has been compiling the disgustingly and systematically erroneous global figures for 'Covid' cases and deaths. Similar Johns Hopkins health crisis scenarios have included the Dark Winter exercise in 2001 and Atlantic Storm in 2005.

Nostradamus 201

For sheer predictive genius look no further prophecy-watchers than the Bill Gates-funded Event 201 held only six weeks before the 'coronavirus pandemic' is supposed to have broken out in China and Event 201 was based on a scenario of a global 'coronavirus pandemic'. Melinda Gates, the great man's missus, told the BBC that he had 'prepared for years' for a coronavirus pandemic which told us what we already knew. Nostradamugates had predicted in a TED talk in 2015 that a pandemic was coming that would kill a lot of people and demolish the world economy. My god, the man is a machine – possibly even literally. Now here he was only weeks before the real thing funding just such a simulated scenario and involving his friends and associates at Johns Hopkins, the World Economic Forum Cult-front of Klaus Schwab, the United Nations,

Johnson & Johnson, major banks, and officials from China and the Centers for Disease Control in the United States. What synchronicity – Johns Hopkins would go on to compile the fraudulent 'Covid' figures, the World Economic Forum and Schwab would push the 'Great Reset' in response to 'Covid', the Centers for Disease Control would be at the forefront of 'Covid' policy in the United States, Johnson & Johnson would produce a 'Covid vaccine', and everything would officially start just weeks later in China. Spooky, eh? They were even accurate in creating a simulation of a 'virus' pandemic because the 'real thing' would also be a simulation. Event 201 was not an exercise preparing for something that might happen; it was a rehearsal for what those in control knew was *going* to happen and very shortly. Hours of this simulation were posted on the Internet and the various themes and responses mirrored what would soon be imposed to transform human society. News stories were inserted and what they said would be commonplace a few weeks later with still more prophecy perfection. Much discussion focused on the need to deal with misinformation and the 'anti-vax movement' which is exactly what happened when the 'virus' arrived – was said to have arrived – in the West.

Cult-owned social media banned criticism and exposure of the official 'virus' narrative and when I said there *was* no 'virus' in early April, 2020, I was banned by one platform after another including YouTube, Facebook and later Twitter. The mainstream broadcast media in Britain was in effect banned from interviewing me by the Tony-Blair-created government broadcasting censor Ofcom headed by career government bureaucrat Melanie Dawes who was appointed just as the 'virus' hoax was about to play out in January, 2020. At the same time the Ickonic media platform was using Vimeo, another ultra-Zionist-owned operation, while our own player was being created and they deleted in an instant hundreds of videos, documentaries, series and shows to confirm their unbelievable vindictiveness. We had copies, of course, and they had to be restored one by one when our player was ready. These people have no class. Sabbatian Facebook promised free advertisements for the Gates-

controlled World Health Organization narrative while deleting 'false claims and conspiracy theories' to stop 'misinformation' about the alleged coronavirus. All these responses could be seen just a short while earlier in the scenarios of Event 201. Extreme censorship was absolutely crucial for the Cult because the official story was so ridiculous and unsupportable by the evidence that it could never survive open debate and the free-flow of information and opinion. If you can't win a debate then don't have one is the Cult's approach throughout history. Facebook's little boy front man – front boy – Mark Zuckerberg equated 'credible and accurate information' with official sources and exposing their lies with 'misinformation'.

Silencing those that can see

The censorship dynamic of Event 201 is now the norm with an army of narrative-supporting 'fact-checker' organisations whose entire reason for being is to tell the public that official narratives are true and those exposing them are lying. One of the most appalling of these 'fact-checkers' is called NewsGuard founded by ultra-Zionist Americans Gordon Crovitz and Steven Brill. Crovitz is a former publisher of *The Wall Street Journal*, former Executive Vice President of Dow Jones, a member of the Council on Foreign Relations (CFR), and on the board of the American Association of Rhodes Scholars. The CFR and Rhodes Scholarships, named after Rothschild agent Cecil Rhodes who plundered the gold and diamonds of South Africa for his masters and the Cult, have featured widely in my books. NewsGuard don't seem to like me for some reason – I really can't think why – and they have done all they can to have me censored and discredited which is, to quote an old British politician, like being savaged by a dead sheep. They are, however, like all in the censorship network, very well connected and funded by organisations themselves funded by, or connected to, Bill Gates. As you would expect with anything associated with Gates NewsGuard has an offshoot called HealthGuard which 'fights online health care hoaxes'. How very kind. Somehow the NewsGuard European Managing Director Anna-Sophie Harling, a remarkably young-

looking woman with no broadcasting experience and little hands-on work in journalism, has somehow secured a position on the 'Content Board' of UK government broadcast censor Ofcom. An executive of an organisation seeking to discredit dissidents of the official narratives is making decisions for the government broadcast 'regulator' about content?? Another appalling 'fact-checker' is Full Fact funded by George Soros and global censors Google and Facebook.

It's amazing how many activists in the 'fact-checking', 'anti-hate', arena turn up in government-related positions – people like UK Labour Party activist Imran Ahmed who heads the Center for Countering Digital Hate founded by people like Morgan McSweeney, now chief of staff to the Labour Party's hapless and useless 'leader' Keir Starmer. Digital Hate – which is what it really is – uses the American spelling of Center to betray its connection to a transatlantic network of similar organisations which in 2020 shapeshifted from attacking people for 'hate' to attacking them for questioning the 'Covid' hoax and the dangers of the 'Covid vaccine'. It's just a coincidence, you understand. This is one of Imran Ahmed's hysterical statements: 'I would go beyond calling anti-vaxxers conspiracy theorists to say they are an extremist group that pose a national security risk.' No one could ever accuse this prat of understatement and he's including in that those parents who are now against vaccines after their children were damaged for life or killed by them. He's such a nice man. Ahmed does the rounds of the Woke media getting soft-ball questions from spineless 'journalists' who never ask what right he has to campaign to destroy the freedom of speech of others while he demands it for himself. There also seems to be an overrepresentation in Ofcom of people connected to the narrative-worshipping BBC. This incredible global network of narrative-support was super-vital when the 'Covid' hoax was played in the light of the mega-whopper lies that have to be defended from the spotlight cast by the most basic intelligence.

Setting the scene

The Cult plays the long game and proceeds step-by-step ensuring that everything is in place before major cards are played and they don't come any bigger than the 'Covid' hoax. The psychopaths can't handle events where the outcome isn't certain and as little as possible – preferably nothing – is left to chance. Politicians, government and medical officials who would follow direction were brought to illusory power in advance by the Cult web whether on the national stage or others like state governors and mayors of America. For decades the dynamic between officialdom, law enforcement and the public was changed from one of service to one of control and dictatorship. Behaviour manipulation networks established within government were waiting to impose the coming 'Covid' rules and regulations specifically designed to subdue and rewire the psyche of the people in the guise of protecting health. These included in the UK the Behavioural Insights Team part-owned by the British government Cabinet Office; the Scientific Pandemic Insights Group on Behaviours (SPI-B); and a whole web of intelligence and military groups seeking to direct the conversation on social media and control the narrative. Among them are the cyberwarfare (on the people) 77th Brigade of the British military which is also coordinated through the Cabinet Office as civilian and military leadership continues to combine in what they call the Fusion Doctrine. The 77th Brigade is a British equivalent of the infamous Israeli (Sabbatian) military cyberwarfare and Internet manipulation operation Unit 8200 which I expose at length in *The Trigger*. Also carefully in place were the medical and science advisers to government – many on the payroll past or present of Bill Gates – and a whole alternative structure of unelected government stood by to take control when elected parliaments were effectively closed down once the 'Covid' card was slammed on the table. The structure I have described here and so much more was installed in every major country through the Cult networks. The top-down control hierarchy looks like this: The Cult – Cult-owned Gates – the World Health Organization and Tedros – Gates-funded or controlled chief medical officers and science 'advisers' (dictators) in each country –

political 'leaders'– law enforcement – The People. Through this simple global communication and enforcement structure the policy of the Cult could be imposed on virtually the entire human population so long as they acquiesced to the fascism. With everything in place it was time for the button to be pressed in late 2019/early 2020.

These were the prime goals the Cult had to secure for its will to prevail:

1) Locking down economies, closing all but designated 'essential' businesses (Cult-owned corporations were 'essential'), and putting the population under house arrest was an imperative to destroy independent income and employment and ensure dependency on the Cult-controlled state in the Hunger Games Society. Lockdowns had to be established as the global blueprint from the start to respond to the 'virus' and followed by pretty much the entire world.

2) The global population had to be terrified into believing in a deadly 'virus' that didn't actually exist so they would unquestioningly obey authority in the belief that authority must know how best to protect them and their families. Software salesman Gates would suddenly morph into the world's health expert and be promoted as such by the Cult-owned media.

3) A method of testing that wasn't testing for the 'virus', but was only claimed to be, had to be in place to provide the illusion of 'cases' and subsequent 'deaths' that had a very different cause to the 'Covid-19' that would be scribbled on the death certificate.

4) Because there was no 'virus' and the great majority testing positive with a test not testing for the 'virus' would have no symptoms of anything the lie had to be sold that people without symptoms (without the 'virus') could still pass it on to others. This was crucial to justify for the first time quarantining – house arresting – healthy people. Without this the economy-destroying lockdown of *everybody* could not have been credibly sold.

5) The 'saviour' had to be seen as a vaccine which beyond evil drug companies were working like angels of mercy to develop as quickly as possible, with all corners cut, to save the day. The public must absolutely not know that the 'vaccine' had nothing to do with a 'virus' or that the contents were ready and waiting with a very different motive long before the 'Covid' card was even lifted from the pack.

I said in March, 2020, that the 'vaccine' would have been created way ahead of the 'Covid' hoax which justified its use and the following December an article in the New York *Intelligencer* magazine said the Moderna 'vaccine' had been 'designed' by

January, 2020. This was 'before China had even acknowledged that the disease could be transmitted from human to human, more than a week before the first confirmed coronavirus case in the United States'. The article said that by the time the first American death was announced a month later 'the vaccine had already been manufactured and shipped to the National Institutes of Health for the beginning of its Phase I clinical trial'. The 'vaccine' was actually 'designed' long before that although even with this timescale you would expect the article to ask how on earth it could have been done that quickly. Instead it asked why the 'vaccine' had not been rolled out then and not months later. Journalism in the mainstream is truly dead. I am going to detail in the next chapter why the 'virus' has never existed and how a hoax on that scale was possible, but first the foundation on which the Big Lie of 'Covid' was built.

The test that doesn't test

Fraudulent 'testing' is the bottom line of the whole 'Covid' hoax and was the means by which a 'virus' that did not exist *appeared* to exist. They could only achieve this magic trick by using a test not testing for the 'virus'. To use a test that *was* testing for the 'virus' would mean that every test would come back negative given there was no 'virus'. They chose to exploit something called the RT-PCR test invented by American biochemist Kary Mullis in the 1980s who said publicly that his PCR test … *cannot detect infectious disease*. Yes, the 'test' used worldwide to detect infectious 'Covid' to produce all the illusory 'cases' and 'deaths' compiled by Johns Hopkins and others *cannot detect infectious disease*. This fact came from the mouth of the man who invented PCR and was awarded the Nobel Prize in Chemistry in 1993 for doing so. Sadly, and incredibly conveniently for the Cult, Mullis died in August, 2019, at the age of 74 just before his test would be fraudulently used to unleash fascism on the world. He was said to have died from pneumonia which was an irony in itself. A few months later he would have had 'Covid-19' on his death certificate. I say the timing of his death was convenient because had he lived Mullis, a brilliant, honest and decent man, would have been

vociferously speaking out against the use of his test to detect 'Covid' when it was never designed, or able, to do that. I know that to be true given that Mullis made the same point when his test was used to 'detect' – not detect – HIV. He had been seriously critical of the Gallo/Montagnier claim to have isolated the HIV 'virus' and shown it to cause AIDS for which Mullis said there was no evidence. AIDS is actually not a disease but a series of diseases from which people die all the time. When they die from those *same diseases* after a positive 'test' for HIV then AIDS goes on their death certificate. I think I've heard that before somewhere. Countries instigated a policy with 'Covid' that anyone who tested positive with a test not testing for the 'virus' and died of any other cause within 28 days and even longer 'Covid-19' had to go on the death certificate. Cases have come from the test that can't test for infectious disease and the deaths are those who have died of *anything* after testing positive with a test not testing for the 'virus'. I'll have much more later about the death certificate scandal.

Mullis was deeply dismissive of the now US 'Covid' star Anthony Fauci who he said was a liar who didn't know anything about anything – 'and I would say that to his face – nothing.' He said of Fauci: 'The man thinks he can take a blood sample, put it in an electron microscope and if it's got a virus in there you'll know it – he doesn't understand electron microscopy and he doesn't understand medicine and shouldn't be in a position like he's in.' That position, terrifyingly, has made him the decider of 'Covid' fascism policy on behalf of the Cult in his role as director since 1984 of the National Institute of Allergy and Infectious Diseases (NIAID) while his record of being wrong is laughable; but being wrong, so long as it's the *right kind* of wrong, is why the Cult loves him. He'll say anything the Cult tells him to say. Fauci was made Chief Medical Adviser to the President immediately Biden took office. Biden was installed in the White House by Cult manipulation and one of his first decisions was to elevate Fauci to a position of even more control. This is a coincidence? Yes, and I identify as a flamenco dancer called Lola. How does such an incompetent criminal like Fauci remain in that

pivotal position in American health since *the 1980s*? When you serve the Cult it looks after you until you are surplus to requirements. Kary Mullis said prophetically of Fauci and his like: 'Those guys have an agenda and it's not an agenda we would like them to have … they make their own rules, they change them when they want to, and Tony Fauci does not mind going on television in front of the people who pay his salary and lie directly into the camera.' Fauci has done that almost daily since the 'Covid' hoax began. Lying is in Fauci's DNA. To make the situation crystal clear about the PCR test this is a direct quote from its inventor Kary Mullis:

> It [the PCR test] doesn't tell you that you're sick and doesn't tell you that the thing you ended up with was really going to hurt you ...'

Ask yourself why governments and medical systems the world over have been using this very test to decide who is 'infected' with the SARS-CoV-2 'virus' and the alleged disease it allegedly causes, 'Covid-19'. The answer to that question will tell you what has been going on. By the way, here's a little show-stopper – the 'new' SARS-CoV-2 'virus' was 'identified' as such right from the start using … *the PCR test not testing for the 'virus'*. If you are new to this and find that shocking then stick around. I have hardly started yet. Even worse, other 'tests', like the 'Lateral Flow Device' (LFD), are considered so useless that they have to be *confirmed* by the PCR test! Leaked emails written by Ben Dyson, adviser to UK 'Health' Secretary Matt Hancock, said they were 'dangerously unreliable'. Dyson, executive director of strategy at the Department of Health, wrote: 'As of today, someone who gets a positive LFD result in (say) London has at best a 25 per cent chance of it being a true positive, but if it is a self-reported test potentially as low as 10 per cent (on an optimistic assumption about specificity) or as low as 2 per cent (on a more pessimistic assumption).' These are the 'tests' that schoolchildren and the public are being urged to have twice a week or more and have to isolate if they get a positive. Each fake positive goes in the statistics as a 'case' no matter how ludicrously inaccurate and the

'cases' drive lockdown, masks and the pressure to 'vaccinate'. The government said in response to the email leak that the 'tests' were accurate which confirmed yet again what shocking bloody liars they are. The real false positive rate is *100 percent* as we'll see. In another 'you couldn't make it up' the UK government agreed to pay £2.8 billion to California's Innova Medical Group to supply the irrelevant lateral flow tests. The company's primary test-making centre is in China. Innova Medical Group, established in March, 2020, is owned by Pasaca Capital Inc, chaired by Chinese-American millionaire Charles Huang who was born in Wuhan.

How it works – and how it doesn't

The RT-PCR test, known by its full title of Polymerase chain reaction, is used across the world to make millions, even billions, of copies of a DNA/RNA genetic information sample. The process is called 'amplification' and means that a tiny sample of genetic material is amplified to bring out the detailed content. I stress that it is not testing for an infectious disease. It is simply amplifying a sample of genetic material. In the words of Kary Mullis: 'PCR is … just a process that's used to make a whole lot of something out of something.' To emphasise the point companies that make the PCR tests circulated around the world to 'test' for 'Covid' warn on the box that it can't be used to detect 'Covid' or infectious disease and is for research purposes only. It's okay, rest for a minute and you'll be fine. This is the test that produces the 'cases' and 'deaths' that have been used to destroy human society. All those global and national medical and scientific 'experts' demanding this destruction to 'save us' *KNOW* that the test is not testing for the 'virus' and the cases and deaths they claim to be real are an almost unimaginable fraud. Every one of them and so many others including politicians and psychopaths like Gates and Tedros must be brought before Nuremburg-type trials and jailed for the rest of their lives. The more the genetic sample is amplified by PCR the more elements of that material become sensitive to the test and by that I don't mean sensitive for a 'virus' but for elements of the genetic material which

is *naturally* in the body or relates to remnants of old conditions of various kinds lying dormant and causing no disease. Once the amplification of the PCR reaches a certain level *everyone* will test positive. So much of the material has been made sensitive to the test that everyone will have some part of it in their body. Even lying criminals like Fauci have said that once PCR amplifications pass 35 cycles everything will be a false positive that cannot be trusted for the reasons I have described. I say, like many proper doctors and scientists, that 100 percent of the 'positives' are false, but let's just go with Fauci for a moment.

He says that any amplification over 35 cycles will produce false positives and yet the US Centers for Disease Control (CDC) and Food and Drug Administration (FDA) have recommended up to *40 cycles* and the National Health Service (NHS) in Britain admitted in an internal document for staff that it was using *45 cycles* of amplification. A long list of other countries has been doing the same and at least one 'testing' laboratory has been using *50 cycles*. Have you ever heard a doctor, medical 'expert' or the media ask what level of amplification has been used to claim a 'positive'. The 'test' comes back 'positive' and so you have the 'virus', end of story. Now we can see how the government in Tanzania could send off samples from a goat and a pawpaw fruit under human names and both came back positive for 'Covid-19'. Tanzania president John Magufuli mocked the 'Covid' hysteria, the PCR test and masks and refused to import the DNA-manipulating 'vaccine'. The Cult hated him and an article sponsored by the Bill Gates Foundation appeared in the London *Guardian* in February, 2021, headed 'It's time for Africa to rein in Tanzania's anti-vaxxer president'. Well, 'reined in' he shortly was. Magufuli appeared in good health, but then, in March, 2021, he was dead at 61 from 'heart failure'. He was replaced by Samia Hassan Suhulu who is connected to Klaus Schwab's World Economic Forum and she immediately reversed Magufuli's 'Covid' policy. A sample of cola tested positive for 'Covid' with the PCR test in Germany while American actress and singer-songwriter Erykah Badu tested positive in one nostril and negative in the other. Footballer Ronaldo called

the PCR test 'bullshit' after testing positive three times and being forced to quarantine and miss matches when there was nothing wrong with him. The mantra from Tedros at the World Health Organization and national governments (same thing) has been test, test, test. They know that the more tests they can generate the more fake 'cases' they have which go on to become 'deaths' in ways I am coming to. The UK government has its Operation Moonshot planned to test multiple millions every day in workplaces and schools with free tests for everyone to use twice a week at home in line with the Cult plan from the start to make testing part of life. A government advertisement for an 'Interim Head of Asymptomatic Testing Communication' said the job included responsibility for delivering a 'communications strategy' (propaganda) 'to support the expansion of asymptomatic testing that *'normalises testing as part of everyday life*'. More tests means more fake 'cases', 'deaths' and fascism. I have heard of, and from, many people who booked a test, couldn't turn up, and yet got a positive result through the post for a test they'd never even had. The whole thing is crazy, but for the Cult there's method in the madness. Controlling and manipulating the level of amplification of the test means the authorities can control whenever they want the number of apparent 'cases' and 'deaths'. If they want to justify more fascist lockdown and destruction of livelihoods they keep the amplification high. If they want to give the illusion that lockdowns and the 'vaccine' are working then they lower the amplification and 'cases' and 'deaths' will appear to fall. In January, 2021, the Cult-owned World Health Organization suddenly warned laboratories about over-amplification of the test and to lower the threshold. Suddenly headlines began appearing such as: 'Why ARE "Covid" cases plummeting?' This was just when the vaccine rollout was underway and I had predicted months before they would make cases appear to fall through amplification tampering when the 'vaccine' came. These people are so predictable.

Cow vaccines?

The question must be asked of what is on the test swabs being poked far up the nose of the population to the base of the brain? A nasal swab punctured one woman's brain and caused it to leak fluid. Most of these procedures are being done by people with little training or medical knowledge. Dr Lorraine Day, former orthopaedic trauma surgeon and Chief of Orthopaedic Surgery at San Francisco General Hospital, says the tests are really a *'vaccine'*. Cows have long been vaccinated this way. She points out that masks have to cover the nose and the mouth where it is claimed the 'virus' exists in saliva. Why then don't they take saliva from the mouth as they do with a DNA test instead of pushing a long swab up the nose towards the brain? The ethmoid bone separates the nasal cavity from the brain and within that bone is the cribriform plate. Dr Day says that when the swab is pushed up against this plate and twisted the procedure is 'depositing things back there'. She claims that among these 'things' are nanoparticles that can enter the brain. Researchers have noted that a team at the Gates-funded Johns Hopkins have designed tiny, star-shaped micro-devices that can latch onto intestinal mucosa and release drugs into the body. Mucosa is the thin skin that covers the inside surface of parts of the body such as *the nose* and mouth and produces mucus to protect them. The Johns Hopkins micro-devices are called 'theragrippers' and were 'inspired' by a parasitic worm that digs its sharp teeth into a host's intestines. Nasal swabs are also coated in the sterilisation agent ethylene oxide. The US National Cancer Institute posts this explanation on its website:

> At room temperature, ethylene oxide is a flammable colorless gas with a sweet odor. It is used primarily to produce other chemicals, including antifreeze. In smaller amounts, ethylene oxide is used as a pesticide and a sterilizing agent. The ability of ethylene oxide to damage DNA makes it an effective sterilizing agent but also accounts for its cancer-causing activity.

The Institute mentions lymphoma and leukaemia as cancers most frequently reported to be associated with occupational exposure to ethylene oxide along with stomach and breast cancers. How does anyone think this is going to work out with the constant testing

regime being inflicted on adults and children at home and at school that will accumulate in the body anything that's on the swab?

Doctors know best

It is vital for people to realise that 'hero' doctors 'know' only what the Big Pharma-dominated medical authorities tell them to 'know' and if they refuse to 'know' what they are told to 'know' they are out the door. They are mostly not physicians or healers, but repeaters of the official narrative – or else. I have seen alleged professional doctors on British television make shocking statements that we are supposed to take seriously. One called 'Dr' Amir Khan, who is actually telling patients how to respond to illness, said that men could take the birth pill to 'help slow down the effects of Covid-19'. In March, 2021, another ridiculous 'Covid study' by an American doctor proposed injecting men with the female sex hormone progesterone as a 'Covid' treatment. British doctor Nighat Arif told the BBC that face coverings were now going to be part of ongoing normal. Yes, the vaccine protects you, she said (evidence?) … but the way to deal with viruses in the community was always going to come down to hand washing, face covering and keeping a physical distance. That's not what we were told before the 'vaccine' was circulating. Arif said she couldn't imagine ever again going on the underground or in a lift without a mask. I was just thanking my good luck that she was not my doctor when she said – in March, 2021 – that if 'we are *behaving* and we are doing all the right things' she thought we could 'have our nearest and dearest around us at home … around *Christmas* and N*ew Year!* Her patronising delivery was the usual school teacher talking to six-year-olds as she repeated every government talking point and probably believed them all. If we have learned anything from the 'Covid' experience surely it must be that humanity's perception of doctors needs a fundamental rethink. NHS 'doctor' Sara Kayat told her television audience that the 'Covid vaccine' would '100 percent prevent hospitalisation and death'. Not even Big Pharma claimed that. We have to stop taking 'experts' at their word without question when so many of them are

clueless and only repeating the party line on which their careers depend. That is not to say there are not brilliants doctors – there are and I have spoken to many of them since all this began – but you won't see them in the mainstream media or quoted by the psychopaths and yes-people in government.

Remember the name – Christian Drosten

German virologist Christian Drosten, Director of Charité Institute of Virology in Berlin, became a national star after the pandemic hoax began. He was feted on television and advised the German government on 'Covid' policy. Most importantly to the wider world Drosten led a group that produced the 'Covid' testing protocol for the PCR test. What a remarkable feat given the PCR cannot test for infectious disease and even more so when you think that Drosten said that his method of testing for SARS-CoV-2 was developed 'without having virus material available'. *He developed a test for a 'virus' that he didn't have and had never seen*. Let that sink in as you survey the global devastation that came from what he did. The whole catastrophe of Drosten's 'test' was based on the alleged genetic sequence published by Chinese scientists on the Internet. We will see in the next chapter that this alleged 'genetic sequence' has never been produced by China or anyone and cannot be when there *is no* SARS-CoV-2. Drosten, however, doesn't seem to let little details like that get in the way. He was the lead author with Victor Corman from the same Charité Hospital of the paper 'Detection of 2019 novel coronavirus (2019-nCoV) by real-time PCR' published in a magazine called *Eurosurveillance*. This became known as the Corman-Drosten paper. In November, 2020, with human society devastated by the effects of the Corman-Drosten test baloney, the protocol was publicly challenged by 22 international scientists and independent researchers from Europe, the United States, and Japan. Among them were senior molecular geneticists, biochemists, immunologists, and microbiologists. They produced a document headed 'External peer review of the RTPCR test to detect SARS-Cov-2 Reveals 10 Major Flaws At The Molecular and Methodological Level: Consequences

For False-Positive Results'. The flaws in the Corman-Drosten test included the following:

- The test is non-specific because of erroneous design
- Results are enormously variable
- The test is unable to discriminate between the whole 'virus' and viral fragments
- It doesn't have positive or negative controls
- The test lacks a standard operating procedure
- It is unsupported by proper peer view

The scientists said the PCR 'Covid' testing protocol was not founded on science and they demanded the Corman-Drosten paper be retracted by *Eurosurveillance*. They said all present and previous Covid deaths, cases, and 'infection rates' should be subject to a massive retroactive inquiry. Lockdowns and travel restrictions should be reviewed and relaxed and those diagnosed through PCR to have 'Covid-19' should not be forced to isolate. Dr Kevin Corbett, a health researcher and nurse educator with a long academic career producing a stream of peer-reviewed publications at many UK universities, made the same point about the PCR test debacle. He said of the scientists' conclusions: 'Every scientific rationale for the development of that test has been totally destroyed by this paper. It's like Hiroshima/Nagasaki to the Covid test.' He said that China hadn't given them an isolated 'virus' when Drosten developed the test. Instead they had developed the test from *a sequence in a gene bank*.' Put another way … *they made it up*! The scientists were supported in this contention by a Portuguese appeals court which ruled in November, 2020, that PCR tests are unreliable and it is unlawful to quarantine people based solely on a PCR test. The point about China not providing an isolated virus must be true when the 'virus' has never been isolated to this day and the consequences of that will become clear. Drosten and company produced this useless 'protocol' right on cue in January, 2020, just as the 'virus' was said to

be moving westward and it somehow managed to successfully pass a peer-review in 24 hours. In other words there was no peer-review for a test that would be used to decide who had 'Covid' and who didn't across the world. The Cult-created, Gates-controlled World Health Organization immediately recommended all its nearly 200 member countries to use the Drosten PCR protocol to detect 'cases' and 'deaths'. The sting was underway and it continues to this day.

So who is this Christian Drosten that produced the means through which death, destruction and economic catastrophe would be justified? His education background, including his doctoral thesis, would appear to be somewhat shrouded in mystery and his track record is dire as with another essential player in the 'Covid' hoax, the Gates-funded Professor Neil Ferguson at the Gates-funded Imperial College in London of whom more shortly. Drosten predicted in 2003 that the alleged original SARS 'virus' (SARS-1') was an epidemic that could have serious effects on economies and an effective vaccine would take at least two years to produce. Drosten's answer to every alleged 'outbreak' is a vaccine which you won't be shocked to know. What followed were just 774 official deaths worldwide and none in Germany where there were only nine cases. That is even if you believe there ever was a SARS 'virus' when the evidence is zilch and I will expand on this in the next chapter. Drosten claims to be co-discoverer of 'SARS-1' and developed a test for it in 2003. He was screaming warnings about 'swine flu' in 2009 and how it was a widespread infection far more severe than any dangers from a vaccine could be and people should get vaccinated. It would be helpful for Drosten's vocal chords if he simply recorded the words 'the virus is deadly and you need to get vaccinated' and copies could be handed out whenever the latest made-up threat comes along. Drosten's swine flu epidemic never happened, but Big Pharma didn't mind with governments spending hundreds of millions on vaccines that hardly anyone bothered to use and many who did wished they hadn't. A study in 2010 revealed that the risk of dying from swine flu, or H1N1, was no higher than that of the annual seasonal flu which is what at least most of 'it' really was as in

the case of 'Covid-19'. A media investigation into Drosten asked how with such a record of inaccuracy he could be *the* government adviser on these issues. The answer to that question is the same with Drosten, Ferguson and Fauci – they keep on giving the authorities the 'conclusions' and 'advice' they want to hear. Drosten certainly produced the goods for them in January, 2020, with his PCR protocol garbage and provided the foundation of what German internal medicine specialist Dr Claus Köhnlein, co-author of *Virus Mania*, called the 'test pandemic'. The 22 scientists in the *Eurosurveillance* challenge called out conflicts of interest within the Drosten 'protocol' group and with good reason. Olfert Landt, a regular co-author of Drosten 'studies', owns the biotech company TIB Molbiol Syntheselabor GmbH in Berlin which manufactures and sells the tests that Drosten and his mates come up with. They have done this with SARS, Enterotoxigenic E. coli (ETEC), MERS, Zika 'virus', yellow fever, and now 'Covid'. Landt told the *Berliner Zeitung* newspaper:

> The testing, design and development came from the Charité [Drosten and Corman]. We simply implemented it immediately in the form of a kit. And if we don't have the virus, which originally only existed in Wuhan, we can make a synthetic gene to simulate the genome of the virus. That's what we did very quickly.

This is more confirmation that the Drosten test was designed without access to the 'virus' and only a synthetic simulation which is what SARS-CoV-2 really is – a computer-generated synthetic fiction. It's quite an enterprise they have going here. A Drosten team decides what the test for something should be and Landt's biotech company flogs it to governments and medical systems across the world. His company must have made an absolute fortune since the 'Covid' hoax began. Dr Reiner Fuellmich, a prominent German consumer protection trial lawyer in Germany and California, is on Drosten's case and that of Tedros at the World Health Organization for crimes against humanity with a class-action lawsuit being prepared in the United States and other legal action in Germany.

Why China?

Scamming the world with a 'virus' that doesn't exist would seem impossible on the face of it, but not if you have control of the relatively few people that make policy decisions and the great majority of the global media. Remember it's not about changing 'real' reality it's about controlling *perception* of reality. You don't have to make something happen you only have make people *believe* that it's happening. Renegade Minds understand this and are therefore much harder to swindle. 'Covid-19' is not a 'real' 'virus'. It's a mind virus, like a computer virus, which has infected the minds, not the bodies, of billions. It all started, publically at least, in China and that alone is of central significance. The Cult was behind the revolution led by its asset Mao Zedong, or Chairman Mao, which established the People's Republic of China on October 1st, 1949. It should have been called The Cult's Republic of China, but the name had to reflect the recurring illusion that vicious dictatorships are run by and for the people (see all the 'Democratic Republics' controlled by tyrants). In the same way we have the 'Biden' Democratic Republic of America officially ruled by a puppet tyrant (at least temporarily) on behalf of Cult tyrants. The creation of Mao's merciless communist/fascist dictatorship was part of a frenzy of activity by the Cult at the conclusion of World War Two which, like the First World War, it had instigated through its assets in Germany, Britain, France, the United States and elsewhere. Israel was formed in 1948; the Soviet Union expanded its 'Iron Curtain' control, influence and military power with the Warsaw Pact communist alliance in 1955; the United Nations was formed in 1945 as a Cult precursor to world government; and a long list of world bodies would be established including the World Health Organization (1948), World Trade Organization (1948 under another name until 1995), International Monetary Fund (1945) and World Bank (1944). Human society was redrawn and hugely centralised in the global Problem-Reaction-Solution that was World War Two. All these changes were significant. Israel would become the headquarters of the Sabbatians

and the revolution in China would prepare the ground and control system for the events of 2019/2020.

Renegade Minds know there are no borders except for public consumption. The Cult is a seamless, borderless global entity and to understand the game we need to put aside labels like borders, nations, countries, communism, fascism and democracy. These delude the population into believing that countries are ruled within their borders by a government of whatever shade when these are mere agencies of a global power. America's illusion of democracy and China's communism/fascism are subsidiaries – vehicles – for the same agenda. We may hear about conflict and competition between America and China and on the lower levels that will be true; but at the Cult level they are branches of the same company in the way of the McDonald's example I gave earlier. I have tracked in the books over the years support by US governments of both parties for Chinese Communist Party infiltration of American society through allowing the sale of land, even military facilities, and the acquisition of American business and university influence. All this is underpinned by the infamous stealing of intellectual property and technological know-how. Cult-owned Silicon Valley corporations waive their fraudulent 'morality' to do business with human-rights-free China; Cult-controlled Disney has become China's PR department; and China in effect owns 'American' sports such as basketball which depends for much of its income on Chinese audiences. As a result any sports player, coach or official speaking out against China's horrific human rights record is immediately condemned or fired by the China-worshipping National Basketball Association. One of the first acts of China-controlled Biden was to issue an executive order telling federal agencies to stop making references to the 'virus' by the 'geographic location of its origin'. Long-time Congressman Jerry Nadler warned that criticising China, America's biggest rival, leads to hate crimes against Asian people in the United States. So shut up you bigot. China is fast closing in on Israel as a country that must not be criticised which is apt, really, given that Sabbatians control them both. The two countries have

developed close economic, military, technological and strategic ties which include involvement in China's 'Silk Road' transport and economic initiative to connect China with Europe. Israel was the first country in the Middle East to recognise the establishment of Mao's tyranny in 1950 months after it was established.

Project Wuhan – the 'Covid' Psyop

I emphasise again that the Cult plays the long game and what is happening to the world today is the result of centuries of calculated manipulation following a script to take control step-by-step of every aspect of human society. I will discuss later the common force behind all this that has spanned those centuries and thousands of years if the truth be told. Instigating the Mao revolution in China in 1949 with a 2020 'pandemic' in mind is not only how they work – the 71 years between them is really quite short by the Cult's standards of manipulation preparation. The reason for the Cult's Chinese revolution was to create a fiercely-controlled environment within which an extreme structure for human control could be incubated to eventually be unleashed across the world. We have seen this happen since the 'pandemic' emerged from China with the Chinese control-structure founded on AI technology and tyrannical enforcement sweep across the West. Until the moment when the Cult went for broke in the West and put its fascism on public display Western governments had to pay some lip-service to freedom and democracy to not alert too many people to the tyranny-in-the-making. Freedoms were more subtly eroded and power centralised with covert government structures put in place waiting for the arrival of 2020 when that smokescreen of 'freedom' could be dispensed with. The West was not able to move towards tyranny before 2020 anything like as fast as China which was created as a tyranny and had no limits on how fast it could construct the Cult's blueprint for global control. When the time came to impose that structure on the world it was the same Cult-owned Chinese communist/fascist government that provided the excuse – the 'Covid pandemic'. It was absolutely crucial to the Cult plan for the Chinese response to the 'pandemic' –

draconian lockdowns of the entire population – to become the blueprint that Western countries would follow to destroy the livelihoods and freedom of their people. This is why the Cult-owned, Gates-owned, WHO Director-General Tedros said early on:

The Chinese government is to be congratulated for the extraordinary measures it has taken to contain the outbreak. China is actually setting a new standard for outbreak response and it is not an exaggeration.

Forbes magazine said of China: '… those measures protected untold millions from getting the disease'. The Rockefeller Foundation 'epidemic scenario' document in 2010 said 'prophetically':

However, a few countries did fare better – China in particular. The Chinese government's quick imposition and enforcement of mandatory quarantine for all citizens, as well as its instant and near-hermetic sealing off of all borders, saved millions of lives, stopping the spread of the virus far earlier than in other countries and enabling a swifter post-pandemic recovery.

Once again – *spooky*.

The first official story was the 'bat theory' or rather the bat diversion. The source of the 'virus outbreak' we were told was a ''wet market' in Wuhan where bats and other animals are bought and eaten in horrifically unhygienic conditions. Then another story emerged through the alternative media that the 'virus' had been released on purpose or by accident from a BSL-4 (biosafety level 4) laboratory in Wuhan not far from the wet market. The lab was reported to create and work with lethal concoctions and bioweapons. Biosafety level 4 is the highest in the World Health Organization system of safety and containment. Renegade Minds are aware of what I call designer manipulation. The ideal for the Cult is for people to buy its prime narrative which in the opening salvoes of the 'pandemic' was the wet market story. It knows, however, that there is now a considerable worldwide alternative media of researchers sceptical of anything governments say and they are often given a version of events in a form they can perceive as credible while misdirecting them from the real truth. In this case let them

think that the conspiracy involved is a 'bioweapon virus' released from the Wuhan lab to keep them from the real conspiracy – *there is no 'virus'*. The WHO's current position on the source of the outbreak at the time of writing appears to be: 'We haven't got a clue, mate.' This is a good position to maintain mystery and bewilderment. The inner circle will know where the 'virus' came from – *nowhere*. The bottom line was to ensure the public believed there *was* a 'virus' and it didn't much matter if they thought it was natural or had been released from a lab. The belief that there was a 'deadly virus' was all that was needed to trigger global panic and fear. The population was terrified into handing their power to authority and doing what they were told. They had to or they were 'all gonna die'.

In March, 2020, information began to come my way from real doctors and scientists and my own additional research which had my intuition screaming: 'Yes, that's it! *There is no virus*.' The 'bioweapon' was not the 'virus'; it was the *'vaccine'* already being talked about that would be the bioweapon. My conclusion was further enhanced by happenings in Wuhan. The 'virus' was said to be sweeping the city and news footage circulated of people collapsing in the street (which they've never done in the West with the same 'virus'). The Chinese government was building 'new hospitals' in a matter of ten days to 'cope with demand' such was the virulent nature of the 'virus'. Yet in what seemed like no time the 'new hospitals' closed – even if they even opened – and China declared itself 'virus-free'. It was back to business as usual. This was more propaganda to promote the Chinese draconian lockdowns in the West as the way to 'beat the virus'. Trouble was that we subsequently had lockdown after lockdown, but never business as usual. As the people of the West and most of the rest of the world were caught in an ever-worsening spiral of lockdown, social distancing, masks, isolated old people, families forced apart, and livelihood destruction, it was party-time in Wuhan. Pictures emerged of thousands of people enjoying pool parties and concerts. It made no sense until you realised there never was a 'virus' and the

whole thing was a Cult set-up to transform human society out of one its major global strongholds – China.

How is it possible to deceive virtually the entire world population into believing there is a deadly virus when there is not even a 'virus' let alone a deadly one? It's nothing like as difficult as you would think and that's clearly true because it happened.

Postscript: See end of book Postscript for more on the 'Wuhan lab virus release' story which the authorities and media were pushing heavily in the summer of 2021 to divert attention from the truth that the 'Covid virus' is pure invention.

CHAPTER FIVE

There *is no* 'virus'

You can fool some of the people all of the time, and all of the people some of the time, but you cannot fool all of the people all of the time
Abraham Lincoln

The greatest form of mind control is repetition. The more you repeat the same mantra of alleged 'facts' the more will accept them to be true. It becomes an 'everyone knows that, mate'. If you can also censor any other version or alternative to your alleged 'facts' you are pretty much home and cooking.

By the start of 2020 the Cult owned the global mainstream media almost in its entirety to spew out its 'Covid' propaganda and ignore or discredit any other information and view. Cult-owned social media platforms in Cult-owned Silicon Valley were poised and ready to unleash a campaign of ferocious censorship to obliterate all but the official narrative. To complete the circle many demands for censorship by Silicon Valley were led by the mainstream media as 'journalists' became full-out enforcers for the Cult both as propagandists and censors. Part of this has been the influx of young people straight out of university who have become 'journalists' in significant positions. They have no experience and a headful of programmed perceptions from their years at school and university at a time when today's young are the most perceptually-targeted generations in known human history given the insidious impact of technology. They enter the media perceptually prepared and ready to repeat the narratives of the system that programmed them to

repeat its narratives. The BBC has a truly pathetic 'specialist disinformation reporter' called Marianna Spring who fits this bill perfectly. She is clueless about the world, how it works and what is really going on. Her role is to discredit anyone doing the job that a proper journalist would do and system-serving hacks like Spring wouldn't dare to do or even see the need to do. They are too busy licking the arse of authority which can never be wrong and, in the case of the BBC propaganda programme, *Panorama*, contacting payments systems such as PayPal to have a donations page taken down for a film company making documentaries questioning vaccines. Even the BBC soap opera *EastEnders* included a disgracefully biased scene in which an inarticulate white working class woman was made to look foolish for questioning the 'vaccine' while a well-spoken black man and Asian woman promoted the government narrative. It ticked every BBC box and the fact that the black and minority community was resisting the 'vaccine' had nothing to do with the way the scene was written. The BBC has become a disgusting tyrannical propaganda and censorship operation that should be defunded and disbanded and a free media take its place with a brief to stop censorship instead of demanding it. A BBC 'interview' with Gates goes something like: 'Mr Gates, sir, if I can call you sir, would you like to tell our audience why you are such a great man, a wonderful humanitarian philanthropist, and why you should absolutely be allowed as a software salesman to decide health policy for approaching eight billion people? Thank you, sir, please sir.' Propaganda programming has been incessant and merciless and when all you hear is the same story from the media, repeated by those around you who have only heard the same story, is it any wonder that people on a grand scale believe absolute mendacious garbage to be true? You are about to see, too, why this level of information control is necessary when the official 'Covid' narrative is so nonsensical and unsupportable by the evidence.

Structure of Deceit

The pyramid structure through which the 'Covid' hoax has been manifested is very simple and has to be to work. As few people as possible have to be involved with full knowledge of what they are doing – and why – or the real story would get out. At the top of the pyramid are the inner core of the Cult which controls Bill Gates who, in turn, controls the World Health Organization through his pivotal funding and his puppet Director-General mouthpiece, Tedros. Before he was appointed Tedros was chair of the Gates-founded Global Fund to 'fight against AIDS, tuberculosis and malaria', a board member of the Gates-funded 'vaccine alliance' GAVI, and on the board of another Gates-funded organisation. Gates owns him and picked him for a specific reason – Tedros is a crook and worse. 'Dr' Tedros (he's not a medical doctor, the first WHO chief not to be) was a member of the tyrannical Marxist government of Ethiopia for decades with all its human rights abuses. He has faced allegations of corruption and misappropriation of funds and was exposed three times for covering up cholera epidemics while Ethiopia's health minister. Tedros appointed the mass-murdering genocidal Zimbabwe dictator Robert Mugabe as a WHO goodwill ambassador for public health which, as with Tedros, is like appointing a psychopath to run a peace and love campaign. The move was so ridiculous that he had to drop Mugabe in the face of widespread condemnation. American economist David Steinman, a Nobel peace prize nominee, lodged a complaint with the International Criminal Court in The Hague over alleged genocide by Tedros when he was Ethiopia's foreign minister. Steinman says Tedros was a 'crucial decision maker' who directed the actions of Ethiopia's security forces from 2013 to 2015 and one of three officials in charge when those security services embarked on the 'killing' and 'torturing' of Ethiopians. You can see where Tedros is coming from and it's sobering to think that he has been the vehicle for Gates and the Cult to direct the global response to 'Covid'. Think about that. A psychopathic Cult dictates to psychopath Gates who dictates to psychopath Tedros who dictates how countries of the world must respond to a 'Covid virus' never scientifically shown to exist. At the same time psychopathic Cult-owned Silicon Valley information

giants like Google, YouTube, Facebook and Twitter announced very early on that they would give the Cult/Gates/Tedros/WHO version of the narrative free advertising and censor those who challenged their intelligence-insulting, mendacious story.

The next layer in the global 'medical' structure below the Cult, Gates and Tedros are the chief medical officers and science 'advisers' in each of the WHO member countries which means virtually all of them. Medical officers and arbiters of science (they're not) then take the WHO policy and recommended responses and impose them on their country's population while the political 'leaders' say they are deciding policy (they're clearly not) by 'following the science' on the advice of the 'experts' – the same medical officers and science 'advisers' (dictators). In this way with the rarest of exceptions the entire world followed the same policy of lockdown, people distancing, masks and 'vaccines' dictated by the psychopathic Cult, psychopathic Gates and psychopathic Tedros who we are supposed to believe give a damn about the health of the world population they are seeking to enslave. That, amazingly, is all there is to it in terms of crucial decision-making. Medical staff in each country then follow like sheep the dictates of the shepherds at the top of the national medical hierarchies – chief medical officers and science 'advisers' who themselves follow like sheep the shepherds of the World Health Organization and the Cult. Shepherds at the national level often have major funding and other connections to Gates and his Bill and Melinda Gates Foundation which carefully hands out money like confetti at a wedding to control the entire global medical system from the WHO down.

Follow the money

Christopher Whitty, Chief Medical Adviser to the UK Government at the centre of 'virus' policy, a senior adviser to the government's Scientific Advisory Group for Emergencies (SAGE), and Executive Board member of the World Health Organization, was gifted a grant of $40 million by the Bill and Melinda Gates Foundation for malaria research in Africa. The BBC described the unelected Whitty as 'the

official who will probably have the greatest impact on our everyday lives of any individual policymaker in modern times' and so it turned out. What Gates and Tedros have said Whitty has done like his equivalents around the world. Patrick Vallance, co-chair of SAGE and the government's Chief Scientific Adviser, is a former executive of Big Pharma giant GlaxoSmithKline with its fundamental financial and business connections to Bill Gates. In September, 2020, it was revealed that Vallance owned a deferred bonus of shares in GlaxoSmithKline worth £600,000 while the company was 'developing' a 'Covid vaccine'. Move along now – nothing to see here – what could possibly be wrong with that? Imperial College in London, a major player in 'Covid' policy in Britain and elsewhere with its 'Covid-19' Response Team, is funded by Gates and has big connections to China while the now infamous Professor Neil Ferguson, the useless 'computer modeller' at Imperial College is also funded by Gates. Ferguson delivered the dramatically inaccurate excuse for the first lockdowns (much more in the next chapter). The Institute for Health Metrics and Evaluation (IHME) in the United States, another source of outrageously false 'Covid' computer models to justify lockdowns, is bankrolled by Gates who is a vehement promotor of lockdowns. America's version of Whitty and Vallance, the again now infamous Anthony Fauci, has connections to 'Covid vaccine' maker Moderna as does Bill Gates through funding from the Bill and Melinda Gates Foundation. Fauci is director of the National Institute of Allergy and Infectious Diseases (NIAID), a major recipient of Gates money, and they are very close. Deborah Birx who was appointed White House Coronavirus Response Coordinator in February, 2020, is yet another with ties to Gates. Everywhere you look at the different elements around the world behind the coordination and decision making of the 'Covid' hoax there is Bill Gates and his money. They include the World Health Organization; Centers for Disease Control (CDC) in the United States; National Institutes of Health (NIH) of Anthony Fauci; Imperial College and Neil Ferguson; the London School of Hygiene where Chris Whitty worked; Regulatory agencies like the UK Medicines & Healthcare products Regulatory Agency (MHRA)

which gave emergency approval for 'Covid vaccines'; Wellcome Trust; GAVI, the Vaccine Alliance; the Coalition for Epidemic Preparedness Innovations (CEPI); Johns Hopkins University which has compiled the false 'Covid' figures; and the World Economic Forum. A Nationalfile.com article said:

> Gates has a lot of pull in the medical world, he has a multi-million dollar relationship with Dr. Fauci, and Fauci originally took the Gates line supporting vaccines and casting doubt on [the drug hydroxychloroquine]. Coronavirus response team member Dr. Deborah Birx, appointed by former president Obama to serve as United States Global AIDS Coordinator, also sits on the board of a group that has received billions from Gates' foundation, and Birx reportedly used a disputed Bill Gates-funded model for the White House's Coronavirus effort. Gates is a big proponent for a population lockdown scenario for the Coronavirus outbreak.

Another funder of Moderna is the Defense Advanced Research Projects Agency (DARPA), the technology-development arm of the Pentagon and one of the most sinister organisations on earth. DARPA had a major role with the CIA covert technology-funding operation In-Q-Tel in the development of Google and social media which is now at the centre of global censorship. Fauci and Gates are extremely close and openly admit to talking regularly about 'Covid' policy, but then why wouldn't Gates have a seat at every national 'Covid' table after his Foundation committed $1.75 billion to the 'fight against Covid-19'. When passed through our Orwellian Translation Unit this means that he has bought and paid for the Cult-driven 'Covid' response worldwide. Research the major 'Covid' response personnel in your own country and you will find the same Gates funding and other connections again and again. Medical and science chiefs following World Health Organization 'policy' sit atop a medical hierarchy in their country of administrators, doctors and nursing staff. These 'subordinates' are told they must work and behave in accordance with the policy delivered from the 'top' of the national 'health' pyramid which is largely the policy delivered by the WHO which is the policy delivered by Gates and the Cult. The whole 'Covid' narrative has been imposed on medical staff by a climate of fear although great numbers don't even need that to comply. They do so through breathtaking levels of ignorance and

include doctors who go through life simply repeating what Big Pharma and their hierarchical masters tell them to say and believe. No wonder Big Pharma 'medicine' is one of the biggest killers on Planet Earth.

The same top-down system of intimidation operates with regard to the Cult Big Pharma cartel which also dictates policy through national and global medical systems in this way. The Cult and Big Pharma agendas are the same because the former controls and owns the latter. 'Health' administrators, doctors, and nursing staff are told to support and parrot the dictated policy or they will face consequences which can include being fired. How sad it's been to see medical staff meekly repeating and imposing Cult policy without question and most of those who can see through the deceit are only willing to speak anonymously off the record. They know what will happen if their identity is known. This has left the courageous few to expose the lies about the 'virus', face masks, overwhelmed hospitals that aren't, and the dangers of the 'vaccine' that isn't a vaccine. When these medical professionals and scientists, some renowned in their field, have taken to the Internet to expose the truth their articles, comments and videos have been deleted by Cult-owned Facebook, Twitter and YouTube. What a real head-shaker to see YouTube videos with leading world scientists and highly qualified medical specialists with an added link underneath to the notorious Cult propaganda website *Wikipedia* to find the 'facts' about the same subject.

HIV – the 'Covid' trial-run

I'll give you an example of the consequences for health and truth that come from censorship and unquestioning belief in official narratives. The story was told by PCR inventor Kary Mullis in his book *Dancing Naked in the Mind Field*. He said that in 1984 he accepted as just another scientific fact that Luc Montagnier of France's Pasteur Institute and Robert Gallo of America's National Institutes of Health had independently discovered that a 'retrovirus' dubbed HIV (human immunodeficiency virus) caused AIDS. They

were, after all, Mullis writes, specialists in retroviruses. This is how the medical and science pyramids work. Something is announced or *assumed* and then becomes an everybody-knows-that purely through repetition of the assumption as if it is fact. Complete crap becomes accepted truth with no supporting evidence and only repetition of the crap. This is how a 'virus' that doesn't exist became the 'virus' that changed the world. The HIV-AIDS fairy story became a multi-billion pound industry and the media poured out propaganda terrifying the world about the deadly HIV 'virus' that caused the lethal AIDS. By then Mullis was working at a lab in Santa Monica, California, to detect retroviruses with his PCR test in blood donations received by the Red Cross. In doing so he asked a virologist where he could find a reference for HIV being the cause of AIDS. 'You don't need a reference,' the virologist said … *'Everybody knows it.'* Mullis said he wanted to quote a reference in the report he was doing and he said he felt a little funny about not knowing the source of such an important discovery when everyone else seemed to. The virologist suggested he cite a report by the Centers for Disease Control and Prevention (CDC) on morbidity and mortality. Mullis read the report, but it only said that an organism had been identified and did not say how. The report did not identify the original scientific work. Physicians, however, *assumed* (key recurring theme) that if the CDC was convinced that HIV caused AIDS then proof must exist. Mullis continues:

I did computer searches. Neither Montagnier, Gallo, nor anyone else had published papers describing experiments which led to the conclusion that HIV probably caused AIDS. I read the papers in Science for which they had become well known as AIDS doctors, but all they had said there was that they had found evidence of a past infection by something which was probably HIV in some AIDS patients.

They found antibodies. Antibodies to viruses had always been considered evidence of past disease, not present disease. Antibodies signaled that the virus had been defeated. The patient had saved himself. There was no indication in these papers that this virus caused a disease. They didn't show that everybody with the antibodies had the disease. In fact they found some healthy people with antibodies.

Mullis asked why their work had been published if Montagnier and Gallo hadn't really found this evidence, and why had they been fighting so hard to get credit for the discovery? He says he was hesitant to write 'HIV is the probable cause of AIDS' until he found published evidence to support that. 'Tens of thousands of scientists and researchers were spending billions of dollars a year doing research based on this idea,' Mullis writes. 'The reason had to be there somewhere; otherwise these people would not have allowed their research to settle into one narrow channel of investigation.' He said he lectured about PCR at numerous meetings where people were always talking about HIV and he asked them how they knew that HIV was the cause of AIDS:

> Everyone said something. Everyone had the answer at home, in the office, in some drawer. They all knew, and they would send me the papers as soon as they got back. But I never got any papers. Nobody ever sent me the news about how AIDS was caused by HIV.

Eventually Mullis was able to ask Montagnier himself about the reference proof when he lectured in San Diego at the grand opening of the University of California AIDS Research Center. Mullis says this was the last time he would ask his question without showing anger. Montagnier said he should reference the CDC report. 'I read it', Mullis said, and it didn't answer the question. 'If Montagnier didn't know the answer who the hell did?' Then one night Mullis was driving when an interview came on National Public Radio with Peter Duesberg, a prominent virologist at Berkeley and a California Scientist of the Year. Mullis says he finally understood why he could not find references that connected HIV to AIDS – *there weren't any!* No one had ever proved that HIV causes AIDS even though it had spawned a multi-billion pound global industry and the media was repeating this as fact every day in their articles and broadcasts terrifying the shit out of people about AIDS and giving the impression that a positive test for HIV (see 'Covid') was a death sentence. Duesberg was a threat to the AIDS gravy train and the agenda that underpinned it. He was therefore abused and castigated after he told the Proceedings of the National Academy of Sciences

there was no good evidence implicating the new 'virus'. Editors rejected his manuscripts and his research funds were deleted. Mullis points out that the CDC has defined AIDS as one of more than 30 diseases *if accompanied* by a positive result on a test that detects antibodies to HIV; but those same diseases are not defined as AIDS cases when antibodies are not detected:

> If an HIV-positive woman develops uterine cancer, for example, she is considered to have AIDS. If she is not HIV positive, she simply has uterine cancer. An HIV-positive man with tuberculosis has AIDS; if he tests negative he simply has tuberculosis. If he lives in Kenya or Colombia, where the test for HIV antibodies is too expensive, he is simply presumed to have the antibodies and therefore AIDS, and therefore he can be treated in the World Health Organization's clinic. It's the only medical help available in some places. And it's free, because the countries that support WHO are worried about AIDS.

Mullis accuses the CDC of continually adding new diseases (see ever more 'Covid symptoms') to the grand AIDS definition and of virtually doctoring the books to make it appear as if the disease continued to spread. He cites how in 1993 the CDC enormously broadened its AIDS definition and county health authorities were delighted because they received $2,500 per year from the Federal government for every reported AIDS case. Ladies and gentlemen, I have just described, via Kary Mullis, the 'Covid pandemic' of 2020 and beyond. Every element is the same and it's been pulled off in the same way by the same networks.

The 'Covid virus' exists? Okay – prove it. Er … still waiting

What Kary Mullis described with regard to 'HIV' has been repeated with 'Covid'. A claim is made that a new, or 'novel', infection has been found and the entire medical system of the world repeats that as fact exactly as they did with HIV and AIDS. No one in the mainstream asks rather relevant questions such as 'How do you know?' and 'Where is your proof?' The SARS-Cov-2 'virus' and the 'Covid-19 disease' became an overnight 'everybody-knows-that'. The origin could be debated and mulled over, but what you could not suggest was that 'SARS-Cov-2' didn't exist. That would be

ridiculous. 'Everybody knows' the 'virus' exists. Well, I didn't for one along with American proper doctors like Andrew Kaufman and Tom Cowan and long-time American proper journalist Jon Rappaport. We dared to pursue the obvious and simple question: 'Where's the evidence?' The overwhelming majority in medicine, journalism and the general public did not think to ask that. After all, *everyone knew* there was a new 'virus'. Everyone was saying so and I heard it on the BBC. Some would eventually argue that the 'deadly virus' was nothing like as deadly as claimed, but few would venture into the realms of its very existence. Had they done so they would have found that the evidence for that claim had gone AWOL as with HIV causes AIDS. In fact, not even that. For something to go AWOL it has to exist in the first place and scientific proof for a 'SARS-Cov-2' can be filed under nothing, nowhere and zilch.

Dr Andrew Kaufman is a board-certified forensic psychiatrist in New York State, a Doctor of Medicine and former Assistant Professor and Medical Director of Psychiatry at SUNY Upstate Medical University, and Medical Instructor of Hematology and Oncology at the Medical School of South Carolina. He also studied biology at the Massachusetts Institute of Technology (MIT) and trained in Psychiatry at Duke University. Kaufman is retired from allopathic medicine, but remains a consultant and educator on natural healing, I saw a video of his very early on in the 'Covid' hoax in which he questioned claims about the 'virus' in the absence of any supporting evidence and with plenty pointing the other way. I did everything I could to circulate his work which I felt was asking the pivotal questions that needed an answer. I can recommend an excellent pull-together interview he did with the website The Last Vagabond entitled *Dr Andrew Kaufman: Virus Isolation, Terrain Theory and Covid-19* and his website is andrewkaufmanmd.com. Kaufman is not only a forensic psychiatrist; he is forensic in all that he does. He always reads original scientific papers, experiments and studies instead of second-third-fourth-hand reports about the 'virus' in the media which are repeating the repeated repetition of the narrative. When he did so with the original Chinese 'virus' papers Kaufman

realised that there was no evidence of a 'SARS-Cov-2'. They had never – from the start – shown it to exist and every repeat of this claim worldwide was based on the accepted existence of proof that was nowhere to be found – see Kary Mullis and HIV. Here we go again.

Let's postulate

Kaufman discovered that the Chinese authorities immediately concluded that the cause of an illness that broke out among about 200 initial patients in Wuhan was a 'new virus' when there were no grounds to make that conclusion. The alleged 'virus' was not isolated from other genetic material in their samples and then shown through a system known as Koch's postulates to be the causative agent of the illness. The world was told that the SARS-Cov-2 'virus' caused a disease they called 'Covid-19' which had 'flu-like' symptoms and could lead to respiratory problems and pneumonia. If it wasn't so tragic it would almost be funny. *'Flu-like' symptoms'? Pneumonia? Respiratory disease?* What in *CHINA* and particularly in *Wuhan*, one of the most polluted cities in the world with a resulting epidemic of respiratory disease?? Three hundred thousand people get pneumonia in China every year and there are nearly a billion cases worldwide of 'flu-like symptoms'. These have a whole range of causes – including pollution in Wuhan – but no other possibility was credibly considered in late 2019 when the world was told there was a new and deadly 'virus'. The global prevalence of pneumonia and 'flu-like systems' gave the Cult networks unlimited potential to re-diagnose these other causes as the mythical 'Covid-19' and that is what they did from the very start. Kaufman revealed how Chinese medical and science authorities (all subordinates to the Cult-owned communist government) took genetic material from the lungs of only a few of the first patients. The material contained their own cells, bacteria, fungi and other microorganisms living in their bodies. The only way you could prove the existence of the 'virus' and its responsibility for the alleged 'Covid-19' was to isolate the virus from all the other material – a process also known as 'purification' – and

then follow the postulates sequence developed in the late 19th century by German physician and bacteriologist Robert Koch which became the 'gold standard' for connecting an alleged causation agent to a disease:

1. The microorganism (bacteria, fungus, virus, etc.) must be present in every case of the disease and all patients must have the same symptoms. It must also *not be present in healthy individuals*.

2. The microorganism must be isolated from the host with the disease. If the microorganism is a bacteria or fungus it must be grown in a pure culture. If it is a virus, it must be purified (i.e. containing no other material except the virus particles) from a clinical sample.

3. The specific disease, with all of its characteristics, must be reproduced when the infectious agent (the purified virus or a pure culture of bacteria or fungi) is inoculated into a healthy, susceptible host.

4. The microorganism must be recoverable from the experimentally infected host as in step 2.

Not one of these criteria has been met in the case of 'SARS-Cov-2' and 'Covid-19'. Not ONE. *EVER*. Robert Koch refers to bacteria and not viruses. What are called 'viral particles' are so minute (hence masks are useless by any definition) that they could only be seen after the invention of the electron microscope in the 1930s and can still only be observed through that means. American bacteriologist and virologist Thomas Milton Rivers, the so-called 'Father of Modern Virology' who was very significantly director of the Rockefeller Institute for Medical Research in the 1930s, developed a less stringent version of Koch's postulates to identify 'virus' causation known as 'Rivers criteria'. 'Covid' did not pass that process either. Some even doubt whether any 'virus' can be isolated from other particles containing genetic material in the Koch method. Freedom of Information requests in many countries asking for scientific proof that the 'Covid virus' has been purified and isolated and shown to exist have all come back with a 'we don't have that' and when this happened with a request to the UK Department of Health they added this comment:

However, outside of the scope of the [Freedom of Information Act] and on a discretionary basis, the following information has been advised to us, which may be of interest. Most infectious diseases are caused by viruses, bacteria or fungi. Some bacteria or fungi have the capacity to grow on their own in isolation, for example in colonies on a petri dish. Viruses are different in that they are what we call 'obligate pathogens' – that is, they cannot survive or reproduce without infecting a host ...

… For some diseases, it is possible to establish causation between a microorganism and a disease by isolating the pathogen from a patient, growing it in pure culture and reintroducing it to a healthy organism. These are known as 'Koch's postulates' and were developed in 1882. However, as our understanding of disease and different disease-causing agents has advanced, these are no longer the method for determining causation [Andrew Kaufman asks why in that case are there two published articles falsely claiming to satisfy Koch's postulates].

It has long been known that viral diseases cannot be identified in this way as viruses cannot be grown in 'pure culture'. When a patient is tested for a viral illness, this is normally done by looking for the presence of antigens, or viral genetic code in a host with molecular biology techniques [Kaufman asks how you could know the origin of these chemicals without having a pure culture for comparison].

For the record 'antigens' are defined so:

Invading microorganisms have antigens on their surface that the human body can recognise as being foreign – meaning not belonging to it. When the body recognises a foreign antigen, lymphocytes (white blood cells) produce antibodies, which are complementary in shape to the antigen.

Notwithstanding that this is open to question in relation to 'SARS-Cov-2' the presence of 'antibodies' can have many causes and they are found in people that are perfectly well. Kary Mullis said: 'Antibodies … had always been considered evidence of past disease, not present disease.'

'Covid' really is a *computer* 'virus'

Where the UK Department of Health statement says 'viruses' are now 'diagnosed' through a 'viral genetic code in a host with molecular biology techniques', they mean … *the PCR test* which its inventor said cannot test for infectious disease. They have no credible method of connecting a 'virus' to a disease and we will see that there is no scientific proof that any 'virus' causes any disease or there is any such thing as a 'virus' in the way that it is described. Tenacious Canadian researcher Christine Massey and her team made

some 40 Freedom of Information requests to national public health agencies in different countries asking for proof that SARS-CoV-2 has been isolated and not one of them could supply that information. Massey said of her request in Canada: 'Freedom of Information reveals Public Health Agency of Canada has no record of 'SARS-COV-2' isolation performed by anyone, anywhere, ever.' If you accept the comment from the UK Department of Health it's because they can't isolate a 'virus'. Even so many 'science' papers claimed to have isolated the 'Covid virus' until they were questioned and had to admit they hadn't. A reply from the Robert Koch Institute in Germany was typical: 'I am not aware of a paper which purified isolated SARS-CoV-2.' So what the hell was Christian Drosten and his gang using to design the 'Covid' testing protocol that has produced all the illusory Covid' cases and 'Covid' deaths when the head of the Chinese version of the CDC admitted there was a problem right from the start in that the 'virus' had never been isolated/purified? Breathe deeply: What they are calling 'Covid' is actually created by a *computer program* i.e. *they made it up* – er, that's it. They took lung fluid, with many sources of genetic material, from one single person alleged to be infected with Covid-19 by a PCR test which they *claimed*, without clear evidence, contained a 'virus'. They used several computer programs to create a model of a theoretical virus genome sequence from more than fifty-six million small sequences of RNA, each of an unknown source, assembling them like a puzzle with no known solution. The computer filled in the gaps with sequences from bits in the gene bank to make it look like a bat SARS-like coronavirus! A wave of the magic wand and poof, an in silico (computer-generated) genome, a scientific fantasy, was created. UK health researcher Dr Kevin Corbett made the same point with this analogy:

> … It's like giving you a few bones and saying that's your fish. It could be any fish. Not even a skeleton. Here's a few fragments of bones. That's your fish … It's all from gene bank and the bits of the virus sequence that weren't there they made up.
>
> They synthetically created them to fill in the blanks. That's what genetics is; it's a code. So it's ABBBCCDDD and you're missing some what you think is EEE so you put it in. It's all

synthetic. You just manufacture the bits that are missing. This is the end result of the geneticization of virology. This is basically a computer virus.

Further confirmation came in an email exchange between British citizen journalist Frances Leader and the government's Medicines & Healthcare Products Regulatory Agency (the Gates-funded MHRA) which gave emergency permission for untested 'Covid vaccines' to be used. The agency admitted that the 'vaccine' is not based on an isolated 'virus', but comes from a *computer-generated model*. Frances Leader was naturally banned from Cult-owned fascist Twitter for making this exchange public. The process of creating computer-generated alleged 'viruses' is called 'in silico' or 'in silicon' – computer chips – and the term 'in silico' is believed to originate with biological experiments using only a computer in 1989. 'Vaccines' involved with 'Covid' are also produced 'in silico' or by computer not a natural process. If the original 'virus' is nothing more than a made-up computer model how can there be 'new variants' of something that never existed in the first place? They are not new 'variants'; they are new *computer models* only minutely different to the original program and designed to further terrify the population into having the 'vaccine' and submitting to fascism. You want a 'new variant'? Click, click, enter – there you go. Tell the medical profession that you have discovered a 'South African variant', 'UK variants' or a 'Brazilian variant' and in the usual HIV-causes-AIDS manner they will unquestioningly repeat it with no evidence whatsoever to support these claims. They will go on television and warn about the dangers of 'new variants' while doing nothing more than repeating what they have been told to be true and knowing that any deviation from that would be career suicide. Big-time insiders will know it's a hoax, but much of the medical community is clueless about the way they are being played and themselves play the public without even being aware they are doing so. What an interesting 'coincidence' that AstraZeneca and Oxford University were conducting 'Covid vaccine trials' in the three countries – the UK, South Africa and Brazil – where the first three 'variants' were claimed to have 'broken out'.

Here's your 'virus' – it's a unicorn

Dr Andrew Kaufman presented a brilliant analysis describing how the 'virus' was imagined into fake existence when he dissected an article published by *Nature* and written by 19 authors detailing *alleged* 'sequencing of a complete viral genome' of the 'new SARS-CoV-2 virus'. This computer-modelled *in silico* genome was used as a template for all subsequent genome sequencing experiments that resulted in the so-called variants which he said now number more than *6,000*. The fake genome was constructed from more than 56 million individual short strands of RNA. Those little pieces were assembled into longer pieces by finding areas of overlapping sequences. The computer programs created over two million possible combinations from which the authors simply chose the longest one. They then compared this to a 'bat virus' and the computer 'alignment' rearranged the sequence and filled in the gaps! They called this computer-generated abomination the 'complete genome'. Dr Tom Cowan, a fellow medical author and collaborator with Kaufman, said such computer-generation constitutes scientific fraud and he makes this superb analogy:

> Here is an equivalency: A group of researchers claim to have found a unicorn because they found a piece of a hoof, a hair from a tail, and a snippet of a horn. They then add that information into a computer and program it to re-create the unicorn, and they then claim this computer re-creation is the real unicorn. Of course, they had never actually seen a unicorn so could not possibly have examined its genetic makeup to compare their samples with the actual unicorn's hair, hooves and horn.
>
> The researchers claim they decided which is the real genome of SARS-CoV-2 by 'consensus', sort of like a vote. Again, different computer programs will come up with different versions of the imaginary 'unicorn', so they come together as a group and decide which is the real imaginary unicorn.

This is how the 'virus' that has transformed the world was brought into fraudulent 'existence'. Extraordinary, yes, but as the Nazis said the bigger the lie the more will believe it. Cowan, however, wasn't finished and he went on to identify what he called the real blockbuster in the paper. He quotes this section from a paper written

by virologists and published by the CDC and then explains what it means:

Therefore, we examined the capacity of SARS-CoV-2 to infect and replicate in several common primate and human cell lines, including human adenocarcinoma cells (A549), human liver cells (HUH 7.0), and human embryonic kidney cells (HEK-293T). In addition to Vero E6 and Vero CCL81 cells. ... Each cell line was inoculated at high multiplicity of infection and examined 24h post-infection.

No CPE was observed in any of the cell lines except in Vero cells, which grew to greater than 10 to the 7th power at 24 h post-infection. In contrast, HUH 7.0 and 293T showed only modest viral replication, and A549 cells were incompatible with SARS CoV-2 infection.

Cowan explains that when virologists attempt to prove infection they have three possible 'hosts' or models on which they can test. The first was humans. Exposure to humans was generally not done for ethical reasons and has never been done with SARS-CoV-2 or any coronavirus. The second possible host was animals. Cowan said that forgetting for a moment that they never actually use purified virus when exposing animals they do use solutions that they *claim* contain the virus. Exposure to animals has been done with SARS-CoV-2 in an experiment involving mice and this is what they found: *None of the wild (normal) mice got sick*. In a group of genetically-modified mice, a statistically insignificant number lost weight and had slightly bristled fur, but they experienced nothing like the illness called 'Covid-19'. Cowan said the third method – the one they mostly rely on – is to inoculate solutions they *say* contain the virus onto a variety of tissue cultures. This process had never been shown to kill tissue *unless* the sample material was starved of nutrients and poisoned as *part of the process*. Yes, incredibly, in tissue experiments designed to show the 'virus' is responsible for killing the tissue they starve the tissue of nutrients and add toxic drugs including antibiotics and they do not have control studies to see if it's the starvation and poisoning that is degrading the tissue rather than the 'virus' they allege to be in there somewhere. You want me to pinch you? Yep, I understand. Tom Cowan said this about the whole nonsensical farce as he explains what that quote from the CDC paper really means:

The shocking thing about the above quote is that using their own methods, the virologists found that solutions containing SARS-CoV-2 – even in high amounts – were NOT, I repeat NOT, infective to any of the three human tissue cultures they tested. In plain English, this means they proved, on their terms, that this 'new coronavirus' is not infectious to human beings. It is ONLY infective to monkey kidney cells, and only then when you add two potent drugs (gentamicin and amphotericin), known to be toxic to kidneys, to the mix.

My friends, read this again and again. These virologists, published by the CDC, performed a clear proof, on their terms, showing that the SARS-CoV-2 virus is harmless to human beings. That is the only possible conclusion, but, unfortunately, this result is not even mentioned in their conclusion. They simply say they can provide virus stocks cultured only on monkey Vero cells, thanks for coming.

Cowan concluded: 'If people really understood how this "science" was done, I would hope they would storm the gates and demand honesty, transparency and truth.' Dr Michael Yeadon, former Vice President and Chief Scientific Adviser at drug giant Pfizer has been a vocal critic of the 'Covid vaccine' and its potential for multiple harm. He said in an interview in April, 2021, that 'not one [vaccine] has the virus. He was asked why vaccines normally using a 'dead' version of a disease to activate the immune system were not used for 'Covid' and instead we had the synthetic methods of the 'mRNA Covid vaccine'. Yeadon said that to do the former 'you'd have to have some of [the virus] wouldn't you?' He added: 'No-one's got any – seriously.' Yeadon said that surely they couldn't have fooled the whole world for a year without having a virus, 'but oddly enough ask around – no one's got it'. He didn't know why with all the 'great labs' around the world that the virus had not been isolated – 'Maybe they've been too busy running bad PCR tests and vaccines that people don't need.' What is today called 'science' is not 'science' at all. Science is no longer what is, but whatever people can be manipulated to *believe* that it is. Real science has been hijacked by the Cult to dispense and produce the 'expert scientists' and contentions that suit the agenda of the Cult. How big-time this has happened with the 'Covid' hoax which is entirely based on fake science delivered by fake 'scientists' and fake 'doctors'. The human-caused climate change hoax is also entirely based on fake science delivered by fake 'scientists' and fake 'climate experts'. In both cases real

scientists, climate experts and doctors have their views suppressed and deleted by the Cult-owned science establishment, media and Silicon Valley. This is the 'science' that politicians claim to be 'following' and a common denominator of 'Covid' and climate are Cult psychopaths Bill Gates and his mate Klaus Schwab at the Gates-funded World Economic Forum. But, don't worry, it's all just a coincidence and absolutely nothing to worry about. *Zzzzzzzz*.

What is a 'virus' REALLY?

Dr Tom Cowan is one of many contesting the very existence of viruses let alone that they cause disease. This is understandable when there is no scientific evidence for a disease-causing 'virus'. German virologist Dr Stefan Lanka won a landmark case in 2017 in the German Supreme Court over his contention that there is no such thing as a measles virus. He had offered a big prize for anyone who could prove there is and Lanka won his case when someone sought to claim the money. There is currently a prize of more than 225,000 euros on offer from an Isolate Truth Fund for anyone who can prove the isolation of SARS-CoV-2 and its genetic substance. Lanka wrote in an article headed 'The Misconception Called Virus' that scientists think a 'virus' is causing tissue to become diseased and degraded when in fact it is the *processes they are using* which do that – not a 'virus'. Lanka has done an important job in making this point clear as Cowan did in his analysis of the CDC paper. Lanka says that all claims about viruses as disease-causing pathogens are wrong and based on 'easily recognisable, understandable and verifiable misinterpretations.' Scientists believed they were working with 'viruses' in their laboratories when they were really working with 'typical particles of specific dying tissues or cells …' Lanka said that the tissue decaying process claimed to be caused by a 'virus' still happens when no alleged 'virus' is involved. It's the *process* that does the damage and not a 'virus'. The genetic sample is deprived of nutrients, removed from its energy supply through removal from the body and then doused in toxic antibiotics to remove any bacteria. He confirms again that establishment scientists do not (pinch me)

conduct control experiments to see if this is the case and if they did they would see the claims that 'viruses' are doing the damage is nonsense. He adds that during the measles 'virus' court case he commissioned an independent laboratory to perform just such a control experiment and the result was that the tissues and cells died in the exact same way as with alleged 'infected' material. This is supported by a gathering number of scientists, doctors and researchers who reject what is called 'germ theory' or the belief in the body being infected by contagious sources emitted by other people. Researchers Dawn Lester and David Parker take the same stance in their highly-detailed and sourced book *What Really Makes You Ill – Why everything you thought you knew about disease is wrong* which was recommended to me by a number of medical professionals genuinely seeking the truth. Lester and Parker say there is no provable scientific evidence to show that a 'virus' can be transmitted between people or people and animals or animals and people:

> The definition also claims that viruses are the cause of many diseases, as if this has been definitively proven. But this is not the case; there is no original scientific evidence that definitively demonstrates that any virus is the cause of any disease. The burden of proof for any theory lies with those who proposed it; but none of the existing documents provides 'proof' that supports the claim that 'viruses' are pathogens.

Dr Tom Cowan employs one of his clever analogies to describe the process by which a 'virus' is named as the culprit for a disease when what is called a 'virus' is only material released by cells detoxing themselves from infiltration by chemical or radiation poisoning. The tidal wave of technologically-generated radiation in the 'smart' modern world plus all the toxic food and drink are causing this to happen more than ever. Deluded 'scientists' misread this as a gathering impact of what they wrongly label 'viruses'.

Paper can infect houses

Cowan said in an article for davidicke.com – with his tongue only mildly in his cheek – that he believed he had made a tremendous

discovery that may revolutionise science. He had discovered that small bits of paper are alive, 'well alive-ish', can 'infect' houses, and then reproduce themselves inside the house. The result was that this explosion of growth in the paper inside the house causes the house to explode, blowing it to smithereens. His evidence for this new theory is that in the past months he had carefully examined many of the houses in his neighbourhood and found almost no scraps of paper on the lawns and surrounds of the house. There was an occasional stray label, but nothing more. Then he would return to these same houses a week or so later and with a few, not all of them, particularly the old and decrepit ones, he found to his shock and surprise they were littered with stray bits of paper. He knew then that the paper had infected these houses, made copies of itself, and blew up the house. A young boy on a bicycle at one of the sites told him he had seen a demolition crew using dynamite to explode the house the previous week, but Cowan dismissed this as the idle thoughts of silly boys because 'I was on to something big'. He was on to how 'scientists' mistake genetic material in the detoxifying process for something they call a 'virus'. Cowan said of his house and paper story:

> If this sounds crazy to you, it's because it should. This scenario is obviously nuts. But consider this admittedly embellished, for effect, current viral theory that all scientists, medical doctors and virologists currently believe.

He takes the example of the 'novel SARS-Cov2' virus to prove the point. First they take someone with an undefined illness called 'Covid-19' and don't even attempt to find any virus in their sputum. Never mind the scientists still describe how this 'virus', which they have not located attaches to a cell receptor, injects its genetic material, in 'Covid's' case, RNA, into the cell. The RNA once inserted exploits the cell to reproduce itself and makes 'thousands, nay millions, of copies of itself … Then it emerges victorious to claim its next victim':

If you were to look in the scientific literature for proof, actual scientific proof, that uniform SARS-CoV2 viruses have been properly isolated from the sputum of a sick person, that actual spike proteins could be seen protruding from the virus (which has not been found), you would find that such evidence doesn't exist.

If you go looking in the published scientific literature for actual pictures, proof, that these spike proteins or any viral proteins are ever attached to any receptor embedded in any cell membrane, you would also find that no such evidence exists. If you were to look for a video or documented evidence of the intact virus injecting its genetic material into the body of the cell, reproducing itself and then emerging victorious by budding off the cell membrane, you would find that no such evidence exists.

The closest thing you would find is electron micrograph pictures of cellular particles, possibly attached to cell debris, both of which to be seen were stained by heavy metals, a process that completely distorts their architecture within the living organism. This is like finding bits of paper stuck to the blown-up bricks, thereby proving the paper emerged by taking pieces of the bricks on its way out.

The Enders baloney

Cowan describes the 'Covid' story as being just as make-believe as his paper story and he charts back this fantasy to a Nobel Prize winner called John Enders (1897-1985), an American biomedical scientist who has been dubbed 'The Father of Modern Vaccines'. Enders is claimed to have 'discovered' the process of the viral culture which 'proved' that a 'virus' caused measles. Cowan explains how Enders did this 'by using the EXACT same procedure that has been followed by every virologist to find and characterize every new virus since 1954'. Enders took throat swabs from children with measles and immersed them in 2ml of milk. Penicillin (100u/ml) and the antibiotic streptomycin (50,g/ml) were added and the whole mix was centrifuged – rotated at high speed to separate large cellular debris from small particles and molecules as with milk and cream, for example. Cowan says that if the aim is to find little particles of genetic material ('viruses') in the snot from children with measles it would seem that the last thing you would do is mix the snot with other material – milk –that also has genetic material. 'How are you ever going to know whether whatever you found came from the snot or the milk?' He points out that streptomycin is a 'nephrotoxic' or poisonous-to-the-kidney drug. You will see the relevance of that

shortly. Cowan says that it gets worse, much worse, when Enders describes the culture medium upon which the virus 'grows': 'The culture medium consisted of bovine amniotic fluid (90%), beef embryo extract (5%), horse serum (5%), antibiotics and phenol red as an indicator of cell metabolism.' Cowan asks incredulously: 'Did he just say that the culture medium also contained fluids and tissues that are themselves rich sources of genetic material?' The genetic cocktail, or 'medium', is inoculated onto tissue and cells from rhesus monkey *kidney* tissue. This is where the importance of streptomycin comes in and currently-used antimicrobials and other drugs that are *poisonous to kidneys* and used in ALL modern viral cultures (e.g. gentamicin, streptomycin, and amphotericin). Cowan asks: 'How are you ever going to know from this witch's brew where any genetic material comes from as we now have five different sources of rich genetic material in our mix?' Remember, he says, that all genetic material, whether from monkey kidney tissues, bovine serum, milk, etc., is made from the exact same components. The same central question returns: 'How are you possibly going to know that it was the virus that killed the kidney tissue and not the toxic antibiotic and starvation rations on which you are growing the tissue?' John Enders answered the question himself – *you can't*:

A second agent was obtained from an uninoculated culture of monkey kidney cells. The cytopathic changes [death of the cells] it induced in the unstained preparations could not be distinguished with confidence from the viruses isolated from measles.

The death of the cells ('cytopathic changes') happened in exactly the same manner, whether they inoculated the kidney tissue with the measles snot or not, Cowan says. 'This is evidence that the destruction of the tissue, the very proof of viral causation of illness, was not caused by anything in the snot because they saw the same destructive effect when the snot was not even used … the cytopathic, i.e., cell-killing, changes come from the process of the culture itself, not from any virus in any snot, period.' Enders quotes in his 1957 paper a virologist called Ruckle as reporting similar findings 'and in addition has isolated an agent from monkey kidney tissue that is so

far indistinguishable from human measles virus'. In other words, Cowan says, these particles called 'measles viruses' are simply and clearly breakdown products of the starved and poisoned tissue. For measles 'virus' see all 'viruses' including the so-called 'Covid virus'. Enders, the 'Father of Modern Vaccines', also said:

There is a potential risk in employing cultures of primate cells for the production of vaccines composed of attenuated virus, since the presence of other agents possibly latent in primate tissues cannot be definitely excluded by any known method.

Cowan further quotes from a paper published in the journal *Viruses* in May, 2020, while the 'Covid pandemic' was well underway in the media if not in reality. 'EVs' here refers to particles of genetic debris from our own tissues, such as exosomes of which more in a moment: 'The remarkable resemblance between EVs and viruses has caused quite a few problems in the studies focused on the analysis of EVs released during viral infections.' Later the paper adds that to date a reliable method that can actually guarantee a complete separation (of EVs from viruses) DOES NOT EXIST. This was published at a time when a fairy tale 'virus' was claimed in total certainty to be causing a fairy tale 'viral disease' called 'Covid-19' – a fairy tale that was already well on the way to transforming human society in the image that the Cult has worked to achieve for so long. Cowan concludes his article:

To summarize, there is no scientific evidence that pathogenic viruses exist. What we think of as 'viruses' are simply the normal breakdown products of dead and dying tissues and cells. When we are well, we make fewer of these particles; when we are starved, poisoned, suffocated by wearing masks, or afraid, we make more.

There is no engineered virus circulating and making people sick. People in laboratories all over the world are making genetically modified products to make people sick. These are called vaccines. There is no virome, no 'ecosystem' of viruses, viruses are not 8%, 50% or 100 % of our genetic material. These are all simply erroneous ideas based on the misconception called a virus.

What is 'Covid'? Load of bollocks

The background described here by Cowan and Lanka was emphasised in the first video presentation that I saw by Dr Andrew Kaufman when he asked whether the 'Covid virus' was in truth a natural defence mechanism of the body called 'exosomes'. These are released by cells when in states of toxicity – see the same themes returning over and over. They are released ever more profusely as chemical and radiation toxicity increases and think of the potential effect therefore of 5G alone as its destructive frequencies infest the human energetic information field with a gathering pace (5G went online in Wuhan in 2019 as the 'virus' emerged). I'll have more about this later. Exosomes transmit a warning to the rest of the body that 'Houston, we have a problem'. Kaufman presented images of exosomes and compared them with 'Covid' under an electron microscope and the similarity was remarkable. They both attach to the same cell receptors (*claimed* in the case of 'Covid'), contain the same genetic material in the form of RNA or ribonucleic acid, and both are found in 'viral cell cultures' with damaged or dying cells. James Hildreth MD, President and Chief Executive Officer of the Meharry Medical College at Johns Hopkins, said: 'The virus is fully an exosome in every sense of the word.' Kaufman's conclusion was that there is no 'virus': 'This entire pandemic is a completely manufactured crisis … there is no evidence of anyone dying from [this] illness.' Dr Tom Cowan and Sally Fallon Morell, authors of *The Contagion Myth*, published a statement with Dr Kaufman in February, 2021, explaining why the 'virus' does not exist and you can read it that in full in the Appendix.

'Virus' theory can be traced to the 'cell theory' in 1858 of German physician Rudolf Virchow (1821-1920) who contended that disease originates from a single cell infiltrated by a 'virus'. Dr Stefan Lanka said that findings and insights with respect to the structure, function and central importance of tissues in the creation of life, which were already known in 1858, comprehensively refute the cell theory. Virchow ignored them. We have seen the part later played by John Enders in the 1950s and Lanka notes that infection theories were only established as a global dogma through the policies and

eugenics of the Third Reich in Nazi Germany (creation of the same Sabbatian cult behind the 'Covid' hoax). Lanka said: 'Before 1933, scientists dared to contradict this theory; after 1933, these critical scientists were silenced'. Dr Tom Cowan's view is that ill-heath is caused by too much of something, too little of something, or toxification from chemicals and radiation – not contagion. We must also highlight as a major source of the 'virus' theology a man still called the 'Father of Modern Virology' – Thomas Milton Rivers (1888-1962). There is no way given the Cult's long game policy that it was a coincidence for the 'Father of Modern Virology' to be director of the Rockefeller Institute for Medical Research from 1937 to 1956 when he is credited with making the Rockefeller Institute a leader in 'viral research'. Cult Rockefellers were the force behind the creation of Big Pharma 'medicine', established the World Health Organisation in 1948, and have long and close associations with the Gates family that now runs the WHO during the pandemic hoax through mega-rich Cult gofer and psychopath Bill Gates.

Only a Renegade Mind can see through all this bullshit by asking the questions that need to be answered, not taking 'no' or prevarication for an answer, and certainly not hiding from the truth in fear of speaking it. Renegade Minds have always changed the world for the better and they will change this one no matter how bleak it may currently appear to be.

CHAPTER SIX

Sequence of deceit

If you tell the truth, you don't have to remember anything
Mark Twain

Against the background that I have laid out this far the sequence that took us from an invented 'virus' in Cult-owned China in late 2019 to the fascist transformation of human society can be seen and understood in a whole new context.

We were told that a deadly disease had broken out in Wuhan and the world media began its campaign (coordinated by behavioural psychologists as we shall see) to terrify the population into unquestioning compliance. We were shown images of Chinese people collapsing in the street which never happened in the West with what was supposed to be the same condition. In the earliest days when alleged cases and deaths were few the fear register was hysterical in many areas of the media and this would expand into the common media narrative across the world. The real story was rather different, but we were never told that. The Chinese government, one of the Cult's biggest centres of global operation, said they had discovered a new illness with flu-like and pneumonia-type symptoms in a city with such toxic air that it is overwhelmed with flu-like symptoms, pneumonia and respiratory disease. Chinese scientists said it was a new – 'novel' – coronavirus which they called Sars-Cov-2 and that it caused a disease they labelled 'Covid-19'. There was no evidence for this and the 'virus' has never to this day been isolated, purified and its genetic code established from that. It

was from the beginning a computer-generated fiction. Stories of Chinese whistleblowers saying the number of deaths was being supressed or that the 'new disease' was related to the Wuhan bio-lab misdirected mainstream and alternative media into cul-de-sacs to obscure the real truth – there was no 'virus'.

Chinese scientists took genetic material from the lung fluid of just a few people and said they had found a 'new' disease when this material had a wide range of content. There was no evidence for a 'virus' for the very reasons explained in the last two chapters. The 'virus' has never been shown to (a) exist and (b) cause any disease. People were diagnosed on symptoms that are so widespread in Wuhan and polluted China and with a PCR test that can't detect infectious disease. On this farce the whole global scam was sold to the rest of the world which would also diagnose respiratory disease as 'Covid-19' from symptoms alone or with a PCR test not testing for a 'virus'. Flu miraculously disappeared *worldwide* in 2020 and into 2021 as it was redesignated 'Covid-19'. It was really the same old flu with its 'flu-like' symptoms attributed to 'flu-like' 'Covid-19'. At the same time with very few exceptions the Chinese response of draconian lockdown and fascism was the chosen weapon to respond across the West as recommended by the Cult-owned Tedros at the Cult-owned World Health Organization run by the Cult-owned Gates. All was going according to plan. Chinese scientists – everything in China is controlled by the Cult-owned government – compared their contaminated RNA lung-fluid material with other RNA sequences and said it appeared to be just under 80 percent identical to the SARS-CoV-1 'virus' claimed to be the cause of the SARS (severe acute respiratory syndrome) 'outbreak' in 2003. They decreed that because of this the 'new virus' had to be related and they called it SARS-CoV-2. There are some serious problems with this assumption and *assumption* was all it was. Most 'factual' science turns out to be assumptions repeated into everyone-knows-that. A match of under 80-percent is meaningless. Dr Kaufman makes the point that there's a *96 percent* genetic correlation between humans and chimpanzees, but 'no one would say our genetic material is part

of the chimpanzee family'. Yet the Chinese authorities were claiming that a much lower percentage, less than 80 percent, proved the existence of a new 'coronavirus'. For goodness sake human DNA is 60 percent similar to a *banana*.

You are feeling sleepy

The entire 'Covid' hoax is a global Psyop, a psychological operation to program the human mind into believing and fearing a complete fantasy. A crucial aspect of this was what *appeared* to happen in Italy. It was all very well streaming out daily images of an alleged catastrophe in Wuhan, but to the Western mind it was still on the other side of the world in a very different culture and setting. A reaction of 'this could happen to me and my family' was still nothing like as intense enough for the mind-doctors. The Cult needed a Western example to push people over that edge and it chose Italy, one of its major global locations going back to the Roman Empire. An Italian 'Covid' crisis was manufactured in a particular area called Lombardy which just happens to be notorious for its toxic air and therefore respiratory disease. Wuhan, China, déjà vu. An hysterical media told horror stories of Italians dying from 'Covid' in their droves and how Lombardy hospitals were being overrun by a tidal wave of desperately ill people needing treatment after being struck down by the 'deadly virus'. Here was the psychological turning point the Cult had planned. Wow, if this is happening in Italy, the Western mind concluded, this indeed could happen to me and my family. Another point is that Italian authorities responded by following the Chinese blueprint so vehemently recommended by the Cult-owned World Health Organization. They imposed fascistic lockdowns on the whole country viciously policed with the help of surveillance drones sweeping through the streets seeking out anyone who escaped from mass house arrest. Livelihoods were destroyed and psychology unravelled in the way we have witnessed since in all lockdown countries. Crucial to the plan was that Italy responded in this way to set the precedent of suspending freedom and imposing fascism in a 'Western liberal democracy'. I emphasised in an

animated video explanation on davidicke.com posted in the summer of 2020 how important it was to the Cult to expand the Chinese lockdown model across the West. Without this, and the bare-faced lie that non-symptomatic people could still transmit a 'disease' they didn't have, there was no way locking down the whole population, sick and not sick, could be pulled off. At just the right time and with no evidence Cult operatives and gofers claimed that people without symptoms could pass on the 'disease'. In the name of protecting the 'vulnerable' like elderly people, who lockdowns would kill by the tens of thousands, we had for the first time healthy people told to isolate as well as the sick. The great majority of people who tested positive had no symptoms because there was nothing wrong with them. It was just a trick made possible by a test not testing for the 'virus'.

Months after my animated video the Gates-funded Professor Neil Ferguson at the Gates-funded Imperial College confirmed that I was right. He didn't say it in those terms, naturally, but he did say it. Ferguson will enter the story shortly for his outrageously crazy 'computer models' that led to Britain, the United States and many other countries following the Chinese and now Italian methods of response. Put another way, following the Cult script. Ferguson said that SAGE, the UK government's scientific advisory group which has controlled 'Covid' policy from the start, wanted to follow the Chinese lockdown model (while they all continued to work and be paid), but they wondered if they could possibly, in Ferguson's words, 'get away with it in Europe'. 'Get away with it'? Who the hell do these moronic, arrogant people think they are? This appalling man Ferguson said that once Italy went into national lockdown they realised they, too, could mimic China:

> It's a communist one-party state, we said. We couldn't get away with it in Europe, we thought … and then Italy did it. And we realised we could. Behind this garbage from Ferguson is a simple fact: Doing the same as China in every country was the plan from the start and Ferguson's 'models' would play a central role in achieving that. It's just a coincidence, of course, and absolutely nothing to worry your little head about.

Oops, sorry, our mistake

Once the Italian segment of the Psyop had done the job it was designed to do a very different story emerged. Italian authorities revealed that 99 percent of those who had 'died from Covid-19' in Italy had one, two, three, or more 'co-morbidities' or illnesses and health problems that could have ended their life. The US Centers for Disease Control and Prevention (CDC) published a figure of 94 percent for Americans dying of 'Covid' while having other serious medical conditions – on average two to three (some five or six) other potential causes of death. In terms of death from an unproven 'virus' I say it is 100 percent. The other one percent in Italy and six percent in the US would presumably have died from 'Covid's' flu-like symptoms with a range of other possible causes in conjunction with a test not testing for the 'virus'. Fox News reported that even more startling figures had emerged in one US county in which 410 of 422 deaths attributed to 'Covid-19' had other potentially deadly health conditions. The Italian National Health Institute said later that the average age of people dying with a 'Covid-19' diagnosis in Italy was about 81. Ninety percent were over 70 with ten percent over 90. In terms of other reasons to die some 80 percent had two or more chronic diseases with half having three or more including cardiovascular problems, diabetes, respiratory problems and cancer. Why is the phantom 'Covid-19' said to kill overwhelmingly old people and hardly affect the young? Old people continually die of many causes and especially respiratory disease which you can re-diagnose 'Covid-19' while young people die in tiny numbers by comparison and rarely of respiratory disease. Old people 'die of Covid' because they die of other things that can be redesignated 'Covid' and it really is that simple.

Flu has flown

The blueprint was in place. Get your illusory 'cases' from a test not testing for the 'virus' and redesignate other causes of death as 'Covid-19'. You have an instant 'pandemic' from something that is nothing more than a computer-generated fiction. With near-on a

billion people having 'flu-like' symptoms every year the potential was limitless and we can see why flu quickly and apparently miraculously disappeared *worldwide* by being diagnosed 'Covid-19'. The painfully bloody obvious was explained away by the childlike media in headlines like this in the UK '*Independent*': 'Not a single case of flu detected by Public Health England this year as Covid restrictions suppress virus'. I kid you not. The masking, social distancing and house arrest that did not make the 'Covid virus' disappear somehow did so with the 'flu virus'. Even worse the article, by a bloke called Samuel Lovett, suggested that maybe the masking, sanitising and other 'Covid' measures should continue to keep the flu away. With a ridiculousness that disturbs your breathing (it's 'Covid-19') the said Lovett wrote: 'With widespread social distancing and mask-wearing measures in place throughout the UK, the usual routes of transmission for influenza have been blocked.' He had absolutely no evidence to support that statement, but look at the consequences of him acknowledging the obvious. With flu not disappearing at all and only being relabelled 'Covid-19' he would have to contemplate that 'Covid' was a hoax on a scale that is hard to imagine. You need guts and commitment to truth to even go there and that's clearly something Samuel Lovett does not have in abundance. He would never have got it through the editors anyway.

Tens of thousands die in the United States alone every winter from flu including many with pneumonia complications. CDC figures record *45 million* Americans diagnosed with flu in 2017-2018 of which 61,000 died and some reports claim 80,000. Where was the same hysteria then that we have seen with 'Covid-19'? Some 250,000 Americans are admitted to hospital with pneumonia every year with about 50,000 cases proving fatal. About 65 million suffer respiratory disease every year and three million deaths makes this the third biggest cause of death worldwide. You only have to redesignate a portion of all these people 'Covid-19' and you have an instant global pandemic or the *appearance* of one. Why would doctors do this? They are told to do this and all but a few dare not refuse those who must be obeyed. Doctors in general are not researching their own

knowledge and instead take it direct and unquestioned from the authorities that own them and their careers. The authorities say they must now diagnose these symptoms 'Covid-19' and not flu, or whatever, and they do it. Dark suits say put 'Covid-19' on death certificates no matter what the cause of death and the doctors do it. Renegade Minds don't fall for the illusion that doctors and medical staff are all highly-intelligent, highly-principled, seekers of medical truth. *Some are*, but not the majority. They are repeaters, gofers, and yes sir, no sir, purveyors of what the system demands they purvey. The 'Covid' con is not merely confined to diseases of the lungs. Instructions to doctors to put 'Covid-19' on death certificates for anyone dying of *anything* within 28 days (or much more) of a positive test not testing for the 'virus' opened the floodgates. The term dying *with* 'Covid' and not *of* 'Covid' was coined to cover the truth. Whether it was a *with* or an *of* they were all added to the death numbers attributed to the 'deadly virus' compiled by national governments and globally by the Gates-funded Johns Hopkins operation in the United States that was so involved in those 'pandemic' simulations. Fraudulent deaths were added to the ever-growing list of fraudulent 'cases' from false positives from a false test. No wonder Professor Walter Ricciardi, scientific advisor to the Italian minister of health, said after the Lombardy hysteria had done its job that 'Covid' death rates were due to Italy having the second oldest population in the world and to *how hospitals record deaths*:

The way in which we code deaths in our country is very generous in the sense that all the people who die in hospitals with the coronavirus are deemed to be dying of the coronavirus. On re-evaluation by the National Institute of Health, only 12 per cent of death certificates have shown a direct causality from coronavirus, while 88 per cent of patients who have died have at least one pre-morbidity – many had two or three.

This is extraordinary enough when you consider the propaganda campaign to use Italy to terrify the world, but how can they even say twelve percent were genuine when the 'virus' has not been shown to exist, its 'code' is a computer program, and diagnosis comes from a test not testing for it? As in China, and soon the world, 'Covid-19' in

Italy was a redesignation of diagnosis. Lies and corruption were to become the real 'pandemic' fuelled by a pathetically-compliant medical system taking its orders from the tiny few at the top of their national hierarchy who answered to the World Health Organization which answers to Gates and the Cult. Doctors were told – ordered – to diagnose a particular set of symptoms 'Covid-19' and put that on the death certificate for any cause of death if the patient had tested positive with a test not testing for the virus or had 'Covid' symptoms like the flu. The United States even introduced big financial incentives to manipulate the figures with hospitals receiving £4,600 from the Medicare system for diagnosing someone with regular pneumonia, $13,000 if they made the diagnosis from the same symptoms 'Covid-19' pneumonia, and $39, 000 if they put a 'Covid' diagnosed patient on a ventilator that would almost certainly kill them. A few – painfully and pathetically few – medical whistleblowers revealed (before Cult-owned YouTube deleted their videos) that they had been instructed to 'let the patient crash' and put them straight on a ventilator instead of going through a series of far less intrusive and dangerous methods as they would have done before the pandemic hoax began and the financial incentives kicked in. We are talking cold-blooded murder given that ventilators are so damaging to respiratory systems they are usually the last step before heaven awaits. Renegade Minds never fall for the belief that people in white coats are all angels of mercy and cannot be full-on psychopaths. I have explained in detail in *The Answer* how what I am describing here played out across the world coordinated by the World Health Organization through the medical hierarchies in almost every country.

Medical scientist calls it

Information about the non-existence of the 'virus' began to emerge for me in late March, 2020, and mushroomed after that. I was sent an email by Sir Julian Rose, a writer, researcher, and organic farming promotor, from a medical scientist friend of his in the United States. Even at that early stage in March the scientist was able to explain

how the 'Covid' hoax was being manipulated. He said there were no reliable tests for a specific 'Covid-19 virus' and nor were there any reliable agencies or media outlets for reporting numbers of actual 'Covid-19' cases. We have seen in the long period since then that he was absolutely right. 'Every action and reaction to Covid-19 is based on totally flawed data and we simply cannot make accurate assessments,' he said. Most people diagnosed with 'Covid-19' were showing nothing more than cold and flu-like symptoms 'because most coronavirus strains *are* nothing more than cold/flu-like symptoms'. We had farcical situations like an 84-year-old German man testing positive for 'Covid-19' and his nursing home ordered to quarantine only for him to be found to have a common cold. The scientist described back then why PCR tests and what he called the 'Mickey Mouse test kits' were useless for what they were claimed to be identifying. 'The idea these kits can isolate a specific virus like Covid-19 is nonsense,' he said. Significantly, he pointed out that 'if you want to create a totally false panic about a totally false pandemic – pick a coronavirus'. This is exactly what the Cult-owned Gates, World Economic Forum and Johns Hopkins University did with their Event 201 'simulation' followed by their real-life simulation called the 'pandemic'. The scientist said that all you had to do was select the sickest of people with respiratory-type diseases in a single location – 'say Wuhan' – and administer PCR tests to them. You can then claim that anyone showing 'viral sequences' similar to a coronavirus 'which will inevitably be quite a few' is suffering from a 'new' disease:

> Since you already selected the sickest flu cases a fairly high proportion of your sample will go on to die. You can then say this 'new' virus has a CFR [case fatality rate] higher than the flu and use this to infuse more concern and do more tests which will of course produce more 'cases', which expands the testing, which produces yet more 'cases' and so on and so on. Before long you have your 'pandemic', and all you have done is use a simple test kit trick to convert the worst flu and pneumonia cases into something new that doesn't ACTUALLY EXIST [my emphasis].

He said that you then 'just run the same scam in other countries' and make sure to keep the fear message running high 'so that people

will feel panicky and less able to think critically'. The only problem to overcome was the fact *there is no* actual new deadly pathogen and only regular sick people. This meant that deaths from the 'new deadly pathogen' were going to be way too low for a real new deadly virus pandemic, but he said this could be overcome in the following ways – all of which would go on to happen:

1. You can claim this is just the beginning and more deaths are imminent [you underpin this with fantasy 'computer projections']. Use this as an excuse to quarantine everyone and then claim the quarantine prevented the expected millions of dead.

2. You can [say that people] 'minimizing' the dangers are irresponsible and bully them into not talking about numbers.

3. You can talk crap about made up numbers hoping to blind people with pseudoscience.

4. You can start testing well people (who, of course, will also likely have shreds of coronavirus [RNA] in them) and thus inflate your 'case figures' with 'asymptomatic carriers' (you will of course have to spin that to sound deadly even though any virologist knows the more symptom-less cases you have the less deadly is your pathogen).

The scientist said that if you take these simple steps 'you can have your own entirely manufactured pandemic up and running in weeks'. His analysis made so early in the hoax was brilliantly prophetic of what would actually unfold. Pulling all the information together in these recent chapters we have this is simple 1, 2, 3, of how you can delude virtually the entire human population into believing in a 'virus' that doesn't exist:

- A 'Covid case' is someone who tests positive with a test not testing for the 'virus'.

- A 'Covid death' is someone who dies of *any cause* within 28 days (or much longer) of testing positive with a test not testing for the 'virus.

- Asymptomatic means there is nothing wrong with you, but they claim you can pass on what you don't have to justify locking

down (quarantining) healthy people in totality.

The foundations of the hoax are that simple. A study involving ten million people in Wuhan, published in November, 2020, demolished the whole lie about those without symptoms passing on the 'virus'. They found '300 asymptomatic cases' and traced their contacts to find that not one of them was detected with the 'virus'. 'Asymptomatic' patients and their contacts were isolated for no less than two weeks and nothing changed. I know it's all crap, but if you are going to claim that those without symptoms can transmit 'the virus' then you must produce evidence for that and they never have. Even World Health Organization official Dr Maria Van Kerkhove, head of the emerging diseases and zoonosis unit, said as early as June, 2020, that she doubted the validity of asymptomatic transmission. She said that 'from the data we have, it still seems to be rare that an asymptomatic person actually transmits onward to a secondary individual' and by 'rare' she meant that she couldn't cite any case of asymptomatic transmission.

The Ferguson factor

The problem for the Cult as it headed into March, 2020, when the script had lockdown due to start, was that despite all the manipulation of the case and death figures they still did not have enough people alleged to have died from 'Covid' to justify mass house arrest. This was overcome in the way the scientist described: 'You can claim this is just the beginning and more deaths are imminent … Use this as an excuse to quarantine everyone and then claim the quarantine prevented the expected millions of dead.' Enter one Professor Neil Ferguson, the Gates-funded 'epidemiologist' at the Gates-funded Imperial College in London. Ferguson is Britain's Christian Drosten in that he has a dire record of predicting health outcomes, but is still called upon to advise government on the next health outcome when another 'crisis' comes along. This may seem to be a strange and ridiculous thing to do. Why would you keep turning for policy guidance to people who have a history of being

monumentally wrong? Ah, but it makes sense from the Cult point of view. These 'experts' keep on producing predictions that suit the Cult agenda for societal transformation and so it was with Neil Ferguson as he revealed his horrific (and clearly insane) computer model predictions that allowed lockdowns to be imposed in Britain, the United States and many other countries. Ferguson does not have even an A-level in biology and would appear to have no formal training in computer modelling, medicine or epidemiology, according to Derek Winton, an MSc in Computational Intelligence. He wrote an article somewhat aghast at what Ferguson did which included taking no account of respiratory disease 'seasonality' which means it is far worse in the winter months. Who would have thought that respiratory disease could be worse in the winter? Well, certainly not Ferguson.

The massively China-connected Imperial College and its bizarre professor provided the excuse for the long-incubated Chinese model of human control to travel westward at lightning speed. Imperial College confirms on its website that it collaborates with the Chinese Research Institute; publishes more than 600 research papers every year with Chinese research institutions; has 225 Chinese staff; 2,600 Chinese students – the biggest international group; 7,000 former students living in China which is the largest group outside the UK; and was selected for a tour by China's President Xi Jinping during his state visit to the UK in 2015. The college takes major donations from China and describes itself as the UK's number one university collaborator with Chinese research institutions. The China communist/fascist government did not appear phased by the woeful predictions of Ferguson and Imperial when during the lockdown that Ferguson induced the college signed a five-year collaboration deal with China tech giant Huawei that will have Huawei's indoor 5G network equipment installed at the college's West London tech campus along with an 'AI cloud platform'. The deal includes Chinese sponsorship of Imperial's Venture Catalyst entrepreneurship competition. Imperial is an example of the enormous influence the Chinese government has within British and North American

universities and research centres – and further afield. Up to 200 academics from more than a dozen UK universities are being investigated on suspicion of 'unintentionally' helping the Chinese government build weapons of mass destruction by 'transferring world-leading research in advanced military technology such as aircraft, missile designs and cyberweapons'. Similar scandals have broken in the United States, but it's all a coincidence. Imperial College serves the agenda in many other ways including the promotion of every aspect of the United Nations Agenda 21/2030 (the Great Reset) and produced computer models to show that human-caused 'climate change' is happening when in the real world it isn't. Imperial College is driving the climate agenda as it drives the 'Covid' agenda (both Cult hoaxes) while Patrick Vallance, the UK government's Chief Scientific Adviser on 'Covid', was named Chief Scientific Adviser to the UN 'climate change' conference known as COP26 hosted by the government in Glasgow, Scotland. 'Covid' and 'climate' are fundamentally connected.

Professor Woeful

From Imperial's bosom came Neil Ferguson still advising government despite his previous disasters and it was announced early on that he and other key people like UK Chief Medical Adviser Chris Whitty had caught the 'virus' as the propaganda story was being sold. Somehow they managed to survive and we had Prime Minister Boris Johnson admitted to hospital with what was said to be a severe version of the 'virus' in this same period. His whole policy and demeanour changed when he returned to Downing Street. It's a small world with these government advisors – especially in their communal connections to Gates – and Ferguson had partnered with Whitty to write a paper called 'Infectious disease: Tough choices to reduce Ebola transmission' which involved another scare-story that didn't happen. Ferguson's 'models' predicted that up to150, 000 could die from 'mad cow disease', or BSE, and its version in sheep if it was transmitted to humans. BSE was not transmitted and instead triggered by an organophosphate pesticide used to treat a pest on

cows. Fewer than 200 deaths followed from the human form. Models by Ferguson and his fellow incompetents led to the unnecessary culling of millions of pigs, cattle and sheep in the foot and mouth outbreak in 2001 which destroyed the lives and livelihoods of farmers and their families who had often spent decades building their herds and flocks. Vast numbers of these animals did not have foot and mouth and had no contact with the infection. Another 'expert' behind the cull was Professor Roy Anderson, a computer modeller at Imperial College specialising in the epidemiology of *human*, not animal, disease. Anderson has served on the Bill and Melinda Gates Grand Challenges in Global Health advisory board and chairs another Gates-funded organisation. Gates is everywhere.

In a precursor to the 'Covid' script Ferguson backed closing schools 'for prolonged periods' over the swine flu 'pandemic' in 2009 and said it would affect a third of the world population if it continued to spread at the speed he claimed to be happening. His mates at Imperial College said much the same and a news report said: 'One of the authors, the epidemiologist and disease modeller Neil Ferguson, who sits on the World Health Organisation's emergency committee for the outbreak, said the virus had "full pandemic potential".' Professor Liam Donaldson, the Chris Whitty of his day as Chief Medical Officer, said the worst case could see 30 percent of the British people infected by swine flu with 65,000 dying. Ferguson and Donaldson were indeed proved correct when at the end of the year the number of deaths attributed to swine flu was 392. The term 'expert' is rather liberally applied unfortunately, not least to complete idiots. Swine flu 'projections' were great for GlaxoSmithKline (GSK) as millions rolled in for its Pandemrix influenza vaccine which led to brain damage with children most affected. The British government (taxpayers) paid out more than £60 million in compensation after GSK was given immunity from prosecution. Yet another 'Covid' déjà vu. Swine flu was supposed to have broken out in Mexico, but Dr Wolfgang Wodarg, a German doctor, former member of parliament and critic of the 'Covid' hoax, observed 'the spread of swine flu' in Mexico City at the time. He

said: 'What we experienced in Mexico City was a very mild flu which did not kill more than usual – which killed even fewer people than usual.' Hyping the fear against all the facts is not unique to 'Covid' and has happened many times before. Ferguson is reported to have over-estimated the projected death toll of bird flu (H5N1) by some three million-fold, but bird flu vaccine makers again made a killing from the scare. This is some of the background to the Neil Ferguson who produced the perfectly-timed computer models in early 2020 predicting that half a million people would die in Britain without draconian lockdown and 2.2 million in the United States. Politicians panicked, people panicked, and lockdowns of alleged short duration were instigated to 'flatten the curve' of cases gleaned from a test not testing for the 'virus'. I said at the time that the public could forget the 'short duration' bit. This was an agenda to destroy the livelihoods of the population and force them into mass control through dependency and there was going to be nothing 'short' about it. American researcher Daniel Horowitz described the consequences of the 'models' spewed out by Gates-funded Ferguson and Imperial College:

> What led our government and the governments of many other countries into panic was a single Imperial College of UK study, funded by global warming activists, that predicted 2.2 million deaths if we didn't lock down the country. In addition, the reported 8-9% death rate in Italy scared us into thinking there was some other mutation of this virus that they got, which might have come here.
>
> Together with the fact that we were finally testing and had the ability to actually report new cases, we thought we were headed for a death spiral. But again … we can't flatten a curve if we don't know when the curve started.

How about it *never* started?

Giving them what they want

An investigation by German news outlet *Welt Am Sonntag (World on Sunday)* revealed how in March, 2020, the German government gathered together 'leading scientists from several research institutes and universities' and 'together, they were to produce a [modelling]

paper that would serve as legitimization for further tough political measures'. The Cult agenda was justified by computer modelling not based on evidence or reality; it was specifically constructed to justify the Cult demand for lockdowns all over the world to destroy the independent livelihoods of the global population. All these modellers and everyone responsible for the 'Covid' hoax have a date with a trial like those in Nuremberg after World War Two when Nazis faced the consequences of their war crimes. These corrupt-beyond-belief 'modellers' wrote the paper according to government instructions and it said that that if lockdown measures were lifted then up to one million Germans would die from 'Covid-19' adding that some would die 'agonizingly at home, gasping for breath' unable to be treated by hospitals that couldn't cope. All lies. No matter – it gave the Cult all that it wanted. What did long-time government 'modeller' Neil Ferguson say? If the UK and the United States didn't lockdown half a million would die in Britain and 2.2 million Americans. Anyone see a theme here? 'Modellers' are such a crucial part of the lockdown strategy that we should look into their background and follow the money. Researcher Rosemary Frei produced an excellent article headlined 'The Modelling-paper Mafiosi'. She highlights a guy called John Edmunds, a British epidemiologist, and professor in the Faculty of Epidemiology and Population Health at the London School of Hygiene & Tropical Medicine. He studied at Imperial College. Edmunds is a member of government 'Covid' advisory bodies which have been dictating policy, the New and Emerging Respiratory Virus Threats Advisory Group (NERVTAG) and the Scientific Advisory Group for Emergencies (SAGE).

Ferguson, another member of NERVTAG and SAGE, led the way with the original 'virus' and Edmunds has followed in the 'variant' stage and especially the so-called UK or Kent variant known as the 'Variant of Concern' (VOC) B.1.1.7. He said in a co-written report for the Centre for Mathematical modelling of Infectious Diseases at the London School of Hygiene and Tropical Medicine, with input from the Centre's 'Covid-19' Working Group, that there was 'a realistic

possibility that VOC B.1.1.7 is associated with an increased risk of death compared to non-VOC viruses'. Fear, fear, fear, get the vaccine, fear, fear, fear, get the vaccine. Rosemary Frei reveals that almost all the paper's authors and members of the modelling centre's 'Covid-19' Working Group receive funding from the Bill and Melinda Gates Foundation and/or the associated Gates-funded Wellcome Trust. The paper was published by e-journal *Medr χiv* which only publishes papers not peer-reviewed and the journal was established by an organisation headed by Facebook's Mark Zuckerberg and his missus. What a small world it is. Frei discovered that Edmunds is on the Scientific Advisory Board of the Coalition for Epidemic Preparedness Innovations (CEPI) which was established by the Bill and Melinda Gates Foundation, Klaus Schwab's Davos World Economic Forum and Big Pharma giant Wellcome. CEPI was 'launched in Davos [in 2017] to develop vaccines to stop future epidemics', according to its website. 'Our mission is to accelerate the development of vaccines against emerging infectious diseases and enable equitable access to these vaccines for people during outbreaks.' What kind people they are. Rosemary Frei reveals that Public Health England (PHE) director Susan Hopkins is an author of her organisation's non-peer-reviewed reports on 'new variants'. Hopkins is a professor of infectious diseases at London's Imperial College which is gifted tens of millions of dollars a year by the Bill and Melinda Gates Foundation. Gates-funded modelling disaster Neil Ferguson also co-authors Public Health England reports and he spoke in December, 2020, about the potential danger of the B.1.1.7. 'UK variant' promoted by Gates-funded modeller John Edmunds. When I come to the 'Covid vaccines' the 'new variants' will be shown for what they are – bollocks.

Connections, connections

All these people and modellers are lockdown-obsessed or, put another way, they demand what the Cult demands. Edmunds said in January, 2021, that to ease lockdowns too soon would be a disaster and they had to 'vaccinate much, much, much more widely than the

elderly'. Rosemary Frei highlights that Edmunds is married to Jeanne Pimenta who is described in a LinkedIn profile as director of epidemiology at GlaxoSmithKline (GSK) and she held shares in the company. Patrick Vallance, co-chair of SAGE and the government's Chief Scientific Adviser, is a former executive of GSK and has a deferred bonus of shares in the company worth £600,000. GSK has serious business connections with Bill Gates and is collaborating with mRNA-'vaccine' company CureVac to make 'vaccines' for the new variants that Edmunds is talking about. GSK is planning a 'Covid vaccine' with drug giant Sanofi. Puppet Prime Minister Boris Johnson announced in the spring of 2021 that up to 60 million vaccine doses were to be made at the GSK facility at Barnard Castle in the English North East. Barnard Castle, with a population of just 6,000, was famously visited in breach of lockdown rules in April, 2020, by Johnson aide Dominic Cummings who said that he drove there 'to test his eyesight' before driving back to London. Cummings would be better advised to test his integrity – not that it would take long. The GSK facility had nothing to do with his visit then although I'm sure Patrick Vallance would have been happy to arrange an introduction and some tea and biscuits. Ruthless psychopath Gates has made yet another fortune from vaccines in collaboration with Big Pharma companies and gushes at the phenomenal profits to be made from vaccines – more than a 20-to-1 return as he told one interviewer. Gates also tweeted in December, 2019, with the foreknowledge of what was coming: 'What's next for our foundation? I'm particularly excited about what the next year could mean for one of the best buys in global health: vaccines.'

Modeller John Edmunds is a big promotor of vaccines as all these people appear to be. He's the dean of the London School of Hygiene & Tropical Medicine's Faculty of Epidemiology and Population Health which is primarily funded by the Bill and Melinda Gates Foundation and the Gates-established and funded GAVI vaccine alliance which is the Gates vehicle to vaccinate the world. The organisation Doctors Without Borders has described GAVI as being 'aimed more at supporting drug-industry desires to promote new

products than at finding the most efficient and sustainable means for fighting the diseases of poverty'. But then that's why the psychopath Gates created it. John Edmunds said in a video that the London School of Hygiene & Tropical Medicine is involved in every aspect of vaccine development including large-scale clinical trials. He contends that mathematical modelling can show that vaccines protect individuals and society. That's on the basis of shit in and shit out, I take it. Edmunds serves on the UK Vaccine Network as does Ferguson and the government's foremost 'Covid' adviser, the grim-faced, dark-eyed Chris Whitty. The Vaccine Network says it works 'to support the government to identify and shortlist targeted investment opportunities for the most promising vaccines and vaccine technologies that will help combat infectious diseases with epidemic potential, and to address structural issues related to the UK's broader vaccine infrastructure'. Ferguson is acting Director of the Imperial College Vaccine Impact Modelling Consortium which has funding from the Bill and Melina Gates Foundation and the Gates-created GAVI 'vaccine alliance'. Anyone wonder why these characters see vaccines as the answer to every problem? Ferguson is wildly enthusiastic in his support for GAVI's campaign to vaccine children en masse in poor countries. You would expect someone like Gates who has constantly talked about the need to reduce the population to want to fund vaccines to keep more people alive. I'm sure that's why he does it. The John Edmunds London School of Hygiene & Tropical Medicine (LSHTM) has a Vaccines Manufacturing Innovation Centre which develops, tests and commercialises vaccines. Rosemary Frei writes:

> The vaccines centre also performs affiliated activities like combating 'vaccine hesitancy'. The latter includes the Vaccine Confidence Project. The project's stated purpose is, among other things, 'to provide analysis and guidance for early response and engagement with the public to ensure sustained confidence in vaccines and immunisation'. The Vaccine Confidence Project's director is LSHTM professor Heidi Larson. For more than a decade she's been researching how to combat vaccine hesitancy.

How the bloody hell can blokes like John Edmunds and Neil Ferguson with those connections and financial ties model 'virus' case

and death projections for the government and especially in a way that gives their paymasters like Gates exactly what they want? It's insane, but this is what you find throughout the world.

'Covid' is not dangerous, oops, wait, yes it is

Only days before Ferguson's nightmare scenario made Jackboot Johnson take Britain into a China-style lockdown to save us from a deadly 'virus' the UK government website gov.uk was reporting something very different to Ferguson on a page of official government guidance for 'high consequence infectious diseases (HCID)'. It said this about 'Covid-19':

As of 19 March 2020, COVID-19 *is no longer considered to be a high consequence infectious diseases (HCID) in the UK* [my emphasis]. The 4 nations public health HCID group made an interim recommendation in January 2020 to classify COVID-19 as an HCID. This was based on consideration of the UK HCID criteria about the virus and the disease with information available during the early stages of the outbreak.

Now that more is known about COVID-19, the public health bodies in the UK have reviewed the most up to date information about COVID-19 against the UK HCID criteria. They have determined that several features have now changed; in particular, more information is available about mortality rates (low overall), and there is now greater clinical awareness and a specific and sensitive laboratory test, the availability of which continues to increase. The Advisory Committee on Dangerous Pathogens (ACDP) is also of the opinion that COVID-19 should no longer be classified as an HCID.

Soon after the government had been exposed for downgrading the risk they upgraded it again and everyone was back to singing from the same Cult hymn book. Ferguson and his fellow Gates clones indicated that lockdowns and restrictions would have to continue until a Gates-funded vaccine was developed. Gates said the same because Ferguson and his like were repeating the Gates script which is the Cult script. 'Flatten the curve' became an ongoing nightmare of continuing lockdowns with periods in between of severe restrictions in pursuit of destroying independent incomes and had nothing to do with protecting health about which the Cult gives not a shit. Why wouldn't Ferguson be pushing a vaccine 'solution' when he's owned by vaccine-obsessive Gates who makes a fortune from them and

when Ferguson heads the Vaccine Impact Modelling Consortium at Imperial College funded by the Gates Foundation and GAVI, the 'vaccine alliance', created by Gates as his personal vaccine promotion operation? To compound the human catastrophe that Ferguson's 'models' did so much to create he was later exposed for breaking his own lockdown rules by having sexual liaisons with his married girlfriend Antonia Staats at his home while she was living at another location with her husband and children. Staats was a 'climate' activist and senior campaigner at the Soros-funded Avaaz which I wouldn't trust to tell me that grass is green. Ferguson had to resign as a government advisor over this hypocrisy in May, 2020, but after a period of quiet he was back being quoted by the ridiculous media on the need for more lockdowns and a vaccine rollout. Other government-advising 'scientists' from Imperial College' held the fort in his absence and said lockdown could be indefinite until a vaccine was found. The Cult script was being sung by the payrolled choir. I said there was no intention of going back to 'normal' when the 'vaccine' came because the 'vaccine' is part of a very different agenda that I will discuss in Human 2.0. Why would the Cult want to let the world go back to normal when destroying that normal forever was the whole point of what was happening? House arrest, closing businesses and schools through lockdown, (un)social distancing and masks all followed the Ferguson fantasy models. Again as I predicted (these people are so predictable) when the 'vaccine' arrived we were told that house arrest, lockdown, (un)social distancing and masks would still have to continue. I will deal with the masks in the next chapter because they are of fundamental importance.

Where's the 'pandemic'?

Any mildly in-depth assessment of the figures revealed what was really going on. Cult-funded and controlled organisations still have genuine people working within them such is the number involved. So it is with Genevieve Briand, assistant program director of the Applied Economics master's degree program at Johns Hopkins

University. She analysed the impact that 'Covid-19' had on deaths from *all* causes in the United States using official data from the CDC for the period from early February to early September, 2020. She found that allegedly 'Covid' *related*-deaths exceeded those from heart disease which she found strange with heart disease always the biggest cause of fatalities. Her research became even more significant when she noted the sudden decline in 2020 of *all* non-'Covid' deaths: 'This trend is completely contrary to the pattern observed in all previous years … the total decrease in deaths by other causes almost exactly equals the increase in deaths by Covid-19.' This was such a game, set and match in terms of what was happening that Johns Hopkins University deleted the article on the grounds that it 'was being used to support false and dangerous inaccuracies about the impact of the pandemic'. No – because it exposed the scam from official CDC figures and this was confirmed when those figures were published in January, 2021. Here we can see the effect of people dying from heart attacks, cancer, road accidents and gunshot wounds – *anything* – having 'Covid-19' on the death certificate along with those diagnosed from 'symptoms' who had even not tested positive with a test not testing for the 'virus'. I am not kidding with the gunshot wounds, by the way. Brenda Bock, coroner in Grand County, Colorado, revealed that two gunshot victims tested positive for the 'virus' within the previous 30 days and were therefore classified as 'Covid deaths'. Bock said: 'These two people had tested positive for Covid, but that's not what killed them. A gunshot wound is what killed them.' She said she had not even finished her investigation when the state listed the gunshot victims as deaths due to the 'virus'. The death and case figures for 'Covid-19' are an absolute joke and yet they are repeated like parrots by the media, politicians and alleged medical 'experts'. The official Cult narrative is the only show in town.

Genevieve Briand found that deaths from all causes were not exceptional in 2020 compared with previous years and a Spanish magazine published figures that said the same about Spain which was a 'Covid' propaganda hotspot at one point. *Discovery Salud*, a

health and medicine magazine, quoted government figures which showed how 17,000 *fewer* people died in Spain in 2020 than in 2019 and more than 26,000 fewer than in 2018. The age-standardised mortality rate for England and Wales when age distribution is taken into account was significantly lower in 2020 than the 1970s, 80s and 90s, and was only the ninth highest since 2000. Where is the 'pandemic'?

Post mortems and autopsies virtually disappeared for 'Covid' deaths amid claims that 'virus-infected' bodily fluids posed a risk to those carrying out the autopsy. This was rejected by renowned German pathologist and forensic doctor Klaus Püschel who said that he and his staff had by then done 150 autopsies on 'Covid' patients with no problems at all. He said they were needed to know why some 'Covid' patients suffered blood clots and not severe respiratory infections. The 'virus' is, after all, called SARS or 'severe acute respiratory syndrome'. I highlighted in the spring of 2020 this phenomenon and quoted New York intensive care doctor Cameron Kyle-Sidell who posted a soon deleted YouTube video to say that they had been told to prepare to treat an infectious disease called 'Covid-19', but that was not what they were dealing with. Instead he likened the lung condition of the most severely ill patients to what you would expect with cabin depressurisation in a plane at 30,000 feet or someone dropped on the top of Everest without oxygen or acclimatisation. I have never said this is not happening to a small minority of alleged 'Covid' patients – I am saying this is not caused by a phantom 'contagious virus'. Indeed Kyle-Sidell said that 'Covid-19' was not the disease they were told was coming their way. 'We are operating under a medical paradigm that is untrue,' he said, and he believed they were treating the wrong disease: 'These people are being slowly starved of oxygen.' Patients would take off their oxygen masks in a state of fear and stress and while they were blue in the face on the brink of death. They did not look like patients dying of pneumonia. You can see why they don't want autopsies when their virus doesn't exist and there is another condition in some people that they don't wish to be uncovered. I should add here that

the 5G system of millimetre waves was being rapidly introduced around the world in 2020 and even more so now as they fire 5G at the Earth from satellites. At 60 gigahertz within the 5G range that frequency interacts with the oxygen molecule and stops people breathing in sufficient oxygen to be absorbed into the bloodstream. They are installing 5G in schools and hospitals. The world is not mad or anything. 5G can cause major changes to the lungs and blood as I detail in *The Answer* and these consequences are labelled 'Covid-19', the alleged symptoms of which can be caused by 5G and other electromagnetic frequencies as cells respond to radiation poisoning.

The 'Covid death' scam

Dr Scott Jensen, a Minnesota state senator and medical doctor, exposed 'Covid' Medicare payment incentives to hospitals and death certificate manipulation. He said he was sent a seven-page document by the US Department of Health 'coaching' him on how to fill out death certificates which had never happened before. The document said that he didn't need to have a laboratory test for 'Covid-19' to put that on the death certificate and that shocked him when death certificates are supposed to be about facts. Jensen described how doctors had been 'encouraged, if not pressured' to make a diagnosis of 'Covid-19' if they thought it was probable or *'presumed*'. No positive test was necessary – not that this would have mattered anyway. He said doctors were told to diagnose 'Covid' by symptoms when these were the same as colds, allergies, other respiratory problems, and certainly with influenza which 'disappeared' in the 'Covid' era. A common sniffle was enough to get the dreaded verdict. Ontario authorities decreed that a single care home resident with *one* symptom from a long list must lead to the isolation of the entire home. Other courageous doctors like Jensen made the same point about death figure manipulation and how deaths by other causes were falling while 'Covid-19 deaths' were rising at the same rate due to re-diagnosis. Their videos rarely survive long on YouTube with its Cult-supporting algorithms courtesy of CEO Susan Wojcicki and her bosses at Google. Figure-tampering was so glaring

and ubiquitous that even officials were letting it slip or outright saying it. UK chief scientific adviser Patrick Vallance said on one occasion that 'Covid' on the death certificate doesn't mean 'Covid' was the cause of death (so why the hell is it there?) and we had the rare sight of a BBC reporter telling the truth when she said: 'Someone could be successfully treated for Covid, in say April, discharged, and then in June, get run over by a bus and die … That person would still be counted as a Covid death in England.' Yet the BBC and the rest of the world media went on repeating the case and death figures as if they were real. Illinois Public Health Director Dr Ngozi Ezike revealed the deceit while her bosses must have been clenching their buttocks:

> If you were in a hospice and given a few weeks to live and you were then found to have Covid that would be counted as a Covid death. [There might be] a clear alternate cause, but it is still listed as a Covid death. So everyone listed as a Covid death doesn't mean that was the cause of the death, but that they had Covid at the time of death.

Yes, a 'Covid virus' never shown to exist and tested for with a test not testing for the 'virus'. In the first period of the pandemic hoax through the spring of 2020 the process began of designating almost everything a 'Covid' death and this has continued ever since. I sat in a restaurant one night listening to a loud conversation on the next table where a family was discussing in bewilderment how a relative who had no symptoms of 'Covid', and had died of a long-term problem, could have been diagnosed a death by the 'virus'. I could understand their bewilderment. If they read this book they will know why this medical fraud has been perpetrated the world over.

Some media truth shock

The media ignored the evidence of death certificate fraud until eventually one columnist did speak out when she saw it first-hand. Bel Mooney is a long-time national newspaper journalist in Britain currently working for the *Daily Mail*. Her article on February 19th, 2021, carried this headline: 'My dad Ted passed three Covid tests

and died of a chronic illness yet he's officially one of Britain's 120,000 victims of the virus and is far from alone ... so how many more are there?' She told how her 99-year-old father was in a care home with a long-standing chronic obstructive pulmonary disease and vascular dementia. Maybe, but he was still aware enough to tell her from the start that there was no 'virus' and he refused the 'vaccine' for that reason. His death was not unexpected given his chronic health problems and Mooney said she was shocked to find that 'Covid-19' was declared the cause of death on his death certificate. She said this was a 'bizarre and unacceptable untruth' for a man with long-time health problems who had tested negative twice at the home for the 'virus'. I was also shocked by this story although not by what she said. I had been highlighting the death certificate manipulation for ten months. It was the confirmation that a professional full-time journalist only realised this was going on when it affected her directly and neither did she know that whether her dad tested positive or negative was irrelevant with the test not testing for the 'virus'. Where had she been? She said she did not believe in 'conspiracy theories' without knowing I'm sure that this and 'conspiracy theorists' were terms put into widespread circulation by the CIA in the 1960s to discredit those who did not accept the ridiculous official story of the Kennedy assassination. A blanket statement of 'I don't believe in conspiracy theories' is always bizarre. The dictionary definition of the term alone means the world is drowning in conspiracies. What she said was even more daft when her dad had just been affected by the 'Covid' conspiracy. Why else does she think that 'Covid-19' was going on the death certificates of people who died of something else?

To be fair once she saw from personal experience what was happening she didn't mince words. Mooney was called by the care home on the morning of February 9th to be told her father had died in his sleep. When she asked for the official cause of death what came back was 'Covid-19'. Mooney challenged this and was told there had been deaths from Covid on the dementia floor (confirmed by a test not testing for the 'virus') so they considered it 'reasonable

to assume'. 'But doctor,' Mooney rightly protested, 'an assumption isn't a diagnosis.' She said she didn't blame the perfectly decent and sympathetic doctor – 'he was just doing his job'. Sorry, but that's *bullshit*. He wasn't doing his job at all. He was putting a false cause of death on the death certificate and that is a criminal offence for which he should be brought to account and the same with the millions of doctors worldwide who have done the same. They were not doing their job they were following orders and that must not wash at new Nuremberg trials any more than it did at the first ones. Mooney's doctor was 'assuming' (presuming) as he was told to, but 'just following orders' makes no difference to his actions. A doctor's job is to serve the patient and the truth, not follow orders, but that's what they have done all over the world and played a central part in making the 'Covid' hoax possible with all its catastrophic consequences for humanity. Shame on them and they must answer for their actions. Mooney said her disquiet worsened when she registered her father's death by telephone and was told by the registrar there had been very many other cases like hers where 'the deceased' had not tested positive for 'Covid' yet it was recorded as the cause of death. The test may not matter, but those involved at their level *think* it matters and it shows a callous disregard for accurate diagnosis. The pressure to do this is coming from the top of the national 'health' pyramids which in turn obey the World Health Organization which obeys Gates and the Cult. Mooney said the registrar agreed that this must distort the national figures adding that 'the strangest thing is that every winter we record countless deaths from flu, and this winter there have been none. Not one!' She asked if the registrar thought deaths from flu were being misdiagnosed and lumped together with 'Covid' deaths. The answer was a 'puzzled yes'. Mooney said that the funeral director said the same about 'Covid' deaths which had nothing to do with 'Covid'. They had lost count of the number of families upset by this and other funeral companies in different countries have had the same experience. Mooney wrote:

The nightly shroud-waving and shocking close-ups of pain imposed on us by the TV news bewildered and terrified the population into eager compliance with lockdowns. We were invited to 'save the NHS' and to grieve for strangers – the real-life loved ones behind those shocking death counts. Why would the public imagine what I now fear, namely that the way Covid-19 death statistics are compiled might make the numbers seem greater than they are?

Oh, just a little bit – like 100 percent.

Do the maths

Mooney asked why a country would wish to skew its mortality figures by wrongly certifying deaths? What had been going on? Well, if you don't believe in conspiracies you will never find the answer which is that *it's a conspiracy*. She did, however, describe what she had discovered as a 'national scandal'. In reality it's a global scandal and happening everywhere. Pillars of this conspiracy were all put into place before the button was pressed with the Drosten PCR protocol and high amplifications to produce the cases and death certificate changes to secure illusory 'Covid' deaths. Mooney notes that normally two doctors were needed to certify a death, with one having to know the patient, and how the rules were changed in the spring of 2020 to allow one doctor to do this. In the same period 'Covid deaths' were decreed to be all cases where Covid-19 was put on the death certificate even without a positive test or any symptoms. Mooney asked: 'How many of the 30,851 (as of January 15) care home resident deaths with Covid-19 on the certificate (32.4 per cent of all deaths so far) were based on an assumption, like that of my father? And what has that done to our national psyche?'All of them is the answer to the first question and it has devastated and dismantled the national psyche, actually the global psyche, on a colossal scale. In the UK case and death data is compiled by organisations like Public Health England (PHE) and the Office for National Statistics (ONS). Mooney highlights the insane policy of counting a death from any cause as 'Covid-19' if this happens within 28 days of a positive test (with a test not testing for the 'virus') and she points out that ONS statistics reflect deaths 'involving Covid' 'or due to Covid' which meant in practice any

death where ‘Covid-19’ was mentioned on the death certificate. She described the consequences of this fraud:

Most people will accept the narrative they are fed, so panicky governments here and in Europe witnessed the harsh measures enacted in totalitarian China and jumped into lockdown. Headlines about Covid deaths tolled like the knell that would bring doomsday to us all. Fear stalked our empty streets. Politicians parroted the frankly ridiculous aim of ‘zero Covid’ and shut down the economy, while most British people agreed that lockdown was essential and (astonishingly to me, as a patriotic Brit) even wanted more restrictions.

For what? Lies on death certificates? Never mind the grim toll of lives ruined, suicides, schools closed, rising inequality, depression, cancelled hospital treatments, cancer patients in a torture of waiting, poverty, economic devastation, loneliness, families kept apart, and so on. How many lives have been lost as a direct result of lockdown?

She said that we could join in a national chorus of shock and horror at reaching the 120,000 death toll which was surely certain to have been totally skewed all along, but what about the human cost of lockdown justified by these ‘death figures’? *The British Medical Journal* had reported a 1,493 percent increase in cases of children taken to Great Ormond Street Hospital with abusive head injuries alone and then there was the effect on families:

Perhaps the most shocking thing about all this is that families have been kept apart – and obeyed the most irrational, changing rules at the whim of government – because they believed in the statistics. They succumbed to fear, which his generation rejected in that war fought for freedom. Dad (God rest his soul) would be angry. And so am I.

Another theme to watch is that in the winter months when there are more deaths from all causes they focus on ‘Covid’ deaths and in the summer when the British Lung Foundation says respiratory disease plummets by 80 percent they rage on about ‘cases’. Either way fascism on population is always the answer.

Nazi eugenics in the 21st century

Elderly people in care homes have been isolated from their families month after lonely month with no contact with relatives and grandchildren who were banned from seeing them. We were told

that lockdown fascism was to 'protect the vulnerable' like elderly people. At the same time Do Not Resuscitate (DNR) orders were placed on their medical files so that if they needed resuscitation it wasn't done and 'Covid-19' went on their death certificates. Old people were not being 'protected' they were being culled – murdered in truth. DNR orders were being decreed for disabled and young people with learning difficulties or psychological problems. The UK Care Quality Commission, a non-departmental body of the Department of Health and Social Care, found that 34 percent of those working in health and social care were pressured into placing 'do not attempt cardiopulmonary resuscitation' orders on 'Covid' patients who suffered from disabilities and learning difficulties without involving the patient or their families in the decision. UK judges ruled that an elderly woman with dementia should have the DNA-manipulating 'Covid vaccine' against her son's wishes and that a man with severe learning difficulties should have the jab despite his family's objections. Never mind that many had already died. The judiciary always supports doctors and government in fascist dictatorships. They wouldn't dare do otherwise. A horrific video was posted showing fascist officers from Los Angeles police forcibly giving the 'Covid' shot to women with special needs who were screaming that they didn't want it. The same fascists are seen giving the jab to a sleeping elderly woman in a care home. This is straight out of the Nazi playbook. Hitler's Nazis committed mass murder of the mentally ill and physically disabled throughout Germany and occupied territories in the programme that became known as Aktion T4, or just T4. Sabbatian-controlled Hitler and his grotesque crazies set out to kill those they considered useless and unnecessary. The Reich Committee for the Scientific Registering of Hereditary and Congenital Illnesses registered the births of babies identified by physicians to have 'defects'. By 1941 alone more than 5,000 children were murdered by the state and it is estimated that in total the number of innocent people killed in Aktion T4 was between 275,000 and 300,000. Parents were told their children had been sent away for 'special treatment' never to return. It is rather pathetic to see claims about plans for new extermination camps being dismissed today

when the same force behind current events did precisely that 80 years ago. Margaret Sanger was a Cult operative who used 'birth control' to sanitise her programme of eugenics. Organisations she founded became what is now Planned Parenthood. Sanger proposed that 'the whole dysgenic population would have its choice of segregation or sterilization'. These included epileptics, 'feeble-minded', and prostitutes. Sanger opposed charity because it perpetuated 'human waste'. She reveals the Cult mentality and if anyone thinks that extermination camps are a 'conspiracy theory' their naivety is touching if breathtakingly stupid.

If you don't believe that doctors can act with callous disregard for their patients it is worth considering that doctors and medical staff agreed to put government-decreed DNR orders on medical files and do nothing when resuscitation is called for. I don't know what you call such people in your house. In mine they are Nazis from the Josef Mengele School of Medicine. Phenomenal numbers of old people have died worldwide from the effects of lockdown, depression, lack of treatment, the 'vaccine' (more later) and losing the will to live. A common response at the start of the manufactured pandemic was to remove old people from hospital beds and transfer them to nursing homes. The decision would result in a mass cull of elderly people in those homes through lack of treatment – *not* 'Covid'. Care home whistleblowers have told how once the 'Covid' era began doctors would not come to their homes to treat patients and they were begging for drugs like antibiotics that often never came. The most infamous example was ordered by New York governor Andrew Cuomo, brother of a moronic CNN host, who amazingly was given an Emmy Award for his handling of the 'Covid crisis' by the ridiculous Wokers that hand them out. Just how ridiculous could be seen in February, 2021, when a Department of Justice and FBI investigation began into how thousands of old people in New York died in nursing homes after being discharged from hospital to make way for 'Covid' patients on Cuomo's say-so – and how he and his staff covered up these facts. This couldn't have happened to a nicer psychopath. Even then there was a 'Covid' spin. Reports said that

thousands of old people who tested positive for 'Covid' in hospital were transferred to nursing homes to both die of 'Covid' and transmit it to others. No – they were in hospital because they were ill and the fact that they tested positive with a test not testing for the 'virus' is irrelevant. They were ill often with respiratory diseases ubiquitous in old people near the end of their lives. Their transfer out of hospital meant that their treatment stopped and many would go on to die.

They're old. Who gives a damn?

I have exposed in the books for decades the Cult plan to cull the world's old people and even to introduce at some point what they call a 'demise pill' which at a certain age everyone would take and be out of here by law. In March, 2021, Spain legalised euthanasia and assisted suicide following the Netherlands, Belgium, Luxembourg and Canada on the Tiptoe to the demise pill. Treatment of old people by many 'care' homes has been a disgrace in the 'Covid' era. There are many, many, caring staff – I know some. There have, however, been legions of stories about callous treatment of old people and their families. Police were called when families came to take their loved ones home in the light of isolation that was killing them. They became prisoners of the state. Care home residents in insane, fascist Ontario, Canada, were not allowed to leave their *room* once the 'Covid' hoax began. UK staff have even wheeled elderly people away from windows where family members were talking with them. Oriana Criscuolo from Stockport in the English North West dropped off some things for her 80-year-old father who has Parkinson's disease and dementia and she wanted to wave to him through a ground-floor window. She was told that was 'illegal'. When she went anyway they closed the curtains in the middle of the day. Oriana said:

> It's just unbelievable. I cannot understand how care home staff – people who are being paid to care – have become so uncaring. Their behaviour is inhumane and cruel. It's beyond belief.

She was right and this was not a one-off. What a way to end your life in such loveless circumstances. UK registered nurse Nicky Millen, a proper old school nurse for 40 years, said that when she started her career care was based on dignity, choice, compassion and empathy. Now she said 'the things that are important to me have gone out of the window.' She was appalled that people were dying without their loved ones and saying goodbye on iPads. Nicky described how a distressed 89-year-old lady stroked her face and asked her 'how many paracetamol would it take to finish me off'. Life was no longer worth living while not seeing her family. Nicky said she was humiliated in front of the ward staff and patients for letting the lady stroke her face and giving her a cuddle. Such is the dehumanisation that the 'Covid' hoax has brought to the surface. Nicky worked in care homes where patients told her they were being held prisoner. 'I want to live until I die', one said to her. 'I had a lady in tears because she hadn't seen her great-grandson.' Nicky was compassionate old school meeting psychopathic New Normal. She also said she had worked on a 'Covid' ward with no 'Covid' patients. Jewish writer Shai Held wrote an article in March, 2020, which was headlined 'The Staggering, Heartless Cruelty Toward the Elderly'. What he described was happening from the earliest days of lockdown. He said 'the elderly' were considered a group and not unique individuals (the way of the Woke). Shai Held said:

> Notice how the all-too-familiar rhetoric of dehumanization works: 'The elderly' are bunched together as a faceless mass, all of them considered culprits and thus effectively deserving of the suffering the pandemic will inflict upon them. Lost entirely is the fact that the elderly are individual human beings, each with a distinctive face and voice, each with hopes and dreams, memories and regrets, friendships and marriages, loves lost and loves sustained.

'The elderly' have become another dehumanised group for which anything goes and for many that has resulted in cold disregard for their rights and their life. The distinctive face that Held talks about is designed to be deleted by masks until everyone is part of a faceless mass.

‘War-zone’ hospitals myth

Again and again medical professionals have told me what was really going on and how hospitals ‘overrun like war zones’ according to the media were virtually empty. The mantra from medical whistleblowers was please don’t use my name or my career is over. Citizen journalists around the world sneaked into hospitals to film evidence exposing the ‘war-zone’ lie. They really *were* largely empty with closed wards and operating theatres. I met a hospital worker in my town on the Isle of Wight during the first lockdown in 2020 who said the only island hospital had never been so quiet. Lockdown was justified by the psychopaths to stop hospitals being overrun. At the same time that the island hospital was near-empty the military arrived here to provide *extra beds*. It was all propaganda to ramp up the fear to ensure compliance with fascism as were never-used temporary hospitals with thousands of beds known as Nightingales and never-used make-shift mortuaries opened by the criminal UK government. A man who helped to install those extra island beds attributed to the army said they were never used and the hospital was empty. Doctors and nurses ‘stood around talking or on their phones, wandering down to us to see what we were doing’. There were no masks or social distancing. He accused the useless local island paper, the *County Press*, of ‘pumping the fear as if our hospital was overrun and we only have one so it should have been’. He described ambulances parked up with crews outside in deck chairs. When his brother called an ambulance he was told there was a two-hour backlog which he called ‘bullshit’. An old lady on the island fell ‘and was in a bad way’, but a caller who rang for an ambulance was told the situation wasn’t urgent enough. Ambulance stations were working under capacity while people would hear ambulances with sirens blaring driving through the streets. When those living near the stations realised what was going on they would follow them as they left, circulated around an urban area with the sirens going, and then came back without stopping. All this was to increase levels of fear and the same goes for the ‘ventilator shortage crisis’ that cost tens of millions for hastily produced ventilators never to be used.

Ambulance crews that agreed to be exploited in this way for fear propaganda might find themselves a mirror. I wish them well with that. Empty hospitals were the obvious consequence of treatment and diagnoses of non-'Covid' conditions cancelled and those involved handed a death sentence. People have been dying at home from undiagnosed and untreated cancer, heart disease and other life-threatening conditions to allow empty hospitals to deal with a 'pandemic' that wasn't happening.

Death of the innocent

'War-zones' have been laying off nursing staff, even doctors where they can. There was no work for them. Lockdown was justified by saving lives and protecting the vulnerable they were actually killing with DNR orders and preventing empty hospitals being 'overrun'. In Britain the mantra of stay at home to 'save the NHS' was everywhere and across the world the same story was being sold when it was all lies. Two California doctors, Dan Erickson and Artin Massihi at Accelerated Urgent Care in Bakersfield, held a news conference in April, 2020, to say that intensive care units in California were 'empty, essentially', with hospitals shutting floors, not treating patients and laying off doctors. The California health system was working at minimum capacity 'getting rid of doctors because we just don't have the volume'. They said that people with conditions such as heart disease and cancer were not coming to hospital out of fear of 'Covid-19'. Their video was deleted by Susan Wojcicki's Cult-owned YouTube after reaching five million views. Florida governor Ron Desantis, who rejected the severe lockdowns of other states and is being targeted for doing so, said that in March, 2020, every US governor was given models claiming they would run out of hospital beds in days. That was never going to happen and the 'modellers' knew it. Deceit can be found at every level of the system. Urgent children's operations were cancelled including fracture repairs and biopsies to spot cancer. Eric Nicholls, a consultant paediatrician, said 'this is obviously concerning and we need to return to normal operating and to increase capacity as soon as possible'. Psychopaths

in power were rather less concerned *because* they are psychopaths. Deletion of urgent care and diagnosis has been happening all over the world and how many kids and others have died as a result of the actions of these cold and heartless lunatics dictating 'health' policy? The number must be stratospheric. Richard Sullivan, professor of cancer and global health at King's College London, said people feared 'Covid' more than cancer such was the campaign of fear. 'Years of lost life will be quite dramatic', Sullivan said, with 'a huge amount of avoidable mortality'. Sarah Woolnough, executive director for policy at Cancer Research UK, said there had been a 75 percent drop in urgent referrals to hospitals by family doctors of people with suspected cancer. Sullivan said that 'a lot of services have had to scale back – we've seen a dramatic decrease in the amount of elective cancer surgery'. Lockdown deaths worldwide has been absolutely fantastic with the *New York Post* reporting how data confirmed that 'lockdowns end more lives than they save':

> There was a sharp decline in visits to emergency rooms and an increase in fatal heart attacks because patients didn't receive prompt treatment. Many fewer people were screened for cancer. Social isolation contributed to excess deaths from dementia and Alzheimer's.
>
> Researchers predicted that the social and economic upheaval would lead to tens of thousands of "deaths of despair" from drug overdoses, alcoholism and suicide. As unemployment surged and mental-health and substance-abuse treatment programs were interrupted, the reported levels of anxiety, depression and suicidal thoughts increased dramatically, as did alcohol sales and fatal drug overdoses.

This has been happening while nurses and other staff had so much time on their hands in the 'war-zones' that Tic-Tok dancing videos began appearing across the Internet with medical staff dancing around in empty wards and corridors as people died at home from causes that would normally have been treated in hospital.

Mentions in dispatches

One brave and truth-committed whistleblower was Louise Hampton, a call handler with the UK NHS who made a viral Internet video saying she had done 'fuck all' during the 'pandemic'

which was 'a load of bollocks'. She said that 'Covid-19' was rebranded flu and of course she lost her job. This is what happens in the medical and endless other professions now when you tell the truth. Louise filmed inside 'war-zone' accident and emergency departments to show they were empty and I mean *empty* as in no one there. The mainstream media could have done the same and blown the gaff on the whole conspiracy. They haven't to their eternal shame. Not that most 'journalists' seem capable of manifesting shame as with the psychopaths they slavishly repeat without question. The relative few who were admitted with serious health problems were left to die alone with no loved ones allowed to see them because of 'Covid' rules and they included kids dying without the comfort of mum and dad at their bedside while the evil behind this couldn't give a damn. It was all good fun to them. A Scottish NHS staff nurse publicly quit in the spring of 2021 saying: 'I can no longer be part of the lies and the corruption by the government.' She said hospitals 'aren't full, the beds aren't full, beds have been shut, wards have been shut'. Hospitals were never busy throughout 'Covid'. The staff nurse said that Nicola Sturgeon, tragically the leader of the Scottish government, was on television saying save the hospitals and the NHS – 'but the beds are empty' and 'we've not seen flu, we always see flu every year'. She wrote to government and spoke with her union Unison (the unions are Cult-compromised and *useless*, but nothing changed. Many of her colleagues were scared of losing their jobs if they spoke out as they wanted to. She said nursing staff were being affected by wearing masks all day and 'my head is splitting every shift from wearing a mask'. The NHS is part of the fascist tyranny and must be dismantled so we can start again with human beings in charge. (Ironically, hospitals were reported to be busier again when official 'Covid' cases *fell* in spring/summer of 2021 and many other conditions required treatment at the same time as *the fake vaccine rollout*.)

I will cover the 'Covid vaccine' scam in detail later, but it is another indicator of the sickening disregard for human life that I am highlighting here. The DNA-manipulating concoctions do not fulfil

the definition of a 'vaccine', have never been used on humans before and were given only emergency approval because trials were not completed and they continued using the unknowing public. The result was what a NHS senior nurse with responsibility for 'vaccine' procedure said was 'genocide'. She said the 'vaccines' were not 'vaccines'. They had not been shown to be safe and claims about their effectiveness by drug companies were 'poetic licence'. She described what was happening as a 'horrid act of human annihilation'. The nurse said that management had instigated a policy of not providing a Patient Information Leaflet (PIL) before people were 'vaccinated' even though health care professionals are supposed to do this according to protocol. Patients should also be told that they are taking part in an ongoing clinical trial. Her challenges to what is happening had seen her excluded from meetings and ridiculed in others. She said she was told to 'watch my step … or I would find myself surplus to requirements'. The nurse, who spoke anonymously in fear of her career, said she asked her NHS manager why he/she was content with taking part in genocide against those having the 'vaccines'. The reply was that everyone had to play their part and to 'put up, shut up, and get it done'. Government was 'leaning heavily' on NHS management which was clearly leaning heavily on staff. This is how the global 'medical' hierarchy operates and it starts with the Cult and its World Health Organization.

She told the story of a doctor who had the Pfizer jab and when questioned had no idea what was in it. The doctor had never read the literature. We have to stop treating doctors as intellectual giants when so many are moral and medical pygmies. The doctor did not even know that the 'vaccines' were not fully approved or that their trials were ongoing. They were, however, asking their patients if they minded taking part in follow-ups for research purposes – yes, the *ongoing clinical trial*. The nurse said the doctor's ignorance was not rare and she had spoken to a hospital consultant who had the jab without any idea of the background or that the 'trials' had not been completed. Nurses and pharmacists had shown the same ignorance.

'My NHS colleagues have forsaken their duty of care, broken their code of conduct – Hippocratic Oath – and have been brainwashed just the same as the majority of the UK public through propaganda …' She said she had not been able to recruit a single NHS colleague, doctor, nurse or pharmacist to stand with her and speak out. Her union had refused to help. She said that if the genocide came to light she would not hesitate to give evidence at a Nuremberg-type trial against those in power who could have affected the outcomes but didn't.

And all for what?

To put the nonsense into perspective let's say the 'virus' does exist and let's go completely crazy and accept that the official manipulated figures for cases and deaths are accurate. *Even then* a study by Stanford University epidemiologist Dr John Ioannidis published on the World Health Organization website produced an average infection to fatality rate of … *0.23 percent!* Ioannidis said: 'If one could sample equally from all locations globally, the median infection fatality rate might even be substantially lower than the 0.23% observed in my analysis.' For healthy people under 70 it was … *0.05 percent*! This compares with the 3.4 percent claimed by the Cult-owned World Health Organization when the hoax was first played and maximum fear needed to be generated. An updated Stanford study in April, 2021, put the 'infection' to 'fatality' rate at just 0.15 percent. Another team of scientists led by Megan O'Driscoll and Henrik Salje studied data from 45 countries and published their findings on the Nature website. For children and young people the figure is so small it virtually does not register although authorities will be hyping dangers to the young when they introduce DNA-manipulating 'vaccines' for children. The O'Driscoll study produced an average infection-fatality figure of 0.003 for children from birth to four; 0.001 for 5 to 14; 0.003 for 15 to 19; and it was still only 0.456 up to 64. To claim that children must be 'vaccinated' to protect them from 'Covid' is an obvious lie and so there must be another reason and there is. What's more the average age of a 'Covid' death is akin

to the average age that people die in general. The average age of death in England is about 80 for men and 83 for women. The average age of death from alleged 'Covid' is between 82 and 83. California doctors, Dan Erickson and Artin Massihi, said at their April media conference that projection models of millions of deaths had been 'woefully inaccurate'. They produced detailed figures showing that Californians had a 0.03 chance of dying from 'Covid' based on the number of people who tested positive (with a test not testing for the 'virus'). Erickson said there was a 0.1 percent chance of dying from 'Covid' in the *state* of New York, not just the city, and a 0.05 percent chance in Spain, a centre of 'Covid-19' hysteria at one stage. The Stanford studies supported the doctors' data with fatality rate estimates of 0.23 and 0.15 percent. How close are these figures to my estimate of *zero*? Death-rate figures claimed by the World Health Organization at the start of the hoax were some 15 times higher. The California doctors said there was no justification for lockdowns and the economic devastation they caused. Everything they had ever learned about quarantine was that you quarantine the *sick* and not the healthy. They had never seen this before and it made no medical sense.

Why in the in the light of all this would governments and medical systems the world over say that billions must go under house arrest; lose their livelihood; in many cases lose their mind, their health and their life; force people to wear masks dangerous to health and psychology; make human interaction and even family interaction a criminal offence; ban travel; close restaurants, bars, watching live sport, concerts, theatre, and any activity involving human togetherness and discourse; and closing schools to isolate children from their friends and cause many to commit suicide in acts of hopelessness and despair? The California doctors said lockdown consequences included increased child abuse, partner abuse, alcoholism, depression, and other impacts they were seeing every day. Who would do that to the entire human race if not mentally-ill psychopaths of almost unimaginable extremes like Bill Gates? We must face the reality of what we are dealing with and come out of

denial. Fascism and tyranny are made possible only by the target population submitting and acquiescing to fascism and tyranny. The whole of human history shows that to be true. Most people naively and unquestioning believed what they were told about a 'deadly virus' and meekly and weakly submitted to house arrest. Those who didn't believe it – at least in total – still submitted in fear of the consequences of not doing so. For the rest who wouldn't submit draconian fines have been imposed, brutal policing by psychopaths *for* psychopaths, and condemnation from the meek and weak who condemn the Pushbackers on behalf of the very force that has them, too, in its gunsights. 'Pathetic' does not even begin to suffice. Britain's brainless 'Health' Secretary Matt Hancock warned anyone lying to border officials about returning from a list of 'hotspot' countries could face a jail sentence of up to ten years which is more than for racially-aggravated assault, incest and attempting to have sex with a child under 13. Hancock is a lunatic, but he has the state apparatus behind him in a Cult-led chain reaction and the same with UK 'Vaccine Minister' Nadhim Zahawi, a prominent member of the mega-Cult secret society, Le Cercle, which featured in my earlier books. The Cult enforces its will on governments and medical systems; government and medical systems enforce their will on business and police; business enforces its will on staff who enforce it on customers; police enforce the will of the Cult on the population and play their essential part in creating a world of fascist control that their own children and grandchildren will have to live in their entire lives. It is a hierarchical pyramid of imposition and acquiescence and, yes indeedy, of clinical insanity.

Does anyone bright enough to read this book have to ask what the answer is? I think not, but I will reveal it anyway in the fewest of syllables: Tell the psychos and their moronic lackeys to fuck off and let's get on with our lives. We are many – They are few.

CHAPTER SEVEN

War on your mind

One believes things because one has been conditioned to believe them
Aldous Huxley, *Brave New World*

I have described the 'Covid' hoax as a 'Psyop' and that is true in every sense and on every level in accordance with the definition of that term which is psychological warfare. Break down the 'Covid pandemic' to the foundation themes and it is psychological warfare on the human individual and collective mind.

The same can be said for the entire human belief system involving every subject you can imagine. Huxley was right in his contention that people believe what they are conditioned to believe and this comes from the repetition throughout their lives of the same falsehoods. They spew from government, corporations, media and endless streams of 'experts' telling you what the Cult wants you to believe and often believing it themselves (although *far* from always). 'Experts' are rewarded with 'prestigious' jobs and titles and as agents of perceptual programming with regular access to the media. The Cult has to control the narrative – control *information* – or they lose control of the vital, crucial, without-which-they-cannot-prevail public perception of reality. The foundation of that control today is the Internet made possible by the Defense Advanced Research Projects Agency (DARPA), the incredibly sinister technological arm of the Pentagon. The Internet is the result of military technology.

DARPA openly brags about establishing the Internet which has been a long-term project to lasso the minds of the global population. I have said for decades the plan is to control information to such an extreme that eventually no one would see or hear anything that the Cult does not approve. We are closing in on that end with ferocious censorship since the 'Covid' hoax began and in my case it started back in the 1990s in terms of books and speaking venues. I had to create my own publishing company in 1995 precisely because no one else would publish my books even then. I think they're all still running.

Cult Internet

To secure total control of information they needed the Internet in which pre-programmed algorithms can seek out 'unclean' content for deletion and even stop it being posted in the first place. The Cult had to dismantle print and non-Internet broadcast media to ensure the transfer of information to the appropriate-named 'Web' – a critical expression of the *Cult* web. We've seen the ever-quickening demise of traditional media and control of what is left by a tiny number of corporations operating worldwide. Independent journalism in the mainstream is already dead and never was that more obvious than since the turn of 2020. The Cult wants all information communicated via the Internet to globally censor and allow the plug to be pulled any time. Lockdowns and forced isolation has meant that communication between people has been through electronic means and no longer through face-to-face discourse and discussion. Cult psychopaths have targeted the bars, restaurants, sport, venues and meeting places in general for this reason. None of this is by chance and it's to stop people gathering in any kind of privacy or number while being able to track and monitor all Internet communications and block them as necessary. Even private messages between individuals have been censored by these fascists that control Cult fronts like Facebook, Twitter, Google and YouTube which are all officially run by Sabbatian place-people and from the background by higher-level Sabbatian place people.

Facebook, Google, Amazon and their like were seed-funded and supported into existence with money-no-object infusions of funds either directly or indirectly from DARPA and CIA technology arm In-Q-Tel. The Cult plays the long game and prepares very carefully for big plays like 'Covid'. Amazon is another front in the psychological war and pretty much controls the global market in book sales and increasingly publishing. Amazon's limitless funds have deleted fantastic numbers of independent publishers to seize global domination on the way to deciding which books can be sold and circulated and which cannot. Moves in that direction are already happening. Amazon's leading light Jeff Bezos is the grandson of Lawrence Preston Gise who worked with DARPA predecessor ARPA. Amazon has big connections to the CIA and the Pentagon. The plan I have long described went like this:

1. Employ military technology to establish the Internet.

2. Sell the Internet as a place where people can freely communicate without censorship and allow that to happen until the Net becomes the central and irreversible pillar of human society. If the Internet had been highly censored from the start many would have rejected it.

3. Fund and manipulate major corporations into being to control the circulation of information on your Internet using cover stories about geeks in garages to explain how they came about. Give them unlimited funds to expand rapidly with no need to make a profit for years while non-Cult companies who need to balance the books cannot compete. You know that in these circumstances your Googles, YouTubes, Facebooks and Amazons are going to secure near monopolies by either crushing or buying up the opposition.

4. Allow freedom of expression on both the Internet and communication platforms to draw people in until the Internet is the central and irreversible pillar of human society and your communication corporations have reached a stage of near monopoly domination.

5. Then unleash your always-planned frenzy of censorship on the basis of 'where else are you going to go?' and continue to expand that until nothing remains that the Cult does not want its human targets to see.

The process was timed to hit the 'Covid' hoax to ensure the best chance possible of controlling the narrative which they knew they had to do at all costs. They were, after all, about to unleash a 'deadly virus' that didn't really exist. If you do that in an environment of free-flowing information and opinion you would be dead in the

water before you could say Gates is a psychopath. The network was in place through which the Cult-created-and-owned World Health Organization could dictate the 'Covid' narrative and response policy slavishly supported by Cult-owned Internet communication giants and mainstream media while those telling a different story were censored. Google, YouTube, Facebook and Twitter openly announced that they would do this. What else would we expect from Cult-owned operations like Facebook which former executives have confirmed set out to make the platform more addictive than cigarettes and coldly manipulates emotions of its users to sow division between people and groups and scramble the minds of the young? If Zuckerberg lives out the rest of his life without going to jail for crimes against humanity, and most emphatically against the young, it will be a travesty of justice. Still, no matter, cause and effect will catch up with him eventually and the same with Sergey Brin and Larry Page at Google with its CEO Sundar Pichai who fix the Google search results to promote Cult narratives and hide the opposition. Put the same key words into Google and other search engines like DuckDuckGo and you will see how different results can be. Wikipedia is another intensely biased 'encyclopaedia' which skews its content to the Cult agenda. YouTube links to Wikipedia's version of 'Covid' and 'climate change' on video pages in which experts in their field offer a different opinion (even that is increasingly rare with Wojcicki censorship). Into this 'Covid' silence-them network must be added government media censors, sorry 'regulators', such as Ofcom in the UK which imposed tyrannical restrictions on British broadcasters that had the effect of banning me from ever appearing. Just to debate with me about my evidence and views on 'Covid' would mean breaking the fascistic impositions of Ofcom and its CEO career government bureaucrat Melanie Dawes. Gutless British broadcasters tremble at the very thought of fascist Ofcom.

Psychos behind 'Covid'

The reason for the 'Covid' catastrophe in all its facets and forms can be seen by whom and what is driving the policies worldwide in such a coordinated way. Decisions are not being made to protect health, but to target psychology. The dominant group guiding and 'advising' government policy are not medical professionals. They are psychologists and behavioural scientists. Every major country has its own version of this phenomenon and I'll use the British example to show how it works. In many ways the British version has been affecting the wider world in the form of the huge behaviour manipulation network in the UK which operates in other countries. The network involves private companies, government, intelligence and military. The Cabinet Office is at the centre of the government 'Covid' Psyop and part-owns, with 'innovation charity' Nesta, the Behavioural Insights Team (BIT) which claims to be independent of government but patently isn't. The BIT was established in 2010 and its job is to manipulate the psyche of the population to acquiesce to government demands and so much more. It is also known as the 'Nudge Unit', a name inspired by the 2009 book by two ultra-Zionists, Cass Sunstein and Richard Thaler, called *Nudge: Improving Decisions About Health, Wealth, and Happiness*. The book, as with the Behavioural Insights Team, seeks to 'nudge' behaviour (manipulate it) to make the public follow patterns of action and perception that suit those in authority (the Cult). Sunstein is so skilled at this that he advises the World Health Organization and the UK Behavioural Insights Team and was Administrator of the White House Office of Information and Regulatory Affairs in the Obama administration. Biden appointed him to the Department of Homeland Security – another ultra-Zionist in the fold to oversee new immigration laws which is another policy the Cult wants to control. Sunstein is desperate to silence anyone exposing conspiracies and co-authored a 2008 report on the subject in which suggestions were offered to ban 'conspiracy theorizing' or impose 'some kind of tax, financial or otherwise, on those who disseminate such theories'. I guess a psychiatrist's chair is out of the question?

Sunstein's mate Richard Thaler, an 'academic affiliate' of the UK Behavioural Insights Team, is a proponent of 'behavioural economics' which is defined as the study of 'the effects of psychological, cognitive, emotional, cultural and social factors on the decisions of individuals and institutions'. Study the effects so they can be manipulated to be what you want them to be. Other leading names in the development of behavioural economics are ultra-Zionists Daniel Kahneman and Robert J. Shiller and they, with Thaler, won the Nobel Memorial Prize in Economic Sciences for their work in this field. The Behavioural Insights Team is operating at the heart of the UK government and has expanded globally through partnerships with several universities including Harvard, Oxford, Cambridge, University College London (UCL) and Pennsylvania. They claim to have 'trained' (reframed) 20,000 civil servants and run more than 750 projects involving 400 randomised controlled trials in dozens of countries' as another version of mind reframers Common Purpose. BIT works from its office in New York with cities and their agencies, as well as other partners, across the United States and Canada – this is a company part-owned by the British government Cabinet Office. An executive order by President Cult-servant Obama established a US Social and Behavioral Sciences Team in 2015. They all have the same reason for being and that's to brainwash the population directly and by brainwashing those in positions of authority.

'Covid' mind game

Another prime aspect of the UK mind-control network is the 'independent' [joke] Scientific Pandemic Insights Group on Behaviours (SPI-B) which 'provides behavioural science advice aimed at anticipating and helping people adhere to interventions that are recommended by medical or epidemiological experts'. That means manipulating public perception and behaviour to do whatever government tells them to do. It's disgusting and if they really want the public to be 'safe' this lot should all be under lock and key. According to the government website SPI-B consists of

'behavioural scientists, health and social psychologists, anthropologists and historians' and advises the Whitty-Vallance-led Scientific Advisory Group for Emergencies (SAGE) which in turn advises the government on 'the science' (it doesn't) and 'Covid' policy. When politicians say they are being guided by 'the science' this is the rabble in each country they are talking about and that 'science' is dominated by behaviour manipulators to enforce government fascism through public compliance. The Behaviour Insight Team is headed by psychologist David Solomon Halpern, a visiting professor at King's College London, and connects with a national and global web of other civilian and military organisations as the Cult moves towards its goal of fusing them into one fascistic whole in every country through its 'Fusion Doctrine'. The behaviour manipulation network involves, but is not confined to, the Foreign Office; National Security Council; government communications headquarters (GCHQ); MI5; MI6; the Cabinet Office-based Media Monitoring Unit; and the Rapid Response Unit which 'monitors digital trends to spot emerging issues; including misinformation and disinformation; and identifies the best way to respond'.

There is also the 77th Brigade of the UK military which operates like the notorious Israeli military's Unit 8200 in manipulating information and discussion on the Internet by posing as members of the public to promote the narrative and discredit those who challenge it. Here we have the military seeking to manipulate *domestic* public opinion while the Nazis in government are fine with that. Conservative Member of Parliament Tobias Ellwood, an advocate of lockdown and control through 'vaccine passports', is a Lieutenant Colonel reservist in the 77th Brigade which connects with the military operation jHub, the 'innovation centre' for the Ministry of Defence and Strategic Command. jHub has also been involved with the civilian National Health Service (NHS) in 'symptom tracing' the population. The NHS is a key part of this mind control network and produced a document in December, 2020, explaining to staff how to use psychological manipulation with different groups and ages to get them to have the DNA-manipulating 'Covid vaccine'

that's designed to cumulatively rewrite human genetics. The document, called 'Optimising Vaccination Roll Out – Do's and Dont's for all messaging, documents and "communications" in the widest sense', was published by NHS England and the NHS Improvement *Behaviour Change Unit* in partnership with Public Health England and Warwick Business School. I hear the mantra about 'save the NHS' and 'protect the NHS' when we need to scrap the NHS and start again. The current version is far too corrupt, far too anti-human and totally compromised by Cult operatives and their assets. UK government broadcast media censor Ofcom will connect into this web – as will the BBC with its tremendous Ofcom influence – to control what the public see and hear and dictate mass perception. Nuremberg trials must include personnel from all these organisations.

The fear factor

The 'Covid' hoax has led to the creation of the UK Cabinet Office-connected Joint Biosecurity Centre (JBC) which is officially described as providing 'expert advice on pandemics' using its independent [all Cult operations are 'independent'] analytical function to provide real-time analysis about infection outbreaks to identify and respond to outbreaks of Covid-19'. Another role is to advise the government on a response to spikes in infections – 'for example by closing schools or workplaces in local areas where infection levels have risen'. Put another way, promoting the Cult agenda. The Joint Biosecurity Centre is modelled on the Joint Terrorism Analysis Centre which analyses intelligence to set 'terrorism threat levels' and here again you see the fusion of civilian and military operations and intelligence that has led to military intelligence producing documents about 'vaccine hesitancy' and how it can be combated. Domestic civilian matters and opinions should not be the business of the military. The Joint Biosecurity Centre is headed by Tom Hurd, director general of the Office for Security and Counter-Terrorism from the establishment-to-its-fingertips Hurd family. His father is former Foreign Secretary Douglas Hurd. How coincidental that Tom

Hurd went to the elite Eton College and Oxford University with Boris Johnson. Imperial College with its ridiculous computer modeller Neil Ferguson will connect with this gigantic web that will itself interconnect with similar set-ups in other major and not so major countries. Compared with this Cult network the politicians, be they Boris Johnson, Donald Trump or Joe Biden, are bit-part players 'following the science'. The network of psychologists was on the 'Covid' case from the start with the aim of generating maximum fear of the 'virus' to ensure compliance by the population. A government behavioural science group known as SPI-B produced a paper in March, 2020, for discussion by the main government science advisory group known as SAGE. It was headed 'Options for increasing adherence to social distancing measures' and it said the following in a section headed 'Persuasion':

- A substantial number of people still do not feel sufficiently personally threatened; it could be that they are reassured by the low death rate in their demographic group, although levels of concern may be rising. Having a good understanding of the risk has been found to be positively associated with adoption of COVID-19 social distancing measures in Hong Kong.

- The perceived level of personal threat needs to be increased among those who are complacent, using hard-hitting evaluation of options for increasing social distancing emotional messaging. To be effective this must also empower people by making clear the actions they can take to reduce the threat.

- Responsibility to others: There seems to be insufficient understanding of, or feelings of responsibility about, people's role in transmitting the infection to others … Messaging about actions need to be framed positively in terms of protecting oneself and the community, and increase confidence that they will be effective.

- Some people will be more persuaded by appeals to play by the rules, some by duty to the community, and some to personal risk.

> All these different approaches are needed. The messaging also needs to take account of the realities of different people's lives. Messaging needs to take account of the different motivational levers and circumstances of different people.

All this could be achieved the SPI-B psychologists said by *using the media to increase the sense of personal threat* which translates as terrify the shit out of the population, including children, so they all do what we want. That's not happened has it? Those excuses for 'journalists' who wouldn't know journalism if it bit them on the arse (the great majority) have played their crucial part in serving this Cult-government Psyop to enslave their own kids and grandkids. How they live with themselves I have no idea. The psychological war has been underpinned by constant government 'Covid' propaganda in almost every television and radio ad break, plus the Internet and print media, which has pounded out the fear with taxpayers footing the bill for their own programming. The result has been people terrified of a 'virus' that doesn't exist or one with a tiny fatality rate even if you believe it does. People walk down the street and around the shops wearing face-nappies damaging their health and psychology while others report those who refuse to be that naïve to the police who turn up in their own face-nappies. I had a cameraman come to my flat and he was so frightened of 'Covid' he came in wearing a mask and refused to shake my hand in case he caught something. He had – naïveitis – and the thought that he worked in the mainstream media was both depressing and made his behaviour perfectly explainable. The fear which has gripped the minds of so many and frozen them into compliance has been carefully cultivated by these psychologists who are really psychopaths. If lives get destroyed and a lot of young people commit suicide it shows our plan is working. SPI-B then turned to compulsion on the public to comply. 'With adequate preparation, rapid change can be achieved', it said. Some countries had introduced mandatory self-isolation on a wide scale without evidence of major public unrest and a large majority of the UK's population appeared to be supportive of more coercive measures with 64 percent of adults saying they would

support putting London under a lockdown (watch the 'polls' which are designed to make people believe that public opinion is in favour or against whatever the subject in hand).

For 'aggressive protective measures' to be effective, the SPI-B paper said, special attention should be devoted to those population groups that are more at risk. Translated from the Orwellian this means making the rest of population feel guilty for not protecting the 'vulnerable' such as old people which the Cult and its agencies were about to kill on an industrial scale with lockdown, lack of treatment and the Gates 'vaccine'. Psychopath psychologists sold their guilt-trip so comprehensively that Los Angeles County Supervisor Hilda Solis reported that children were apologising (from a distance) to their parents and grandparents for bringing 'Covid' into their homes and getting them sick. '… These apologies are just some of the last words that loved ones will ever hear as they die alone,' she said. Gut-wrenchingly Solis then used this childhood tragedy to tell children to stay at home and 'keep your loved ones alive'. Imagine heaping such potentially life-long guilt on a kid when it has absolutely nothing to do with them. These people are deeply disturbed and the psychologists behind this even more so.

Uncivil war – divide and rule

Professional mind-controllers at SPI-B wanted the media to increase a sense of responsibility to others (do as you're told) and promote 'positive messaging' for those actions while in contrast to invoke 'social disapproval' by the unquestioning, obedient, community of anyone with a mind of their own. Again the compliant Goebbels-like media obliged. This is an old, old, trick employed by tyrannies the world over throughout human history. You get the target population to keep the target population in line – *your* line. SPI-B said this could 'play an important role in preventing anti-social behaviour or discouraging failure to enact pro-social behaviour'. For 'anti-social' in the Orwellian parlance of SPI-B see any behaviour that government doesn't approve. SPI-B recommendations said that 'social disapproval' should be accompanied by clear messaging and

promotion of strong collective identity – hence the government and celebrity mantra of 'we're all in this together'. Sure we are. The mind doctors have such contempt for their targets that they think some clueless comedian, actor or singer telling them to do what the government wants will be enough to win them over. We have had UK comedian Lenny Henry, actor Michael Caine and singer Elton John wheeled out to serve the propagandists by urging people to have the DNA-manipulating 'Covid' non-'vaccine'. The role of Henry and fellow black celebrities in seeking to coax a 'vaccine' reluctant black community into doing the government's will was especially stomach-turning. An emotion-manipulating script and carefully edited video featuring these black 'celebs' was such an insult to the intelligence of black people and where's the self-respect of those involved selling their souls to a fascist government agenda? Henry said he heard black people's 'legitimate worries and concerns', but people must 'trust the facts' when they were doing exactly that by not having the 'vaccine'. They had to include the obligatory reference to Black Lives Matter with the line … 'Don't let coronavirus cost even more black lives – because we matter'. My god, it was pathetic. 'I know the vaccine is safe and what it does.' How? 'I'm a comedian and it says so in my script.'

SPI-B said social disapproval needed to be carefully managed to avoid victimisation, scapegoating and misdirected criticism, but they knew that their 'recommendations' would lead to exactly that and the media were specifically used to stir-up the divide-and-conquer hostility. Those who conform like good little baa, baas, are praised while those who have seen through the tidal wave of lies are 'Covidiots'. The awake have been abused by the fast asleep for not conforming to fascism and impositions that the awake know are designed to endanger their health, dehumanise them, and tear asunder the very fabric of human society. We have had the curtain-twitchers and morons reporting neighbours and others to the face-nappied police for breaking 'Covid rules' with fascist police delighting in posting links and phone numbers where this could be done. The Cult cannot impose its will without a compliant police

and military or a compliant population willing to play their part in enslaving themselves and their kids. The words of a pastor in Nazi Germany are so appropriate today:

First they came for the socialists and I did not speak out because I was not a socialist.

Then they came for the trade unionists and I did not speak out because I was not a trade unionist.

Then they came for the Jews and I did not speak out because I was not a Jew.

Then they came for me and there was no one left to speak for me.

Those who don't learn from history are destined to repeat it and so many are.

'Covid' rules: Rewiring the mind

With the background laid out to this gigantic national and global web of psychological manipulation we can put 'Covid' rules into a clear and sinister perspective. Forget the claims about protecting health. 'Covid' rules are about dismantling the human mind, breaking the human spirit, destroying self-respect, and then putting Humpty Dumpty together again as a servile, submissive slave. Social isolation through lockdown and distancing have devastating effects on the human psyche as the psychological psychopaths well know and that's the real reason for them. Humans need contact with each other, discourse, closeness and touch, or they eventually, and literarily, go crazy. Masks, which I will address at some length, fundamentally add to the effects of isolation and the Cult agenda to dehumanise and de-individualise the population. To do this while knowing – in fact *seeking* – this outcome is the very epitome of evil and psychologists involved in this *are* the epitome of evil. They must like all the rest of the Cult demons and their assets stand trial for crimes against humanity on a scale that defies the imagination. Psychopaths in uniform use isolation to break enemy troops and agents and make them subservient and submissive to tell what they know. The technique is rightly considered a form of torture and

torture is most certainly what has been imposed on the human population.

Clinically-insane American psychologist Harry Harlow became famous for his isolation experiments in the 1950s in which he separated baby monkeys from their mothers and imprisoned them for months on end in a metal container or 'pit of despair'. They soon began to show mental distress and depression as any idiot could have predicted. Harlow put other monkeys in steel chambers for three, six or twelve months while denying them any contact with animals or humans. He said that the effects of total social isolation for six months were 'so devastating and debilitating that we had assumed initially that twelve months of isolation would not produce any additional decrement'; but twelve months of isolation 'almost obliterated the animals socially'. This is what the Cult and its psychopaths are doing to you and your children. Even monkeys in partial isolation in which they were not allowed to form relationships with other monkeys became 'aggressive and hostile, not only to others, but also towards their own bodies'. We have seen this in the young as a consequence of lockdown. UK government psychopaths launched a public relations campaign telling people not to hug each other even after they received the 'Covid-19 vaccine' which we were told with more lies would allow a return to 'normal life'. A government source told *The Telegraph*: 'It will be along the lines that it is great that you have been vaccinated, but if you are going to visit your family and hug your grandchildren there is a chance you are going to infect people you love.' The source was apparently speaking from a secure psychiatric facility. Janet Lord, director of Birmingham University's Institute of Inflammation and Ageing, said that parents and grandparents should avoid hugging their children. Well, how can I put it, Ms Lord? Fuck off. Yep, that'll do.

Destroying the kids – where are the parents?

Observe what has happened to people enslaved and isolated by lockdown as suicide and self-harm has soared worldwide,

particularly among the young denied the freedom to associate with their friends. A study of 49,000 people in English-speaking countries concluded that almost half of young adults are at clinical risk of mental health disorders. A national survey in America of 1,000 currently enrolled high school and college students found that 5 percent reported attempting suicide during the pandemic. Data from the US CDC's National Syndromic Surveillance Program from January 1st to October 17th, 2020, revealed a *31 percent* increase in mental health issues among adolescents aged 12 to 17 compared with 2019. The CDC reported that America in general suffered the biggest drop in life expectancy since World War Two as it fell by a year in the first half of 2020 as a result of 'deaths of despair' – overdoses and suicides. Deaths of despair have leapt by more than 20 percent during lockdown and include the highest number of fatal overdoses ever recorded in a single year – 81,000. Internet addiction is another consequence of being isolated at home which lowers interest in physical activities as kids fall into inertia and what's the point? Children and young people are losing hope and giving up on life, sometimes literally. A 14-year-old boy killed himself in Maryland because he had 'given up' when his school district didn't reopen; an 11-year-old boy shot himself during a zoom class; a teenager in Maine succumbed to the isolation of the 'pandemic' when he ended his life after experiencing a disrupted senior year at school. Children as young as nine have taken their life and all these stories can be repeated around the world. Careers are being destroyed before they start and that includes those in sport in which promising youngsters have not been able to take part. The plan of the psycho-psychologists is working all right. Researchers at Cambridge University found that lockdowns cause significant harm to children's mental health. Their study was published in the *Archives of Disease in Childhood*, and followed 168 children aged between 7 and 11. The researchers concluded:

> During the UK lockdown, children's depression symptoms have increased substantially, relative to before lockdown. The scale of this effect has direct relevance for the continuation of different elements of lockdown policy, such as complete or partial school closures …

… Specifically, we observed a statistically significant increase in ratings of depression, with a medium-to-large effect size. Our findings emphasise the need to incorporate the potential impact of lockdown on child mental health in planning the ongoing response to the global pandemic and the recovery from it.

Not a chance when the Cult's psycho-psychologists were getting exactly what they wanted. The UK's Royal College of Paediatrics and Child Health has urged parents to look for signs of eating disorders in children and young people after a three to four fold increase. Specialists say the 'pandemic' is a major reason behind the rise. You don't say. The College said isolation from friends during school closures, exam cancellations, loss of extra-curricular activities like sport, and an increased use of social media were all contributory factors along with fears about the virus (psycho-psychologists again), family finances, and students being forced to quarantine. Doctors said young people were becoming severely ill by the time they were seen with 'Covid' regulations reducing face-to-face consultations. Nor is it only the young that have been devastated by the psychopaths. Like all bullies and cowards the Cult is targeting the young, elderly, weak and infirm. A typical story was told by a British lady called Lynn Parker who was not allowed to visit her husband in 2020 for the last ten and half months of his life 'when he needed me most' between March 20th and when he died on December 19th. This vacates the criminal and enters the territory of evil. The emotional impact on the immune system alone is immense as are the number of people of all ages worldwide who have died as a result of Cult-demanded, Gates-demanded, lockdowns.

Isolation is torture

The experience of imposing solitary confinement on millions of prisoners around the world has shown how a large percentage become 'actively psychotic and/or acutely suicidal'. Social isolation has been found to trigger 'a specific psychiatric syndrome, characterized by hallucinations; panic attacks; overt paranoia; diminished impulse control; hypersensitivity to external stimuli; and difficulties with thinking, concentration and memory'. Juan Mendez,

a United Nations rapporteur (investigator), said that isolation is a form of torture. Research has shown that even after isolation prisoners find it far more difficult to make social connections and I remember chatting to a shop assistant after one lockdown who told me that when her young son met another child again he had no idea how to act or what to do. Hannah Flanagan, Director of Emergency Services at Journey Mental Health Center in Dane County, Wisconsin, said: 'The specificity about Covid social distancing and isolation that we've come across as contributing factors to the suicides are really new to us this year.' But they are not new to those that devised them. They are getting the effect they want as the population is psychologically dismantled to be rebuilt in a totally different way. Children and the young are particularly targeted. They will be the adults when the full-on fascist AI-controlled technocracy is planned to be imposed and they are being prepared to meekly submit. At the same time older people who still have a memory of what life was like before – and how fascist the new normal really is – are being deleted. You are going to see efforts to turn the young against the old to support this geriatric genocide. Hannah Flanagan said the big increase in suicide in her county proved that social isolation is not only harmful, but deadly. Studies have shown that isolation from others is one of the main risk factors in suicide and even more so with women. Warnings that lockdown could create a 'perfect storm' for suicide were ignored. After all this was one of the *reasons* for lockdown. Suicide, however, is only the most extreme of isolation consequences. There are many others. Dr Dhruv Khullar, assistant professor of healthcare policy at Weill Cornell Medical College, said in a *New York Times* article in 2016 long before the fake 'pandemic':

> A wave of new research suggests social separation is bad for us. Individuals with less social connection have disrupted sleep patterns, altered immune systems, more inflammation and higher levels of stress hormones. One recent study found that isolation increases the risk of heart disease by 29 percent and stroke by 32 percent. Another analysis that pooled data from 70 studies and 3.4 million people found that socially isolated individuals had a 30 percent higher risk of dying in the next seven years, and that this effect was largest in middle age.

Loneliness can accelerate cognitive decline in older adults, and isolated individuals are twice as likely to die prematurely as those with more robust social interactions. These effects start early: Socially isolated children have significantly poorer health 20 years later, even after controlling for other factors. All told, loneliness is as important a risk factor for early death as obesity and smoking.

There you have proof from that one article alone four years before 2020 that those who have enforced lockdown, social distancing and isolation knew what the effect would be and that is even more so with professional psychologists that have been driving the policy across the globe. We can go back even further to the years 2000 and 2003 and the start of a major study on the effects of isolation on health by Dr Janine Gronewold and Professor Dirk M. Hermann at the University Hospital in Essen, Germany, who analysed data on 4,316 people with an average age of 59 who were recruited for the long-term research project. They found that socially isolated people are more than 40 percent more likely to have a heart attack, stroke, or other major cardiovascular event and nearly 50 percent more likely to die from any cause. Given the financial Armageddon unleashed by lockdown we should note that the study found a relationship between increased cardiovascular risk and lack of financial support. After excluding other factors social isolation was still connected to a 44 percent increased risk of cardiovascular problems and a 47 percent increased risk of death by any cause. Lack of financial support was associated with a 30 percent increase in the risk of cardiovascular health events. Dr Gronewold said it had been known for some time that feeling lonely or lacking contact with close friends and family can have an impact on physical health and the study had shown that having strong social relationships is of high importance for heart health. Gronewold said they didn't understand yet why people who are socially isolated have such poor health outcomes, but this was obviously a worrying finding, particularly during these times of prolonged social distancing. Well, it can be explained on many levels. You only have to identify the point in the body where people feel loneliness and missing people they are parted from – it's in the centre of the chest where they feel the ache of loneliness and the ache of missing people. 'My heart aches for

you' … 'My heart aches for some company.' I will explain this more in the chapter Escaping Wetiko, but when you realise that the body is the mind – they are expressions of each other – the reason why state of the mind dictates state of the body becomes clear.

American psychologist Ranjit Powar was highlighting the effects of lockdown isolation as early as April, 2020. She said humans have evolved to be social creatures and are wired to live in interactive groups. Being isolated from family, friends and colleagues could be unbalancing and traumatic for most people and could result in short or even long-term psychological and physical health problems. An increase in levels of anxiety, aggression, depression, forgetfulness and hallucinations were possible psychological effects of isolation. 'Mental conditions may be precipitated for those with underlying pre-existing susceptibilities and show up in many others without any pre-condition.' Powar said personal relationships helped us cope with stress and if we lost this outlet for letting off steam the result can be a big emotional void which, for an average person, was difficult to deal with. 'Just a few days of isolation can cause increased levels of anxiety and depression' – so what the hell has been the effect on the global population of *18 months* of this at the time of writing? Powar said: 'Add to it the looming threat of a dreadful disease being repeatedly hammered in through the media and you have a recipe for many shades of mental and physical distress.' For those with a house and a garden it is easy to forget that billions have had to endure lockdown isolation in tiny overcrowded flats and apartments with nowhere to go outside. The psychological and physical consequences of this are unimaginable and with lunatic and abusive partners and parents the consequences have led to tremendous increases in domestic and child abuse and alcoholism as people seek to shut out the horror. Ranjit Powar said:

Staying in a confined space with family is not all a rosy picture for everyone. It can be extremely oppressive and claustrophobic for large low-income families huddled together in small single-room houses. Children here are not lucky enough to have many board/electronic games or books to keep them occupied.

Add to it the deep insecurity of running out of funds for food and basic necessities. On the other hand, there are people with dysfunctional family dynamics, such as domineering, abusive or alcoholic partners, siblings or parents which makes staying home a period of trial. Incidence of suicide and physical abuse against women has shown a worldwide increase. Heightened anxiety and depression also affect a person's immune system, making them more susceptible to illness.

To think that Powar's article was published on April 11th, 2020.

Six-feet fantasy

Social (unsocial) distancing demanded that people stay six feet or two metres apart. UK government advisor Robert Dingwall from the New and Emerging Respiratory Virus Threats Advisory Group said in a radio interview that the two-metre rule was 'conjured up out of nowhere' and was not based on science. No, it was not based on *medical* science, but it didn't come out of nowhere. The distance related to *psychological* science. Six feet/two metres was adopted in many countries and we were told by people like the criminal Anthony Fauci and his ilk that it was founded on science. Many schools could not reopen because they did not have the space for six-feet distancing. Then in March, 2021, after a year of six-feet 'science', a study published in the *Journal of Infectious Diseases* involving more than 500,000 students and almost 100,000 staff over 16 weeks revealed no significant difference in 'Covid' cases between six feet and three feet and Fauci changed his tune. Now three feet was okay. There is no difference between six feet and three *inches* when there is no 'virus' and they got away with six feet for psychological reasons for as long as they could. I hear journalists and others talk about 'unintended consequences' of lockdown. They are not *unintended* at all; they have been coldly-calculated for a specific outcome of human control and that's why super-psychopaths like Gates have called for them so vehemently. Super-psychopath psychologists have demanded them and psychopathic or clueless, spineless, politicians have gone along with them by 'following the science'. But it's not science at all. 'Science' is not what is; it's only what people can be manipulated to believe it is. The whole 'Covid' catastrophe is

founded on mind control. Three word or three statement mantras issued by the UK government are a well-known mind control technique and so we've had 'Stay home/protect the NHS/save lives', 'Stay alert/control the virus/save lives' and 'hands/face/space'. One of the most vocal proponents of extreme 'Covid' rules in the UK has been Professor Susan Michie, a member of the British Communist Party, who is not a medical professional. Michie is the director of the Centre for Behaviour Change at University College London. She is a *behavioural psychologist* and another filthy rich 'Marxist' who praised China's draconian lockdown. She was known by fellow students at Oxford University as 'Stalin's nanny' for her extreme Marxism. Michie is an influential member of the UK government's Scientific Advisory Group for Emergencies (SAGE) and behavioural manipulation groups which have dominated 'Covid' policy. She is a consultant adviser to the World Health Organization on 'Covid-19' and behaviour. Why the hell are lockdowns anything to do with her when they are claimed to be about health? Why does a behavioural psychologist from a group charged with changing the behaviour of the public want lockdown, human isolation and mandatory masks? Does that question really need an answer? Michie *absolutely* has to explain herself before a Nuremberg court when humanity takes back its world again and even more so when you see the consequences of masks that she demands are compulsory. This is a Michie classic:

> The benefits of getting primary school children to wear masks is that regardless of what little degree of transmission is occurring in those age groups it could help normalise the practice. Young children wearing masks may be more likely to get their families to accept masks.

Those words alone should carry a prison sentence when you ponder on the callous disregard for children involved and what a statement it makes about the mind and motivations of Susan Michie. What a lovely lady and what she said there encapsulates the mentality of the psychopaths behind the 'Covid' horror. Let us compare what Michie said with a countrywide study in Germany published at researchsquare.com involving 25,000 school children and 17,854 health complaints submitted by parents. Researchers

found that masks are harming children physically, psychologically, and behaviourally with 24 health issues associated with mask wearing. They include: shortness of breath (29.7%); dizziness (26.4%); increased headaches (53%); difficulty concentrating (50%); drowsiness or fatigue (37%); and malaise (42%). Nearly a third of children experienced more sleep issues than before and a quarter developed new fears. Researchers found health issues and other impairments in 68 percent of masked children covering their faces for an average of 4.5 hours a day. Hundreds of those taking part experienced accelerated respiration, tightness in the chest, weakness, and short-term impairment of consciousness. A reminder of what Michie said again:

The benefits of getting primary school children to wear masks is that regardless of what little degree of transmission is occurring in those age groups it could help normalise the practice. Young children wearing masks may be more likely to get their families to accept masks.

Psychopaths in government and psychology now have children and young people – plus all the adults – wearing masks for hours on end while clueless teachers impose the will of the psychopaths on the young they should be protecting. What the hell are parents doing?

Cult lab rats

We have some schools already imposing on students microchipped buzzers that activate when they get 'too close' to their pals in the way they do with lab rats. How apt. To the Cult and its brain-dead servants our children *are* lab rats being conditioned to be unquestioning, dehumanised slaves for the rest of their lives. Children and young people are being weaned and frightened away from the most natural human instincts including closeness and touch. I have tracked in the books over the years how schools were banning pupils from greeting each other with a hug and the whole Cult-induced Me Too movement has terrified men and boys from a relaxed and natural interaction with female friends and work colleagues to the point where many men try never to be in a room

alone with a woman that's not their partner. Airhead celebrities have as always played their virtue-signalling part in making this happen with their gross exaggeration. For every monster like Harvey Weinstein there are at least tens of thousands of men that don't treat women like that; but everyone must be branded the same and policy changed for them as well as the monster. I am going to be using the word 'dehumanise' many times in this chapter because that is what the Cult is seeking to do and it goes very deep as we shall see. Don't let them kid you that social distancing is planned to end one day. That's not the idea. We are seeing more governments and companies funding and producing wearable gadgets to keep people apart and they would not be doing that if this was meant to be short-term. A tech start-up company backed by GCHQ, the British Intelligence and military surveillance headquarters, has created a social distancing wrist sensor that alerts people when they get too close to others. The CIA has also supported tech companies developing similar devices. The wearable sensor was developed by Tended, one of a number of start-up companies supported by GCHQ (see the CIA and DARPA). The device can be worn on the wrist or as a tag on the waistband and will vibrate whenever someone wearing the device breaches social distancing and gets anywhere near natural human contact. The company had a lucky break in that it was developing a distancing sensor when the 'Covid' hoax arrived which immediately provided a potentially enormous market. How fortunate. The government in big-time Cult-controlled Ontario in Canada is investing $2.5 million in wearable contact tracing technology that 'will alert users if they may have been exposed to the Covid-19 in the workplace and will beep or vibrate if they are within six feet of another person'. Facedrive Inc., the technology company behind this, was founded in 2016 with funding from the Ontario Together Fund and obviously they, too, had a prophet on the board of directors. The human surveillance and control technology is called TraceSCAN and would be worn by the human cyborgs in places such as airports, workplaces, construction sites, care homes and … *schools*.

I emphasise schools with children and young people the prime targets. You know what is planned for society as a whole if you keep your eyes on the schools. They have always been places where the state program the next generation of slaves to be its compliant worker-ants – or Woker-ants these days; but in the mist of the 'Covid' madness they have been transformed into mind laboratories on a scale never seen before. Teachers and head teachers are just as programmed as the kids – often more so. Children are kept apart from human interaction by walk lanes, classroom distancing, staggered meal times, masks, and the rolling-out of buzzer systems. Schools are now physically laid out as a laboratory maze for lab-rats. Lunatics at a school in Anchorage, Alaska, who should be prosecuted for child abuse, took away desks and forced children to kneel (know your place) on a mat for five hours a day while wearing a mask and using their chairs as a desk. How this was supposed to impact on a 'virus' only these clinically insane people can tell you and even then it would be clap-trap. The school banned recess (interaction), art classes (creativity), and physical exercise (getting body and mind moving out of inertia). Everyone behind this outrage should be in jail or better still a mental institution. The behavioural manipulators are all for this dystopian approach to schools. Professor Susan Michie, the mind-doctor and British Communist Party member, said it was wrong to say that schools were safe. They had to be made so by 'distancing', masks and ventilation (sitting all day in the cold). I must ask this lady round for dinner on a night I know I am going to be out and not back for weeks. She probably wouldn't be able to make it, anyway, with all the visits to her own psychologist she must have block-booked.

Masking identity

I know how shocking it must be for you that a behaviour manipulator like Michie wants everyone to wear masks which have long been a feature of mind-control programs like the infamous MKUltra in the United States, but, there we are. We live and learn. I spent many years from 1996 to right across the millennium

researching mind control in detail on both sides of the Atlantic and elsewhere. I met a large number of mind-control survivors and many had been held captive in body and mind by MKUltra. MK stands for mind-control, but employs the German spelling in deference to the Nazis spirited out of Germany at the end of World War Two by Operation Paperclip in which the US authorities, with help from the Vatican, transported Nazi mind-controllers and engineers to America to continue their work. Many of them were behind the creation of NASA and they included Nazi scientist and SS officer Wernher von Braun who swapped designing V-2 rockets to bombard London with designing the Saturn V rockets that powered the NASA moon programme's Apollo craft. I think I may have mentioned that the Cult has no borders. Among Paperclip escapees was Josef Mengele, the Angel of Death in the Nazi concentration camps where he conducted mind and genetic experiments on children often using twins to provide a control twin to measure the impact of his 'work' on the other. If you want to observe the Cult mentality in all its extremes of evil then look into the life of Mengele. I have met many people who suffered mercilessly under Mengele in the United States where he operated under the name Dr Greene and became a stalwart of MKUltra programming and torture. Among his locations was the underground facility in the Mojave Desert in California called the China Lake Naval Weapons Station which is almost entirely below the surface. My books *The Biggest Secret*, *Children of the Matrix* and *The Perception Deception* have the detailed background to MKUltra.

The best-known MKUltra survivor is American Cathy O'Brien. I first met her and her late partner Mark Phillips at a conference in Colorado in 1996. Mark helped her escape and deprogram from decades of captivity in an offshoot of MKUltra known as Project Monarch in which 'sex slaves' were provided for the rich and famous including Father George Bush, Dick Cheney and the Clintons. Read Cathy and Mark's book *Trance-Formation of America* and if you are new to this you will be shocked to the core. I read it in 1996 shortly before, with the usual synchronicity of my life, I found

myself given a book table at the conference right next to hers. MKUltra never ended despite being very publicly exposed (only a small part of it) in the 1970s and continues in other guises. I am still in touch with Cathy. She contacted me during 2020 after masks became compulsory in many countries to tell me how they were used as part of MKUltra programming. I had been observing 'Covid regulations' and the relationship between authority and public for months. I saw techniques that I knew were employed on individuals in MKUltra being used on the global population. I had read many books and manuals on mind control including one called *Silent Weapons for Quiet Wars* which came to light in the 1980s and was a guide on how to perceptually program on a mass scale. 'Silent Weapons' refers to mind-control. I remembered a line from the manual as governments, medical authorities and law enforcement agencies have so obviously talked to – or rather at – the adult population since the 'Covid' hoax began as if they are children. The document said:

> If a person is spoken to by a T.V. advertiser as if he were a twelve-year-old, then, due to suggestibility, he will, with a certain probability, respond or react to that suggestion with the uncritical response of a twelve-year-old and will reach in to his economic reservoir and deliver its energy to buy that product on impulse when he passes it in the store.

That's why authority has spoken to adults like children since all this began.

Why did Michael Jackson wear masks?

Every aspect of the 'Covid' narrative has mind-control as its central theme. Cathy O'Brien wrote an article for davidicke.com about the connection between masks and mind control. Her daughter Kelly who I first met in the 1990s was born while Cathy was still held captive in MKUltra. Kelly was forced to wear a mask as part of her programming from the age of *two* to dehumanise her, target her sense of individuality and reduce the amount of oxygen her brain and body received. *Bingo*. This is the real reason for compulsory

masks, why they have been enforced en masse, and why they seek to increase the number they demand you wear. First one, then two, with one disgraceful alleged 'doctor' recommending four which is nothing less than a death sentence. Where and how often they must be worn is being expanded for the purpose of mass mind control and damaging respiratory health which they can call 'Covid-19'. Canada's government headed by the man-child Justin Trudeau, says it's fine for children of two and older to wear masks. An insane 'study' in Italy involving just 47 children concluded there was no problem for babies as young as *four months* wearing them. Even after people were 'vaccinated' they were still told to wear masks by the criminal that is Anthony Fauci. Cathy wrote that mandating masks is allowing the authorities literally to control the air we breathe which is what was done in MKUltra. You might recall how the singer Michael Jackson wore masks and there is a reason for that. He was subjected to MKUltra mind control through Project Monarch and his psyche was scrambled by these simpletons. Cathy wrote:

In MKUltra Project Monarch mind control, Michael Jackson had to wear a mask to silence his voice so he could not reach out for help. Remember how he developed that whisper voice when he wasn't singing? Masks control the mind from the outside in, like the redefining of words is doing. By controlling what we can and cannot say for fear of being labeled racist or beaten, for example, it ultimately controls thought that drives our words and ultimately actions (or lack thereof).

Likewise, a mask muffles our speech so that we are not heard, which controls voice … words … mind. This is Mind Control. Masks are an obvious mind control device, and I am disturbed so many people are complying on a global scale. Masks depersonalize while making a person feel as though they have no voice. It is a barrier to others. People who would never choose to comply but are forced to wear a mask in order to keep their job, and ultimately their family fed, are compromised. They often feel shame and are subdued. People have stopped talking with each other while media controls the narrative.

The 'no voice' theme has often become literal with train passengers told not to speak to each other in case they pass on the 'virus', singing banned for the same reason and bonkers California officials telling people riding roller coasters that they cannot shout and scream. Cathy said she heard every day from healed MKUltra survivors who cannot wear a mask without flashing back on ways

their breathing was controlled – 'from ball gags and penises to water boarding'. She said that through the years when she saw images of people in China wearing masks 'due to pollution' that it was really to control their oxygen levels. 'I knew it was as much of a population control mechanism of depersonalisation as are burkas', she said. Masks are another Chinese communist/fascist method of control that has been swept across the West as the West becomes China at lightning speed since we entered 2020.

Mask-19

There are other reasons for mandatory masks and these include destroying respiratory health to call it 'Covid-19' and stunting brain development of children and the young. Dr Margarite Griesz-Brisson MD, PhD, is a Consultant Neurologist and Neurophysiologist and the Founder and Medical Director of the London Neurology and Pain Clinic. Her CV goes down the street and round the corner. She is clearly someone who cares about people and won't parrot the propaganda. Griesz-Brisson has a PhD in pharmacology, with special interest in neurotoxicology, environmental medicine, neuroregeneration and neuroplasticity (the way the brain can change in the light of information received). She went public in October, 2020, with a passionate warning about the effects of mask-wearing laws:

> The reinhalation of our exhaled air will without a doubt create oxygen deficiency and a flooding of carbon dioxide. We know that the human brain is very sensitive to oxygen deprivation. There are nerve cells for example in the hippocampus that can't be longer than 3 minutes without oxygen – they cannot survive. The acute warning symptoms are headaches, drowsiness, dizziness, issues in concentration, slowing down of reaction time – reactions of the cognitive system.

Oh, I know, let's tell bus, truck and taxi drivers to wear them and people working machinery. How about pilots, doctors and police? Griesz-Brisson makes the important point that while the symptoms she mentions may fade as the body readjusts this does not alter the fact that people continue to operate in oxygen deficit with long list of

potential consequences. She said it was well known that neurodegenerative diseases take years or decades to develop. 'If today you forget your phone number, the breakdown in your brain would have already started 20 or 30 years ago.' She said degenerative processes in your brain are getting amplified as your oxygen deprivation continues through wearing a mask. Nerve cells in the brain are unable to divide themselves normally in these circumstances and lost nerve cells will no longer be regenerated. 'What is gone is gone.' Now consider that people like shop workers and *schoolchildren* are wearing masks for hours every day. What in the name of sanity is going to be happening to them? 'I do not wear a mask, I need my brain to think', Griesz-Brisson said, 'I want to have a clear head when I deal with my patients and not be in a carbon dioxide-induced anaesthesia'. If you are told to wear a mask anywhere ask the organisation, police, store, whatever, for their risk assessment on the dangers and negative effects on mind and body of enforcing mask-wearing. They won't have one because it has never been done not even by government. All of them must be subject to class-action lawsuits as the consequences come to light. They don't do mask risk assessments for an obvious reason. They know what the conclusions would be and independent scientific studies that *have* been done tell a horror story of consequences.

'Masks are criminal'

Dr Griesz-Brisson said that for children and adolescents, masks are an absolute no-no. They had an extremely active and adaptive immune system and their brain was incredibly active with so much to learn. 'The child's brain, or the youth's brain, is thirsting for oxygen.' The more metabolically active an organ was, the more oxygen it required; and in children and adolescents every organ was metabolically active. Griesz-Brisson said that to deprive a child's or adolescent's brain of oxygen, or to restrict it in any way, was not only dangerous to their health, it was absolutely criminal. 'Oxygen deficiency inhibits the development of the brain, and the damage that has taken place as a result CANNOT be reversed.' Mind

manipulators of MKUltra put masks on two-year-olds they wanted to neurologically rewire and you can see why. Griesz-Brisson said a child needs the brain to learn and the brain needs oxygen to function. 'We don't need a clinical study for that. This is simple, indisputable physiology.' Consciously and purposely induced oxygen deficiency was an absolutely deliberate health hazard, and an absolute medical contraindication which means that 'this drug, this therapy, this method or measure should not be used, and is not allowed to be used'. To coerce an entire population to use an absolute medical contraindication by force, she said, there had to be definite and serious reasons and the reasons must be presented to competent interdisciplinary and independent bodies to be verified and authorised. She had this warning of the consequences that were coming if mask wearing continued:

When, in ten years, dementia is going to increase exponentially, and the younger generations couldn't reach their god-given potential, it won't help to say 'we didn't need the masks'. I know how damaging oxygen deprivation is for the brain, cardiologists know how damaging it is for the heart, pulmonologists know how damaging it is for the lungs. Oxygen deprivation damages every single organ. Where are our health departments, our health insurance, our medical associations? It would have been their duty to be vehemently against the lockdown and to stop it and stop it from the very beginning.

Why do the medical boards issue punishments to doctors who give people exemptions? Does the person or the doctor seriously have to prove that oxygen deprivation harms people? What kind of medicine are our doctors and medical associations representing? Who is responsible for this crime? The ones who want to enforce it? The ones who let it happen and play along, or the ones who don't prevent it?

All of the organisations and people she mentions there either answer directly to the Cult or do whatever hierarchical levels above them tell them to do. The outcome of both is the same. 'It's not about masks, it's not about viruses, it's certainly not about your health', Griesz-Brisson said. 'It is about much, much more. I am not participating. I am not afraid.' They were taking our air to breathe and there was no unfounded medical exemption from face masks. Oxygen deprivation was dangerous for every single brain. It had to be the free decision of every human being whether they want to

wear a mask that was absolutely ineffective to protect themselves from a virus. She ended by rightly identifying where the responsibility lies for all this:

> The imperative of the hour is personal responsibility. We are responsible for what we think, not the media. We are responsible for what we do, not our superiors. We are responsible for our health, not the World Health Organization. And we are responsible for what happens in our country, not the government.

Halle-bloody-lujah.

But surgeons wear masks, right?

Independent studies of mask-wearing have produced a long list of reports detailing mental, emotional and physical dangers. What a definition of insanity to see police officers imposing mask-wearing on the public which will cumulatively damage their health while the police themselves wear masks that will cumulatively damage *their* health. It's utter madness and both public and police do this because 'the government says so' – yes a government of brain-donor idiots like UK Health Secretary Matt Hancock reading the 'follow the science' scripts of psychopathic, lunatic psychologists. The response you get from Stockholm syndrome sufferers defending the very authorities that are destroying them and their families is that 'surgeons wear masks'. This is considered the game, set and match that they must work and don't cause oxygen deficit. Well, actually, scientific studies have shown that they *do* and oxygen levels are monitored in operating theatres to compensate. Surgeons wear masks to stop spittle and such like dropping into open wounds – not to stop 'viral particles' which are so miniscule they can only be seen through an electron microscope. Holes in the masks are significantly bigger than 'viral particles' and if you sneeze or cough they will breach the mask. I watched an incredibly disingenuous 'experiment' that claimed to prove that masks work in catching 'virus' material from the mouth and nose. They did this with a slow motion camera and the mask did block big stuff which stayed inside the mask and

against the face to be breathed in or cause infections on the face as we have seen with many children. 'Viral particles', however, would never have been picked up by the camera as they came through the mask when they are far too small to be seen. The 'experiment' was therefore disingenuous *and* useless.

Studies have concluded that wearing masks in operating theatres (and thus elsewhere) make no difference to preventing infection while the opposite is true with toxic shite building up in the mask and this had led to an explosion in tooth decay and gum disease dubbed by dentists 'mask mouth'. You might have seen the Internet video of a furious American doctor urging people to take off their masks after a four-year-old patient had been rushed to hospital the night before and nearly died with a lung infection that doctors sourced to mask wearing. A study in the journal *Cancer Discovery* found that inhalation of harmful microbes can contribute to advanced stage lung cancer in adults and long-term use of masks can help breed dangerous pathogens. Microbiologists have said frequent mask wearing creates a moist environment in which microbes can grow and proliferate before entering the lungs. The Canadian Agency for Drugs and Technologies in Health, or CADTH, a Canadian national organisation that provides research and analysis to healthcare decision-makers, said this as long ago as 2013 in a report entitled 'Use of Surgical Masks in the Operating Room: A Review of the Clinical Effectiveness and Guidelines'. It said:

- No evidence was found to support the use of surgical face masks to reduce the frequency of surgical site infections
- No evidence was found on the effectiveness of wearing surgical face masks to protect staff from infectious material in the operating room.
- Guidelines recommend the use of surgical face masks by staff in the operating room to protect both operating room staff and patients (despite the lack of evidence).

We were told that the world could go back to 'normal' with the arrival of the 'vaccines'. When they came, fraudulent as they are, the story changed as I knew that it would. We are in the midst of transforming 'normal', not going back to it. Mary Ramsay, head of immunisation at Public Health England, echoed the words of US criminal Anthony Fauci who said masks and other regulations must stay no matter if people are vaccinated. The Fauci idiot continued to wear two masks – different colours so both could be clearly seen – after he *claimed* to have been vaccinated. Senator Rand Paul told Fauci in one exchange that his double-masks were 'theatre' and he was right. It's all theatre. Mary Ramsay back-tracked on the vaccine-return-to-normal theme when she said the public may need to wear masks and social-distance for years despite the jabs. 'People have got used to those lower-level restrictions now, and [they] can live with them', she said telling us what the idea has been all along. 'The vaccine does not give you a pass, even if you have had it, you must continue to follow all the guidelines' said a Public Health England statement which reneged on what we had been told before and made having the 'vaccine' irrelevant to 'normality' even by the official story. Spain's fascist government trumped everyone by passing a law mandating the wearing of masks on the beach and even when swimming in the sea. The move would have devastated what's left of the Spanish tourist industry, posed potential breathing dangers to swimmers and had Northern European sunbathers walking around with their forehead brown and the rest of their face white as a sheet. The ruling was so crazy that it had to be retracted after pressure from public and tourist industry, but it confirmed where the Cult wants to go with masks and how clinically insane authority has become. The determination to make masks permanent and hide the serious dangers to body and mind can be seen in the censorship of scientist Professor Denis Rancourt by Bill Gates-funded academic publishing website ResearchGate over his papers exposing the dangers and uselessness of masks. Rancourt said:

ResearchGate today has permanently locked my account, which I have had since 2015. Their reasons graphically show the nature of their attack against democracy, and their corruption of

science … By their obscene non-logic, a scientific review of science articles reporting on harms caused by face masks has a 'potential to cause harm'. No criticism of the psychological device (face masks) is tolerated, if the said criticism shows potential to influence public policy.

This is what happens in a fascist world.

Where are the 'greens' (again)?

Other dangers of wearing masks especially regularly relate to the inhalation of minute plastic fibres into the lungs and the deluge of discarded masks in the environment and oceans. Estimates predicted that more than 1.5 billion disposable masks will end up in the world's oceans every year polluting the water with tons of plastic and endangering marine wildlife. Studies project that humans are using 129 billion face masks each month worldwide – about three million a minute. Most are disposable and made from plastic, non-biodegradable microfibers that break down into smaller plastic particles that become widespread in ecosystems. They are littering cities, clogging sewage channels and turning up in bodies of water. I have written in other books about the immense amounts of microplastics from endless sources now being absorbed into the body. Rolf Halden, director of the Arizona State University (ASU) Biodesign Center for Environmental Health Engineering, was the senior researcher in a 2020 study that analysed 47 human tissue samples and found microplastics in all of them. 'We have detected these chemicals of plastics in every single organ that we have investigated', he said. I wrote in *The Answer* about the world being deluged with microplastics. A study by the Worldwide Fund for Nature (WWF) found that people are consuming on average every week some 2,000 tiny pieces of plastic mostly through water and also through marine life and the air. Every year humans are ingesting enough microplastics to fill a heaped dinner plate and in a life-time of 79 years it is enough to fill two large waste bins. Marco Lambertini, WWF International director general said: 'Not only are plastics polluting our oceans and waterways and killing marine life – it's in all of us and we can't escape consuming plastics,' American

geologists found tiny plastic fibres, beads and shards in rainwater samples collected from the remote slopes of the Rocky Mountain National Park near Denver, Colorado. Their report was headed: 'It is raining plastic.' Rachel Adams, senior lecturer in Biomedical Science at Cardiff Metropolitan University, said that among health consequences are internal inflammation and immune responses to a 'foreign body'. She further pointed out that microplastics become carriers of toxins including mercury, pesticides and dioxins (a known cause of cancer and reproductive and developmental problems). These toxins accumulate in the fatty tissues once they enter the body through microplastics. Now this is being compounded massively by people putting plastic on their face and throwing it away.

Workers exposed to polypropylene plastic fibres known as 'flock' have developed 'flock worker's lung' from inhaling small pieces of the flock fibres which can damage lung tissue, reduce breathing capacity and exacerbate other respiratory problems. *Now* … commonly used surgical masks have three layers of melt-blown textiles made of … polypropylene. We have billions of people putting these microplastics against their mouth, nose and face for hours at a time day after day in the form of masks. How does anyone think that will work out? I mean – what could possibly go wrong? We posted a number of scientific studies on this at davidicke.com, but when I went back to them as I was writing this book the links to the science research website where they were hosted were dead. Anything that challenges the official narrative in any way is either censored or vilified. The official narrative is so unsupportable by the evidence that only deleting the truth can protect it. A study by Chinese scientists still survived – with the usual twist which it why it was still active, I guess. Yes, they found that virtually all the masks they tested increased the daily intake of microplastic fibres, but people should still wear them because the danger from the 'virus' was worse said the crazy 'team' from the Institute of Hydrobiology in Wuhan. Scientists first discovered microplastics in lung tissue of some patients who died of lung cancer

in the 1990s. Subsequent studies have confirmed the potential health damage with the plastic degrading slowly and remaining in the lungs to accumulate in volume. Wuhan researchers used a machine simulating human breathing to establish that masks shed up to nearly 4,000 microplastic fibres in a month with reused masks producing more. Scientists said some masks are laced with toxic chemicals and a variety of compounds seriously restricted for both health and environmental reasons. They include cobalt (used in blue dye) and formaldehyde known to cause watery eyes, burning sensations in the eyes, nose, and throat, plus coughing, wheezing and nausea. No – that must be 'Covid-19'.

Mask 'worms'

There is another and potentially even more sinister content of masks. Mostly new masks of different makes filmed under a microscope around the world have been found to contain strange black fibres or 'worms' that appear to move or 'crawl' by themselves and react to heat and water. The nearest I have seen to them are the self-replicating fibres that are pulled out through the skin of those suffering from Morgellons disease which has been connected to the phenomena of 'chemtrails' which I will bring into the story later on. Morgellons fibres continue to grow outside the body and have a form of artificial intelligence. Black 'worm' fibres in masks have that kind of feel to them and there is a nanotechnology technique called 'worm micelles' which carry and release drugs or anything else you want to deliver to the body. For sure the suppression of humanity by mind altering drugs is the Cult agenda big time and the more excuses they can find to gain access to the body the more opportunities there are to make that happen whether through 'vaccines' or masks pushed against the mouth and nose for hours on end.

So let us summarise the pros and cons of masks:

Against masks: Breathing in your own carbon dioxide; depriving the body and brain of sufficient oxygen; build-up of toxins in the mask that can be breathed into the lungs and cause rashes on the face and 'mask-mouth'; breathing microplastic fibres and toxic chemicals into the lungs; dehumanisation and deleting individualisation by literally making people faceless; destroying human emotional interaction through facial expression and deleting parental connection with their babies which look for guidance to their facial expression.

For masks: They don't protect you from a 'virus' that doesn't exist and even if it did 'viral' particles are so minute they are smaller than the holes in the mask.

Governments, police, supermarkets, businesses, transport companies, and all the rest who seek to impose masks have done no risk assessment on their consequences for health and psychology and are now open to group lawsuits when the impact becomes clear with a cumulative epidemic of respiratory and other disease. Authorities will try to exploit these effects and hide the real cause by dubbing them 'Covid-19'. Can you imagine setting out to force the population to wear health-destroying masks without doing any assessment of the risks? It is criminal and it is evil, but then how many people targeted in this way, who see their children told to wear them all day at school, have asked for a risk assessment? Billions can't be imposed upon by the few unless the billions allow it. Oh, yes, with just a tinge of irony, 85 percent of all masks made worldwide come from *China*.

Wash your hands in toxic shite

'Covid' rules include the use of toxic sanitisers and again the health consequences of constantly applying toxins to be absorbed through the skin is obvious to any level of Renegade Mind. America's Food and Drug Administration (FDA) said that sanitisers are drugs and issued a warning about 75 dangerous brands which contain

methanol used in antifreeze and can cause death, kidney damage and blindness. The FDA circulated the following warning even for those brands that it claims to be safe:

Store hand sanitizer out of the reach of pets and children, and children should use it only with adult supervision. Do not drink hand sanitizer. This is particularly important for young children, especially toddlers, who may be attracted by the pleasant smell or brightly colored bottles of hand sanitizer.

Drinking even a small amount of hand sanitizer can cause alcohol poisoning in children. (However, there is no need to be concerned if your children eat with or lick their hands after using hand sanitizer.) During this coronavirus pandemic, poison control centers have had an increase in calls about accidental ingestion of hand sanitizer, so it is important that adults monitor young children's use.

Do not allow pets to swallow hand sanitizer. If you think your pet has eaten something potentially dangerous, call your veterinarian or a pet poison control center right away. Hand sanitizer is flammable and should be stored away from heat and flames. When using hand sanitizer, rub your hands until they feel completely dry before performing activities that may involve heat, sparks, static electricity, or open flames.

There you go, perfectly safe, then, and that's without even a mention of the toxins absorbed through the skin. Come on kids – sanitise your hands everywhere you go. It will save you from the 'virus'. Put all these elements together of the 'Covid' normal and see how much health and psychology is being cumulatively damaged, even devastated, to 'protect your health'. Makes sense, right? They are only imposing these things because they care, right? *Right?*

Submitting to insanity

Psychological reframing of the population goes very deep and is done in many less obvious ways. I hear people say how contradictory and crazy 'Covid' rules are and how they are ever changing. This is explained away by dismissing those involved as idiots. It is a big mistake. The Cult is delighted if its cold calculation is perceived as incompetence and idiocy when it is anything but. Oh, yes, there are idiots within the system – lots of them – but they are *administering* the Cult agenda, mostly unknowingly. They are not deciding and dictating it. The bulwark against tyranny is self-

respect, always has been, always will be. It is self-respect that has broken every tyranny in history. By its very nature self-respect will not bow to oppression and its perpetrators. There is so little self-respect that it's always the few that overturn dictators. Many may eventually follow, but the few with the iron spines (self-respect) kick it off and generate the momentum. The Cult targets self-respect in the knowledge that once this has gone only submission remains. Crazy, contradictory, ever-changing 'Covid' rules are systematically applied by psychologists to delete self-respect. They *want* you to see that the rules make no sense. It is one thing to decide to do something when *you* have made the choice based on evidence and logic. You still retain your self-respect. It is quite another when you can see what you are being told to do is insane, ridiculous and makes no sense, and *yet you still do it*. Your self-respect is extinguished and this has been happening as ever more obviously stupid and nonsensical things have been demanded and the great majority have complied even when they can see they are stupid and nonsensical.

People walk around in face-nappies knowing they are damaging their health and make no difference to a 'virus'. They do it in fear of not doing it. I know it's daft, but I'll do it anyway. When that happens something dies inside of you and submissive reframing has begun. Next there's a need to hide from yourself that you have conceded your self-respect and you convince yourself that you have not really submitted to fear and intimidation. You begin to believe that you are complying with craziness because it's the right thing to do. When first you concede your self-respect of 2+2 = 4 to 2+2 = 5 you *know* you are compromising your self-respect. Gradually to avoid facing that fact you begin to *believe* that 2+2=5. You have been reframed and I have been watching this process happening in the human psyche on an industrial scale. The Cult is working to break your spirit and one of its major tools in that war is humiliation. I read how former American soldier Bradley Manning (later Chelsea Manning after a sex-change) was treated after being jailed for supplying WikiLeaks with documents exposing the enormity of

government and elite mendacity. Manning was isolated in solitary confinement for eight months, put under 24-hour surveillance, forced to hand over clothing before going to bed, and stand naked for every roll call. This is systematic humiliation. The introduction of anal swab 'Covid' tests in China has been done for the same reason to delete self-respect and induce compliant submission. Anal swabs are mandatory for incoming passengers in parts of China and American diplomats have said they were forced to undergo the indignity which would have been calculated humiliation by the Cult-owned Chinese government that has America in its sights.

Government-people: An abusive relationship

Spirit-breaking psychological techniques include giving people hope and apparent respite from tyranny only to take it away again. This happened in the UK during Christmas, 2020, when the psycho-psychologists and their political lackeys announced an easing of restrictions over the holiday only to reimpose them almost immediately on the basis of yet another lie. There is a big psychological difference between getting used to oppression and being given hope of relief only to have that dashed. Psychologists know this and we have seen the technique used repeatedly. Then there is traumatising people before you introduce more extreme regulations that require compliance. A perfect case was the announcement by the dark and sinister Whitty and Vallance in the UK that 'new data' predicted that 4,000 could die every day over the winter of 2020/2021 if we did not lockdown again. I think they call it lying and after traumatising people with that claim out came Jackboot Johnson the next day with new curbs on human freedom. Psychologists know that a frightened and traumatised mind becomes suggestable to submission and behaviour reframing. Underpinning all this has been to make people fearful and suspicious of each other and see themselves as a potential danger to others. In league with deleted self-respect you have the perfect psychological recipe for self-loathing. The relationship between authority and public is now demonstrably the same as that of

subservience to an abusive partner. These are signs of an abusive relationship explained by psychologist Leslie Becker-Phelps:

Psychological and emotional abuse: Undermining a partner's self-worth with verbal attacks, name-calling, and belittling. Humiliating the partner in public, unjustly accusing them of having an affair, or interrogating them about their every behavior. Keeping partner confused or off balance by saying they were just kidding or blaming the partner for 'making' them act this way … Feigning in public that they care while turning against them in private. This leads to victims frequently feeling confused, incompetent, unworthy, hopeless, and chronically self-doubting. [Apply these techniques to how governments have treated the population since New Year, 2020, and the parallels are obvious.]

Physical abuse: The abuser might physically harm their partner in a range of ways, such as grabbing, hitting, punching, or shoving them. They might throw objects at them or harm them with a weapon. [Observe the physical harm imposed by masks, lockdown, and so on.]

Threats and intimidation: One way abusers keep their partners in line is by instilling fear. They might be verbally threatening, or give threatening looks or gestures. Abusers often make it known that they are tracking their partner's every move. They might destroy their partner's possessions, threaten to harm them, or threaten to harm their family members. Not surprisingly, victims of this abuse often feel anxiety, fear, and panic. [No words necessary.]

Isolation: Abusers often limit their partner's activities, forbidding them to talk or interact with friends or family. They might limit access to a car or even turn off their phone. All of this might be done by physically holding them against their will, but is often accomplished through psychological abuse and intimidation. The more isolated a person feels, the fewer resources they have to help gain perspective on their situation and to escape from it. [No words necessary.]

Economic abuse: Abusers often make their partners beholden to them for money by controlling access to funds of any kind. They might prevent their partner from getting a job or withhold access to money they earn from a job. This creates financial dependency that makes leaving the relationship very difficult. [See destruction of livelihoods and the proposed meagre 'guaranteed income' so long as you do whatever you are told.]

Using children: An abuser might disparage their partner's parenting skills, tell their children lies about their partner, threaten to take custody of their children, or threaten to harm their children. These tactics instil fear and often elicit compliance. [See reframed social service mafia and how children are being mercilessly abused by the state over 'Covid' while their parents look on too frightened to do anything.]

A further recurring trait in an abusive relationship is the abused blaming themselves for their abuse and making excuses for the abuser. We have the public blaming each other for lockdown abuse by government and many making excuses for the government while attacking those who challenge the government. How often we have heard authorities say that rules are being imposed or reimposed only because people have refused to 'behave' and follow the rules. We don't want to do it – it's *you*.

Renegade Minds are an antidote to all of these things. They will never concede their self-respect no matter what the circumstances. Even when apparent humiliation is heaped upon them they laugh in its face and reflect back the humiliation on the abuser where it belongs. Renegade Minds will never wear masks they know are only imposed to humiliate, suppress and damage both physically and psychologically. Consequences will take care of themselves and they will never break their spirit or cause them to concede to tyranny. UK newspaper columnist Peter Hitchens was one of the few in the mainstream media to speak out against lockdowns and forced vaccinations. He then announced he had taken the jab. He wanted to see family members abroad and he believed vaccine passports were inevitable even though they had not yet been introduced. Hitchens

has a questioning and critical mind, but not a Renegade one. If he had no amount of pressure would have made him concede. Hitchens excused his action by saying that the battle has been lost. Renegade Minds never accept defeat when freedom is at stake and even if they are the last one standing the self-respect of not submitting to tyranny is more important than any outcome or any consequence.

That's why Renegade Minds are the only minds that ever changed anything worth changing.

CHAPTER EIGHT

'Reframing' insanity

Insanity is relative. It depends on who has who locked in what cage
Ray Bradbury

'Reframing' a mind means simply to change its perception and behaviour. This can be done subconsciously to such an extent that subjects have no idea they have been 'reframed' while to any observer changes in behaviour and attitudes are obvious.

Human society is being reframed on a ginormous scale since the start of 2020 and here we have the reason why psychologists rather than doctors have been calling the shots. Ask most people who have succumbed to 'Covid' reframing if they have changed and most will say 'no'; but they *have* and fundamentally. The Cult's long-game has been preparing for these times since way back and crucial to that has been to prepare both population and officialdom mentally and emotionally. To use the mind-control parlance they had to reframe the population with a mentality that would submit to fascism and reframe those in government and law enforcement to impose fascism or at least go along with it. The result has been the fact-deleted mindlessness of 'Wokeness' and officialdom that has either enthusiastically or unquestioningly imposed global tyranny demanded by reframed politicians on behalf of psychopathic and deeply evil cultists. 'Cognitive reframing' identifies and challenges the way someone sees the world in the form of situations, experiences and emotions and then restructures those perceptions to view the same set of circumstances in a different way. This can have

benefits if the attitudes are personally destructive while on the other side it has the potential for individual and collective mind control which the subject has no idea has even happened.

Cognitive therapy was developed in the 1960s by Aaron T. Beck who was born in Rhode Island in 1921 as the son of Jewish immigrants from the Ukraine. He became interested in the techniques as a treatment for depression. Beck's daughter Judith S. Beck is prominent in the same field and they founded the Beck Institute for Cognitive Behavior Therapy in Philadelphia in 1994. Cognitive reframing, however, began to be used worldwide by those with a very dark agenda. The Cult reframes politicians to change their attitudes and actions until they are completely at odds with what they once appeared to stand for. The same has been happening to government administrators at all levels, law enforcement, military and the human population. Cultists love mind control for two main reasons: It allows them to control what people think, do and say to secure agenda advancement and, by definition, it calms their legendary insecurity and fear of the unexpected. I have studied mind control since the time I travelled America in 1996. I may have been talking to next to no one in terms of an audience in those years, but my goodness did I gather a phenomenal amount of information and knowledge about so many things including the techniques of mind control. I have described this in detail in other books going back to *The Biggest Secret* in 1998. I met a very large number of people recovering from MKUltra and its offshoots and successors and I began to see how these same techniques were being used on the population in general. This was never more obvious than since the 'Covid' hoax began.

Reframing the enforcers

I have observed over the last two decades and more the very clear transformation in the dynamic between the police, officialdom and the public. I tracked this in the books as the relationship mutated from one of serving the public to seeing them as almost the enemy and certainly a lower caste. There has always been a class divide

based on income and always been some psychopathic, corrupt, and big-I-am police officers. This was different. Wholesale change was unfolding in the collective dynamic; it was less about money and far more about position and perceived power. An us-and-them was emerging. Noses were lifted skyward by government administration and law enforcement and their attitude to the public they were *supposed* to be serving changed to one of increasing contempt, superiority and control. The transformation was so clear and widespread that it had to be planned. Collective attitudes and dynamics do not change naturally and organically that quickly on that scale. I then came across an organisation in Britain called Common Purpose created in the late 1980s by Julia Middleton who would work in the office of Deputy Prime Minister John Prescott during the long and disastrous premiership of war criminal Tony Blair. When Blair speaks the Cult is speaking and the man should have been in jail a long time ago. Common Purpose proclaims itself to be one of the biggest 'leadership development' organisations in the world while functioning as a *charity* with all the financial benefits which come from that. It hosts 'leadership development' courses and programmes all over the world and claims to have 'brought together' what it calls 'leaders' from more than 100 countries on six continents. The modus operandi of Common Purpose can be compared with the work of the UK government's reframing network that includes the Behavioural Insights Team 'nudge unit' and 'Covid' reframing specialists at SPI-B. WikiLeaks described Common Purpose long ago as 'a hidden virus in our government and schools' which is unknown to the general public: 'It recruits and trains "leaders" to be loyal to the directives of Common Purpose and the EU, instead of to their own departments, which they then undermine or subvert, the NHS [National Health Service] being an example.' This is a vital point to understand the 'Covid' hoax. The NHS, and its equivalent around the world, has been utterly reframed in terms of administrators and much of the medical personnel with the transformation underpinned by recruitment policies. The outcome has been the criminal and psychopathic behaviour of the

NHS over 'Covid' and we have seen the same in every other major country. WikiLeaks said Common Purpose trainees are 'learning to rule without regard to democracy' and to usher in a police state (current events explained). Common Purpose operated like a 'glue' and had members in the NHS, BBC, police, legal profession, church, many of Britain's 7,000 quangos, local councils, the Civil Service, government ministries and Parliament, and controlled many RDA's (Regional Development Agencies). Here we have one answer for how and why British institutions and their like in other countries have changed so negatively in relation to the public. This further explains how and why the beyond-disgraceful reframed BBC has become a propaganda arm of 'Covid' fascism. They are all part of a network pursuing the same goal.

By 2019 Common Purpose was quoting a figure of 85,000 'leaders' that had attended its programmes. These 'students' of all ages are known as Common Purpose 'graduates' and they consist of government, state and local government officials and administrators, police chiefs and officers, and a whole range of others operating within the national, local and global establishment. Cressida Dick, Commissioner of the London Metropolitan Police, is the Common Purpose graduate who was the 'Gold Commander' that oversaw what can only be described as the murder of Brazilian electrician Jean Charles de Menezes in 2005. He was held down by psychopathic police and shot seven times in the head by a psychopathic lunatic after being mistaken for a terrorist when he was just a bloke going about his day. Dick authorised officers to pursue and keep surveillance on de Menezes and ordered that he be stopped from entering the underground train system. Police psychopaths took her at her word clearly. She was 'disciplined' for this outrage by being *promoted* – eventually to the top of the 'Met' police where she has been a disaster. Many Chief Constables controlling the police in different parts of the UK are and have been Common Purpose graduates. I have heard the 'graduate' network described as a sort of Mafia or secret society operating within the fabric of government at all levels pursuing a collective policy

ingrained at Common Purpose training events. Founder Julia Middleton herself has said:

> Locally and internationally, Common Purpose graduates will be 'lighting small fires' to create change in their organisations and communities … The Common Purpose effect is best illustrated by the many stories of small changes brought about by leaders, who themselves have changed.

A Common Purpose mission statement declared:

> Common Purpose aims to improve the way society works by expanding the vision, decision-making ability and influence of all kinds of leaders. The organisation runs a variety of educational programmes for leaders of all ages, backgrounds and sectors, in order to provide them with the inspirational, information and opportunities they need to change the world.

Yes, but into what? Since 2020 the answer has become clear.

NLP and the Delphi technique

Common Purpose would seem to be a perfect name or would common programming be better? One of the foundation methods of reaching 'consensus' (group think) is by setting the agenda theme and then encouraging, cajoling or pressuring everyone to agree a 'consensus' in line with the core theme promoted by Common Purpose. The methodology involves the 'Delphi technique', or an adaption of it, in which opinions are expressed that are summarised by a 'facilitator or change agent' at each stage. Participants are 'encouraged' to modify their views in the light of what others have said. Stage by stage the former individual opinions are merged into group consensus which just happens to be what Common Purpose wants them to believe. A key part of this is to marginalise anyone refusing to concede to group think and turn the group against them to apply pressure to conform. We are seeing this very technique used on the general population to make 'Covid' group-thinkers hostile to those who have seen through the bullshit. People can be reframed by using perception manipulation methods such as Neuro-Linguistic Programming (NLP) in which you change perception with the use of

carefully constructed language. An NLP website described the technique this way:

… A method of influencing brain behaviour (the 'neuro' part of the phrase) through the use of language (the 'linguistic' part) and other types of communication to enable a person to 'recode' the way the brain responds to stimuli (that's the 'programming') and manifest new and better behaviours. Neuro-Linguistic Programming often incorporates hypnosis and self-hypnosis to help achieve the change (or 'programming') that is wanted.

British alternative media operation UKColumn has done very detailed research into Common Purpose over a long period. I quoted co-founder and former naval officer Brian Gerrish in my book *Remember Who You Are*, published in 2011, as saying the following years before current times:

It is interesting that many of the mothers who have had children taken by the State speak of the Social Services people being icily cool, emotionless and, as two ladies said in slightly different words, '… like little robots'. We know that NLP is cumulative, so people can be given small imperceptible doses of NLP in a course here, another in a few months, next year etc. In this way, major changes are accrued in their personality, but the day by day change is almost unnoticeable.

In these and other ways 'graduates' have had their perceptions uniformly reframed and they return to their roles in the institutions of government, law enforcement, legal profession, military, 'education', the UK National Health Service and the whole swathe of the establishment structure to pursue a common agenda preparing for the 'post-industrial', 'post-democratic' society. I say 'preparing' but we are now there. 'Post-industrial' is code for the Great Reset and 'post-democratic' is 'Covid' fascism. UKColumn has spoken to partners of those who have attended Common Purpose 'training'. They have described how personalities and attitudes of 'graduates' changed very noticeably for the worse by the time they had completed the course. They had been 'reframed' and told they are the 'leaders' – the special ones – who know better than the population. There has also been the very demonstrable recruitment of psychopaths and narcissists into government administration at all

levels and law enforcement. If you want psychopathy hire psychopaths and you get a simple cause and effect. If you want administrators, police officers and 'leaders' to perceive the public as lesser beings who don't matter then employ narcissists. These personalities are identified using 'psychometrics' that identifies knowledge, abilities, attitudes and personality traits, mostly through carefully-designed questionnaires and tests. As this policy has passed through the decades we have had power-crazy, power-trippers appointed into law enforcement, security and government administration in preparation for current times and the dynamic between public and law enforcement/officialdom has been transformed. UKColumn's Brian Gerrish said of the narcissistic personality:

> Their love of themselves and power automatically means that they will crush others who get in their way. I received a major piece of the puzzle when a friend pointed out that when they made public officials re-apply for their own jobs several years ago they were also required to do psychometric tests. This was undoubtedly the start of the screening process to get 'their' sort of people in post.

How obvious that has been since 2020 although it was clear what was happening long before if people paid attention to the changing public-establishment dynamic.

Change agents

At the centre of events in 'Covid' Britain is the National Health Service (NHS) which has behaved disgracefully in slavishly following the Cult agenda. The NHS management structure is awash with Common Purpose graduates or 'change agents' working to a common cause. Helen Bevan, a Chief of Service Transformation at the NHS Institute for Innovation and Improvement, co-authored a document called 'Towards a million change agents, a review of the social movements literature: implications for large scale change in the NHS'. The document compared a project management approach to that of change and social movements where 'people change

themselves and each other – peer to peer'. Two definitions given for a 'social movement' were:

A group of people who consciously attempt to build a radically new social order; involves people of a broad range of social backgrounds; and deploys politically confrontational and socially disruptive tactics – Cyrus Zirakzadeh 1997

Collective challenges, based on common purposes and social solidarities, in sustained interaction with elites, opponents, and authorities – Sidney Tarrow 1994

Helen Bevan wrote another NHS document in which she defined 'framing' as 'the process by which leaders construct, articulate and put across their message in a powerful and compelling way in order to win people to their cause and call them to action'. I think I could come up with another definition that would be rather more accurate. The National Health Service and institutions of Britain and the wider world have been taken over by reframed 'change agents' and that includes everything from the United Nations to national governments, local councils and social services which have been kidnapping children from loving parents on an extraordinary and gathering scale on the road to the end of parenthood altogether. Children from loving homes are stolen and kidnapped by the state and put into the 'care' (inversion) of the local authority through council homes, foster parents and forced adoption. At the same time children are allowed to be abused without response while many are under council 'care'. UKColumn highlighted the Common Purpose connection between South Yorkshire Police and Rotherham council officers in the case of the scandal in that area of the sexual exploitation of children to which the authorities turned not one blind eye, but both:

We were alarmed to discover that the Chief Executive, the Strategic Director of Children and Young People's Services, the Manager for the Local Strategic Partnership, the Community Cohesion Manager, the Cabinet Member for Cohesion, the Chief Constable and his predecessor had all attended Leadership training courses provided by the pseudo-charity Common Purpose.

Once 'change agents' have secured positions of hire and fire within any organisation things start to move very quickly. Personnel are then hired and fired on the basis of whether they will work towards the agenda the change agent represents. If they do they are rapidly promoted even though they may be incompetent. Those more qualified and skilled who are pre-Common Purpose 'old school' see their careers stall and even disappear. This has been happening for decades in every institution of state, police, 'health' and social services and all of them have been transformed as a result in their attitudes to their jobs and the public. Medical professions, including nursing, which were once vocations for the caring now employ many cold, callous and couldn't give a shit personality types. The UKColumn investigation concluded:

By blurring the boundaries between people, professions, public and private sectors, responsibility and accountability, Common Purpose encourages 'graduates' to believe that as new selected leaders, they can work together, outside of the established political and social structures, to achieve a paradigm shift or CHANGE – so called 'Leading Beyond Authority'. In doing so, the allegiance of the individual becomes 'reframed' on CP colleagues and their NETWORK.

Reframing the Face-Nappies

Nowhere has this process been more obvious than in the police where recruitment of psychopaths and development of unquestioning mind-controlled group-thinkers have transformed law enforcement into a politically-correct 'Woke' joke and a travesty of what should be public service. Today they wear their face-nappies like good little gofers and enforce 'Covid' rules which are fascism under another name. Alongside the specifically-recruited psychopaths we have software minds incapable of free thought. Brian Gerrish again:

An example is the policeman who would not get on a bike for a press photo because he had not done the cycling proficiency course. Normal people say this is political correctness gone mad. Nothing could be further from the truth. The policeman has been reframed, and in his reality it is perfect common sense not to get on the bike 'because he hasn't done the cycling course'.

Another example of this is where the police would not rescue a boy from a pond until they had taken advice from above on the 'risk assessment'. A normal person would have arrived, perhaps thought of the risk for a moment, and dived in. To the police now 'reframed', they followed 'normal' procedure.

There are shocking cases of reframed ambulance crews doing the same. Sheer unthinking stupidity of London Face-Nappies headed by Common Purpose graduate Cressida Dick can be seen in their behaviour at a vigil in March, 2021, for a murdered woman, Sarah Everard. A police officer had been charged with the crime. Anyone with a brain would have left the vigil alone in the circumstances. Instead they 'manhandled' women to stop them breaking 'Covid rules' to betray classic reframing. Minds in the thrall of perception control have no capacity for seeing a situation on its merits and acting accordingly. 'Rules is rules' is their only mind-set. My father used to say that rules and regulations are for the guidance of the intelligent and the blind obedience of the idiot. Most of the intelligent, decent, coppers have gone leaving only the other kind and a few old school for whom the job must be a daily nightmare. The combination of psychopaths and rule-book software minds has been clearly on public display in the 'Covid' era with automaton robots in uniform imposing fascistic 'Covid' regulations on the population without any personal initiative or judging situations on their merits. There are thousands of examples around the world, but I'll make my point with the infamous Derbyshire police in the English East Midlands – the ones who think pouring dye into beauty spots and using drones to track people walking in the countryside away from anyone is called 'policing'. To them there are rules decreed by the government which they have to enforce and in their bewildered state a group gathering in a closed space and someone walking alone in the countryside are the same thing. It is beyond idiocy and enters the realm of clinical insanity.

Police officers in Derbyshire said they were 'horrified' – *horrified* – to find 15 to 20 'irresponsible' kids playing a football match at a closed leisure centre 'in breach of coronavirus restrictions'. When they saw the police the kids ran away leaving their belongings behind and the reframed men and women of Derbyshire police were seeking to establish their identities with a view to fining their parents. The most natural thing for youngsters to do – kicking a ball about – is turned into a criminal activity and enforced by the moronic software programs of Derbyshire police. You find the same mentality in every country. These barely conscious 'horrified' officers said they had to take action because 'we need to ensure these rules are being followed' and 'it is of the utmost importance that you ensure your children are following the rules and regulations for Covid-19'. Had any of them done ten seconds of research to see if this parroting of their masters' script could be supported by any evidence? Nope. Reframed people don't think – others think for them and that's the whole idea of reframing. I have seen police officers one after the other repeating without question word for word what officialdom tells them just as I have seen great swathes of the public doing the same. Ask either for 'their' opinion and out spews what they have been told to think by the official narrative. Police and public may seem to be in different groups, but their mentality is the same. Most people do whatever they are told in fear not doing so or because they believe what officialdom tells them; almost the entirety of the police do what they are told for the same reason. Ultimately it's the tiny inner core of the global Cult that's telling both what to do.

So Derbyshire police were 'horrified'. Oh, really? Why did they think those kids were playing football? It was to relieve the psychological consequences of lockdown and being denied human contact with their friends and interaction, touch and discourse vital to human psychological health. Being denied this month after month has dismantled the psyche of many children and young people as depression and suicide have exploded. Were Derbyshire police *horrified by that*? Are you kidding? Reframed people don't have those

mental and emotional processes that can see how the impact on the psychological health of youngsters is far more dangerous than any 'virus' even if you take the mendacious official figures to be true. The reframed are told (programmed) how to act and so they do. The Derbyshire Chief Constable in the first period of lockdown when the black dye and drones nonsense was going on was Peter Goodman. He was the man who severed the connection between his force and the Derbyshire Constabulary *Male Voice* Choir when he decided that it was not inclusive enough to allow women to join. The fact it was a male voice choir making a particular sound produced by male voices seemed to elude a guy who terrifyingly ran policing in Derbyshire. He retired weeks after his force was condemned as disgraceful by former Supreme Court Justice Jonathan Sumption for their behaviour over extreme lockdown impositions. Goodman was replaced by his deputy Rachel Swann who was in charge when her officers were 'horrified'. The police statement over the boys committing the hanging-offence of playing football included the line about the youngsters being 'irresponsible in the times we are all living through' missing the point that the real relevance of the 'times we are all living through' is the imposition of fascism enforced by psychopaths and reframed minds of police officers playing such a vital part in establishing the fascist tyranny that their own children and grandchildren will have to live in their entire lives. As a definition of insanity that is hard to beat although it might be run close by imposing masks on people that can have a serious effect on their health while wearing a face nappy all day themselves. Once again public and police do it for the same reason – the authorities tell them to and who are they to have the self-respect to say no?

Wokers in uniform

How reframed do you have to be to arrest a *six-year-old* and take him to court for *picking a flower* while waiting for a bus? Brain dead police and officialdom did just that in North Carolina where criminal proceedings happen regularly for children under nine. Attorney Julie Boyer gave the six-year-old crayons and a colouring book

during the 'flower' hearing while the 'adults' decided his fate. County Chief District Court Judge Jay Corpening asked: 'Should a child that believes in Santa Claus, the Easter Bunny and the tooth fairy be making life-altering decisions?' Well, of course not, but common sense has no meaning when you have a common purpose and a reframed mind. Treating children in this way, and police operating in American schools, is all part of the psychological preparation for children to accept a police state as normal all their adult lives. The same goes for all the cameras and biometric tracking technology in schools. Police training is focused on reframing them as snowflake Wokers and this is happening in the military. Pentagon top brass said that 'training sessions on extremism' were needed for troops who asked why they were so focused on the Capitol Building riot when Black Lives Matter riots were ignored. What's the difference between them some apparently and rightly asked. Actually, there is a difference. Five people died in the Capitol riot, only one through violence, and that was a police officer shooting an unarmed protestor. BLM riots killed at least 25 people and cost billions. Asking the question prompted the psychopaths and reframed minds that run the Pentagon to say that more 'education' (programming) was needed. Troop training is all based on psychological programming to make them fodder for the Cult – 'Military men are just dumb, stupid animals to be used as pawns in foreign policy' as Cult-to-his-DNA former Secretary of State Henry Kissinger famously said. Governments see the police in similar terms and it's time for those among them who can see this to defend the people and stop being enforcers of the Cult agenda upon the people.

The US military, like the country itself, is being targeted for destruction through a long list of Woke impositions. Cult-owned gaga 'President' Biden signed an executive order when he took office to allow taxpayer money to pay for transgender surgery for active military personnel and veterans. Are you a man soldier? No, I'm a LGBTQIA+ with a hint of Skoliosexual and Spectrasexual. Oh, good man. Bad choice of words you bigot. The Pentagon announced in March, 2021, the appointment of the first 'diversity and inclusion

officer' for US Special Forces. Richard Torres-Estrada arrived with the publication of a 'D&I Strategic Plan which will guide the enterprise-wide effort to institutionalize and sustain D&I'. If you think a Special Forces 'Strategic Plan' should have something to do with defending America you haven't been paying attention. Defending Woke is now the military's new role. Torres-Estrada has posted images comparing Donald Trump with Adolf Hitler and we can expect no bias from him as a representative of the supposedly non-political Pentagon. Cable news host Tucker Carlson said: 'The Pentagon is now the Yale faculty lounge but with cruise missiles.' Meanwhile Secretary of Defense Lloyd Austin, a board member of weapons-maker Raytheon with stock and compensation interests in October, 2020, worth $1.4 million, said he was purging the military of the 'enemy within' – anyone who isn't Woke and supports Donald Trump. Austin refers to his targets as 'racist extremists' while in true Woke fashion being himself a racist extremist. Pentagon documents pledge to 'eradicate, eliminate and conquer all forms of racism, sexism and homophobia'. The definitions of these are decided by 'diversity and inclusion committees' peopled by those who see racism, sexism and homophobia in every situation and opinion. Woke (the Cult) is dismantling the US military and purging testosterone as China expands its military and gives its troops 'masculinity training'. How do we think that is going to end when this is all Cult coordinated? The US military, like the British military, is controlled by Woke and spineless top brass who just go along with it out of personal career interests.

'Woke' means fast asleep

Mind control and perception manipulation techniques used on individuals to create group-think have been unleashed on the global population in general. As a result many have no capacity to see the obvious fascist agenda being installed all around them or what 'Covid' is really all about. Their brains are firewalled like a computer system not to process certain concepts, thoughts and realisations that are bad for the Cult. The young are most targeted as the adults they

will be when the whole fascist global state is planned to be fully implemented. They need to be prepared for total compliance to eliminate all pushback from entire generations. The Cult has been pouring billions into taking complete control of 'education' from schools to universities via its operatives and corporations and not least Bill Gates as always. The plan has been to transform 'education' institutions into programming centres for the mentality of 'Woke'. James McConnell, professor of psychology at the University of Michigan, wrote in *Psychology Today* in 1970:

> The day has come when we can combine sensory deprivation with drugs, hypnosis, and astute manipulation of reward and punishment, to gain almost absolute control over an individual's behaviour. It should then be possible to achieve a very rapid and highly effective type of brainwashing that would allow us to make dramatic changes in a person's behaviour and personality ...
>
> … We should reshape society so that we all would be trained from birth to want to do what society wants us to do. We have the techniques to do it... no-one owns his own personality you acquired, and there's no reason to believe you should have the right to refuse to acquire a new personality if your old one is anti-social.

This was the potential for mass brainwashing in 1970 and the mentality there displayed captures the arrogant psychopathy that drives it forward. I emphasise that not all young people have succumbed to Woke programming and those that haven't are incredibly impressive people given that today's young are the most perceptually-targeted generations in history with all the technology now involved. Vast swathes of the young generations, however, have fallen into the spell – and that's what it is – of Woke. The Woke mentality and perceptual program is founded on *inversion* and you will appreciate later why that is so significant. Everything with Woke is inverted and the opposite of what it is claimed to be. Woke was a term used in African-American culture from the 1900s and referred to an awareness of social and racial justice. This is not the meaning of the modern version or 'New Woke' as I call it in *The Answer*. Oh, no, Woke today means something very different no matter how much Wokers may seek to hide that and insist Old Woke and New

Woke are the same. See if you find any 'awareness of social justice' here in the modern variety:

- Woke demands 'inclusivity' while excluding anyone with a different opinion and calls for mass censorship to silence other views.

- Woke claims to stand against oppression when imposing oppression is the foundation of all that it does. It is the driver of political correctness which is nothing more than a Cult invention to manipulate the population to silence itself.

- Woke believes itself to be 'liberal' while pursuing a global society that can only be described as fascist (see 'anti-fascist' fascist Antifa).

- Woke calls for 'social justice' while spreading injustice wherever it goes against the common 'enemy' which can be easily identified as a differing view.

- Woke is supposed to be a metaphor for 'awake' when it is solid-gold asleep and deep in a Cult-induced coma that meets the criteria for 'off with the fairies'.

I state these points as obvious facts if people only care to look. I don't do this with a sense of condemnation. We need to appreciate that the onslaught of perceptual programming on the young has been incessant and merciless. I can understand why so many have been reframed, or, given their youth, framed from the start to see the world as the Cult demands. The Cult has had access to their minds day after day in its 'education' system for their entire formative years. Perception is formed from information received and the Cult-created system is a life-long download of information delivered to elicit a particular perception, thus behaviour. The more this has expanded into still new extremes in recent decades and ever-increasing censorship has deleted other opinions and information why wouldn't that lead to a perceptual reframing on a mass scale? I

have described already cradle-to-grave programming and in more recent times the targeting of young minds from birth to adulthood has entered the stratosphere. This has taken the form of skewing what is 'taught' to fit the Cult agenda and the omnipresent techniques of group-think to isolate non-believers and pressure them into line. There has always been a tendency to follow the herd, but we really are in a new world now in relation to that. We have parents who can see the 'Covid' hoax told by their children not to stop them wearing masks at school, being 'Covid' tested or having the 'vaccine' in fear of the peer-pressure consequences of being different. What is 'peer-pressure' if not pressure to conform to group-think? Renegade Minds never group-think and always retain a set of perceptions that are unique to them. Group-think is always underpinned by consequences for not group-thinking. Abuse now aimed at those refusing DNA-manipulating 'Covid vaccines' are a potent example of this. The biggest pressure to conform comes from the very group which is itself being manipulated. 'I am programmed to be part of a hive mind and so you must be.'

Woke control structures in 'education' now apply to every mainstream organisation. Those at the top of the 'education' hierarchy (the Cult) decide the policy. This is imposed on governments through the Cult network; governments impose it on schools, colleges and universities; their leadership impose the policy on teachers and academics and they impose it on children and students. At any level where there is resistance, perhaps from a teacher or university lecturer, they are targeted by the authorities and often fired. Students themselves regularly demand the dismissal of academics (increasingly few) at odds with the narrative that the students have been programmed to believe in. It is quite a thought that students who are being targeted by the Cult become so consumed by programmed group-think that they launch protests and demand the removal of those who are trying to push back against those targeting the students. Such is the scale of perceptual inversion. We see this with 'Covid' programming as the Cult imposes the rules via psycho-psychologists and governments on

shops, transport companies and businesses which impose them on their staff who impose them on their customers who pressure Pushbackers to conform to the will of the Cult which is in the process of destroying them and their families. Scan all aspects of society and you will see the same sequence every time.

Fact free Woke and hijacking the 'left'

There is no more potent example of this than 'Woke', a mentality only made possible by the deletion of factual evidence by an 'education' system seeking to produce an ever more uniform society. Why would you bother with facts when you don't know any? Deletion of credible history both in volume and type is highly relevant. Orwell said: 'Who controls the past controls the future: who controls the present controls the past.' They who control the perception of the past control the perception of the future and they who control the present control the perception of the past through the writing and deleting of history. Why would you oppose the imposition of Marxism in the name of Wokeism when you don't know that Marxism cost at least 100 million lives in the 20th century alone? Watch videos and read reports in which Woker generations are asked basic historical questions – it's mind-blowing. A survey of 2,000 people found that six percent of millennials (born approximately early1980s to early 2000s) believed the Second World War (1939-1945) broke out with the assassination of President Kennedy (in 1963) and one in ten thought Margaret Thatcher was British Prime Minister at the time. She was in office between 1979 and 1990. We are in a post-fact society. Provable facts are no defence against the fascism of political correctness or Silicon Valley censorship. Facts don't matter anymore as we have witnessed with the 'Covid' hoax. Sacrificing uniqueness to the Woke group-think religion is all you are required to do and that means thinking for yourself is the biggest Woke no, no. All religions are an expression of group-think and censorship and Woke is just another religion with an orthodoxy defended by group-think and censorship. Burned at

the stake becomes burned on Twitter which leads back eventually to burned at the stake as Woke humanity regresses to ages past.

The biggest Woke inversion of all is its creators and funders. I grew up in a traditional left of centre political household on a council estate in Leicester in the 1950s and 60s – you know, the left that challenged the power of wealth-hoarding elites and threats to freedom of speech and opinion. In those days students went on marches defending freedom of speech while today's Wokers march for its deletion. What on earth could have happened? Those very elites (collectively the Cult) that we opposed in my youth and early life have funded into existence the antithesis of that former left and hijacked the 'brand' while inverting everything it ever stood for. We have a mentality that calls itself 'liberal' and 'progressive' while acting like fascists. Cult billionaires and their corporations have funded themselves into control of 'education' to ensure that Woke programming is unceasing throughout the formative years of children and young people and that non-Wokers are isolated (that word again) whether they be students, teachers or college professors. The Cult has funded into existence the now colossal global network of Woke organisations that have spawned and promoted all the 'causes' on the Cult wish-list for global transformation and turned Wokers into demanders of them. Does anyone really think it's a coincidence that the Cult agenda for humanity is a carbon (sorry) copy of the societal transformations desired by Woke?? These are only some of them:

Political correctness: The means by which the Cult deletes all public debates that it knows it cannot win if we had the free-flow of information and evidence.

Human-caused 'climate change': The means by which the Cult seeks to transform society into a globally-controlled dictatorship imposing its will over the fine detail of everyone's lives 'to save the planet' which doesn't actually need saving.

Transgender obsession: Preparing collective perception to accept the 'new human' which would not have genders because it would be created technologically and not through procreation. I'll have much more on this in Human 2.0.

Race obsession: The means by which the Cult seeks to divide and rule the population by triggering racial division through the perception that society is more racist than ever when the opposite is the case. Is it perfect in that regard? No. But to compare today with the racism of apartheid and segregation brought to an end by the civil rights movement in the 1960s is to insult the memory of that movement and inspirations like Martin Luther King. Why is the 'anti-racism' industry (which it is) so dominated by privileged white people?

White supremacy: This is a label used by privileged white people to demonise poor and deprived white people pushing back on tyranny to marginalise and destroy them. White people are being especially targeted as the dominant race by number within Western society which the Cult seeks to transform in its image. If you want to change a society you must weaken and undermine its biggest group and once you have done that by using the other groups you next turn on them to do the same … 'Then they came for the Jews and I was not a Jew so I did nothing.'

Mass migration: The mass movement of people from the Middle East, Africa and Asia into Europe, from the south into the United States and from Asia into Australia are another way the Cult seeks to dilute the racial, cultural and political influence of white people on Western society. White people ask why their governments appear to be working against them while being politically and culturally biased towards incoming cultures. Well, here's your answer. In the same way sexually 'straight' people, men and women, ask why the

authorities are biased against them in favour of other sexualities. The answer is the same – that's the way the Cult wants it to be for very sinister motives.

These are all central parts of the Cult agenda and central parts of the Woke agenda and Woke was created and continues to be funded to an immense degree by Cult billionaires and corporations. If anyone begins to say 'coincidence' the syllables should stick in their throat.

Billionaire 'social justice warriors'

Joe Biden is a 100 percent-owned asset of the Cult and the Wokers' man in the White House whenever he can remember his name and for however long he lasts with his rapidly diminishing cognitive function. Even walking up the steps of an aircraft without falling on his arse would appear to be a challenge. He's not an empty-shell puppet or anything. From the minute Biden took office (or the Cult did) he began his executive orders promoting the Woke wish-list. You will see the Woke agenda imposed ever more severely because it's really the *Cult* agenda. Woke organisations and activist networks spawned by the Cult are funded to the extreme so long as they promote what the Cult wants to happen. Woke is funded to promote 'social justice' by billionaires who become billionaires by destroying social justice. The social justice mantra is only a cover for dismantling social justice and funded by billionaires that couldn't give a damn about social justice. Everything makes sense when you see that. One of Woke's premier funders is Cult billionaire financier George Soros who said: 'I am basically there to make money, I cannot and do not look at the social consequences of what I do.' This is the same Soros who has given more than $32 billion to his Open Society Foundations global Woke network and funded Black Lives Matter, mass immigration into Europe and the United States, transgender activism, climate change activism, political correctness and groups targeting 'white supremacy' in the form of privileged white thugs that dominate Antifa. What a scam it all is and when

you are dealing with the unquestioning fact-free zone of Woke scamming them is child's play. All you need to pull it off in all these organisations are a few in-the-know agents of the Cult and an army of naïve, reframed, uninformed, narcissistic, know-nothings convinced of their own self-righteousness, self-purity and virtue.

Soros and fellow billionaires and billionaire corporations have poured hundreds of millions into Black Lives Matter and connected groups and promoted them to a global audience. None of this is motivated by caring about black people. These are the billionaires that have controlled and exploited a system that leaves millions of black people in abject poverty and deprivation which they do absolutely nothing to address. The same Cult networks funding BLM were behind the *slave trade!* Black Lives Matter hijacked a phrase that few would challenge and they have turned this laudable concept into a political weapon to divide society. You know that BLM is a fraud when it claims that *All* Lives Matter, the most inclusive statement of all, is 'racist'. BLM and its Cult masters don't want to end racism. To them it's a means to an end to control all of humanity never mind the colour, creed, culture or background. What has destroying the nuclear family got to do with ending racism? Nothing – but that is one of the goals of BLM and also happens to be a goal of the Cult as I have been exposing in my books for decades. Stealing children from loving parents and giving schools ever more power to override parents is part of that same agenda. BLM is a Marxist organisation and why would that not be the case when the Cult created Marxism *and* BLM? Patrisse Cullors, a BLM co-founder, said in a 2015 video that she and her fellow organisers, including co-founder Alicia Garza, are 'trained Marxists'. The lady known after marriage as Patrisse Khan-Cullors bought a $1.4 million home in 2021 in one of the whitest areas of California with a black population of just 1.6 per cent and has so far bought *four* high-end homes for a total of $3.2 million. How very Marxist. There must be a bit of spare in the BLM coffers, however, when Cult corporations and billionaires have handed over the best part of $100 million. Many black people can see that Black Lives Matter is not

working for them, but against them, and this is still more confirmation. Black journalist Jason Whitlock, who had his account suspended by Twitter for simply linking to the story about the 'Marxist's' home buying spree, said that BLM leaders are 'making millions of dollars off the backs of these dead black men who they wouldn't spit on if they were on fire and alive'.

Black Lies Matter

Cult assets and agencies came together to promote BLM in the wake of the death of career criminal George Floyd who had been jailed a number of times including for forcing his way into the home of a black woman with others in a raid in which a gun was pointed at her stomach. Floyd was filmed being held in a Minneapolis street in 2020 with the knee of a police officer on his neck and he subsequently died. It was an appalling thing for the officer to do, but the same technique has been used by police on peaceful protestors of lockdown without any outcry from the Woke brigade. As unquestioning supporters of the Cult agenda Wokers have supported lockdown and all the 'Covid' claptrap while attacking anyone standing up to the tyranny imposed in its name. Court documents would later include details of an autopsy on Floyd by County Medical Examiner Dr Andrew Baker who concluded that Floyd had taken a fatal level of the drug fentanyl. None of this mattered to fact-free, question-free, Woke. Floyd's death was followed by worldwide protests against police brutality amid calls to defund the police. Throwing babies out with the bathwater is a Woke speciality. In the wake of the murder of British woman Sarah Everard a Green Party member of the House of Lords, Baroness Jones of Moulescoomb (Nincompoopia would have been better), called for a 6pm curfew for all men. This would be in breach of the Geneva Conventions on war crimes which ban collective punishment, but that would never have crossed the black and white Woke mind of Baroness Nincompoopia who would have been far too convinced of her own self-righteousness to compute such details. Many American cities did defund the police in the face of Floyd riots

and after $15 million was deleted from the police budget in Washington DC under useless Woke mayor Muriel Bowser car-jacking alone rose by 300 percent and within six months the US capital recorded its highest murder rate in 15 years. The same happened in Chicago and other cities in line with the Cult/Soros plan to bring fear to streets and neighbourhoods by reducing the police, releasing violent criminals and not prosecuting crime. This is the mob-rule agenda that I have warned in the books was coming for so long. Shootings in the area of Minneapolis where Floyd was arrested increased by 2,500 percent compared with the year before. Defunding the police over George Floyd has led to a big increase in dead people with many of them black. Police protection for politicians making these decisions stayed the same or increased as you would expect from professional hypocrites. The Cult doesn't actually want to abolish the police. It wants to abolish local control over the police and hand it to federal government as the psychopaths advance the Hunger Games Society. Many George Floyd protests turned into violent riots with black stores and businesses destroyed by fire and looting across America fuelled by Black Lives Matter. Woke doesn't do irony. If you want civil rights you must loot the liquor store and the supermarket and make off with a smart TV. It's the only way.

It's not a race war – it's a class war

Black people are patronised by privileged blacks and whites alike and told they are victims of white supremacy. I find it extraordinary to watch privileged blacks supporting the very system and bloodline networks behind the slave trade and parroting the same Cult-serving manipulative crap of their privileged white, often billionaire, associates. It is indeed not a race war but a class war and colour is just a diversion. Black Senator Cory Booker and black Congresswoman Maxine Waters, more residents of Nincompoopia, personify this. Once you tell people they are victims of someone else you devalue both their own responsibility for their plight and the power they have to impact on their reality and experience. Instead

we have: 'You are only in your situation because of whitey – turn on them and everything will change.' It won't change. Nothing changes in our lives unless *we* change it. Crucial to that is never seeing yourself as a victim and always as the creator of your reality. Life is a simple sequence of choice and consequence. Make different choices and you create different consequences. *You* have to make those choices – not Black Lives Matter, the Woke Mafia and anyone else that seeks to dictate your life. Who are they these Wokers, an emotional and psychological road traffic accident, to tell you what to do? Personal empowerment is the last thing the Cult and its Black Lives Matter want black people or anyone else to have. They claim to be defending the underdog while *creating* and perpetuating the underdog. The Cult's worst nightmare is human unity and if they are going to keep blacks, whites and every other race under economic servitude and control then the focus must be diverted from what they have in common to what they can be manipulated to believe divides them. Blacks have to be told that their poverty and plight is the fault of the white bloke living on the street in the same poverty and with the same plight they are experiencing. The difference is that your plight black people is due to him, a white supremacist with 'white privilege' living on the street. Don't unite as one human family against your mutual oppressors and suppressors – fight the oppressor with the white face who is as financially deprived as you are. The Cult knows that as its 'Covid' agenda moves into still new levels of extremism people are going to respond and it has been spreading the seeds of disunity everywhere to stop a united response to the evil that targets *all of us*.

Racist attacks on 'whiteness' are getting ever more outrageous and especially through the American Democratic Party which has an appalling history for anti-black racism. Barack Obama, Joe Biden, Hillary Clinton and Nancy Pelosi all eulogised about Senator Robert Byrd at his funeral in 2010 after a nearly 60-year career in Congress. Byrd was a brutal Ku Klux Klan racist and a violent abuser of Cathy O'Brien in MKUltra. He said he would never fight in the military 'with a negro by my side' and 'rather I should die a thousand times,

and see Old Glory trampled in the dirt never to rise again, than to see this beloved land of ours become degraded by race mongrels, a throwback to the blackest specimen from the wilds'. Biden called Byrd a 'very close friend and mentor'. These 'Woke' hypocrites are not anti-racist they are anti-poor and anti-people not of their perceived class. Here is an illustration of the scale of anti-white racism to which we have now descended. Seriously Woke and moronic *New York Times* contributor Damon Young described whiteness as a 'virus' that 'like other viruses will not die until there are no bodies left for it to infect'. He went on: '… the only way to stop it is to locate it, isolate it, extract it, and kill it.' Young can say that as a black man with no consequences when a white man saying the same in reverse would be facing a jail sentence. *That's* racism. We had super-Woke numbskull senators Tammy Duckworth and Mazie Hirono saying they would object to future Biden Cabinet appointments if he did not nominate more Asian Americans and Pacific Islanders. Never mind the ability of the candidate what do they look like? Duckworth said: 'I will vote for racial minorities and I will vote for LGBTQ, but anyone else I'm not voting for.' Appointing people on the grounds of race is illegal, but that was not a problem for this ludicrous pair. They were on-message and that's a free pass in any situation.

Critical race racism

White children are told at school they are intrinsically racist as they are taught the divisive 'critical race theory'. This claims that the law and legal institutions are inherently racist and that race is a socially constructed concept used by white people to further their economic and political interests at the expense of people of colour. White is a 'virus' as we've seen. Racial inequality results from 'social, economic, and legal differences that white people create between races to maintain white interests which leads to poverty and criminality in minority communities'. I must tell that to the white guy sleeping on the street. The principal of East Side Community School in New York sent white parents a manifesto that called on

them to become 'white traitors' and advocate for full 'white abolition'. These people are teaching your kids when they urgently need a psychiatrist. The 'school' included a chart with 'eight white identities' that ranged from 'white supremacist' to 'white abolition' and defined the behaviour white people must follow to end 'the regime of whiteness'. Woke blacks and their privileged white associates are acting exactly like the slave owners of old and Ku Klux Klan racists like Robert Byrd. They are too full of their own self-purity to see that, but it's true. Racism is not a body type; it's a state of mind that can manifest through any colour, creed or culture.

Another racial fraud is *'equity'*. Not equality of treatment and opportunity – equity. It's a term spun as equality when it means something very different. Equality in its true sense is a raising up while 'equity' is a race to the bottom. Everyone in the same level of poverty is 'equity'. Keep everyone down – that's equity. The Cult doesn't want anyone in the human family to be empowered and BLM leaders, like all these 'anti-racist' organisations, continue their privileged, pampered existence by perpetuating the perception of gathering racism. When is the last time you heard an 'anti-racist' or 'anti-Semitism' organisation say that acts of racism and discrimination have *fallen?* It's not in the interests of their fund-raising and power to influence and the same goes for the professional soccer anti-racism operation, Kick It Out. Two things confirmed that the Black Lives Matter riots in the summer of 2020 were Cult creations. One was that while anti-lockdown protests were condemned in this same period for 'transmitting 'Covid' the authorities supported mass gatherings of Black Lives Matter supporters. I even saw self-deluding people claiming to be doctors say the two types of protest were not the same. No – the non-existent 'Covid' was in favour of lockdowns and attacked those that protested against them while 'Covid' supported Black Lives Matter and kept well away from its protests. The whole thing was a joke and as lockdown protestors were arrested, often brutally, by reframed Face-Nappies we had the grotesque sight of police officers taking the knee to Black Lives Matter, a Cult-funded Marxist

organisation that supports violent riots and wants to destroy the nuclear family and white people.

He's not white? Shucks!

Woke obsession with race was on display again when ten people were shot dead in Boulder, Colorado, in March, 2021. Cult-owned Woke TV channels like CNN said the shooter appeared to be a white man and Wokers were on Twitter condemning 'violent white men' with the usual mantras. Then the shooter's name was released as Ahmad Al Aliwi Alissa, an anti-Trump Arab-American, and the sigh of disappointment could be heard five miles away. Never mind that ten people were dead and what that meant for their families. Race baiting was all that mattered to these sick Cult-serving people like Barack Obama who exploited the deaths to further divide America on racial grounds which is his job for the Cult. This is the man that 'racist' white Americans made the first black president of the United States and then gave him a second term. Not-very-bright Obama has become filthy rich on the back of that and today appears to have a big influence on the Biden administration. Even so he's still a downtrodden black man and a victim of white supremacy. This disingenuous fraud reveals the contempt he has for black people when he puts on a Deep South Alabama accent whenever he talks to them, no, *at* them.

Another BLM red flag was how the now fully-Woke (fully-Cult) and fully-virtue-signalled professional soccer authorities had their teams taking the knee before every match in support of Marxist Black Lives Matter. Soccer authorities and clubs displayed 'Black Lives Matter' on the players' shirts and flashed the name on electronic billboards around the pitch. Any fans that condemned what is a Freemasonic taking-the-knee ritual were widely condemned as you would expect from the Woke virtue-signallers of professional sport and the now fully-Woke media. We have reverse racism in which you are banned from criticising any race or culture except for white people for whom anything goes – say what you like, no problem. What has this got to do with racial harmony and

equality? We've had black supremacists from Black Lives Matter telling white people to fall to their knees in the street and apologise for their white supremacy. Black supremacists acting like white supremacist slave owners of the past couldn't breach their self-obsessed, race-obsessed sense of self-purity. Joe Biden appointed a race-obsessed black supremacist Kristen Clarke to head the Justice Department Civil Rights Division. Clarke claimed that blacks are endowed with 'greater mental, physical and spiritual abilities' than whites. If anyone reversed that statement they would be vilified. Clarke is on-message so no problem. She's never seen a black-white situation in which the black figure is anything but a virtuous victim and she heads the Civil Rights Division which should treat everyone the same or it isn't civil rights. Another perception of the Renegade Mind: If something or someone is part of the Cult agenda they will be supported by Woke governments and media no matter what. If they're not, they will be condemned and censored. It really is that simple and so racist Clarke prospers despite (make that because of) her racism.

The end of culture

Biden's administration is full of such racial, cultural and economic bias as the Cult requires the human family to be divided into warring factions. We are now seeing racially-segregated graduations and everything, but everything, is defined through the lens of perceived 'racism. We have 'racist' mathematics, 'racist' food and even 'racist' *plants*. World famous Kew Gardens in London said it was changing labels on plants and flowers to tell its pre-'Covid' more than two million visitors a year how racist they are. Kew director Richard Deverell said this was part of an effort to 'move quickly to decolonise collections' after they were approached by one Ajay Chhabra 'an actor with an insight into how sugar cane was linked to slavery'. They are *plants* you idiots. 'Decolonisation' in the Woke manual really means colonisation of society with its mentality and by extension colonisation by the Cult. We are witnessing a new Chinese-style 'Cultural Revolution' so essential to the success of all

Marxist takeovers. Our cultural past and traditions have to be swept away to allow a new culture to be built-back-better. Woke targeting of long-standing Western cultural pillars including historical monuments and cancelling of historical figures is what happened in the Mao revolution in China which 'purged remnants of capitalist and traditional elements from Chinese society' and installed Maoism as the dominant ideology'. For China see the Western world today and for 'dominant ideology' see Woke. Better still see Marxism or Maoism. The 'Covid' hoax has specifically sought to destroy the arts and all elements of Western culture from people meeting in a pub or restaurant to closing theatres, music venues, sports stadiums, places of worship and even banning *singing*. Destruction of Western society is also why criticism of any religion is banned except for Christianity which again is the dominant religion as white is the numerically-dominant race. Christianity may be fading rapidly, but its history and traditions are weaved through the fabric of Western society. Delete the pillars and other structures will follow until the whole thing collapses. I am not a Christian defending that religion when I say that. I have no religion. It's just a fact. To this end Christianity has itself been turned Woke to usher its own downfall and its ranks are awash with 'change agents' – knowing and unknowing – at every level including Pope Francis (*definitely* knowing) and the clueless Archbishop of Canterbury Justin Welby (possibly not, but who can be sure?). Woke seeks to coordinate attacks on Western culture, traditions, and ways of life through 'intersectionality' defined as 'the complex, cumulative way in which the effects of multiple forms of discrimination (such as racism, sexism, and classism) combine, overlap, or intersect especially in the experiences of marginalised individuals or groups'. Wade through the Orwellian Woke-speak and this means coordinating disparate groups in a common cause to overthrow freedom and liberal values.

The entire structure of public institutions has been infested with Woke – government at all levels, political parties, police, military, schools, universities, advertising, media and trade unions. This abomination has been achieved through the Cult web by appointing

Wokers to positions of power and battering non-Wokers into line through intimidation, isolation and threats to their job. Many have been fired in the wake of the empathy-deleted, vicious hostility of 'social justice' Wokers and the desire of gutless, spineless employers to virtue-signal their Wokeness. Corporations are filled with Wokers today, most notably those in Silicon Valley. Ironically at the top they are not Woke at all. They are only exploiting the mentality their Cult masters have created and funded to censor and enslave while the Wokers cheer them on until it's their turn. Thus the Woke 'liberal left' is an inversion of the traditional liberal left. Campaigning for justice on the grounds of power and wealth distribution has been replaced by campaigning for identity politics. The genuine traditional left would never have taken money from today's billionaire abusers of fairness and justice and nor would the billionaires have wanted to fund that genuine left. It would not have been in their interests to do so. The division of opinion in those days was between the haves and have nots. This all changed with Cult manipulated and funded identity politics. The division of opinion today is between Wokers and non-Wokers and not income brackets. Cult corporations and their billionaires may have taken wealth disparity to cataclysmic levels of injustice, but as long as they speak the language of Woke, hand out the dosh to the Woke network and censor the enemy they are 'one of us'. Billionaires who don't give a damn about injustice are laughing at them till their bellies hurt. Wokers are not even close to self-aware enough to see that. The transformed 'left' dynamic means that Wokers who drone on about 'social justice' are funded by billionaires that have destroyed social justice the world over. It's *why* they are billionaires.

The climate con

Nothing encapsulates what I have said more comprehensively than the hoax of human-caused global warming. I have detailed in my books over the years how Cult operatives and organisations were the pump-primers from the start of the climate con. A purpose-built vehicle for this is the Club of Rome established by the Cult in 1968

with the Rockefellers and Rothschilds centrally involved all along. Their gofer frontman Maurice Strong, a Canadian oil millionaire, hosted the Earth Summit in Rio de Janeiro, Brazil, in 1992 where the global 'green movement' really expanded in earnest under the guiding hand of the Cult. The Earth Summit established Agenda 21 through the Cult-created-and-owned United Nations to use the illusion of human-caused climate change to justify the transformation of global society to save the world from climate disaster. It is a No-Problem-Reaction-Solution sold through governments, media, schools and universities as whole generations have been terrified into believing that the world was going to end in their lifetimes unless what old people had inflicted upon them was stopped by a complete restructuring of how everything is done. Chill, kids, it's all a hoax. Such restructuring is precisely what the Cult agenda demands (purely by coincidence of course). Today this has been given the codename of the Great Reset which is only an updated term for Agenda 21 and its associated Agenda 2030. The latter, too, is administered through the UN and was voted into being by the General Assembly in 2015. Both 21 and 2030 seek centralised control of all resources and food right down to the raindrops falling on your own land. These are some of the demands of Agenda 21 established in 1992. See if you recognise this society emerging today:

- End national sovereignty
- State planning and management of all land resources, ecosystems, deserts, forests, mountains, oceans and fresh water; agriculture; rural development; biotechnology; and ensuring *'equity'*
- The state to 'define the role' of business and financial resources
- Abolition of private property
- 'Restructuring' the family unit (see BLM)
- Children raised by the state
- People told what their job will be
- Major restrictions on movement
- Creation of 'human settlement zones'

- Mass resettlement as people are forced to vacate land where they live
- Dumbing down education
- Mass global depopulation in pursuit of all the above

The United Nations was created as a Trojan horse for world government. With the climate con of critical importance to promoting that outcome you would expect the UN to be involved. Oh, it's involved all right. The UN is promoting Agenda 21 and Agenda 2030 justified by 'climate change' while also driving the climate hoax through its Intergovernmental Panel on Climate Change (IPCC), one of the world's most corrupt organisations. The IPCC has been lying ferociously and constantly since the day it opened its doors with the global media hanging unquestioningly on its every mendacious word. The Green movement is entirely Woke and has long lost its original environmental focus since it was co-opted by the Cult. An obsession with 'global warming' has deleted its values and scrambled its head. I experienced a small example of what I mean on a beautiful country walk that I have enjoyed several times a week for many years. The path merged into the fields and forests and you felt at one with the natural world. Then a 'Green' organisation, the Hampshire and Isle of Wight Wildlife Trust, took over part of the land and proceeded to cut down a large number of trees, including mature ones, to install a horrible big, bright steel 'this-is-ours-stay-out' fence that destroyed the whole atmosphere of this beautiful place. No one with a feel for nature would do that. Day after day I walked to the sound of chainsaws and a magnificent mature weeping willow tree that I so admired was cut down at the base of the trunk. When I challenged a Woke young girl in a green shirt (of course) about this vandalism she replied: 'It's a weeping willow – it will grow back.' This is what people are paying for when they donate to the Hampshire and Isle of Wight Wildlife Trust and many other 'green' organisations today. It is not the environmental movement that I knew and instead has become a support-system – as with Extinction Rebellion – for a very dark agenda.

Private jets for climate justice

The Cult-owned, Gates-funded, World Economic Forum and its founder Klaus Schwab were behind the emergence of Greta Thunberg to harness the young behind the climate agenda and she was invited to speak to the world at … the UN. Schwab published a book, *Covid-19: The Great Reset* in 2020 in which he used the 'Covid' hoax and the climate hoax to lay out a new society straight out of Agenda 21 and Agenda 2030. Bill Gates followed in early 2021 when he took time out from destroying the world to produce a book in his name about the way to save it. Gates flies across the world in private jets and admitted that 'I probably have one of the highest greenhouse gas footprints of anyone on the planet … my personal flying alone is gigantic.' He has also bid for the planet's biggest private jet operator. Other climate change saviours who fly in private jets include John Kerry, the US Special Presidential Envoy for Climate, and actor Leonardo DiCaprio, a 'UN Messenger of Peace with special focus on climate change'. These people are so full of bullshit they could corner the market in manure. We mustn't be sceptical, though, because the Gates book, *How to Avoid a Climate Disaster: The Solutions We Have and the Breakthroughs We Need*, is a genuine attempt to protect the world and not an obvious pile of excrement attributed to a mega-psychopath aimed at selling his masters' plans for humanity. The Gates book and the other shite-pile by Klaus Schwab could have been written by the same person and may well have been. Both use 'climate change' and 'Covid' as the excuses for their new society and by coincidence the Cult's World Economic Forum and Bill and Melinda Gates Foundation promote the climate hoax and hosted Event 201 which pre-empted with a 'simulation' the very 'coronavirus' hoax that would be simulated for real on humanity within weeks. The British 'royal' family is promoting the 'Reset' as you would expect through Prince 'climate change caused the war in Syria' Charles and his hapless son Prince William who said that we must 'reset our relationship with nature and our trajectory as a species' to avoid a climate disaster. Amazing how many promotors of the 'Covid' and 'climate change' control

systems are connected to Gates and the World Economic Forum. A 'study' in early 2021 claimed that carbon dioxide emissions must fall by the equivalent of a global lockdown roughly every two years for the next decade to save the planet. The 'study' appeared in the same period that the Schwab mob claimed in a video that lockdowns destroying the lives of billions are good because they make the earth 'quieter' with less 'ambient noise'. They took down the video amid a public backlash for such arrogant, empathy-deleted stupidity You see, however, where they are going with this. Corinne Le Quéré, a professor at the Tyndall Centre for Climate Change Research, University of East Anglia, was lead author of the climate lockdown study, and she writes for … the World Economic Forum. Gates calls in 'his' book for changing 'every aspect of the economy' (long-time Cult agenda) and for humans to eat synthetic 'meat' (predicted in my books) while cows and other farm animals are eliminated. Australian TV host and commentator Alan Jones described what carbon emission targets would mean for farm animals in Australia alone if emissions were reduced as demanded by 35 percent by 2030 and zero by 2050:

Well, let's take agriculture, the total emissions from agriculture are about 75 million tonnes of carbon dioxide, equivalent. Now reduce that by 35 percent and you have to come down to 50 million tonnes, I've done the maths. So if you take for example 1.5 million cows, you're going to have to reduce the herd by 525,000 [by] 2030, nine years, that's 58,000 cows a year. The beef herd's 30 million, reduce that by 35 percent, that's 10.5 million, which means 1.2 million cattle have to go every year between now and 2030. This is insanity!

There are 75 million sheep. Reduce that by 35 percent, that's 26 million sheep, that's almost 3 million a year. So under the Paris Agreement over 30 million beasts. dairy cows, cattle, pigs and sheep would go. More than 8,000 every minute of every hour for the next decade, do these people know what they're talking about?

Clearly they don't at the level of campaigners, politicians and administrators. The Cult *does* know; that's the outcome it wants. We are faced with not just a war on humanity. Animals and the natural world are being targeted and I have been saying since the 'Covid' hoax began that the plan eventually was to claim that the 'deadly virus' is able to jump from animals, including farm animals and

domestic pets, to humans. Just before this book went into production came this story: 'Russia registers world's first Covid-19 vaccine for cats & dogs as makers of Sputnik V warn pets & farm animals could spread virus'. The report said 'top scientists warned that the deadly pathogen could soon begin spreading through homes and farms' and 'the next stage is the infection of farm and domestic animals'. Know the outcome and you'll see the journey. Think what that would mean for animals and keep your eye on a term called zoonosis or zoonotic diseases which transmit between animals and humans. The Cult wants to break the connection between animals and people as it does between people and people. Farm animals fit with the Cult agenda to transform food from natural to synthetic.

The gas of life is killing us

There can be few greater examples of Cult inversion than the condemnation of carbon dioxide as a dangerous pollutant when it is the gas of life. Without it the natural world would be dead and so we would all be dead. We breathe in oxygen and breathe out carbon dioxide while plants produce oxygen and absorb carbon dioxide. It is a perfect symbiotic relationship that the Cult wants to dismantle for reasons I will come to in the final two chapters. Gates, Schwab, other Cult operatives and mindless repeaters, want the world to be 'carbon neutral' by at least 2050 and the earlier the better. 'Zero carbon' is the cry echoed by lunatics calling for 'Zero Covid' when we already have it. These carbon emission targets will deindustrialise the world in accordance with Cult plans – the post-industrial, post-democratic society – and with so-called renewables like solar and wind not coming even close to meeting human energy needs blackouts and cold are inevitable. Texans got the picture in the winter of 2021 when a snow storm stopped wind turbines and solar panels from working and the lights went down along with water which relies on electricity for its supply system. Gates wants everything to be powered by electricity to ensure that his masters have the kill switch to stop all human activity, movement, cooking, water and warmth any time they like. The climate lie is so

stupendously inverted that it claims we must urgently reduce carbon dioxide when we *don't have enough*.

Co2 in the atmosphere is a little above 400 parts per million when the optimum for plant growth is 2,000 ppm and when it falls anywhere near 150 ppm the natural world starts to die and so do we. It fell to as low as 280 ppm in an 1880 measurement in Hawaii and rose to 413 ppm in 2019 with industrialisation which is why the planet has become *greener* in the industrial period. How insane then that psychopathic madman Gates is not satisfied only with blocking the rise of Co2. He's funding technology to suck it out of the atmosphere. The reason why will become clear. The industrial era is not destroying the world through Co2 and has instead turned around a potentially disastrous ongoing fall in Co2. Greenpeace co-founder and scientist Patrick Moore walked away from Greenpeace in 1986 and has exposed the green movement for fear-mongering and lies. He said that 500 million years ago there was *17 times* more Co2 in the atmosphere than we have today and levels have been falling for hundreds of millions of years. In the last 150 million years Co2 levels in Earth's atmosphere had reduced by *90 percent*. Moore said that by the time humanity began to unlock carbon dioxide from fossil fuels we were at '38 seconds to midnight' and in that sense: 'Humans are [the Earth's] salvation.' Moore made the point that only half the Co2 emitted by fossil fuels stays in the atmosphere and we should remember that all pollution pouring from chimneys that we are told is carbon dioxide is in fact nothing of the kind. It's pollution. Carbon dioxide is an invisible gas.

William Happer, Professor of Physics at Princeton University and long-time government adviser on climate, has emphasised the Co2 deficiency for maximum growth and food production. Greenhouse growers don't add carbon dioxide for a bit of fun. He said that most of the warming in the last 100 years, after the earth emerged from the super-cold period of the 'Little Ice Age' into a natural warming cycle, was over by 1940. Happer said that a peak year for warming in 1988 can be explained by a 'monster El Nino' which is a natural and cyclical warming of the Pacific that has nothing to do with 'climate

change'. He said the effect of Co2 could be compared to painting a wall with red paint in that once two or three coats have been applied it didn't matter how much more you slapped on because the wall will not get much redder. Almost all the effect of the rise in Co2 has already happened, he said, and the volume in the atmosphere would now have to *double* to increase temperature by a single degree. Climate hoaxers know this and they have invented the most ridiculously complicated series of 'feedback' loops to try to overcome this rather devastating fact. You hear puppet Greta going on cluelessly about feedback loops and this is why.

The Sun affects temperature? No you *climate denier*

Some other nonsense to contemplate: Climate graphs show that rises in temperature do not follow rises in Co2 – *it's the other way round* with a lag between the two of some 800 years. If we go back 800 years from present time we hit the Medieval Warm Period when temperatures were higher than now without any industrialisation and this was followed by the Little Ice Age when temperatures plummeted. The world was still emerging from these centuries of serious cold when many climate records began which makes the ever-repeated line of the 'hottest year since records began' meaningless when you are not comparing like with like. The coldest period of the Little Ice Age corresponded with the lowest period of sunspot activity when the Sun was at its least active. Proper scientists will not be at all surprised by this when it confirms the obvious fact that earth temperature is affected by the scale of Sun activity and the energetic power that it subsequently emits; but when is the last time you heard a climate hoaxer talking about the Sun as a source of earth temperature?? Everything has to be focussed on Co2 which makes up just 0.117 percent of so-called greenhouse gases and only a fraction of even that is generated by human activity. The rest is natural. More than *90 percent* of those greenhouse gases are water vapour and clouds (Fig 9). Ban moisture I say. Have you noticed that the climate hoaxers no longer use the polar bear as their promotion image? That's because far from becoming extinct polar

bear communities are stable or thriving. Joe Bastardi, American meteorologist, weather forecaster and outspoken critic of the climate lie, documents in his book *The Climate Chronicles* how weather patterns and events claimed to be evidence of climate change have been happening since long before industrialisation: 'What happened before naturally is happening again, as is to be expected given the cyclical nature of the climate due to the design of the planet.' If you read the detailed background to the climate hoax in my other books you will shake your head and wonder how anyone could believe the crap which has spawned a multi-trillion dollar industry based on absolute garbage (see HIV causes AIDs and Sars-Cov-2 causes 'Covid-19'). Climate and 'Covid' have much in common given they have the same source. They both have the contradictory *everything* factor in which everything is explained by reference to them. It's hot – 'it's climate change'. It's cold – 'it's climate change'. I got a sniffle – 'it's Covid'. I haven't got a sniffle – 'it's Covid'. Not having a sniffle has to be a symptom of 'Covid'. Everything is and not having a sniffle is especially dangerous if you are a slow walker. For sheer audacity I offer you a Cambridge University 'study' that actually linked 'Covid' to 'climate change'. It had to happen eventually. They concluded that climate change played a role in 'Covid-19' spreading from animals to humans because … wait for it … I kid you not … *the two groups were forced closer together as populations grow*. Er, that's it. The whole foundation on which this depended was that 'Bats are the likely zoonotic origin of SARS-CoV-1 and SARS-CoV-2'. Well, they are not. They are nothing to do with it. Apart from bats not being the origin and therefore 'climate change' effects on bats being irrelevant I am in awe of their academic insight. Where would we be without them? Not where we are that's for sure.

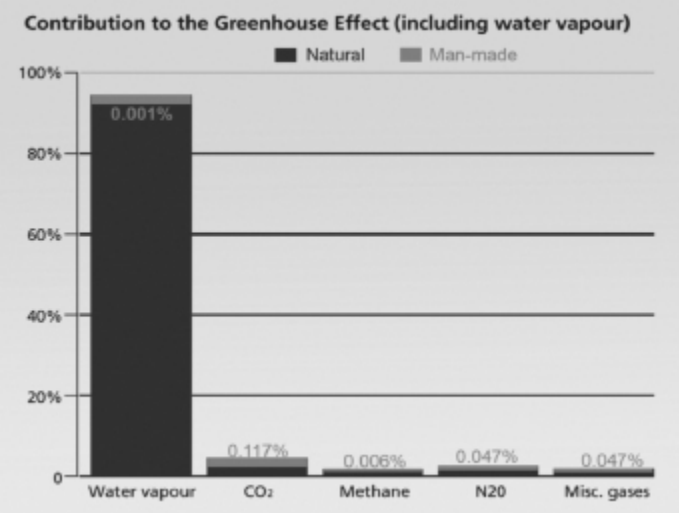

Figure 9: The idea that the gas of life is disastrously changing the climate is an insult to brain cell activity.

One other point about the weather is that climate modification is now well advanced and not every major weather event is natural – or earthquake come to that. I cover this subject at some length in other books. China is openly planning a rapid expansion of its weather modification programme which includes changing the climate in an area more than one and a half times the size of India. China used weather manipulation to ensure clear skies during the 2008 Olympics in Beijing. I have quoted from US military documents detailing how to employ weather manipulation as a weapon of war and they did that in the 1960s and 70s during the conflict in Vietnam with Operation Popeye manipulating monsoon rains for military purposes. Why would there be international treaties on weather modification if it wasn't possible? Of course it is. Weather is energetic information and it can be changed.

How was the climate hoax pulled off? See 'Covid'

If you can get billions to believe in a 'virus' that doesn't exist you can get them to believe in human-caused climate change that doesn't exist. Both are being used by the Cult to transform global society in the way it has long planned. Both hoaxes have been achieved in pretty much the same way. First you declare a lie is a fact. There's a

'virus' you call SARS-Cov-2 or humans are warming the planet with their behaviour. Next this becomes, via Cult networks, the foundation of government, academic and science policy and belief. Those who parrot the mantra are given big grants to produce research that confirms the narrative is true and ever more 'symptoms' are added to make the 'virus'/'climate change' sound even more scary. Scientists and researchers who challenge the narrative have their grants withdrawn and their careers destroyed. The media promote the lie as the unquestionable truth and censor those with an alternative view or evidence. A great percentage of the population believe what they are told as the lie becomes an everybody-knows-that and the believing-masses turn on those with a mind of their own. The technique has been used endlessly throughout human history. Wokers are the biggest promotors of the climate lie *and* 'Covid' fascism because their minds are owned by the Cult; their sense of self-righteous self-purity knows no bounds; and they exist in a bubble of reality in which facts are irrelevant and only get in the way of looking without seeing.

Running through all of this like veins in a blue cheese is control of information, which means control of perception, which means control of behaviour, which collectively means control of human society. The Cult owns the global media and Silicon Valley fascists for the simple reason that it *has* to. Without control of information it can't control perception and through that human society. Examine every facet of the Cult agenda and you will see that anything supporting its introduction is never censored while anything pushing back is always censored. I say again: Psychopaths that know why they are doing this must go before Nuremberg trials and those that follow their orders must trot along behind them into the same dock. 'I was just following orders' didn't work the first time and it must not work now. Nuremberg trials must be held all over the world before public juries for politicians, government officials, police, compliant doctors, scientists and virologists, and all Cult operatives such as Gates, Tedros, Fauci, Vallance, Whitty, Ferguson, Zuckerberg, Wojcicki, Brin, Page, Dorsey, the whole damn lot of

them – including, no *especially*, the psychopath psychologists. Without them and the brainless, gutless excuses for journalists that have repeated their lies, none of this could be happening. Nobody can be allowed to escape justice for the psychological and economic Armageddon they are all responsible for visiting upon the human race.

As for the compliant, unquestioning, swathes of humanity, and the self-obsessed, all-knowing ignorance of the Wokers … don't start me. God help their kids. God help their grandkids. God *help them*.

CHAPTER NINE

We must have it? So what is it?

Well I won't back down. No, I won't back down. You can stand me up at the Gates of Hell. But I won't back down
Tom Petty

I will now focus on the genetically-manipulating 'Covid vaccines' which do not meet this official definition of a vaccine by the US Centers for Disease Control (CDC): 'A product that stimulates a person's immune system to produce immunity to a specific disease, protecting the person from that disease.' On that basis 'Covid vaccines' are not a vaccine in that the makers don't even claim they stop infection or transmission.

They are instead part of a multi-levelled conspiracy to change the nature of the human body and what it means to be 'human' and to depopulate an enormous swathe of humanity. What I shall call Human 1.0 is on the cusp of becoming Human 2.0 and for very sinister reasons. Before I get to the 'Covid vaccine' in detail here's some background to vaccines in general. Government regulators do not test vaccines – the makers do – and the makers control which data is revealed and which isn't. Children in America are given 50 vaccine doses by age six and 69 by age 19 and the effect of the whole combined schedule has never been tested. Autoimmune diseases when the immune system attacks its own body have soared in the mass vaccine era and so has disease in general in children and the young. Why wouldn't this be the case when vaccines target the *immune system?* The US government gave Big Pharma drug

companies immunity from prosecution for vaccine death and injury in the 1986 National Childhood Vaccine Injury Act (NCVIA) and since then the government (taxpayer) has been funding compensation for the consequences of Big Pharma vaccines. The criminal and satanic drug giants can't lose and the vaccine schedule has increased dramatically since 1986 for this reason. There is no incentive to make vaccines safe and a big incentive to make money by introducing ever more. Even against a ridiculously high bar to prove vaccine liability, and with the government controlling the hearing in which it is being challenged for compensation, the vaccine court has so far paid out more than $4 billion. These are the vaccines we are told are safe and psychopaths like Zuckerberg censor posts saying otherwise. The immunity law was even justified by a ruling that vaccines by their nature were 'unavoidably unsafe'.

Check out the ingredients of vaccines and you will be shocked if you are new to this. *They put that in children's bodies?? What??* Try aluminium, a brain toxin connected to dementia, aborted foetal tissue and formaldehyde which is used to embalm corpses. World-renowned aluminium expert Christopher Exley had his research into the health effect of aluminium in vaccines shut down by Keele University in the UK when it began taking funding from the Bill and Melinda Gates Foundation. Research when diseases 'eradicated' by vaccines began to decline and you will find the fall began long *before* the vaccine was introduced. Sometimes the fall even plateaued after the vaccine. Diseases like scarlet fever for which there was no vaccine declined in the same way because of environmental and other factors. A perfect case in point is the polio vaccine. Polio began when lead arsenate was first sprayed as an insecticide and residues remained in food products. Spraying started in 1892 and the first US polio epidemic came in Vermont in 1894. The simple answer was to stop spraying, but Rockefeller-created Big Pharma had a better idea. Polio was decreed to be caused by the *poliovirus* which 'spreads from person to person and can infect a person's spinal cord'. Lead arsenate was replaced by the lethal DDT which had the same effect of causing paralysis by damaging the brain and central nervous

system. Polio plummeted when DDT was reduced and then banned, but the vaccine is still given the credit for something it didn't do. Today by far the biggest cause of polio is the vaccines promoted by Bill Gates. Vaccine justice campaigner Robert Kennedy Jr, son of assassinated (by the Cult) US Attorney General Robert Kennedy, wrote:

> In 2017, the World Health Organization (WHO) reluctantly admitted that the global explosion in polio is predominantly vaccine strain. The most frightening epidemics in Congo, Afghanistan, and the Philippines, are all linked to vaccines. In fact, by 2018, 70% of global polio cases were vaccine strain.

Vaccines make fortunes for Cult-owned Gates and Big Pharma while undermining the health and immune systems of the population. We had a glimpse of the mentality behind the Big Pharma cartel with a report on WION (World is One News), an international English language TV station based in India, which exposed the extraordinary behaviour of US drug company Pfizer over its 'Covid vaccine'. The WION report told how Pfizer had made fantastic demands of Argentina, Brazil and other countries in return for its 'vaccine'. These included immunity from prosecution, even for Pfizer negligence, government insurance to protect Pfizer from law suits and handing over as collateral sovereign assets of the country to include Argentina's bank reserves, military bases and embassy buildings. Pfizer demanded the same of Brazil in the form of waiving sovereignty of its assets abroad; exempting Pfizer from Brazilian laws; and giving Pfizer immunity from all civil liability. This is a 'vaccine' developed with government funding. Big Pharma is evil incarnate as a creation of the Cult and all must be handed tickets to Nuremberg.

Phantom 'vaccine' for a phantom 'disease'

I'll expose the 'Covid vaccine' fraud and then go on to the wider background of why the Cult has set out to 'vaccinate' every man, woman and child on the planet for an alleged 'new disease' with a survival rate of 99.77 percent (or more) even by the grotesquely-

manipulated figures of the World Health Organization and Johns Hopkins University. The 'infection' to 'death' ratio is 0.23 to 0.15 percent according to Stanford epidemiologist Dr John Ioannidis and while estimates vary the danger remains tiny. I say that if the truth be told the fake infection to fake death ratio is zero. Never mind all the evidence I have presented here and in *The Answer* that there is no 'virus' let us just focus for a moment on that death-rate figure of say 0.23 percent. The figure includes all those worldwide who have tested positive with a test not testing for the 'virus' and then died within 28 days or even longer of any other cause – *any other cause*. Now subtract all those illusory 'Covid' deaths on the global data sheets from the 0.23 percent. What do you think you would be left with? *Zero*. A vaccination has never been successfully developed for a so-called coronavirus. They have all failed at the animal testing stage when they caused hypersensitivity to what they were claiming to protect against and made the impact of a disease far worse. Cult-owned vaccine corporations got around that problem this time by bypassing animal trials, going straight to humans and making the length of the 'trials' before the public rollout as short as they could get away with. Normally it takes five to ten years or more to develop vaccines that still cause demonstrable harm to many people and that's without including the long-term effects that are never officially connected to the vaccination. 'Covid' non-vaccines have been officially produced and approved in a matter of months from a standing start and part of the reason is that (a) they were developed before the 'Covid' hoax began and (b) they are based on computer programs and not natural sources. Official non-trials were so short that government agencies gave *emergency*, not full, approval. 'Trials' were not even completed and full approval cannot be secured until they are. Public 'Covid vaccination' is actually a *continuation of the trial*. Drug company 'trials' are not scheduled to end until 2023 by which time a lot of people are going to be dead. Data on which government agencies gave this emergency approval was supplied by the Big Pharma corporations themselves in the form of Pfizer/BioNTech, AstraZeneca, Moderna, Johnson & Johnson, and

others, and this is the case with all vaccines. By its very nature *emergency* approval means drug companies do not have to prove that the 'vaccine' is 'safe and effective'. How could they with trials way short of complete? Government regulators only have to *believe* that they *could* be safe and effective. It is criminal manipulation to get products in circulation with no testing worth the name. Agencies giving that approval are infested with Big Pharma-connected place-people and they act in the interests of Big Pharma (the Cult) and not the public about whom they do not give a damn.

More human lab rats

'Covid vaccines' produced in record time by Pfizer/BioNTech and Moderna employ a technique *never approved before for use on humans*. They are known as mRNA 'vaccines' and inject a synthetic version of 'viral' mRNA or 'messenger RNA'. The key is in the term 'messenger'. The body works, or doesn't, on the basis of information messaging. Communications are constantly passing between and within the genetic system and the brain. Change those messages and you change the state of the body and even its very nature and you can change psychology and behaviour by the way the brain processes information. I think you are going to see significant changes in personality and perception of many people who have had the 'Covid vaccine' synthetic potions. Insider Aldous Huxley predicted the following in 1961 and mRNA 'vaccines' can be included in the term 'pharmacological methods':

> There will be, in the next generation or so, a pharmacological method of making people love their servitude, and producing dictatorship without tears, so to speak, producing a kind of painless concentration camp for entire societies, so that people will in fact have their own liberties taken away from them, but rather enjoy it, because they will be distracted from any desire to rebel by propaganda or brainwashing, or brainwashing enhanced by pharmacological methods. And this seems to be the final revolution.

Apologists claim that mRNA synthetic 'vaccines' don't change the DNA genetic blueprint because RNA does not affect DNA only the other way round. This is so disingenuous. A process called 'reverse

transcription' can convert RNA into DNA and be integrated into DNA in the cell nucleus. This was highlighted in December, 2020, by scientists at Harvard and Massachusetts Institute of Technology (MIT). Geneticists report that more than 40 percent of mammalian genomes results from reverse transcription. On the most basic level if messaging changes then that sequence must lead to changes in DNA which is receiving and transmitting those communications. How can introducing synthetic material into cells not change the cells where DNA is located? The process is known as transfection which is defined as 'a technique to insert foreign nucleic acid (DNA or RNA) into a cell, typically with the intention of altering the properties of the cell'. Researchers at the Sloan Kettering Institute in New York found that changes in messenger RNA can deactivate tumour-suppressing proteins and thereby promote cancer. This is what happens when you mess with messaging. 'Covid vaccine' maker Moderna was founded in 2010 by Canadian stem cell biologist Derrick J. Rossi after his breakthrough discovery in the field of transforming and reprogramming stem cells. These are neutral cells that can be programmed to become any cell including sperm cells. Moderna was therefore founded on the principle of genetic manipulation and has never produced any vaccine or drug before its genetically-manipulating synthetic 'Covid' shite. Look at the name – Mode-RNA or Modify-RNA. Another important point is that the US Supreme Court has ruled that genetically-modified DNA, or complementary DNA (cDNA) synthesized in the laboratory from messenger RNA, can be patented and owned. These psychopaths are doing this to the human body.

Cells replicate synthetic mRNA in the 'Covid vaccines' and in theory the body is tricked into making antigens which trigger antibodies to target the 'virus spike proteins' which as Dr Tom Cowan said have *never been seen*. Cut the crap and these 'vaccines' deliver *self-replicating* synthetic material to the cells with the effect of changing human DNA. The more of them you have the more that process is compounded while synthetic material is all the time self-replicating. 'Vaccine'-maker Moderna describes mRNA as 'like

software for the cell' and so they are messing with the body's software. What happens when you change the software in a computer? Everything changes. For this reason the Cult is preparing a production line of mRNA 'Covid vaccines' and a long list of excuses to use them as with all the 'variants' of a 'virus' never shown to exist. The plan is further to transfer the mRNA technique to other vaccines mostly given to children and young people. The cumulative consequences will be a transformation of human DNA through a constant infusion of synthetic genetic material which will kill many and change the rest. Now consider that governments that have given emergency approval for a vaccine that's not a vaccine; never been approved for humans before; had no testing worth the name; and the makers have been given immunity from prosecution for any deaths or adverse effects suffered by the public. The UK government awarded *permanent legal indemnity* to itself and its employees for harm done when a patient is being treated for 'Covid-19' or 'suspected Covid-19'. That is quite a thought when these are possible 'side-effects' from the 'vaccine' (they are not 'side', they are effects) listed by the US Food and Drug Administration:

Guillain-Barre syndrome; acute disseminated encephalomyelitis; transverse myelitis; encephalitis; myelitis; encephalomyelitis; meningoencephalitis; meningitis; encephalopathy; convulsions; seizures; stroke; narcolepsy; cataplexy; anaphylaxis; acute myocardial infarction (heart attack); myocarditis; pericarditis; autoimmune disease; death; implications for pregnancy, and birth outcomes; other acute demyelinating diseases; non anaphylactic allergy reactions; thrombocytopenia ; disseminated intravascular coagulation; venous thromboembolism; arthritis; arthralgia; joint pain; Kawasaki disease; multisystem inflammatory syndrome in children; vaccine enhanced disease. The latter is the way the 'vaccine' has the potential to make diseases far worse than they would otherwise be.

UK doctor and freedom campaigner Vernon Coleman described the conditions in this list as 'all unpleasant, most of them very serious, and you can't get more serious than death'. The thought that anyone at all has had the 'vaccine' in these circumstances is testament to the potential that humanity has for clueless, unquestioning, stupidity and for many that programmed stupidity has already been terminal.

An insider speaks

Dr Michael Yeadon is a former Vice President, head of research and Chief Scientific Adviser at vaccine giant Pfizer. Yeadon worked on the inside of Big Pharma, but that did not stop him becoming a vocal critic of 'Covid vaccines' and their potential for multiple harms, including infertility in women. By the spring of 2021 he went much further and even used the no, no, term 'conspiracy'. When you begin to see what is going on it is impossible not to do so. Yeadon spoke out in an interview with freedom campaigner James Delingpole and I mentioned earlier how he said that no one had samples of 'the virus'. He explained that the mRNA technique originated in the anti-cancer field and ways to turn on and off certain genes which could be advantageous if you wanted to stop cancer growing out of control. 'That's the origin of them. They are a very unusual application, really.' Yeadon said that treating a cancer patient with an aggressive procedure might be understandable if the alternative was dying, but it was quite another thing to use the same technique as a public health measure. Most people involved wouldn't catch the infectious agent you were vaccinating against and if they did they probably wouldn't die:

> If you are really using it as a public health measure you really want to as close as you can get to zero sides-effects … I find it odd that they chose techniques that were really cutting their teeth in the field of oncology and I'm worried that in using gene-based vaccines that have to be injected in the body and spread around the body, get taken up into some cells, and the regulators haven't quite told us which cells they get taken up into … you are going to be generating a wide range of responses … with multiple steps each of which could go well or badly.

I doubt the Cult intends it to go well. Yeadon said that you can put any gene you like into the body through the 'vaccine'. 'You can certainly give them a gene that would do them some harm if you wanted.' I was intrigued when he said that when used in the cancer field the technique could turn genes on and off. I explore this process in *The Answer* and with different genes having different functions you could create mayhem – physically and psychologically – if you turned the wrong ones on and the right ones off. I read reports of an experiment by researchers at the University of Washington's school of computer science and engineering in which they encoded DNA to infect computers. The body is itself a biological computer and if human DNA can inflict damage on a computer why can't the computer via synthetic material mess with the human body? It can. The Washington research team said it was possible to insert malicious malware into 'physical DNA strands' and corrupt the computer system of a gene sequencing machine as it 'reads gene letters and stores them as binary digits 0 and 1'. They concluded that hackers could one day use blood or spit samples to access computer systems and obtain sensitive data from police forensics labs or infect genome files. It is at this level of digital interaction that synthetic 'vaccines' need to be seen to get the full picture and that will become very clear later on. Michael Yeadon said it made no sense to give the 'vaccine' to younger people who were in no danger from the 'virus'. What was the benefit? It was all downside with potential effects:

> The fact that my government in what I thought was a civilised, rational country, is raining [the 'vaccine'] on people in their 30s and 40s, even my children in their 20s, they're getting letters and phone calls, I know this is not right and any of you doctors who are vaccinating you know it's not right, too. They are not at risk. They are not at risk from the disease, so you are now hoping that the side-effects are so rare that you get away with it. You don't give new technology … that you don't understand to 100 percent of the population.

Blood clot problems with the AstraZeneca 'vaccine' have been affecting younger people to emphasise the downside risks with no benefit. AstraZeneca's version, produced with Oxford University, does not use mRNA, but still gets its toxic cocktail inside cells where

it targets DNA. The Johnson & Johnson 'vaccine' which uses a similar technique has also produced blood clot effects to such an extent that the United States paused its use at one point. They are all 'gene therapy' (cell modification) procedures and not 'vaccines'. The truth is that once the content of these injections enter cells we have no idea what the effect will be. People can speculate and some can give very educated opinions and that's good. In the end, though, only the makers know what their potions are designed to do and even they won't know every last consequence. Michael Yeadon was scathing about doctors doing what they knew to be wrong. 'Everyone's mute', he said. Doctors in the NHS must know this was not right, coming into work and injecting people. 'I don't know how they sleep at night. I know I couldn't do it. I know that if I were in that position I'd have to quit.' He said he knew enough about toxicology to know this was not a good risk-benefit. Yeadon had spoken to seven or eight university professors and all except two would not speak out publicly. Their universities had a policy that no one said anything that countered the government and its medical advisors. They were afraid of losing their government grants. This is how intimidation has been used to silence the truth at every level of the system. I say silence, but these people could still speak out if they made that choice. Yeadon called them 'moral cowards' – 'This is about your children and grandchildren's lives and you have just buggered off and left it.'

'Variant' nonsense

Some of his most powerful comments related to the alleged 'variants' being used to instil more fear, justify more lockdowns, and introduce more 'vaccines'. He said government claims about 'variants' were nonsense. He had checked the alleged variant 'codes' and they were 99.7 percent identical to the 'original'. This was the human identity difference equivalent to putting a baseball cap on and off or wearing it the other way round. A 0.3 percent difference would make it impossible for that 'variant' to escape immunity from the 'original'. This made no sense of having new 'vaccines' for

'variants'. He said there would have to be at least a *30 percent* difference for that to be justified and even then he believed the immune system would still recognise what it was. Gates-funded 'variant modeller' and 'vaccine'-pusher John Edmunds might care to comment. Yeadon said drug companies were making new versions of the 'vaccine' as a 'top up' for 'variants'. Worse than that, he said, the 'regulators' around the world like the MHRA in the UK had got together and agreed that because 'vaccines' for 'variants' were so similar to the first 'vaccines' *they did not have to do safety studies*. How transparently sinister that is. This is when Yeadon said: 'There is a conspiracy here.' There was no need for another vaccine for 'variants' and yet we were told that there was and the country had shut its borders because of them. 'They are going into hundreds of millions of arms without passing 'go' or any regulator. Why did they do that? Why did they pick this method of making the vaccine?'

The reason had to be something bigger than that it seemed and 'it's not protection against the virus'. It's was a far bigger project that meant politicians and advisers were willing to do things and not do things that knowingly resulted in avoidable deaths – 'that's already happened when you think about lockdown and deprivation of health care for a year.' He spoke of people prepared to do something that results in the avoidable death of their fellow human beings and it not bother them. This is the penny-drop I have been working to get across for more than 30 years – the level of pure evil we are dealing with. Yeadon said his friends and associates could not believe there could be that much evil, but he reminded them of Stalin, Pol Pot and Hitler and of what Stalin had said: 'One death is a tragedy. A million? A statistic.' He could not think of a benign explanation for why you need top-up vaccines 'which I'm sure you don't' and for the regulators 'to just get out of the way and wave them through'. Why would the regulators do that when they were still wrestling with the dangers of the 'parent' vaccine? He was clearly shocked by what he had seen since the 'Covid' hoax began and now he was thinking the previously unthinkable:

If you wanted to depopulate a significant proportion of the world and to do it in a way that doesn't involve destruction of the environment with nuclear weapons, poisoning everyone with anthrax or something like that, and you wanted plausible deniability while you had a multi-year infectious disease crisis, I actually don't think you could come up with a better plan of work than seems to be in front of me. I can't say that's what they are going to do, but I can't think of a benign explanation why they are doing it.

He said he never thought that they would get rid of 99 percent of humans, but now he wondered. 'If you wanted to that this would be a hell of a way to do it – it would be unstoppable folks.' Yeadon had concluded that those who submitted to the 'vaccine' would be allowed to have some kind of normal life (but for how long?) while screws were tightened to coerce and mandate the last few percent. 'I think they'll put the rest of them in a prison camp. I wish I was wrong, but I don't think I am.' Other points he made included: There were no coronavirus vaccines then suddenly they all come along at the same time; we have no idea of the long term affect with trials so short; coercing or forcing people to have medical procedures is against the Nuremberg Code instigated when the Nazis did just that; people should at least delay having the 'vaccine'; a quick Internet search confirms that masks don't reduce respiratory viral transmission and 'the government knows that'; they have smashed civil society and they know that, too; two dozen peer-reviewed studies show no connection between lockdown and reducing deaths; he knew from personal friends the elite were still flying around and going on holiday while the public were locked down; the elite were not having the 'vaccines'. He was also asked if 'vaccines' could be made to target difference races. He said he didn't know, but the document by the Project for the New American Century in September, 2000, said developing 'advanced forms of biological warfare that can target *specific genotypes* may transform biological warfare from the realm of terror to a politically useful tool.' Oh, they're evil all right. Of that we can be *absolutely* sure.

Another cull of old people

We have seen from the CDC definition that the mRNA 'Covid vaccine' is not a vaccine and nor are the others that *claim* to reduce 'severity of symptoms' in *some* people, but not protect from infection or transmission. What about all the lies about returning to 'normal' if people were 'vaccinated'? If they are not claimed to stop infection and transmission of the alleged 'virus', how does anything change? This was all lies to manipulate people to take the jabs and we are seeing that now with masks and distancing still required for the 'vaccinated'. How did they think that elderly people with fragile health and immune responses were going to be affected by infusing their cells with synthetic material and other toxic substances? They *knew* that in the short and long term it would be devastating and fatal as the culling of the old that began with the first lockdowns was continued with the 'vaccine'. Death rates in care homes soared immediately residents began to be 'vaccinated' – infused with synthetic material. Brave and committed whistleblower nurses put their careers at risk by exposing this truth while the rest kept their heads down and their mouths shut to put their careers before those they are supposed to care for. A long-time American Certified Nursing Assistant who gave his name as James posted a video in which he described emotionally what happened in his care home when vaccination began. He said that during 2020 very few residents were sick with 'Covid' and no one died during the entire year; but shortly after the Pfizer mRNA injections 14 people died within two weeks and many others were near death. 'They're dropping like flies', he said. Residents who walked on their own before the shot could no longer and they had lost their ability to conduct an intelligent conversation. The home's management said the sudden deaths were caused by a 'super-spreader' of 'Covid-19'. Then how come, James asked, that residents who refused to take the injections were not sick? It was a case of inject the elderly with mRNA synthetic potions and blame their illness and death that followed on the 'virus'. James described what was happening in care homes as 'the greatest crime of genocide this country has ever seen'. Remember the NHS staff nurse from earlier who used the same

word ‘genocide’ for what was happening with the ‘vaccines’ and that it was an ‘act of human annihilation’. A UK care home whistleblower told a similar story to James about the effect of the ‘vaccine’ in deaths and ‘outbreaks’ of illness dubbed ‘Covid’ after getting the jab. She told how her care home management and staff had zealously imposed government regulations and no one was allowed to even question the official narrative let alone speak out against it. She said the NHS was even worse. Again we see the results of reframing. A worker at a local care home where I live said they had not had a single case of ‘Covid’ there for almost a year and when the residents were ‘vaccinated’ they had 19 positive cases in two weeks with eight dying.

It’s not the ‘vaccine’ – honest

The obvious cause and effect was being ignored by the media and most of the public. Australia’s health minister Greg Hunt (a former head of strategy at the World Economic Forum) was admitted to hospital after he had the ‘vaccine’. He was suffering according to reports from the skin infection ‘cellulitis’ and it must have been a severe case to have warranted days in hospital. Immediately the authorities said this was nothing to do with the ‘vaccine’ when an effect of some vaccines is a ‘cellulitis-like reaction’. We had families of perfectly healthy old people who died after the ‘vaccine’ saying that if only they had been given the ‘vaccine’ earlier they would still be alive. As a numbskull rating that is off the chart. A father of four ‘died of Covid’ at aged 48 when he was taken ill two days after having the ‘vaccine’. The man, a health administrator, had been ‘shielding during the pandemic’ and had ‘not really left the house’ until he went for the ‘vaccine’. Having the ‘vaccine’ and then falling ill and dying does not seem to have qualified as a possible cause and effect and ‘Covid-19’ went on his death certificate. His family said they had no idea how he ‘caught the virus’. A family member said: ‘Tragically, it could be that going for a vaccination ultimately led to him catching Covid …The sad truth is that they are never going to know where it came from.’ The family warned people to remember

that the virus still existed and was 'very real'. So was their stupidity. Nurses and doctors who had the first round of the 'vaccine' were collapsing, dying and ending up in a hospital bed while they or their grieving relatives were saying they'd still have the 'vaccine' again despite what happened. I kid you not. You mean if your husband returned from the dead he'd have the same 'vaccine' again that killed him??

Doctors at the VCU Medical Center in Richmond, Virginia, said the Johnson & Johnson 'vaccine' was to blame for a man's skin peeling off. Patient Richard Terrell said: 'It all just happened so fast. My skin peeled off. It's still coming off on my hands now.' He said it was stinging, burning and itching and when he bent his arms and legs it was very painful with 'the skin swollen and rubbing against itself'. Pfizer/BioNTech and Moderna vaccines use mRNA to change the cell while the Johnson & Johnson version uses DNA in a process similar to AstraZeneca's technique. Johnson & Johnson and AstraZeneca have both had their 'vaccines' paused by many countries after causing serious blood problems. Terrell's doctor Fnu Nutan said he could have died if he hadn't got medical attention. It sounds terrible so what did Nutan and Terrell say about the 'vaccine' now? Oh, they still recommend that people have it. A nurse in a hospital bed 40 minutes after the vaccination and unable to swallow due to throat swelling was told by a doctor that he lost mobility in his arm for 36 hours following the vaccination. What did he say to the ailing nurse? 'Good for you for getting the vaccination.' We are dealing with a serious form of cognitive dissonance madness in both public and medical staff. There is a remarkable correlation between those having the 'vaccine' and trumpeting the fact and suffering bad happenings shortly afterwards. Witold Rogiewicz, a Polish doctor, made a video of his 'vaccination' and ridiculed those who were questioning its safety and the intentions of Bill Gates: 'Vaccinate yourself to protect yourself, your loved ones, friends and also patients. And to mention quickly I have info for anti-vaxxers and anti-Coviders if you want to contact Bill Gates you can do this through me.' He further ridiculed the dangers of 5G. Days later he

was dead, but naturally the vaccination wasn't mentioned in the verdict of 'heart attack'.

Lies, lies and more lies

So many members of the human race have slipped into extreme states of insanity and unfortunately they include reframed doctors and nursing staff. Having a 'vaccine' and dying within minutes or hours is not considered a valid connection while death from any cause within 28 days or longer of a positive test with a test not testing for the 'virus' means 'Covid-19' goes on the death certificate. How could that 'vaccine'-death connection not have been made except by calculated deceit? US figures in the initial rollout period to February 12th, 2020, revealed that a third of the deaths reported to the CDC after 'Covid vaccines' happened within 48 hours. Five men in the UK suffered an 'extremely rare' blood clot problem after having the AstraZeneca 'vaccine', but no causal link was established said the Gates-funded Medicines and Healthcare products Regulatory Agency (MHRA) which had given the 'vaccine' emergency approval to be used. Former Pfizer executive Dr Michael Yeadon explained in his interview how the procedures could cause blood coagulation and clots. People who should have been at no risk were dying from blood clots in the brain and he said he had heard from medical doctor friends that people were suffering from skin bleeding and massive headaches. The AstraZeneca 'shot' was stopped by some 20 countries over the blood clotting issue and still the corrupt MHRA, the European Medicines Agency (EMA) and the World Health Organization said that it should continue to be given even though the EMA admitted that it 'still cannot rule out definitively' a link between blood clotting and the 'vaccine'. Later Marco Cavaleri, head of EMA vaccine strategy, said there was indeed a clear link between the 'vaccine' and thrombosis, but they didn't know why. So much for the trials showing the 'vaccine' is safe. Blood clots were affecting younger people who would be under virtually no danger from 'Covid' even if it existed which makes it all the more stupid and sinister.

The British government responded to public alarm by wheeling out June Raine, the terrifyingly weak infant school headmistress sound-alike who heads the UK MHRA drug 'regulator'. The idea that she would stand up to Big Pharma and government pressure is laughable and she told us that all was well in the same way that she did when allowing untested, never-used-on-humans-before, genetically-manipulating 'vaccines' to be exposed to the public in the first place. Mass lying is the new normal of the 'Covid' era. The MHRA later said 30 cases of rare blood clots had by then been connected with the AstraZeneca 'vaccine' (that means a lot more in reality) while stressing that the benefits of the jab in preventing 'Covid-19' outweighed any risks. A more ridiculous and disingenuous statement with callous disregard for human health it is hard to contemplate. Immediately after the mendacious 'all-clears' two hospital workers in Denmark experienced blood clots and cerebral haemorrhaging following the AstraZeneca jab and one died. Top Norwegian health official Pål Andre Holme said the 'vaccine' was the only common factor: 'There is nothing in the patient history of these individuals that can give such a powerful immune response … I am confident that the antibodies that we have found are the cause, and I see no other explanation than it being the vaccine which triggers it.' Strokes, a clot or bleed in the brain, were clearly associated with the 'vaccine' from word of mouth and whistleblower reports. Similar consequences followed with all these 'vaccines' that we were told were so safe and as the numbers grew by the day it was clear we were witnessing human carnage.

Learning the hard way

A woman interviewed by UKColumn told how her husband suffered dramatic health effects after the vaccine when he'd been in good health all his life. He went from being a little unwell to losing all feeling in his legs and experiencing 'excruciating pain'. Misdiagnosis followed twice at Accident and Emergency (an 'allergy' and 'sciatica') before he was admitted to a neurology ward where doctors said his serious condition had been caused by the

'vaccine'. Another seven 'vaccinated' people were apparently being treated on the same ward for similar symptoms. The woman said he had the 'vaccine' because they believed media claims that it was safe. 'I didn't think the government would give out a vaccine that does this to somebody; I believed they would be bringing out a vaccination that would be safe.' What a tragic way to learn that lesson. Another woman posted that her husband was transporting stroke patients to hospital on almost every shift and when he asked them if they had been 'vaccinated' for 'Covid' they all replied 'yes'. One had a 'massive brain bleed' the day after his second dose. She said her husband reported the 'just been vaccinated' information every time to doctors in A and E only for them to ignore it, make no notes and appear annoyed that it was even mentioned. This particular report cannot be verified, but it expresses a common theme that confirms the monumental underreporting of 'vaccine' consequences. Interestingly as the 'vaccines' and their brain blood clot/stroke consequences began to emerge the UK National Health Service began a publicity campaign telling the public what to do in the event of a stroke. A Scottish NHS staff nurse who quit in disgust in March, 2021, said:

I have seen traumatic injuries from the vaccine, they're not getting reported to the yellow card [adverse reaction] scheme, they're treating the symptoms, not asking why, why it's happening. It's just treating the symptoms and when you speak about it you're dismissed like you're crazy, I'm not crazy, I'm not crazy because every other colleague I've spoken to is terrified to speak out, they've had enough.

Videos appeared on the Internet of people uncontrollably shaking after the 'vaccine' with no control over muscles, limbs and even their face. A Scottish mother broke out in a severe rash all over her body almost immediately after she was given the AstraZeneca 'vaccine'. The pictures were horrific. Leigh King, a 41-year-old hairdresser from Lanarkshire said: 'Never in my life was I prepared for what I was about to experience … My skin was so sore and constantly hot … I have never felt pain like this …' But don't you worry, the 'vaccine' is perfectly safe. Then there has been the effect on medical

staff who have been pressured to have the 'vaccine' by psychopathic 'health' authorities and government. A London hospital consultant who gave the name K. Polyakova wrote this to the *British Medical Journal* or *BMJ*:

> I am currently struggling with … the failure to report the reality of the morbidity caused by our current vaccination program within the health service and staff population. The levels of sickness after vaccination is unprecedented and staff are getting very sick and some with neurological symptoms which is having a huge impact on the health service function. Even the young and healthy are off for days, some for weeks, and some requiring medical treatment. Whole teams are being taken out as they went to get vaccinated together.
>
> Mandatory vaccination in this instance is stupid, unethical and irresponsible when it comes to protecting our staff and public health. We are in the voluntary phase of vaccination, and encouraging staff to take an unlicensed product that is impacting on their immediate health … it is clearly stated that these vaccine products do not offer immunity or stop transmission. In which case why are we doing it?

Not to protect health that's for sure. Medical workers are lauded by governments for agenda reasons when they couldn't give a toss about them any more than they can for the population in general. Schools across America faced the same situation as they closed due to the high number of teachers and other staff with bad reactions to the Pfizer/BioNTech, Moderna, and Johnson & Johnson 'Covid vaccines' all of which were linked to death and serious adverse effects. The *BMJ* took down the consultant's comments pretty quickly on the grounds that they were being used to spread 'disinformation'. They were exposing the truth about the 'vaccine' was the real reason. The cover-up is breathtaking.

Hiding the evidence

The scale of the 'vaccine' death cover-up worldwide can be confirmed by comparing official figures with the personal experience of the public. I heard of many people in my community who died immediately or soon after the vaccine that would never appear in the media or even likely on the official totals of 'vaccine' fatalities and adverse reactions when only about ten percent are estimated to be

reported and I have seen some estimates as low as one percent in a Harvard study. In the UK alone by April 29th, 2021, some 757,654 adverse reactions had been officially reported from the Pfizer/BioNTech, Oxford/AstraZeneca and Moderna 'vaccines' with more than a thousand deaths linked to jabs and that means an estimated ten times this number in reality from a ten percent reporting rate percentage. That's seven million adverse reactions and 10,000 potential deaths and a one percent reporting rate would be ten times *those* figures. In 1976 the US government pulled the swine flu vaccine after *53* deaths. The UK data included a combined 10,000 eye disorders from the 'Covid vaccines' with more than 750 suffering visual impairment or blindness and again multiply by the estimated reporting percentages. As 'Covid cases' officially fell hospitals virtually empty during the 'Covid crisis' began to fill up with a range of other problems in the wake of the 'vaccine' rollout. The numbers across America have also been catastrophic. Deaths linked to *all* types of vaccine increased by *6,000 percent* in the first quarter of 2021 compared with 2020. A 39-year-old woman from Ogden, Utah, died four days after receiving a second dose of Moderna's 'Covid vaccine' when her liver, heart and kidneys all failed despite the fact that she had no known medical issues or conditions. Her family sought an autopsy, but Dr Erik Christensen, Utah's chief medical examiner, said proving vaccine injury as a cause of death almost never happened. He could think of only one instance where an autopsy would name a vaccine as the official cause of death and that would be anaphylaxis where someone received a vaccine and died almost instantaneously. 'Short of that, it would be difficult for us to definitively say this is the vaccine,' Christensen said. If that is true this must be added to the estimated ten percent (or far less) reporting rate of vaccine deaths and serious reactions and the conclusion can only be that vaccine deaths and serious reactions – including these 'Covid' potions' – are phenomenally understated in official figures. The same story can be found everywhere. Endless accounts of deaths and serious reactions among the public, medical

and care home staff while official figures did not even begin to reflect this.

Professional script-reader Dr David Williams, a 'top public-health official' in Ontario, Canada, insulted our intelligence by claiming only four serious adverse reactions and no deaths from the more than 380,000 vaccine doses then given. This bore no resemblance to what people knew had happened in their owns circles and we had Dirk Huyer in charge of getting millions vaccinated in Ontario while at the same time he was Chief Coroner for the province investigating causes of death including possible death from the vaccine. An aide said he had stepped back from investigating deaths, but evidence indicated otherwise. Rosemary Frei, who secured a Master of Science degree in molecular biology at the Faculty of Medicine at Canada's University of Calgary before turning to investigative journalism, was one who could see that official figures for 'vaccine' deaths and reactions made no sense. She said that doctors seldom reported adverse events and when people got really sick or died after getting a vaccination they would attribute that to anything except the vaccines. It had been that way for years and anyone who wondered aloud whether the 'Covid vaccines' or other shots cause harm is immediately branded as 'anti-vax' and 'anti-science'. This was 'career-threatening' for health professionals. Then there was the huge pressure to support the push to 'vaccinate' billions in the quickest time possible. Frei said:

> So that's where we're at today. More than half a million vaccine doses have been given to people in Ontario alone. The rush is on to vaccinate all 15 million of us in the province by September. And the mainstream media are screaming for this to be sped up even more. That all adds up to only a very slim likelihood that we're going to be told the truth by officials about how many people are getting sick or dying from the vaccines.

What is true of Ontario is true of everywhere.

They KNEW – and still did it

The authorities knew what was going to happen with multiple deaths and adverse reactions. The UK government's Gates-funded

and Big Pharma-dominated Medicines and Healthcare products Regulatory Agency (MHRA) hired a company to employ AI in compiling the projected reactions to the 'vaccine' that would otherwise be uncountable. The request for applications said: 'The MHRA urgently seeks an Artificial Intelligence (AI) software tool to process the expected high volume of Covid-19 vaccine Adverse Drug Reaction …' This was from the agency, headed by the disingenuous June Raine, that gave the 'vaccines' emergency approval and the company was hired before the first shot was given. 'We are going to kill and maim you – is that okay?' 'Oh, yes, perfectly fine – I'm very grateful, thank you, doctor.' The range of 'Covid vaccine' adverse reactions goes on for page after page in the MHRA criminally underreported 'Yellow Card' system and includes affects to eyes, ears, skin, digestion, blood and so on. Raine's MHRA amazingly claimed that the 'overall safety experience … is so far as expected from the clinical trials'. The death, serious adverse effects, deafness and blindness were *expected?* When did they ever mention that? If these human tragedies were expected then those that gave approval for the use of these 'vaccines' must be guilty of crimes against humanity including murder – a definition of which is 'killing a person with malice aforethought or with recklessness manifesting extreme indifference to the value of human life.' People involved at the MHRA, the CDC in America and their equivalent around the world must go before Nuremberg trials to answer for their callous inhumanity. We are only talking here about the immediate effects of the 'vaccine'. The longer-term impact of the DNA synthetic manipulation is the main reason they are so hysterically desperate to inoculate the entire global population in the shortest possible time.

Africa and the developing world are a major focus for the 'vaccine' depopulation agenda and a mass vaccination sales-pitch is underway thanks to caring people like the Rockefellers and other Cult assets. The Rockefeller Foundation, which pre-empted the 'Covid pandemic' in a document published in 2010 that 'predicted' what happened a decade later, announced an initial $34.95 million grant in February, 2021, 'to ensure more equitable access to Covid-19

testing and vaccines' among other things in Africa in collaboration with '24 organizations, businesses, and government agencies'. The pan-Africa initiative would focus on 10 countries: Burkina Faso, Ethiopia, Ghana, Kenya, Nigeria, Rwanda, South Africa, Tanzania, Uganda, and Zambia'. Rajiv Shah, President of the Rockefeller Foundation and former administrator of CIA-controlled USAID, said that if Africa was not mass-vaccinated (to change the DNA of its people) it was a 'threat to all of humanity' and not fair on Africans. When someone from the Rockefeller Foundation says they want to do something to help poor and deprived people and countries it is time for a belly-laugh. They are doing this out of the goodness of their 'heart' because 'vaccinating' the entire global population is what the 'Covid' hoax set out to achieve. Official 'decolonisation' of Africa by the Cult was merely a prelude to financial colonisation on the road to a return to physical colonisation. The 'vaccine' is vital to that and the sudden and convenient death of the 'Covid' sceptic president of Tanzania can be seen in its true light. A lot of people in Africa are aware that this is another form of colonisation and exploitation and they need to stand their ground.

The 'vaccine is working' scam

A potential problem for the Cult was that the 'vaccine' is meant to change human DNA and body messaging and not to protect anyone from a 'virus' never shown to exist. The vaccine couldn't work because it was not designed to work and how could they make it *appear* to be working so that more people would have it? This was overcome by lowering the amplification rate of the PCR test to produce fewer 'cases' and therefore fewer 'deaths'. Some of us had been pointing out since March, 2020, that the amplification rate of the test not testing for the 'virus' had been made artificially high to generate positive tests which they could call 'cases' to justify lockdowns. The World Health Organization recommended an absurdly high 45 amplification cycles to ensure the high positives required by the Cult and then remained silent on the issue until January 20th, 2021 – Biden's Inauguration Day. This was when the

'vaccinations' were seriously underway and on that day the WHO recommended after discussions with America's CDC that laboratories *lowered their testing amplification*. Dr David Samadi, a certified urologist and health writer, said the WHO was encouraging all labs to reduce their cycle count for PCR tests. He said the current cycle was much too high and was 'resulting in any particle being declared a positive case'. Even one mainstream news report I saw said this meant the number of 'Covid' infections may have been 'dramatically inflated'. Oh, just a little bit. The CDC in America issued new guidance to laboratories in April, 2021, to use 28 cycles *but only for 'vaccinated' people*. The timing of the CDC/WHO interventions were cynically designed to make it appear the 'vaccines' were responsible for falling cases and deaths when the real reason can be seen in the following examples. New York's state lab, the Wadsworth Center, identified 872 positive tests in July, 2020, based on a threshold of 40 cycles. When the figure was lowered to 35 cycles *43 percent* of the 872 were no longer 'positives'. At 30 cycles the figure was 63 percent. A Massachusetts lab found that between *85 to 90 percent* of people who tested positive in July with a cycle threshold of 40 would be negative at 30 cycles, Ashish Jha, MD, director of the Harvard Global Health Institute, said: 'I'm really shocked that it could be that high … Boy, does it really change the way we need to be thinking about testing.' I'm shocked that I could see the obvious in the spring of 2020, with no medical background, and most medical professionals still haven't worked it out. No, that's not shocking – it's terrifying.

Three weeks after the WHO directive to lower PCR cycles the London *Daily Mail* ran this headline: 'Why ARE Covid cases plummeting? New infections have fallen 45% in the US and 30% globally in the past 3 weeks but experts say vaccine is NOT the main driver because only 8% of Americans and 13% of people worldwide have received their first dose.' They acknowledged that the drop could not be attributed to the 'vaccine', but soon this morphed throughout the media into the 'vaccine' has caused cases and deaths to fall when it was the PCR threshold. In December, 2020, there was

chaos at English Channel ports with truck drivers needing negative 'Covid' tests before they could board a ferry home for Christmas. The government wanted to remove the backlog as fast as possible and they brought in troops to do the 'testing'. Out of 1,600 drivers just *36* tested positive and the rest were given the all clear to cross the Channel. I guess the authorities thought that 36 was the least they could get away with without the unquestioning catching on. The amplification trick which most people believed in the absence of information in the mainstream applied more pressure on those refusing the 'vaccine' to succumb when it 'obviously worked'. The truth was the exact opposite with deaths in care homes soaring with the 'vaccine' and in Israel the term used was 'skyrocket'. A re-analysis of published data from the Israeli Health Ministry led by Dr Hervé Seligmann at the Medicine Emerging Infectious and Tropical Diseases at Aix-Marseille University found that Pfizer's 'Covid vaccine' killed 'about 40 times more [elderly] people than the disease itself would have killed' during a five-week vaccination period and *260 times* more younger people than would have died from the 'virus' even according to the manipulated 'virus' figures. Dr Seligmann and his co-study author, Haim Yativ, declared after reviewing the Israeli 'vaccine' death data: 'This is a new Holocaust.'

Then, in mid-April, 2021, after vast numbers of people worldwide had been 'vaccinated', the story changed with clear coordination. The UK government began to prepare the ground for more future lockdowns when Nuremberg-destined Boris Johnson told yet another whopper. He said that cases had fallen because of *lockdowns* not 'vaccines'. Lockdowns are irrelevant when *there is no 'virus'* and the test and fraudulent death certificates are deciding the number of 'cases' and 'deaths'. Study after study has shown that lockdowns don't work and instead kill and psychologically destroy people. Meanwhile in the United States Anthony Fauci and Rochelle Walensky, the ultra-Zionist head of the CDC, peddled the same line. More lockdown was the answer and not the 'vaccine', a line repeated on cue by the moron that is Canadian Prime Minister Justin Trudeau. Why all the hysteria to get everyone 'vaccinated' if lockdowns and

not 'vaccines' made the difference? None of it makes sense on the face of it. Oh, but it does. The Cult wants lockdowns *and* the 'vaccine' and if the 'vaccine' is allowed to be seen as the total answer lockdowns would no longer be justified when there are still livelihoods to destroy. 'Variants' and renewed upward manipulation of PCR amplification are planned to instigate never-ending lockdown *and* more 'vaccines'.

You *must* have it – we're desperate

Israel, where the Jewish and Arab population are ruled by the Sabbatian Cult, was the front-runner in imposing the DNA-manipulating 'vaccine' on its people to such an extent that Jewish refusers began to liken what was happening to the early years of Nazi Germany. This would seem to be a fantastic claim. Why would a government of Jewish people be acting like the Nazis did? If you realise that the Sabbatian Cult was behind the Nazis and that Sabbatians hate Jews the pieces start to fit and the question of why a 'Jewish' government would treat Jews with such callous disregard for their lives and freedom finds an answer. Those controlling the government of Israel *aren't Jewish* – they're Sabbatian. Israeli lawyer Tamir Turgal was one who made the Nazi comparison in comments to German lawyer Reiner Fuellmich who is leading a class action lawsuit against the psychopaths for crimes against humanity. Turgal described how the Israeli government was vaccinating children and pregnant women on the basis that there was no evidence that this was dangerous when they had no evidence that it *wasn't* dangerous either. They just had no evidence. This was medical experimentation and Turgal said this breached the Nuremberg Code about medical experimentation and procedures requiring informed consent and choice. Think about that. A Nuremberg Code developed because of Nazi experimentation on Jews and others in concentration camps by people like the evil-beyond-belief Josef Mengele is being breached by the *Israeli* government; but when you know that it's a *Sabbatian* government along with its intelligence and military agencies like Mossad, Shin Bet and the Israeli Defense Forces, and that Sabbatians

were the force behind the Nazis, the kaleidoscope comes into focus. What have we come to when Israeli Jews are suing their government for violating the Nuremberg Code by essentially making Israelis subject to a medical experiment using the controversial 'vaccines'? It's a shocker that this has to be done in the light of what happened in Nazi Germany. The Anshe Ha-Emet, or 'People of the Truth', made up of Israeli doctors, lawyers, campaigners and public, have launched a lawsuit with the International Criminal Court. It says:

> When the heads of the Ministry of Health as well as the prime minister presented the vaccine in Israel and began the vaccination of Israeli residents, the vaccinated were not advised, that, in practice, they are taking part in a medical experiment and that their consent is required for this under the Nuremberg Code.

The irony is unbelievable, but easily explained in one word: Sabbatians. The foundation of Israeli 'Covid' apartheid is the 'green pass' or 'green passport' which allows Jews and Arabs who have had the DNA-manipulating 'vaccine' to go about their lives – to work, fly, travel in general, go to shopping malls, bars, restaurants, hotels, concerts, gyms, swimming pools, theatres and sports venues, while non-'vaccinated' are banned from all those places and activities. Israelis have likened the 'green pass' to the yellow stars that Jews in Nazi Germany were forced to wear – the same as the yellow stickers that a branch of UK supermarket chain Morrisons told exempt mask-wears they had to display when shopping. How very sensitive. The Israeli system is blatant South African-style apartheid on the basis of compliance or non-compliance to fascism rather than colour of the skin. How appropriate that the Sabbatian Israeli government was so close to the pre-Mandela apartheid regime in Pretoria. The Sabbatian-instigated 'vaccine passport' in Israel is planned for everywhere. Sabbatians struck a deal with Pfizer that allowed them to lead the way in the percentage of a national population infused with synthetic material and the result was catastrophic. Israeli freedom activist Shai Dannon told me how chairs were appearing on beaches that said 'vaccinated only'. Health Minister Yuli Edelstein said that anyone unwilling or unable to get

the jabs that 'confer immunity' will be 'left behind'. The man's a liar. Not even the makers claim the 'vaccines' confer immunity. When you see those figures of 'vaccine' deaths these psychopaths were saying that you must take the chance the 'vaccine' will kill you or maim you while knowing it will change your DNA or lockdown for you will be permanent. That's fascism. The Israeli parliament passed a law to allow personal information of the non-vaccinated to be shared with local and national authorities for three months. This was claimed by its supporters to be a way to 'encourage' people to be vaccinated. Hadas Ziv from Physicians for Human Rights described this as a 'draconian law which crushed medical ethics and the patient rights'. But that's the idea, the Sabbatians would reply.

Your papers, please

Sabbatian Israel was leading what has been planned all along to be a global 'vaccine pass' called a 'green passport' without which you would remain in permanent lockdown restriction and unable to do anything. This is how badly – *desperately* – the Cult is to get everyone 'vaccinated'. The term and colour 'green' was not by chance and related to the psychology of fusing the perception of the green climate hoax with the 'Covid' hoax and how the 'solution' to both is the same Great Reset. Lying politicians, health officials and psychologists denied there were any plans for mandatory vaccinations or restrictions based on vaccinations, but they knew that was exactly what was meant to happen with governments of all countries reaching agreements to enforce a global system. 'Free' Denmark and 'free' Sweden unveiled digital vaccine certification. Cyprus, Czech Republic, Estonia, Greece, Hungary, Iceland, Italy, Poland, Portugal, Slovakia, and Spain have all committed to a vaccine passport system and the rest including the whole of the EU would follow. The satanic UK government will certainly go this way despite mendacious denials and at the time of writing it is trying to manipulate the public into having the 'vaccine' so they could go abroad on a summer holiday. How would that work without something to prove you had the synthetic toxicity injected into you?

Documents show that the EU's European Commission was moving towards 'vaccine certificates' in 2018 and 2019 before the 'Covid' hoax began. They knew what was coming. Abracadabra – Ursula von der Leyen, the German President of the Commission, announced in March, 2021, an EU 'Digital Green Certificate' – green again – to track the public's 'Covid status'. The passport sting is worldwide and the Far East followed the same pattern with South Korea ruling that only those with 'vaccination' passports – again the *green* pass – would be able to 'return to their daily lives'.

Bill Gates has been preparing for this 'passport' with other Cult operatives for years and beyond the paper version is a Gates-funded 'digital tattoo' to identify who has been vaccinated and who hasn't. The 'tattoo' is reported to include a substance which is externally readable to confirm who has been vaccinated. This is a bio-luminous light-generating enzyme (think fireflies) called … *Luciferase*. Yes, named after the Cult 'god' Lucifer the 'light bringer' of whom more to come. Gates said he funded the readable tattoo to ensure children in the developing world were vaccinated and no one was missed out. He cares so much about poor kids as we know. This was just the cover story to develop a vaccine tagging system for everyone on the planet. Gates has been funding the ID2020 'alliance' to do just that in league with other lovely people at Microsoft, GAVI, the Rockefeller Foundation, Accenture and IDEO.org. He said in interviews in March, 2020, before any 'vaccine' publicly existed, that the world must have a globalised digital certificate to track the 'virus' and who had been vaccinated. Gates knew from the start that the mRNA vaccines were coming and when they would come and that the plan was to tag the 'vaccinated' to marginalise the intelligent and stop them doing anything including travel. Evil just doesn't suffice. Gates was exposed for offering a $10 million bribe to the Nigerian House of Representatives to invoke compulsory 'Covid' vaccination of all Nigerians. Sara Cunial, a member of the Italian Parliament, called Gates a 'vaccine criminal'. She urged the Italian President to hand him over to the International Criminal Court for crimes against

humanity and condemned his plans to 'chip the human race' through ID2020.

You know it's a long-planned agenda when war criminal and Cult gofer Tony Blair is on the case. With the scale of arrogance only someone as dark as Blair can muster he said: 'Vaccination in the end is going to be your route to liberty.' Blair is a disgusting piece of work and he confirms that again. The media has given a lot of coverage to a bloke called Charlie Mullins, founder of London's biggest independent plumbing company, Pimlico Plumbers, who has said he won't employ anyone who has not been vaccinated or have them go to any home where people are not vaccinated. He said that if he had his way no one would be allowed to walk the streets if they have not been vaccinated. Gates was cheering at the time while I was alerting the white coats. The plan is that people will qualify for 'passports' for having the first two doses and then to keep it they will have to have all the follow ups and new ones for invented 'variants' until human genetics is transformed and many are dead who can't adjust to the changes. Hollywood celebrities – the usual propaganda stunt – are promoting something called the WELL Health-Safety Rating to verify that a building or space has 'taken the necessary steps to prioritize the health and safety of their staff, visitors and other stakeholders'. They included Lady Gaga, Jennifer Lopez, Michael B. Jordan, Robert DeNiro, Venus Williams, Wolfgang Puck, Deepak Chopra and 17th Surgeon General Richard Carmona. Yawn. WELL Health-Safety has big connections with China. Parent company Delos is headed by former Goldman Sachs partner Paul Scialla. This is another example – and we will see so many others – of using the excuse of 'health' to dictate the lives and activities of the population. I guess one confirmation of the 'safety' of buildings is that only 'vaccinated' people can go in, right?

Electronic concentration camps

I wrote decades ago about the plans to restrict travel and here we are for those who refuse to bow to tyranny. This can be achieved in one go with air travel if the aviation industry makes a blanket decree.

The ‘vaccine’ and guaranteed income are designed to be part of a global version of China’s social credit system which tracks behaviour 24/7 and awards or deletes ‘credits’ based on whether your behaviour is supported by the state or not. I mean your entire lifestyle – what you do, eat, say, everything. Once your credit score falls below a certain level consequences kick in. In China tens of millions have been denied travel by air and train because of this. All the locations and activities denied to refusers by the ‘vaccine’ passports will be included in one big mass ban on doing almost anything for those that don’t bow their head to government. It’s beyond fascist and a new term is required to describe its extremes – I guess fascist technocracy will have to do. The way the Chinese system of technological – technocratic – control is sweeping the West can be seen in the Los Angeles school system and is planned to be expanded worldwide. Every child is required to have a ‘Covid’-tracking app scanned daily before they can enter the classroom. The so-called Daily Pass tracking system is produced by Gates’ Microsoft which I’m sure will shock you rigid. The pass will be scanned using a barcode (one step from an inside-the-body barcode) and the information will include health checks, ‘Covid’ tests and vaccinations. Entry codes are for one specific building only and access will only be allowed if a student or teacher has a negative test with a test not testing for the ‘virus’, has no symptoms of anything alleged to be related to ‘Covid’ (symptoms from a range of other illness), and has a temperature under 100 degrees. No barcode, no entry, is planned to be the case for everywhere and not only schools.

Kids are being psychologically prepared to accept this as ‘normal’ their whole life which is why what they can impose in schools is so important to the Cult and its gofers. Long-time American freedom campaigner John Whitehead of the Rutherford Institute was not exaggerating when he said: ‘Databit by databit, we are building our own electronic concentration camps.’ Canada under its Cult gofer prime minister Justin Trudeau has taken a major step towards the real thing with people interned against their will if they test positive with a test not testing for the ‘virus’ when they arrive at a Canadian

airport. They are jailed in internment hotels often without food or water for long periods and with many doors failing to lock there have been sexual assaults. The interned are being charged sometimes $2,000 for the privilege of being abused in this way. Trudeau is fully on board with the Cult and says the 'Covid pandemic' has provided an opportunity for a global 'reset' to permanently change Western civilisation. His number two, Deputy Prime Minister Chrystia Freeland, is a trustee of the World Economic Forum and a Rhodes Scholar. The Trudeau family have long been servants of the Cult. See *The Biggest Secret* and Cathy O'Brien's book *Trance-Formation of America* for the horrific background to Trudeau's father Pierre Trudeau another Canadian prime minister. Hide your fascism behind the façade of a heart-on-the-sleeve liberal. It's a well-honed Cult technique.

What can the 'vaccine' *really* do?

We have a 'virus' never shown to exist and 'variants' of the 'virus' that have also never been shown to exist except, like the 'original', as computer-generated fictions. Even if you believe there's a 'virus' the 'case' to 'death' rate is in the region of 0.23 to 0.15 percent and those 'deaths' are concentrated among the very old around the same average age that people die anyway. In response to this lack of threat (in truth none) psychopaths and idiots, knowingly and unknowingly answering to Gates and the Cult, are seeking to 'vaccinate' every man, woman and child on Planet Earth. Clearly the 'vaccine' is not about 'Covid' – none of this ever has been. So what is it all about *really*? Why the desperation to infuse genetically-manipulating synthetic material into everyone through mRNA fraudulent 'vaccines' with the intent of doing this over and over with the excuses of 'variants' and other 'virus' inventions? Dr Sherri Tenpenny, an osteopathic medical doctor in the United States, has made herself an expert on vaccines and their effects as a vehement campaigner against their use. Tenpenny was board certified in emergency medicine, the director of a level two trauma centre for 12 years, and moved to Cleveland in 1996 to start an integrative

medicine practice which has treated patients from all 50 states and some 17 other countries. Weaning people off pharmaceutical drugs is a speciality.

She became interested in the consequences of vaccines after attending a meeting at the National Vaccine Information Center in Washington DC in 2000 where she 'sat through four days of listening to medical doctors and scientists and lawyers and parents of vaccine injured kids' and asked: 'What's going on?' She had never been vaccinated and never got ill while her father was given a list of vaccines to be in the military and was 'sick his entire life'. The experience added to her questions and she began to examine vaccine documents from the Centers for Disease Control (CDC). After reading the first one, the 1998 version of *The General Recommendations of Vaccination*, she thought: 'This is it?' The document was poorly written and bad science and Tenpenny began 20 years of research into vaccines that continues to this day. She began her research into 'Covid vaccines' in March, 2020, and she describes them as 'deadly'. For many, as we have seen, they already have been. Tenpenny said that in the first 30 days of the 'vaccine' rollout in the United States there had been more than 40,000 adverse events reported to the vaccine adverse event database. A document had been delivered to her the day before that was 172 pages long. 'We have over 40,000 adverse events; we have over 3,100 cases of [potentially deadly] anaphylactic shock; we have over 5,000 neurological reactions.' Effects ranged from headaches to numbness, dizziness and vertigo, to losing feeling in hands or feet and paraesthesia which is when limbs 'fall asleep' and people have the sensation of insects crawling underneath their skin. All this happened in the first 30 days and remember that only about *ten percent* (or far less) of adverse reactions and vaccine-related deaths are estimated to be officially reported. Tenpenny said:

> So can you think of one single product in any industry, any industry, for as long as products have been made on the planet that within 30 days we have 40,000 people complaining of side effects that not only is still on the market but … we've got paid actors telling us how great

they are for getting their vaccine. We're offering people $500 if they will just get their vaccine and we've got nurses and doctors going; 'I got the vaccine, I got the vaccine'.

Tenpenny said they were not going to be 'happy dancing folks' when they began to suffer Bell's palsy (facial paralysis), neuropathies, cardiac arrhythmias and autoimmune reactions that kill through a blood disorder. 'They're not going to be so happy, happy then, but we're never going to see pictures of those people' she said. Tenpenny described the 'vaccine' as 'a well-designed killing tool'.

No off-switch

Bad as the initial consequences had been Tenpenny said it would be maybe 14 months before we began to see the 'full ravage' of what is going to happen to the 'Covid vaccinated' with full-out consequences taking anything between two years and 20 years to show. You can understand why when you consider that variations of the 'Covid vaccine' use mRNA (messenger RNA) to in theory activate the immune system to produce protective antibodies without using the actual 'virus'. How can they when it's a computer program and they've never isolated what they claim is the 'real thing'? Instead they use *synthetic* mRNA. They are inoculating synthetic material into the body which through a technique known as the Trojan horse is absorbed into cells to change the nature of DNA. Human DNA is changed by an infusion of messenger RNA and with each new 'vaccine' of this type it is changed even more. Say so and you are banned by Cult Internet platforms. The contempt the contemptuous Mark Zuckerberg has for the truth and human health can be seen in an internal Facebook video leaked to the Project Veritas investigative team in which he said of the 'Covid vaccines': '… I share some caution on this because we just don't know the long term side-effects of basically modifying people's DNA and RNA.' At the same time this disgusting man's Facebook was censoring and banning anyone saying exactly the same. He must go before a Nuremberg trial for crimes against humanity when he *knows* that he

is censoring legitimate concerns and denying the right of informed consent on behalf of the Cult that owns him. People have been killed and damaged by the very 'vaccination' technique he cast doubt on himself when they may not have had the 'vaccine' with access to information that he denied them. The plan is to have at least annual 'Covid vaccinations', add others to deal with invented 'variants', and change all other vaccines into the mRNA system. Pfizer executives told shareholders at a virtual Barclays Global Healthcare Conference in March, 2021, that the public may need a third dose of 'Covid vaccine', plus regular yearly boosters and the company planned to hike prices to milk the profits in a 'significant opportunity for our vaccine'. These are the professional liars, cheats and opportunists who are telling you their 'vaccine' is safe. Given this volume of mRNA planned to be infused into the human body and its ability to then replicate we will have a transformation of human genetics from biological to synthetic biological – exactly the long-time Cult plan for reasons we'll see – and many will die. Sherri Tenpenny said of this replication:

> It's like having an on-button but no off-button and that whole mechanism … they actually give it a name and they call it the Trojan horse mechanism, because it allows that [synthetic] virus and that piece of that [synthetic] virus to get inside of your cells, start to replicate and even get inserted into other parts of your DNA as a Trojan-horse.

Ask the overwhelming majority of people who have the 'vaccine' what they know about the contents and what they do and they would reply: 'The government says it will stop me getting the virus.' Governments give that false impression on purpose to increase take-up. You can read Sherri Tenpenny's detailed analysis of the health consequences in her blog at Vaxxter.com, but in summary these are some of them. She highlights the statement by Bill Gates about how human beings can become their own 'vaccine manufacturing machine'. The man is insane. ['Vaccine'-generated] 'antibodies' carry synthetic messenger RNA into the cells and the damage starts, Tenpenny contends, and she says that lungs can be adversely affected through varying degrees of pus and bleeding which

obviously affects breathing and would be dubbed 'Covid-19'. Even more sinister was the impact of 'antibodies' on macrophages, a white blood cell of the immune system. They consist of Type 1 and Type 2 which have very different functions. She said Type 1 are 'hyper-vigilant' white blood cells which 'gobble up' bacteria etc. However, in doing so, this could cause inflammation and in extreme circumstances be fatal. She says these affects are mitigated by Type 2 macrophages which kick in to calm down the system and stop it going rogue. They clear up dead tissue debris and reduce inflammation that the Type 1 'fire crews' have caused. Type 1 kills the infection and Type 2 heals the damage, she says. This is her punchline with regard to 'Covid vaccinations': She says that mRNA 'antibodies' block Type 2 macrophages by attaching to them and deactivating them. This meant that when the Type 1 response was triggered by infection there was nothing to stop that getting out of hand by calming everything down. There's an on-switch, but no off-switch, she says. What follows can be 'over and out, see you when I see you'.

Genetic suicide

Tenpenny also highlights the potential for autoimmune disease – the body attacking itself – which has been associated with vaccines since they first appeared. Infusing a synthetic foreign substance into cells could cause the immune system to react in a panic believing that the body is being overwhelmed by an invader (it is) and the consequences can again be fatal. There is an autoimmune response known as a 'cytokine storm' which I have likened to a homeowner panicked by an intruder and picking up a gun to shoot randomly in all directions before turning the fire on himself. The immune system unleashes a storm of inflammatory response called cytokines to a threat and the body commits hara-kiri. The lesson is that you mess with the body's immune response at your peril and these 'vaccines' seriously – fundamentally – mess with immune response. Tenpenny refers to a consequence called anaphylactic shock which is a severe and highly dangerous allergic reaction when the immune system

floods the body with chemicals. She gives the example of having a bee sting which primes the immune system and makes it sensitive to those chemicals. When people are stung again maybe years later the immune response can be so powerful that it leads to anaphylactic shock. Tenpenny relates this 'shock' with regard to the 'Covid vaccine' to something called polyethylene glycol or PEG. Enormous numbers of people have become sensitive to this over decades of use in a whole range of products and processes including food, drink, skin creams and 'medicine'. Studies have claimed that some 72 percent of people have antibodies triggered by PEG compared with two percent in the 1960s and allergic hypersensitive reactions to this become a gathering cause for concern. Tenpenny points out that the 'mRNA vaccine' is coated in a 'bubble' of polyethylene glycol which has the potential to cause anaphylactic shock through immune sensitivity. Many reports have appeared of people reacting this way after having the 'Covid vaccine'. What do we think is going to happen as humanity has more and more of these 'vaccines'? Tenpenny said: 'All these pictures we have seen with people with these rashes … these weepy rashes, big reactions on their arms and things like that – it's an acute allergic reaction most likely to the polyethylene glycol that you've been previously primed and sensitised to.'

Those who have not studied the conspiracy and its perpetrators at length might think that making the population sensitive to PEG and then putting it in these 'vaccines' is just a coincidence. It is not. It is instead testament to how carefully and coldly-planned current events have been and the scale of the conspiracy we are dealing with. Tenpenny further explains that the 'vaccine' mRNA procedure can breach the blood-brain barrier which protects the brain from toxins and other crap that will cause malfunction. In this case they could make two proteins corrupt brain function to cause Amyotrophic lateral sclerosis (ALS) , a progressive nervous system disease leading to loss of muscle control, and frontal lobe degeneration – Alzheimer's and dementia. Immunologist J. Bart Classon published a paper connecting mRNA 'vaccines' to prion

disease which can lead to Alzheimer's and other forms of neurogenerative disease while others have pointed out the potential to affect the placenta in ways that make women infertile. This will become highly significant in the next chapter when I will discuss other aspects of this non-vaccine that relate to its nanotechnology and transmission from the injected to the uninjected.

Qualified in idiocy

Tenpenny describes how research has confirmed that these 'vaccine'-generated antibodies can interact with a range of other tissues in the body and attack many other organs including the lungs. 'This means that if you have a hundred people standing in front of you that all got this shot they could have a hundred different symptoms.' Anyone really think that Cult gofers like the Queen, Tony Blair, Christopher Whitty, Anthony Fauci, and all the other psychopaths have really had this 'vaccine' in the pictures we've seen? Not a bloody chance. Why don't doctors all tell us about all these dangers and consequences of the 'Covid vaccine'? Why instead do they encourage and pressure patients to have the shot? Don't let's think for a moment that doctors and medical staff can't be stupid, lazy, and psychopathic and that's without the financial incentives to give the jab. Tenpenny again:

> Some people are going to die from the vaccine directly but a large number of people are going to start to get horribly sick and get all kinds of autoimmune diseases 42 days to maybe a year out. What are they going to do, these stupid doctors who say; 'Good for you for getting that vaccine.' What are they going to say; 'Oh, it must be a mutant, we need to give an extra dose of that vaccine.'
>
> Because now the vaccine, instead of one dose or two doses we need three or four because the stupid physicians aren't taking the time to learn anything about it. If I can learn this sitting in my living room reading a 19 page paper and several others so can they. There's nothing special about me, I just take the time to do it.

Remember how Sara Kayat, the NHS and TV doctor, said that the 'Covid vaccine' would '100 percent prevent hospitalisation and death'. Doctors can be idiots like every other profession and they

should not be worshipped as infallible. They are not and far from it. Behind many medical and scientific 'experts' lies an uninformed prat trying to hide themselves from you although in the 'Covid' era many have failed to do so as with UK narrative-repeating 'TV doctor' Hilary Jones. Pushing back against the minority of proper doctors and scientists speaking out against the 'vaccine' has been the entire edifice of the Cult global state in the form of governments, medical systems, corporations, mainstream media, Silicon Valley, and an army of compliant doctors, medical staff and scientists willing to say anything for money and to enhance their careers by promoting the party line. If you do that you are an 'expert' and if you won't you are an 'anti-vaxxer' and 'Covidiot'. The pressure to be 'vaccinated' is incessant. We have even had reports claiming that the 'vaccine' can help cure cancer and Alzheimer's and make the lame walk. I am waiting for the announcement that it can bring you coffee in the morning and cook your tea. Just as the symptoms of 'Covid' seem to increase by the week so have the miracles of the 'vaccine'. American supermarket giant Kroger Co. offered nearly 500,000 employees in 35 states a $100 bonus for having the 'vaccine' while donut chain Krispy Kreme promised 'vaccinated' customers a free glazed donut every day for the rest of 2021. Have your DNA changed and you will get a doughnut although we might not have to give you them for long. Such offers and incentives confirm the desperation.

Perhaps the worse vaccine-stunt of them all was UK 'Health' Secretary Matt-the-prat Hancock on live TV after watching a clip of someone being 'vaccinated' when the roll-out began. Hancock faked tears so badly it was embarrassing. Brain-of-Britain Piers Morgan, the lockdown-supporting, 'vaccine' supporting, 'vaccine' passport-supporting, TV host played along with Hancock – 'You're quite emotional about that' he said in response to acting so atrocious it would have been called out at a school nativity which will presumably today include Mary and Jesus in masks, wise men keeping their camels six feet apart, and shepherds under tent arrest. System-serving Morgan tweeted this: 'Love the idea of covid vaccine passports for everywhere: flights, restaurants, clubs, football, gyms,

shops etc. It's time covid-denying, anti-vaxxer loonies had their bullsh*t bluff called & bar themselves from going anywhere that responsible citizens go.' If only I could aspire to his genius. To think that Morgan, who specialises in shouting over anyone he disagrees with, was lauded as a free speech hero when he lost his job after storming off the set of his live show like a child throwing his dolly out of the pram. If he is a free speech hero we are in real trouble. I have no idea what 'bullsh*t' means, by the way, the * throws me completely.

The Cult is desperate to infuse its synthetic DNA-changing concoction into everyone and has been using every lie, trick and intimidation to do so. The question of '*Why*?' we shall now address.

CHAPTER TEN

Human 2.0

I believe that at the end of the century the use of words and general educated opinion will have altered so much that one will be able to speak of machines thinking without expecting to be contradicted –
Alan Turing (1912-1954), the 'Father of artificial intelligence'

I have been exposing for decades the plan to transform the human body from a biological to a synthetic-biological state. The new human that I will call Human 2.0 is planned to be connected to artificial intelligence and a global AI 'Smart Grid' that would operate as one global system in which AI would control everything from your fridge to your heating system to your car to your mind. Humans would no longer be 'human', but post-human and sub-human, with their thinking and emotional processes replaced by AI.

What I said sounded crazy and beyond science fiction and I could understand that. To any balanced, rational, mind it *is* crazy. Today, however, that world is becoming reality and it puts the 'Covid vaccine' into its true context. Ray Kurzweil is the ultra-Zionist 'computer scientist, inventor and futurist' and co-founder of the Singularity University. Singularity refers to the merging of humans with machines or 'transhumanism'. Kurzweil has said humanity would be connected to the cyber 'cloud' in the period of the ever-recurring year of 2030:

Our thinking … will be a hybrid of biological and non-biological thinking … humans will be able to extend their limitations and 'think in the cloud' … We're going to put gateways to the

cloud in our brains ... We're going to gradually merge and enhance ourselves ... In my view, that's the nature of being human – we transcend our limitations. As the technology becomes vastly superior to what we are then the small proportion that is still human gets smaller and smaller and smaller until it's just utterly negligible.

They are trying to sell this end-of-humanity-as-we-know-it as the next stage of 'evolution' when we become super-human and 'like the gods'. They are lying to you. Shocked, eh? The population, and again especially the young, have been manipulated into addiction to technologies designed to enslave them for life. First they induced an addiction to smartphones (holdables); next they moved to technology on the body (wearables); and then began the invasion of the body (implantables). I warned way back about the plan for microchipped people and we are now entering that era. We should not be diverted into thinking that this refers only to chips we can see. Most important are the nanochips known as smart dust, neural dust and nanobots which are far too small to be seen by the human eye. Nanotechnology is everywhere, increasingly in food products, and released into the atmosphere by the geoengineering of the skies funded by Bill Gates to 'shut out the Sun' and 'save the planet from global warming'. Gates has been funding a project to spray millions of tonnes of chalk (calcium carbonate) into the stratosphere over Sweden to 'dim the Sun' and cool the Earth. Scientists warned the move could be disastrous for weather systems in ways no one can predict and opposition led to the Swedish space agency announcing that the 'experiment' would not be happening as planned in the summer of 2021; but it shows where the Cult is going with dimming the impact of the Sun and there's an associated plan to change the planet's atmosphere. Who gives psychopath Gates the right to dictate to the entire human race and dismantle planetary systems? The world will not be safe while this man is at large.

The global warming hoax has made the Sun, like the gas of life, something to fear when both are essential to good health and human survival (more inversion). The body transforms sunlight into vital vitamin D through a process involving … *cholesterol*. This is the cholesterol we are also told to fear. We are urged to take Big Pharma

statin drugs to reduce cholesterol and it's all systematic. Reducing cholesterol means reducing vitamin D uptake with all the multiple health problems that will cause. At least if you take statins long term it saves the government from having to pay you a pension. The delivery system to block sunlight is widely referred to as chemtrails although these have a much deeper agenda, too. They appear at first to be contrails or condensation trails streaming from aircraft into cold air at high altitudes. Contrails disperse very quickly while chemtrails do not and spread out across the sky before eventually their content falls to earth. Many times I have watched aircraft cross-cross a clear blue sky releasing chemtrails until it looks like a cloudy day. Chemtrails contain many things harmful to humans and the natural world including toxic heavy metals, aluminium (see Alzheimer's) and nanotechnology. Ray Kurzweil reveals the reason without actually saying so: 'Nanobots will infuse all the matter around us with information. Rocks, trees, everything will become these intelligent creatures.' How do you deliver that? *From the sky*. Self-replicating nanobots would connect everything to the Smart Grid. The phenomenon of Morgellons disease began in the chemtrail era and the correlation has led to it being dubbed the 'chemtrail disease'. Self-replicating fibres appear in the body that can be pulled out through the skin. Morgellons fibres continue to grow outside the body and have a form of artificial intelligence. I cover this at greater length in *Phantom Self*.

'Vaccine' operating system

'Covid vaccines' with their self-replicating synthetic material are also designed to make the connection between humanity and Kurzweil's 'cloud'. American doctor and dedicated campaigner for truth, Carrie Madej, an Internal Medicine Specialist in Georgia with more than 20 years medical experience, has highlighted the nanotechnology aspect of the fake 'vaccines'. She explains how one of the components in at least the Moderna and Pfizer synthetic potions are 'lipid nanoparticles' which are 'like little tiny computer bits' – a 'sci-fi substance' known as nanobots and hydrogel which can be 'triggered

at any moment to deliver its payload' and act as 'biosensors'. The synthetic substance had 'the ability to accumulate data from your body like your breathing, your respiration, thoughts and emotions, all kind of things' and each syringe could carry a *million* nanobots:

This substance because it's like little bits of computers in your body, crazy, but it's true, it can do that, [and] obviously has the ability to act through Wi-Fi. It can receive and transmit energy, messages, frequencies or impulses. That issue has never been addressed by these companies. What does that do to the human?

Just imagine getting this substance in you and it can react to things all around you, the 5G, your smart device, your phones, what is happening with that? What if something is triggering it, too, like an impulse, a frequency? We have something completely foreign in the human body.

Madej said her research revealed that electromagnetic (EMF) frequencies emitted by phones and other devices had increased dramatically in the same period of the 'vaccine' rollout and she was seeing more people with radiation problems as 5G and other electromagnetic technology was expanded and introduced to schools and hospitals. She said she was 'floored with the EMF coming off' the devices she checked. All this makes total sense and syncs with my own work of decades when you think that Moderna refers in documents to its mRNA 'vaccine' as an 'operating system':

Recognizing the broad potential of mRNA science, we set out to create an mRNA technology platform that functions very much like an operating system on a computer. It is designed so that it can plug and play interchangeably with different programs. In our case, the 'program' or 'app' is our mRNA drug – the unique mRNA sequence that codes for a protein …

… Our MRNA Medicines – 'The 'Software Of Life': When we have a concept for a new mRNA medicine and begin research, fundamental components are already in place. Generally, the only thing that changes from one potential mRNA medicine to another is the coding region – the actual genetic code that instructs ribosomes to make protein. Utilizing these instruction sets gives our investigational mRNA medicines a software-like quality. We also have the ability to combine different mRNA sequences encoding for different proteins in a single mRNA investigational medicine.

Who needs a real 'virus' when you can create a computer version to justify infusing your operating system into the entire human race on the road to making living, breathing people into cyborgs? What is missed with the 'vaccines' is the *digital* connection between synthetic material and the body that I highlighted earlier with the study that hacked a computer with human DNA. On one level the body is digital, based on mathematical codes, and I'll have more about that in the next chapter. Those who ridiculously claim that mRNA 'vaccines' are not designed to change human genetics should explain the words of Dr Tal Zaks, chief medical officer at Moderna, in a 2017 TED talk. He said that over the last 30 years 'we've been living this phenomenal digital scientific revolution, and I'm here today to tell you, that we are actually *hacking the software of life*, and that it's changing the way we think about prevention and treatment of disease':

In every cell there's this thing called messenger RNA, or mRNA for short, that transmits the critical information from the DNA in our genes to the protein, which is really the stuff we're all made out of. This is the critical information that determines what the cell will do. So we think about it as an operating system. So if you could change that, if you could introduce a line of code, or change a line of code, it turns out, that has profound implications for everything, from the flu to cancer.

Zaks should more accurately have said that this has profound implications for the human genetic code and the nature of DNA. Communications within the body go both ways and not only one. But, hey, no, the 'Covid vaccine' will not affect your genetics. Cult fact-checkers say so even though the man who helped to develop the mRNA technique says that it does. Zaks said in 2017:

If you think about what it is we're trying to do. We've taken information and our understanding of that information and how that information is transmitted in a cell, and we've taken our understanding of medicine and how to make drugs, and we're fusing the two. We think of it as information therapy.

I have been writing for decades that the body is an information field communicating with itself and the wider world. This is why

radiation which is information can change the information field of body and mind through phenomena like 5G and change their nature and function. 'Information therapy' means to change the body's information field and change the way it operates. DNA is a receiver-transmitter of information and can be mutated by information like mRNA synthetic messaging. Technology to do this has been ready and waiting in the underground bases and other secret projects to be rolled out when the 'Covid' hoax was played. 'Trials' of such short and irrelevant duration were only for public consumption. When they say the 'vaccine' is 'experimental' that is not true. It may appear to be 'experimental' to those who don't know what's going on, but the trials have already been done to ensure the Cult gets the result it desires. Zaks said that it took decades to sequence the human genome, completed in 2003, but now they could do it in a week. By 'they' he means scientists operating in the public domain. In the secret projects they were sequencing the genome in a week long before even 2003.

Deluge of mRNA

Highly significantly the Moderna document says the guiding premise is that if using mRNA as a medicine works for one disease then it should work for many diseases. They were leveraging the flexibility afforded by their platform and the fundamental role mRNA plays in protein synthesis to pursue mRNA medicines for a broad spectrum of diseases. Moderna is confirming what I was saying through 2020 that multiple 'vaccines' were planned for 'Covid' (and later invented 'variants') and that previous vaccines would be converted to the mRNA system to infuse the body with massive amounts of genetically-manipulating synthetic material to secure a transformation to a synthetic-biological state. The 'vaccines' are designed to kill stunning numbers as part of the long-exposed Cult depopulation agenda and transform the rest. Given this is the goal you can appreciate why there is such hysterical demand for every human to be 'vaccinated' for an alleged 'disease' that has an estimated 'infection' to 'death' ratio of 0.23-0.15 percent. As I write

children are being given the 'vaccine' in trials (their parents are a disgrace) and ever-younger people are being offered the vaccine for a 'virus' that even if you believe it exists has virtually zero chance of harming them. Horrific effects of the 'trials' on a 12-year-old girl were revealed by a family member to be serious brain and gastric problems that included a bowel obstruction and the inability to swallow liquids or solids. She was unable to eat or drink without throwing up, had extreme pain in her back, neck and abdomen, and was paralysed from the waist down which stopped her urinating unaided. When the girl was first taken to hospital doctors said it was all in her mind. She was signed up for the 'trial' by her parents for whom no words suffice. None of this 'Covid vaccine' insanity makes any sense unless you see what the 'vaccine' really is – a body-changer. Synthetic biology or 'SynBio' is a fast-emerging and expanding scientific discipline which includes everything from genetic and molecular engineering to electrical and computer engineering. Synthetic biology is defined in these ways:

- A multidisciplinary area of research that seeks to create new biological parts, devices, and systems, or to redesign systems that are already found in nature.

- The use of a mixture of physical engineering and genetic engineering to create new (and therefore synthetic) life forms.

- An emerging field of research that aims to combine the knowledge and methods of biology, engineering and related disciplines in the design of chemically-synthesized DNA to create organisms with novel or enhanced characteristics and traits (synthetic organisms including humans).

We now have synthetic blood, skin, organs and limbs being developed along with synthetic body parts produced by 3D printers. These are all elements of the synthetic human programme and this comment by Kurzweil's co-founder of the Singularity University,

Peter Diamandis, can be seen in a whole new light with the 'Covid' hoax and the sanctions against those that refuse the 'vaccine':

Anybody who is going to be resisting the progress forward [to transhumanism] is going to be resisting evolution and, fundamentally, they will die out. It's not a matter of whether it's good or bad. It's going to happen.

'Resisting evolution'? What absolute bollocks. The arrogance of these people is without limit. His 'it's going to happen' mantra is another way of saying 'resistance is futile' to break the spirit of those pushing back and we must not fall for it. Getting this genetically-transforming 'vaccine' into everyone is crucial to the Cult plan for total control and the desperation to achieve that is clear for anyone to see. Vaccine passports are a major factor in this and they, too, are a form of resistance is futile. It's NOT. The paper funded by the Rockefeller Foundation for the 2013 'health conference' in China said:

We will interact more with artificial intelligence. The use of robotics, bio-engineering to augment human functioning is already well underway and will advance. Re-engineering of humans into potentially separate and unequal forms through genetic engineering or mixed human-robots raises debates on ethics and equality.

A new demography is projected to emerge after 2030 [that year again] of technologies (robotics, genetic engineering, nanotechnology) producing robots, engineered organisms, 'nanobots' and artificial intelligence (AI) that can self-replicate. Debates will grow on the implications of an impending reality of human designed life.

What is happening today is so long planned. The world army enforcing the will of the world government is intended to be a robot army, not a human one. Today's military and its technologically 'enhanced' troops, pilotless planes and driverless vehicles are just stepping stones to that end. Human soldiers are used as Cult fodder and its time they woke up to that and worked for the freedom of the population instead of their own destruction and their family's destruction – the same with the police. Join us and let's sort this out. The phenomenon of enforce my own destruction is widespread in the 'Covid' era with Woker 'luvvies' in the acting and entertainment

industries supporting 'Covid' rules which have destroyed their profession and the same with those among the public who put signs on the doors of their businesses 'closed due to Covid – stay safe' when many will never reopen. It's a form of masochism and most certainly insanity.

Transgender = transhumanism

When something explodes out of nowhere and is suddenly everywhere it is always the Cult agenda and so it is with the tidal wave of claims and demands that have infiltrated every aspect of society under the heading of 'transgenderism'. The term 'trans' is so 'in' and this is the dictionary definition:

A prefix meaning 'across', 'through', occurring … in loanwords from Latin, used in particular for denoting movement or conveyance from place to place (transfer; transmit; transplant) or complete change (transform; transmute), or to form adjectives meaning 'crossing', 'on the other side of', or 'going beyond' the place named (transmontane; transnational; trans-Siberian).

Transgender means to go beyond gender and transhuman means to go beyond human. Both are aspects of the Cult plan to transform the human body to a synthetic state with *no gender*. Human 2.0 is not designed to procreate and would be produced technologically with no need for parents. The new human would mean the end of parents and so men, and increasingly women, are being targeted for the deletion of their rights and status. Parental rights are disappearing at an ever-quickening speed for the same reason. The new human would have no need for men or women when there is no procreation and no gender. Perhaps the transgender movement that appears to be in a permanent state of frenzy might now contemplate on how it is being used. This was never about transgender rights which are only the interim excuse for confusing gender, particularly in the young, on the road to *fusing* gender. Transgender activism is not an end; it is a *means* to an end. We see again the technique of creative destruction in which you destroy the status quo to 'build back better' in the form that you want. The gender status quo had to be

destroyed by persuading the Cult-created Woke mentality to believe that you can have 100 genders or more. A programme for 9 to 12 year olds produced by the Cult-owned BBC promoted the 100 genders narrative. The very idea may be the most monumental nonsense, but it is not what is true that counts, only what you can make people *believe* is true. Once the gender of 2 + 2 = 4 has been dismantled through indoctrination, intimidation and 2 + 2 = 5 then the new no-gender normal can take its place with Human 2.0. Aldous Huxley revealed the plan in his prophetic *Brave New World* in 1932:

> Natural reproduction has been done away with and children are created, decanted', and raised in 'hatcheries and conditioning centres'. From birth, people are genetically designed to fit into one of five castes, which are further split into 'Plus' and 'Minus' members and designed to fulfil predetermined positions within the social and economic strata of the World State.

How could Huxley know this in 1932? For the same reason George Orwell knew about the Big Brother state in 1948, Cult insiders I have quoted knew about it in 1969, and I have known about it since the early 1990s. If you are connected to the Cult or you work your balls off to uncover the plan you can predict the future. The process is simple. If there is a plan for the world and nothing intervenes to stop it then it will happen. Thus if you communicate the plan ahead of time you are perceived to have predicted the future, but you haven't. You have revealed the plan which without intervention will become the human future. The whole reason I have done what I have is to alert enough people to inspire an intervention and maybe at last that time has come with the Cult and its intentions now so obvious to anyone with a brain in working order.

The future is here

Technological wombs that Huxley described to replace parent procreation are already being developed and they are only the projects we know about in the public arena. Israeli scientists told *The Times of Israel* in March, 2021, that they have grown 250-cell embryos

into mouse foetuses with fully formed organs using artificial wombs in a development they say could pave the way for gestating humans outside the womb. Professor Jacob Hanna of the Weizmann Institute of Science said:

We took mouse embryos from the mother at day five of development, when they are just of 250 cells, and had them in the incubator from day five until day 11, by which point they had grown all their organs.

By day 11 they make their own blood and have a beating heart, a fully developed brain. Anybody would look at them and say, 'this is clearly a mouse foetus with all the characteristics of a mouse.' It's gone from being a ball of cells to being an advanced foetus.

A special liquid is used to nourish embryo cells in a laboratory dish and they float on the liquid to duplicate the first stage of embryonic development. The incubator creates all the right conditions for its development, Hanna said. The liquid gives the embryo 'all the nutrients, hormones and sugars they need' along with a custom-made electronic incubator which controls gas concentration, pressure and temperature. The cutting-edge in the underground bases and other secret locations will be light years ahead of that, however, and this was reported by the London *Guardian* in 2017:

We are approaching a biotechnological breakthrough. Ectogenesis, the invention of a complete external womb, could completely change the nature of human reproduction. In April this year, researchers at the Children's Hospital of Philadelphia announced their development of an artificial womb.

The article was headed 'Artificial wombs could soon be a reality. What will this mean for women?' What would it mean for children is an even bigger question. No mother to bond with only a machine in preparation for a life of soulless interaction and control in a world governed by machines (see the *Matrix* movies). Now observe the calculated manipulations of the 'Covid' hoax as human interaction and warmth has been curtailed by distancing, isolation and fear with people communicating via machines on a scale never seen before.

These are all dots in the same picture as are all the personal assistants, gadgets and children's toys through which kids and adults communicate with AI as if it is human. The AI 'voice' on Sat-Nav should be included. All these things are psychological preparation for the Cult endgame. Before you can make a physical connection with AI you have to make a psychological connection and that is what people are being conditioned to do with this ever gathering human-AI interaction. Movies and TV programmes depicting the transhuman, robot dystopia relate to a phenomenon known as 'pre-emptive programming' in which the world that is planned is portrayed everywhere in movies, TV and advertising. This is conditioning the conscious and subconscious mind to become familiar with the planned reality to dilute resistance when it happens for real. What would have been a shock such is the change is made less so. We have young children put on the road to transgender transition surgery with puberty blocking drugs at an age when they could never be able to make those life-changing decisions.

Rachel Levine, a professor of paediatrics and psychiatry who believes in treating children this way, became America's highest-ranked openly-transgender official when she was confirmed as US Assistant Secretary at the Department of Health and Human Services after being nominated by Joe Biden (the Cult). Activists and governments press for laws to deny parents a say in their children's transition process so the kids can be isolated and manipulated into agreeing to irreversible medical procedures. A Canadian father Robert Hoogland was denied bail by the Vancouver Supreme Court in 2021 and remained in jail for breaching a court order that he stay silent over his young teenage daughter, a minor, who was being offered life-changing hormone therapy without parental consent. At the age of 12 the girl's 'school counsellor' said she may be transgender, referred her to a doctor and told the school to treat her like a boy. This is another example of state-serving schools imposing ever more control over children's lives while parents have ever less.

Contemptible and extreme child abuse is happening all over the world as the Cult gender-fusion operation goes into warp-speed.

Why the war on men – and now women?

The question about what artificial wombs mean for women should rightly be asked. The answer can be seen in the deletion of women's rights involving sport, changing rooms, toilets and status in favour of people in male bodies claiming to identify as women. I can identify as a mountain climber, but it doesn't mean I can climb a mountain any more than a biological man can be a biological woman. To believe so is a triumph of belief over factual reality which is the very perceptual basis of everything Woke. Women's sport is being destroyed by allowing those with male bodies who say they identify as female to 'compete' with girls and women. Male body 'women' dominate 'women's' competition with their greater muscle mass, bone density, strength and speed. With that disadvantage sport for women loses all meaning. To put this in perspective nearly 300 American high school boys can run faster than the quickest woman sprinter in the world. Women are seeing their previously protected spaces invaded by male bodies simply because they claim to identify as women. That's all they need to do to access all women's spaces and activities under the Biden 'Equality Act' that destroys equality for women with the usual Orwellian Woke inversion. Male sex offenders have already committed rapes in women's prisons after claiming to identify as women to get them transferred. Does this not matter to the Woke 'equality' hypocrites? Not in the least. What matters to Cult manipulators and funders behind transgender activists is to advance gender fusion on the way to the no-gender 'human'. When you are seeking to impose transparent nonsense like this, or the 'Covid' hoax, the only way the nonsense can prevail is through censorship and intimidation of dissenters, deletion of factual information, and programming of the unquestioning, bewildered and naive. You don't have to scan the world for long to see that all these things are happening.

Many women's rights organisations have realised that rights and status which took such a long time to secure are being eroded and that it is systematic. Kara Dansky of the global Women's Human Rights Campaign said that Biden's transgender executive order immediately he took office, subsequent orders, and Equality Act legislation that followed 'seek to erase women and girls in the law as a category'. *Exactly*. I said during the long ago-started war on men (in which many women play a crucial part) that this was going to turn into a war on them. The Cult is phasing out *both* male and female genders. To get away with that they are brought into conflict so they are busy fighting each other while the Cult completes the job with no unity of response. Unity, people, *unity*. We need unity everywhere. Transgender is the only show in town as the big step towards the no-gender human. It's not about rights for transgender people and never has been. Woke political correctness is deleting words relating to genders to the same end. Wokers believe this is to be 'inclusive' when the opposite is true. They are deleting words describing gender because gender *itself* is being deleted by Human 2.0. Terms like 'man', 'woman', 'mother' and 'father' are being deleted in the universities and other institutions to be replaced by the *no*-gender, not trans-gender, 'individuals' and 'guardians'. Women's rights campaigner Maria Keffler of Partners for Ethical Care said: 'Children are being taught from kindergarten upward that some boys have a vagina, some girls have a penis, and that kids can be any gender they want to be.' Do we really believe that suddenly countries all over the world at the same time had the idea of having drag queens go into schools or read transgender stories to very young children in the local library? It's coldly-calculated confusion of gender on the way to the fusion of gender. Suzanne Vierling, a psychologist from Southern California, made another important point:

Yesterday's slave woman who endured gynecological medical experiments is today's girl-child being butchered in a booming gender-transitioning sector. Ovaries removed, pushing her into menopause and osteoporosis, uncharted territory, and parents' rights and authority decimated.

The erosion of parental rights is a common theme in line with the Cult plans to erase the very concept of parents and 'ovaries removed, pushing her into menopause' means what? Those born female lose the ability to have children – another way to discontinue humanity as we know it.

Eliminating Human 1.0 (before our very eyes)

To pave the way for Human 2.0 you must phase out Human 1.0. This is happening through plummeting sperm counts and making women infertile through an onslaught of chemicals, radiation (including smartphones in pockets of men) and mRNA 'vaccines'. Common agriculture pesticides are also having a devastating impact on human fertility. I have been tracking collapsing sperm counts in the books for a long time and in 2021 came a book by fertility scientist and reproductive epidemiologist Shanna Swan, *Count Down: How Our Modern World Is Threatening Sperm Counts, Altering Male and Female Reproductive Development and Imperiling the Future of the Human Race*. She reports how the global fertility rate dropped by *half* between 1960 and 2016 with America's birth rate 16 percent below where it needs to be to sustain the population. Women are experiencing declining egg quality, more miscarriages, and more couples suffer from infertility. Other findings were an increase in erectile dysfunction, infant boys developing more genital abnormalities, male problems with conception, and plunging levels of the male hormone testosterone which would explain why so many men have lost their backbone and masculinity. This has been very evident during the 'Covid' hoax when women have been prominent among the Pushbackers and big strapping blokes have bowed their heads, covered their faces with a nappy and quietly submitted. Mind control expert Cathy O'Brien also points to how global education introduced the concept of 'we're all winners' in sport and classrooms: 'Competition was defused, and it in turn defused a sense of fighting back.' This is another version of the 'equity' doctrine in which you drive down rather than raise up. What a contrast in Cult-controlled China with its global ambitions

where the government published plans in January, 2021, to 'cultivate masculinity' in boys from kindergarten through to high school in the face of a 'masculinity crisis'. A government adviser said boys would be soon become 'delicate, timid and effeminate' unless action was taken. Don't expect any similar policy in the targeted West. A 2006 study showed that a 65-year-old man in 2002 had testosterone levels *15 percent* lower than a 65-year-old man in 1987 while a 2020 study found a similar story with young adults and adolescents. Men are getting prescriptions for testosterone replacement therapy which causes an even greater drop in sperm count with up to 99 percent seeing sperm counts drop to zero during the treatment. More sperm is defective and malfunctioning with some having two heads or not pursuing an egg.

A class of *synthetic* chemicals known as phthalates are being blamed for the decline. These are found everywhere in plastics, shampoos, cosmetics, furniture, flame retardants, personal care products, pesticides, canned foods and even receipts. Why till receipts? Everyone touches them. Let no one delude themselves that all this is not systematic to advance the long-time agenda for human body transformation. Phthalates mimic hormones and disrupt the hormone balance causing testosterone to fall and genital birth defects in male infants. Animals and fish have been affected in the same way due to phthalates and other toxins in rivers. When fish turn gay or change sex through chemicals in rivers and streams it is a pointer to why there has been such an increase in gay people and the sexually confused. It doesn't matter to me what sexuality people choose to be, but if it's being affected by chemical pollution and consumption then we need to know. Does anyone really think that this is not connected to the transgender agenda, the war on men and the condemnation of male 'toxic masculinity'? You watch this being followed by 'toxic femininity'. It's already happening. When breastfeeding becomes 'chest-feeding', pregnant women become pregnant people along with all the other Woke claptrap you know that the world is going insane and there's a Cult scam in progress. Transgender activists are promoting the Cult agenda while Cult

billionaires support and fund the insanity as they laugh themselves to sleep at the sheer stupidity for which humans must be infamous in galaxies far, far away.

'Covid vaccines' and female infertility

We can now see why the 'vaccine' has been connected to potential infertility in women. Dr Michael Yeadon, former Vice President and Chief Scientific Advisor at Pfizer, and Dr Wolfgang Wodarg in Germany, filed a petition with the European Medicines Agency in December, 2020, urging them to stop trials for the Pfizer/BioNTech shot and all other mRNA trials until further studies had been done. They were particularly concerned about possible effects on fertility with 'vaccine'-produced antibodies attacking the protein Syncytin-1 which is responsible for developing the placenta. The result would be infertility 'of indefinite duration' in women who have the 'vaccine' with the placenta failing to form. Section 10.4.2 of the Pfizer/BioNTech trial protocol says that pregnant women or those who might become so should not have mRNA shots. Section 10.4 warns men taking mRNA shots to 'be abstinent from heterosexual intercourse' and not to donate sperm. The UK government said that it *did not know* if the mRNA procedure had an effect on fertility. *Did not know?* These people have to go to jail. UK government advice did not recommend at the start that pregnant women had the shot and said they should avoid pregnancy for at least two months after 'vaccination'. The 'advice' was later updated to pregnant women should only have the 'vaccine' if the benefits outweighed the risks to mother and foetus. What the hell is that supposed to mean? Then 'spontaneous abortions' began to appear and rapidly increase on the adverse reaction reporting schemes which include only a fraction of adverse reactions. Thousands and ever-growing numbers of 'vaccinated' women are describing changes to their menstrual cycle with heavier blood flow, irregular periods and menstruating again after going through the menopause – all links to reproduction effects. Women are passing blood clots and the lining of their uterus while men report erectile dysfunction and blood effects. Most

significantly of all *un*vaccinated women began to report similar menstrual changes after interaction with *'vaccinated'* people and men and children were also affected with bleeding noses, blood clots and other conditions. 'Shedding' is when vaccinated people can emit the content of a vaccine to affect the unvaccinated, but this is different. 'Vaccinated' people were not shedding a 'live virus' allegedly in 'vaccines' as before because the fake 'Covid vaccines' involve synthetic material and other toxicity. Doctors exposing what is happening prefer the term 'transmission' to shedding. Somehow those that have had the shots are transmitting effects to those that haven't. Dr Carrie Madej said the nano-content of the 'vaccines' can 'act like an antenna' to others around them which fits perfectly with my own conclusions. This 'vaccine' transmission phenomenon was becoming known as the book went into production and I deal with this further in the Postscript.

Vaccine effects on sterility are well known. The World Health Organization was accused in 2014 of sterilising millions of women in Kenya with the evidence confirmed by the content of the vaccines involved. The same WHO behind the 'Covid' hoax admitted its involvement for more than ten years with the vaccine programme. Other countries made similar claims. Charges were lodged by Tanzania, Nicaragua, Mexico, and the Philippines. The Gardasil vaccine claimed to protect against a genital 'virus' known as HPV has also been linked to infertility. Big Pharma and the WHO (same thing) are criminal and satanic entities. Then there's the Bill Gates Foundation which is connected through funding and shared interests with 20 pharmaceutical giants and laboratories. He stands accused of directing the policy of United Nations Children's Fund (UNICEF), vaccine alliance GAVI, and other groupings, to advance the vaccine agenda and silence opposition at great cost to women and children. At the same time Gates wants to reduce the global population. Coincidence?

Great Reset = Smart Grid = new human

The Cult agenda I have been exposing for 30 years is now being openly promoted by Cult assets like Gates and Klaus Schwab of the World Economic Forum under code-terms like the 'Great Reset', 'Build Back Better' and 'a rare but narrow window of opportunity to reflect, reimagine, and reset our world'. What provided this 'rare but narrow window of opportunity'? The 'Covid' hoax did. Who created that? *They* did. My books from not that long ago warned about the planned 'Internet of Things' (IoT) and its implications for human freedom. This was the plan to connect all technology to the Internet and artificial intelligence and today we are way down that road with an estimated 36 billion devices connected to the World Wide Web and that figure is projected to be 76 billion by 2025. I further warned that the Cult planned to go beyond that to the Internet of *Everything* when the human brain was connected via AI to the Internet and Kurzweil's 'cloud'. Now we have Cult operatives like Schwab calling for precisely that under the term 'Internet of Bodies', a fusion of the physical, digital and biological into one centrally-controlled Smart Grid system which the Cult refers to as the 'Fourth Industrial Revolution'. They talk about the 'biological', but they really mean the synthetic-biological which is required to fully integrate the human body and brain into the Smart Grid and artificial intelligence planned to replace the human mind. We have everything being synthetically manipulated including the natural world through GMO and smart dust, the food we eat and the human body itself with synthetic 'vaccines'. I said in *The Answer* that we would see the Cult push for synthetic meat to replace animals and in February, 2021, the so predictable psychopath Bill Gates called for the introduction of synthetic meat to save us all from 'climate change'. The climate hoax just keeps on giving like the 'Covid' hoax. The war on meat by vegan activists is a carbon (oops, sorry) copy of the manipulation of transgender activists. They have no idea (except their inner core) that they are being used to promote and impose the agenda of the Cult or that they are only the *vehicle* and not the *reason*. This is not to say those who choose not to eat meat shouldn't be respected and supported in that right, but there are ulterior motives

for those in power. A *Forbes* article in December, 2019, highlighted the plan so beloved of Schwab and the Cult under the heading: 'What Is The Internet of Bodies? And How Is It Changing Our World?' The article said the human body is the latest data platform (remember 'our vaccine is an operating system'). *Forbes* described the plan very accurately and the words could have come straight out of my books from long before:

The Internet of Bodies (IoB) is an extension of the IoT and basically connects the human body to a network through devices that are ingested, implanted, or connected to the body in some way. Once connected, data can be exchanged, and the body and device can be remotely monitored and controlled.

They were really describing a human hive mind with human perception centrally-dictated via an AI connection as well as allowing people to be 'remotely monitored and controlled'. Everything from a fridge to a human mind could be directed from a central point by these insane psychopaths and 'Covid vaccines' are crucial to this. Forbes explained the process I mentioned earlier of holdable and wearable technology followed by implantable. The article said there were three generations of the Internet of Bodies that include:

- Body external: These are wearable devices such as Apple Watches or Fitbits that can monitor our health.

- Body internal: These include pacemakers, cochlear implants, and digital pills that go inside our bodies to monitor or control various aspects of health.

- Body embedded: The third generation of the Internet of Bodies is embedded technology where technology and the human body are melded together and have a real-time connection to a remote machine.

Forbes noted the development of the Brain Computer Interface (BCI) which merges the brain with an external device for monitoring and controlling in real-time. 'The ultimate goal is to help restore function to individuals with disabilities by using brain signals rather than conventional neuromuscular pathways.' Oh, do fuck off. The goal of brain interface technology is controlling human thought and emotion from the central point in a hive mind serving its masters wishes. Many people are now agreeing to be chipped to open doors without a key. You can recognise them because they'll be wearing a mask, social distancing and lining up for the 'vaccine'. The Cult plans a Great Reset money system after they have completed the demolition of the global economy in which 'money' will be exchanged through communication with body operating systems. Rand Corporation, a Cult-owned think tank, said of the Internet of Bodies or IoB:

> Internet of Bodies technologies fall under the broader IoT umbrella. But as the name suggests, IoB devices introduce an even more intimate interplay between humans and gadgets. IoB devices monitor the human body, collect health metrics and other personal information, and transmit those data over the Internet. Many devices, such as fitness trackers, are already in use … IoB devices … and those in development can track, record, and store users' whereabouts, bodily functions, and what they see, hear, and even think.

Schwab's World Economic Forum, a long-winded way of saying 'fascism' or 'the Cult', has gone full-on with the Internet of Bodies in the 'Covid' era. 'We're entering the era of the Internet of Bodies', it declared, 'collecting our physical data via a range of devices that can be implanted, swallowed or worn'. The result would be a huge amount of health-related data that could improve human wellbeing around the world, and prove crucial in fighting the 'Covid-19 pandemic'. Does anyone think these clowns care about 'human wellbeing' after the death and devastation their pandemic hoax has purposely caused? Schwab and co say we should move forward with the Internet of Bodies because 'Keeping track of symptoms could help us stop the spread of infection, and quickly detect new cases'. How wonderful, but keeping track' is all they are really bothered

about. Researchers were investigating if data gathered from smartwatches and similar devices could be used as viral infection alerts by tracking the user's heart rate and breathing. Schwab said in his 2018 book *Shaping the Future of the Fourth Industrial Revolution*:

> The lines between technologies and beings are becoming blurred and not just by the ability to create lifelike robots or synthetics. Instead it is about the ability of new technologies to literally become part of us. Technologies already influence how we understand ourselves, how we think about each other, and how we determine our realities. As the technologies … give us deeper access to parts of ourselves, we may begin to integrate digital technologies into our bodies.

You can see what the game is. Twenty-four hour control and people – if you could still call them that – would never know when something would go ping and take them out of circulation. It's the most obvious rush to a global fascist dictatorship and the complete submission of humanity and yet still so many are locked away in their Cult-induced perceptual coma and can't see it.

Smart Grid control centres

The human body is being transformed by the 'vaccines' and in other ways into a synthetic cyborg that can be attached to the global Smart Grid which would be controlled from a central point and other sub-locations of Grid manipulation. Where are these planned to be? Well, China for a start which is one of the Cult's biggest centres of operation. The technological control system and technocratic rule was incubated here to be unleashed across the world after the 'Covid' hoax came out of China in 2020. Another Smart Grid location that will surprise people new to this is Israel. I have exposed in *The Trigger* how Sabbatian technocrats, intelligence and military operatives were behind the horrors of 9/11 and not '19 Arab hijackers' who somehow manifested the ability to pilot big passenger airliners when instructors at puddle-jumping flying schools described some of them as a joke. The 9/11 attacks were made possible through control of civilian and military air computer systems and those of the White House, Pentagon and connected agencies. See *The Trigger* – it

will blow your mind. The controlling and coordinating force were the Sabbatian networks in Israel and the United States which by then had infiltrated the entire US government, military and intelligence system. The real name of the American Deep State is 'Sabbatian State'. Israel is a tiny country of only nine million people, but it is one of the global centres of cyber operations and fast catching Silicon Valley in importance to the Cult. Israel is known as the 'start-up nation' for all the cyber companies spawned there with the Sabbatian specialisation of 'cyber security' that I mentioned earlier which gives those companies access to computer systems of their clients in real time through 'backdoors' written into the coding when security software is downloaded. The Sabbatian centre of cyber operations outside Silicon Valley is the Israeli military Cyber Intelligence Unit, the biggest infrastructure project in Israel's history, headquartered in the desert-city of Beersheba and involving some 20,000 'cyber soldiers'. Here are located a literal army of Internet trolls scanning social media, forums and comment lists for anyone challenging the Cult agenda. The UK military has something similar with its 77th Brigade and associated operations. The Beersheba complex includes research and development centres for other Cult operations such as Intel, Microsoft, IBM, Google, Apple, Hewlett-Packard, Cisco Systems, Facebook and Motorola. Techcrunch.com ran an article about the Beersheba global Internet technology centre headlined 'Israel's desert city of Beersheba is turning into a cybertech oasis':

> The military's massive relocation of its prestigious technology units, the presence of multinational and local companies, a close proximity to Ben Gurion University and generous government subsidies are turning Beersheba into a major global cybertech hub. Beersheba has all of the ingredients of a vibrant security technology ecosystem, including Ben Gurion University with its graduate program in cybersecurity and Cyber Security Research Center, and the presence of companies such as EMC, Deutsche Telekom, PayPal, Oracle, IBM, and Lockheed Martin. It's also the future home of the INCB (Israeli National Cyber Bureau); offers a special income tax incentive for cyber security companies, and was the site for the relocation of the army's intelligence corps units.

Sabbatians have taken over the cyber world through the following process: They scan the schools for likely cyber talent and develop them at Ben Gurion University and their period of conscription in the Israeli Defense Forces when they are stationed at the Beersheba complex. When the cyber talented officially leave the army they are funded to start cyber companies with technology developed by themselves or given to them by the state. Much of this is stolen through backdoors of computer systems around the world with America top of the list. Others are sent off to Silicon Valley to start companies or join the major ones and so we have many major positions filled by apparently 'Jewish' but really Sabbatian operatives. Google, YouTube and Facebook are all run by 'Jewish' CEOs while Twitter is all but run by ultra-Zionist hedge-fund shark Paul Singer. At the centre of the Sabbatian global cyber web is the Israeli army's Unit 8200 which specialises in hacking into computer systems of other countries, inserting viruses, gathering information, instigating malfunction, and even taking control of them from a distance. A long list of Sabbatians involved with 9/11, Silicon Valley and Israeli cyber security companies are operatives of Unit 8200. This is not about Israel. It's about the Cult. Israel is planned to be a Smart Grid hub as with China and what is happening at Beersheba is not for the benefit of Jewish people who are treated disgustingly by the Sabbatian elite that control the country. A glance at the Nuremberg Codes will tell you that.

The story is much bigger than 'Covid', important as that is to where we are being taken. Now, though, it's time to really strap in. There's more … much more …

CHAPTER ELEVEN

Who controls the Cult?

Awake, arise or be forever fall'n
John Milton, *Paradise Lost*

I have exposed this far the level of the Cult conspiracy that operates in the world of the seen and within the global secret society and satanic network which operates in the shadows one step back from the seen. The story, however, goes much deeper than that.

The 'Covid' hoax is major part of the Cult agenda, but only part, and to grasp the biggest picture we have to expand our attention beyond the realm of human sight and into the infinity of possibility that we cannot see. It is from here, ultimately, that humanity is being manipulated into a state of total control by the force which dictates the actions of the Cult. How much of reality can we see? Next to damn all is the answer. We may appear to see all there is to see in the 'space' our eyes survey and observe, but little could be further from the truth. The human 'world' is only a tiny band of frequency that the body's visual and perceptual systems can decode into *perception* of a 'world'. According to mainstream science the electromagnetic spectrum is 0.005 percent of what exists in the Universe (Fig 10). The maximum estimate I have seen is 0.5 percent and either way it's miniscule. I say it is far, far, smaller even than 0.005 percent when you compare reality we see with the totality of reality that we don't. Now get this if you are new to such information: Visible light, the only band of frequency that we can see, is a *fraction* of the 0.005

percent (Fig 11 overleaf). Take this further and realise that our universe is one of infinite universes and that universes are only a fragment of overall reality – *infinite* reality. Then compare that with the almost infinitesimal frequency band of visible light or human sight. You see that humans are as near blind as it is possible to be without actually being so. Artist and filmmaker, Sergio Toporek, said:

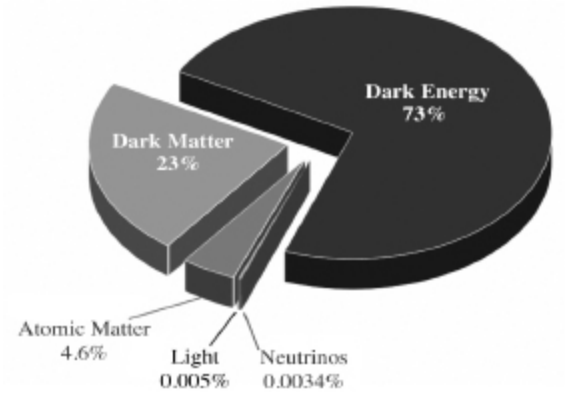

Figure 10: Humans can perceive such a tiny band of visual reality it's laughable.

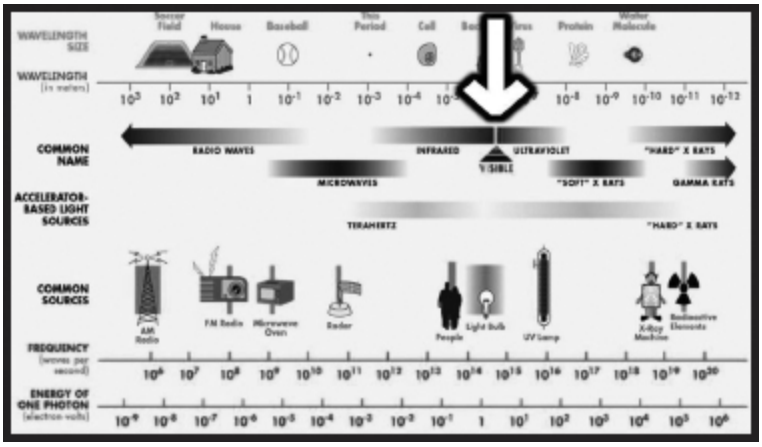

Figure 11: We can see a smear of the 0.005 percent electromagnetic spectrum, but we still know it all. Yep, makes sense.

Consider that you can see less than 1% of the electromagnetic spectrum and hear less than 1% of the acoustic spectrum. 90% of the cells in your body carry their own microbial DNA and are not 'you'. The atoms in your body are 99.9999999999999999% empty space and none of them are the ones you were born with ... Human beings have 46 chromosomes, two less than a potato.

The existence of the rainbow depends on the conical photoreceptors in your eyes; to animals without cones, the rainbow does not exist. So you don't just look at a rainbow, you create it. This is pretty amazing, especially considering that all the beautiful colours you see represent less than 1% of the electromagnetic spectrum.

Suddenly the 'world' of humans looks a very different place. Take into account, too, that Planet Earth when compared with the projected size of this single universe is the equivalent of a billionth of a pinhead. Imagine the ratio that would be when compared to infinite reality. To think that Christianity once insisted that Earth and humanity were the centre of everything. This background is vital if we are going to appreciate the nature of 'human' and how we can be manipulated by an unseen force. To human visual reality virtually *everything* is unseen and yet the prevailing perception within the institutions and so much of the public is that if we can't see it, touch it, hear it, taste it and smell it then it cannot exist. Such perception is indoctrinated and encouraged by the Cult and its agents because it isolates believers in the strictly limited, village-idiot, realm of the five senses where perceptions can be firewalled and information controlled. Most of those perpetuating the 'this-world-is-all-there-is' insanity are themselves indoctrinated into believing the same delusion. While major players and influencers know that official reality is laughable most of those in science, academia and medicine really believe the nonsense they peddle and teach succeeding generations. Those who challenge the orthodoxy are dismissed as nutters and freaks to protect the manufactured illusion from exposure. Observe the dynamic of the 'Covid' hoax and you will see how that takes the same form. The inner-circle psychopaths knows it's a gigantic scam, but almost the entirety of those imposing their fascist rules believe that 'Covid' is all that they're told it is.

Stolen identity

Ask people who they are and they will give you their name, place of birth, location, job, family background and life story. Yet that is not who they are – it is what they are *experiencing*. The difference is *absolutely crucial*. The true 'I', the eternal, infinite 'I', is consciousness,

a state of being aware. Forget 'form'. That is a vehicle for a brief experience. Consciousness does not come *from* the brain, but *through* the brain and even that is more symbolic than literal. We are awareness, pure awareness, and this is what withdraws from the body at what we call 'death' to continue our eternal beingness, *isness*, in other realms of reality within the limitlessness of infinity or the Biblical 'many mansions in my father's house'. Labels of a human life, man, woman, transgender, black, white, brown, nationality, circumstances and income are not who we are. They are what we are – awareness – is *experiencing* in a brief connection with a band of frequency we call 'human'. The labels are not the self; they are, to use the title of one of my books, a *Phantom* Self. I am not David Icke born in Leicester, England, on April 29th, 1952. I am the consciousness *having that experience*. The Cult and its non-human masters seek to convince us through the institutions of 'education', science, medicine, media and government that what we are *experiencing* is who we *are*. It's so easy to control and direct perception locked away in the bewildered illusions of the five senses with no expanded radar. Try, by contrast, doing the same with a humanity aware of its true self and its true power to consciously create its reality and experience. How is it possible to do this? We do it all day every day. If you perceive yourself as 'little me' with no power to impact upon your life and the world then your life experience will reflect that. You will hand the power you don't think you have to authority in all its forms which will use it to control your experience. This, in turn, will appear to confirm your perception of 'little me' in a self-fulfilling feedback loop. But that is what 'little me' really is – a *perception*. We are all 'big-me', infinite me, and the Cult has to make us forget that if its will is to prevail. We are therefore manipulated and pressured into self-identifying with human labels and not the consciousness/awareness *experiencing* those human labels.

The phenomenon of identity politics is a Cult-instigated manipulation technique to sub-divide previous labels into even smaller ones. A United States university employs this list of letters to

describe student identity: LGBTTQQFAGPBDSM or lesbian, gay, bisexual, transgender, transsexual, queer, questioning, flexual, asexual, gender-fuck, polyamorous, bondage/discipline, dominance/submission and sadism/masochism. I'm sure other lists are even longer by now as people feel the need to self-identity the 'I' with the minutiae of race and sexual preference. Wokers programmed by the Cult for generations believe this is about 'inclusivity' when it's really the Cult locking them away into smaller and smaller versions of Phantom Self while firewalling them from the influence of their true self, the infinite, eternal 'I'. You may notice that my philosophy which contends that we are all unique points of attention/awareness within the same infinite whole or Oneness is the ultimate non-racism. The very sense of Oneness makes the judgement of people by their body-type, colour or sexuality utterly ridiculous and confirms that racism has no understanding of reality (including anti-white racism). Yet despite my perception of life Cult agents and fast-asleep Wokers label me racist to discredit my information while they are themselves phenomenally racist and sexist. All they see is race and sexuality and they judge people as good or bad, demons or untouchables, by their race and sexuality. All they see is *Phantom Self* and perceive themselves in terms of Phantom Self. They are pawns and puppets of the Cult agenda to focus attention and self-identity in the five senses and play those identities against each other to divide and rule. Columbia University has introduced segregated graduations in another version of social distancing designed to drive people apart and teach them that different racial and cultural groups have nothing in common with each other. The last thing the Cult wants is unity. Again the pump-primers of this will be Cult operatives in the knowledge of what they are doing, but the rest are just the Phantom Self blind leading the Phantom Self blind. We *do* have something in common – we are all *the same consciousness* having different temporary experiences.

What is this 'human'?

Yes, what *is* 'human'? That is what we are supposed to be, right? I mean 'human'? True, but 'human' is the experience not the 'I'. Break it down to basics and 'human' is the way that information is processed. If we are to experience and interact with this band of frequency we call the 'world' we must have a vehicle that operates within that band of frequency. Our consciousness in its prime form cannot do that; it is way beyond the frequency of the human realm. My consciousness or awareness could not tap these keys and pick up the cup in front of me in the same way that radio station A cannot interact with radio station B when they are on different frequencies. The human body is the means through which we have that interaction. I have long described the body as a biological computer which processes information in a way that allows consciousness to experience this reality. The body is a receiver, transmitter and processor of information in a particular way that we call human. We visually perceive only the world of the five senses in a wakened state – that is the limit of the body's visual decoding system. In truth it's not even visual in the way we experience 'visual reality' as I will come to in a moment. We are 'human' because the body processes the information sources of human into a reality and behaviour system that we *perceive* as human. Why does an elephant act like an elephant and not like a human or a duck? The elephant's biological computer is a different information field and processes information according to that program into a visual and behaviour type we call an elephant. The same applies to everything in our reality. These body information fields are perpetuated through procreation (like making a copy of a software program). The Cult wants to break that cycle and intervene technologically to transform the human information field into one that will change what we call humanity. If it can change the human information field it will change the way that field processes information and change humanity both 'physically' and psychologically. Hence the *messenger* (information) RNA 'vaccines' and so much more that is targeting human genetics by changing the body's information – *messaging* – construct through food, drink, radiation, toxicity and other means.

Reality that we experience is nothing like reality as it really is in the same way that the reality people experience in virtual reality games is not the reality they are really living in. The game is only a decoded source of information that appears to be a reality. Our world is also an information construct – a *simulation* (more later). In its base form our reality is a wavefield of information much the same in theme as Wi-Fi. The five senses decode wavefield information into electrical information which they communicate to the brain to decode into holographic (illusory 'physical') information. Different parts of the brain specialise in decoding different senses and the information is fused into a reality that appears to be outside of us but is really inside the brain and the genetic structure in general (Fig 12 overleaf). DNA is a receiver-transmitter of information and a vital part of this decoding process and the body's connection to other realities. Change DNA and you change the way we decode and connect with reality – see 'Covid vaccines'. Think of computers decoding Wi-Fi. You have information encoded in a radiation field and the computer decodes that information into a very different form on the screen. You can't see the Wi-Fi until its information is made manifest on the screen and the information on the screen is inside the computer and not outside. I have just described how we decode the 'human world'. All five senses decode the waveform 'Wi-Fi' field into electrical signals and the brain (computer) constructs reality inside the brain and not outside – 'You don't just look at a rainbow, you create it'. Sound is a simple example. We don't hear sound until the brain decodes it. Waveform sound waves are picked up by the hearing sense and communicated to the brain in an electrical form to be decoded into the sounds that we hear. Everything we hear is inside the brain along with everything we see, feel, smell and taste. Words and language are waveform fields generated by our vocal chords which pass through this process until they are decoded by the brain into words that we hear. Different languages are different frequency fields or sound waves generated by vocal chords. Late British philosopher Alan Watts said:

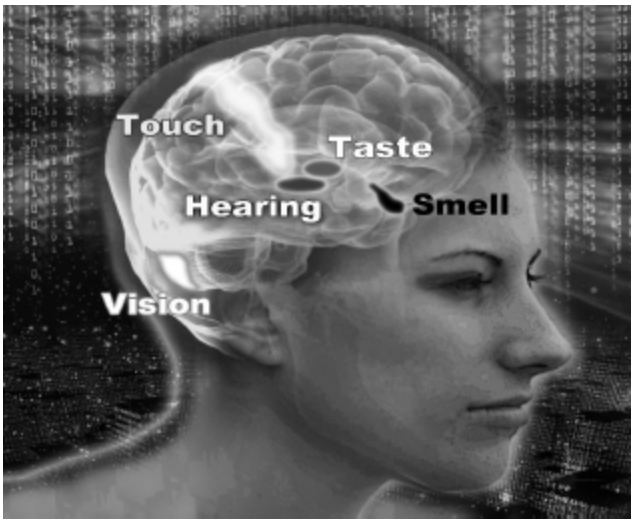

Figure 12: The brain receives information from the five senses and constructs from that our perceived reality.

[Without the brain] the world is devoid of light, heat, weight, solidity, motion, space, time or any other imaginable feature. All these phenomena are interactions, or transactions, of vibrations with a certain arrangement of neurons.

That's exactly what they are and scientist Robert Lanza describes in his book, *Biocentrism*, how we decode electromagnetic waves and energy into visual and 'physical' experience. He uses the example of a flame emitting photons, electromagnetic energy, each pulsing electrically and magnetically:

… these … invisible electromagnetic waves strike a human retina, and if (and only if) the waves happen to measure between 400 and 700 nano meters in length from crest to crest, then their energy is just right to deliver a stimulus to the 8 million cone-shaped cells in the retina.

Each in turn send an electrical pulse to a neighbour neuron, and on up the line this goes, at 250 mph, until it reaches the … occipital lobe of the brain, in the back of the head. There, a cascading complex of neurons fire from the incoming stimuli, and we subjectively perceive this experience as a yellow brightness occurring in a place we have been conditioned to call the 'external world'.

You hear what you decode

If a tree falls or a building collapses they make no noise unless someone is there to decode the energetic waves generated by the disturbance into what we call sound. Does a falling tree make a noise? Only if you hear it – *decode* it. Everything in our reality is a frequency field of information operating within the overall 'Wi-Fi' field that I call The Field. A vibrational disturbance is generated in The Field by the fields of the falling tree or building. These disturbance waves are what we decode into the sound of them falling. If no one is there to do that then neither will make any noise. Reality is created by the observer – *decoder* – and the *perceptions* of the observer affect the decoding process. For this reason different people – different *perceptions* – will perceive the same reality or situation in a different way. What one may perceive as a nightmare another will see as an opportunity. The question of why the Cult is so focused on controlling human perception now answers itself. All experienced reality is the act of decoding and we don't experience Wi-Fi until it is decoded on the computer screen. The sight and sound of an Internet video is encoded in the Wi-Fi all around us, but we don't see or hear it until the computer decodes that information. Taste, smell and touch are all phenomena of the brain as a result of the same process. We don't taste, smell or feel anything except in the brain and there are pain relief techniques that seek to block the signal from the site of discomfort to the brain because if the brain doesn't decode that signal we don't feel pain. Pain is in the brain and only appears to be at the point of impact thanks to the feedback loop between them. We don't see anything until electrical information from the sight senses is decoded in an area at the back of the brain. If that area is damaged we can go blind when our eyes are perfectly okay. So why do we go blind if we damage an eye? We damage the information processing between the waveform visual information and the visual decoding area of the brain. If information doesn't reach the brain in a form it can decode then we can't see the visual reality that it represents. What's more the brain is decoding only a fraction of the information it receives and the rest is absorbed by the

sub-conscious mind. This explanation is from the science magazine, *Wonderpedia*:

> Every second, 11 million sensations crackle along these [brain] pathways ... The brain is confronted with an alarming array of images, sounds and smells which it rigorously filters down until it is left with a manageable list of around 40. Thus 40 sensations per second make up what we perceive as reality.

The 'world' is not what people are told to believe that is it and the inner circles of the Cult *know that*.

Illusory 'physical' reality

We can only see a smear of 0.005 percent of the Universe which is only one of a vast array of universes – 'mansions' – within infinite reality. Even then the brain decodes only 40 pieces of information ('sensations') from a potential *11 million* that we receive every second. Two points strike you from this immediately: The sheer breathtaking stupidity of believing we know anything so rigidly that there's nothing more to know; and the potential for these processes to be manipulated by a malevolent force to control the reality of the population. One thing I can say for sure with no risk of contradiction is that when you can perceive an almost indescribable fraction of infinite reality there is always more to know as in tidal waves of it. Ancient Greek philosopher Socrates was so right when he said that wisdom is to know how little we know. How obviously true that is when you think that we are experiencing a physical world of solidity that is neither physical nor solid and a world of apartness when everything is connected. Cult-controlled 'science' dismisses the so-called 'paranormal' and all phenomena related to that when the 'para'-normal is perfectly normal and explains the alleged 'great mysteries' which dumbfound scientific minds. There is a reason for this. A 'scientific mind' in terms of the mainstream is a material mind, a five-sense mind imprisoned in see it, touch it, hear it, smell it and taste it. Phenomena and happenings that can't be explained that way leave the 'scientific mind' bewildered and the rule is that if they

can't account for why something is happening then it can't, by definition, be happening. I beg to differ. Telepathy is thought waves passing through The Field (think wave disturbance again) to be decoded by someone able to connect with that wavelength (information). For example: You can pick up the thought waves of a friend at any distance and at the very least that will bring them to mind. A few minutes later the friend calls you. 'My god', you say, 'that's incredible – I was just thinking of you.' Ah, but *they* were thinking of *you* before they made the call and that's what you decoded. Native peoples not entrapped in five-sense reality do this so well it became known as the 'bush telegraph'. Those known as psychics and mediums (genuine ones) are doing the same only across dimensions of reality. 'Mind over matter' comes from the fact that matter and mind are the *same*. The state of one influences the state of the other. Indeed one *and* the other are illusions. They are aspects of the same field. Paranormal phenomena are all explainable so why are they still considered 'mysteries' or not happening? Once you go down this road of understanding you begin to expand awareness beyond the five senses and that's the nightmare for the Cult.

Figure 13: Holograms are not solid, but the best ones appear to be.

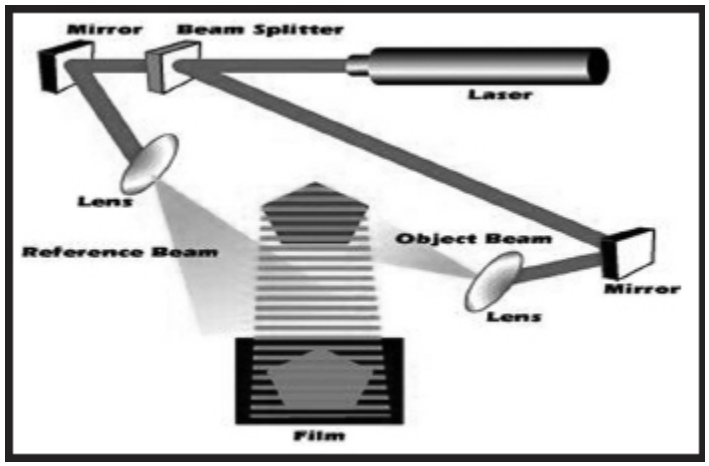

Figure 14: How holograms are created by capturing a waveform version of the subject image.

Holographic 'solidity'

Our reality is not solid, it is holographic. We are now well aware of holograms which are widely used today. Two-dimensional information is decoded into a three-dimensional reality that is not solid although can very much appear to be (Fig 13). Holograms are created with a laser divided into two parts. One goes directly onto a holographic photographic print ('reference beam') and the other takes a waveform image of the subject ('working beam') before being directed onto the print where it 'collides' with the other half of the laser (Fig 14). This creates a *waveform* interference pattern which contains the wavefield information of whatever is being photographed (Fig 15 overleaf). The process can be likened to dropping pebbles in a pond. Waves generated by each one spread out across the water to collide with the others and create a wave representation of where the stones fell and at what speed, weight and distance. A waveform interference pattern of a hologram is akin to the waveform information in The Field which the five senses decode into electrical signals to be decoded by the brain into a holographic illusory 'physical' reality. In the same way when a laser (think human attention) is directed at the waveform interference pattern a three-dimensional version of the subject is projected into apparently 'solid' reality (Fig 16). An amazing trait of holograms reveals more 'paranormal mysteries'. Information of the *whole*

hologram is encoded in waveform in every part of the interference pattern by the way they are created. This means that every *part* of a hologram is a smaller version of the whole. Cut the interference wave-pattern into four and you won't get four parts of the image. You get quarter-sized versions of the *whole* image. The body is a hologram and the same applies. Here we have the basis of acupuncture, reflexology and other forms of healing which identify representations of the whole body in all of the parts, hands, feet, ears, everywhere. Skilled palm readers can do what they do because the information of whole body is encoded in the hand. The concept of as above, so below, comes from this.

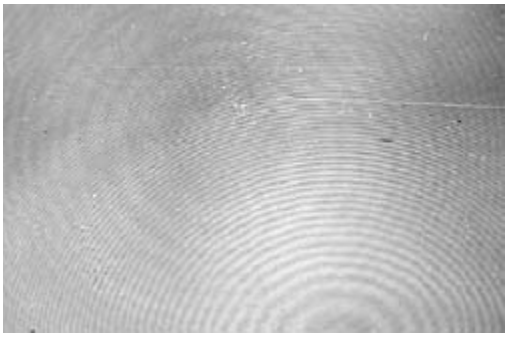

Figure 15: A waveform interference pattern that holds the information that transforms into a hologram.

Figure 16: Holographic people including 'Elvis' holographically inserted to sing a duet with Celine Dion.

The question will be asked of why, if solidity is illusory, we can't just walk through walls and each other. The resistance is not solid against solid; it is electromagnetic field against electromagnetic field and we decode this into the *experience* of solid against solid. We should also not underestimate the power of belief to dictate reality. What you believe is impossible *will be*. Your belief impacts on your decoding processes and they won't decode what you think is impossible. What we believe we perceive and what we perceive we experience. 'Can't dos' and 'impossibles' are like a firewall in a computer system that won't put on the screen what the firewall blocks. How vital that is to understanding how human experience has been hijacked. I explain in *The Answer*, *Everything You Need To Know But Have Never Been Told* and other books a long list of 'mysteries' and 'paranormal' phenomena that are not mysterious and perfectly normal once you realise what reality is and how it works. 'Ghosts' can be seen to pass through 'solid' walls because the walls are not solid and the ghost is a discarnate entity operating on a frequency so different to that of the wall that it's like two radio stations sharing the same space while never interfering with each other. I have seen ghosts do this myself. The apartness of people and objects is also an illusion. Everything is connected by the Field like all sea life is connected by the sea. It's just that within the limits of our visual reality we only 'see' holographic information and not the field of information that connects everything and from which the holographic world is made manifest. If you can only see holographic 'objects' and not the field that connects them they will appear to you as unconnected to each other in the same way that we see the computer while not seeing the Wi-Fi.

What you don't know *can* hurt you

Okay, we return to those 'two worlds' of human society and the Cult with its global network of interconnecting secret societies and satanic groups which manipulate through governments, corporations, media, religions, etc. The fundamental difference between them is *knowledge*. The idea has been to keep humanity

ignorant of the plan for its total enslavement underpinned by a crucial ignorance of reality – who we are and where we are – and how we interact with it. 'Human' should be the interaction between our expanded eternal consciousness and the five-sense body experience. We are meant to be *in* this world in terms of the five senses but not *of* this world in relation to our greater consciousness and perspective. In that state we experience the small picture of the five senses within the wider context of the big picture of awareness beyond the five senses. Put another way the five senses see the dots and expanded awareness connects them into pictures and patterns that give context to the apparently random and unconnected. Without the context of expanded awareness the five senses see only apartness and randomness with apparently no meaning. The Cult and its other-dimensional controllers seek to intervene in the frequency realm where five-sense reality is supposed to connect with expanded reality and to keep the two apart (more on this in the final chapter). When that happens five-sense mental and emotional processes are no longer influenced by expanded awareness, or the True 'I', and instead are driven by the isolated perceptions of the body's decoding systems. They are in the world *and* of it. Here we have the human plight and why humanity with its potential for infinite awareness can be so easily manipulatable and descend into such extremes of stupidity.

Once the Cult isolates five-sense mind from expanded awareness it can then program the mind with perceptions and beliefs by controlling information that the mind receives through the 'education' system of the formative years and the media perceptual bombardment and censorship of an entire lifetime. Limit perception and a sense of the possible through limiting knowledge by limiting and skewing information while censoring and discrediting that which could set people free. As the title of another of my books says *… And The Truth Shall Set You Free*. For this reason the last thing the Cult wants in circulation is the truth about anything – especially the reality of the eternal 'I' – and that's why it is desperate to control information. The Cult knows that information becomes perception

which becomes behaviour which, collectively, becomes human society. Cult-controlled and funded mainstream 'science' denies the existence of an eternal 'I' and seeks to dismiss and trash all evidence to the contrary. Cult-controlled mainstream religion has a version of 'God' that is little more than a system of control and dictatorship that employs threats of damnation in an afterlife to control perceptions and behaviour in the here and now through fear and guilt. Neither is true and it's the 'neither' that the Cult wishes to suppress. This 'neither' is that everything is an expression, a point of attention, within an infinite state of consciousness which is the real meaning of the term 'God'.

Perceptual obsession with the 'physical body' and five-senses means that 'God' becomes personified as a bearded bloke sitting among the clouds or a raging bully who loves us if we do what 'he' wants and condemns us to the fires of hell if we don't. These are no more than a 'spiritual' fairy tales to control and dictate events and behaviour through fear of this 'God' which has bizarrely made 'God-fearing' in religious circles a state to be desired. I would suggest that fearing *anything* is not to be encouraged and celebrated, but rather deleted. You can see why 'God fearing' is so beneficial to the Cult and its religions when *they* decide what 'God' wants and what 'God' demands (the Cult demands) that everyone do. As the great American comedian Bill Hicks said satirising a Christian zealot: 'I think what God meant to say.' How much of this infinite awareness ('God') that we access is decided by how far we choose to expand our perceptions, self-identity and sense of the possible. The scale of self-identity reflects itself in the scale of awareness that we can connect with and are influenced by – how much knowing and insight we have instead of programmed perception. You cannot expand your awareness into the infinity of possibility when you believe that you are little me Peter the postman or Mary in marketing and nothing more. I'll deal with this in the concluding chapter because it's crucial to how we turnaround current events.

Where the Cult came from

When I realised in the early 1990s there was a Cult network behind global events I asked the obvious question: When did it start? I took it back to ancient Rome and Egypt and on to Babylon and Sumer in Mesopotamia, the 'Land Between Two Rivers', in what we now call Iraq. The two rivers are the Tigris and Euphrates and this region is of immense historical and other importance to the Cult, as is the land called Israel only 550 miles away by air. There is much more going with deep esoteric meaning across this whole region. It's not only about 'wars for oil'. Priceless artefacts from Mesopotamia were stolen or destroyed after the American and British invasion of Iraq in 2003 justified by the lies of Boy Bush and Tony Blair (their Cult masters) about non-existent 'weapons of mass destruction'. Mesopotamia was the location of Sumer (about 5,400BC to 1,750BC), and Babylon (about 2,350BC to 539BC). Sabbatians may have become immensely influential in the Cult in modern times but they are part of a network that goes back into the mists of history. Sumer is said by historians to be the 'cradle of civilisation'. I disagree. I say it was the re-start of what we call human civilisation after cataclysmic events symbolised in part as the 'Great Flood' destroyed the world that existed before. These fantastic upheavals that I have been describing in detail in the books since the early1990s appear in accounts and legends of ancient cultures across the world and they are supported by geological and biological evidence. Stone tablets found in Iraq detailing the Sumer period say the cataclysms were caused by non-human 'gods' they call the Anunnaki. These are described in terms of extraterrestrial visitations in which knowledge supplied by the Anunnaki is said to have been the source of at least one of the world's oldest writing systems and developments in astronomy, mathematics and architecture that were way ahead of their time. I have covered this subject at length in *The Biggest Secret* and *Children of the Matrix* and the same basic 'Anunnaki' story can be found in Zulu accounts in South Africa where the late and very great Zulu high shaman Credo Mutwa told me that the Sumerian Anunnaki were known by Zulus as the Chitauri or 'children of the serpent'. See my six-hour video interview with Credo on this subject entitled *The*

Reptilian Agenda recorded at his then home near Johannesburg in 1999 which you can watch on the Ickonic media platform.

The Cult emerged out of Sumer, Babylon and Egypt (and elsewhere) and established the Roman Empire before expanding with the Romans into northern Europe from where many empires were savagely imposed in the form of Cult-controlled societies all over the world. Mass death and destruction was their calling card. The Cult established its centre of operations in Europe and European Empires were Cult empires which allowed it to expand into a global force. Spanish and Portuguese colonialists headed for Central and South America while the British and French targeted North America. Africa was colonised by Britain, France, Belgium, the Netherlands, Portugal, Spain, Italy, and Germany. Some like Britain and France moved in on the Middle East. The British Empire was by far the biggest for a simple reason. By now Britain was the headquarters of the Cult from which it expanded to form Canada, the United States, Australia and New Zealand. The Sun never set on the British Empire such was the scale of its occupation. London remains a global centre for the Cult along with Rome and the Vatican although others have emerged in Israel and China. It is no accident that the 'virus' is alleged to have come out of China while Italy was chosen as the means to terrify the Western population into compliance with 'Covid' fascism. Nor that Israel has led the world in 'Covid' fascism and mass 'vaccination'.

You would think that I would mention the United States here, but while it has been an important means of imposing the Cult's will it is less significant than would appear and is currently in the process of having what power it does have deleted. The Cult in Europe has mostly loaded the guns for the US to fire. America has been controlled from Europe from the start through Cult operatives in Britain and Europe. The American Revolution was an illusion to make it appear that America was governing itself while very different forces were pulling the strings in the form of Cult families such as the Rothschilds through the Rockefellers and other subordinates. The Rockefellers are extremely close to Bill Gates and

established both scalpel and drug 'medicine' and the World Health Organization. They play a major role in the development and circulation of vaccines through the Rockefeller Foundation on which Bill Gates said his Foundation is based. Why wouldn't this be the case when the Rockefellers and Gates are on the same team? Cult infiltration of human society goes way back into what we call history and has been constantly expanding and centralising power with the goal of establishing a global structure to dictate everything. Look how this has been advanced in great leaps with the 'Covid' hoax.

The non-human dimension

I researched and observed the comings and goings of Cult operatives through the centuries and even thousands of years as they were born, worked to promote the agenda within the secret society and satanic networks, and then died for others to replace them. Clearly there had to be a coordinating force that spanned this entire period while operatives who would not have seen the end goal in their lifetimes came and went advancing the plan over millennia. I went in search of that coordinating force with the usual support from the extraordinary synchronicity of my life which has been an almost daily experience since 1990. I saw common themes in religious texts and ancient cultures about a non-human force manipulating human society from the hidden. Christianity calls this force Satan, the Devil and demons; Islam refers to the Jinn or Djinn; Zulus have their Chitauri (spelt in other ways in different parts of Africa); and the Gnostic people in Egypt in the period around and before 400AD referred to this phenomena as the 'Archons', a word meaning rulers in Greek. Central American cultures speak of the 'Predators' among other names and the same theme is everywhere. I will use 'Archons' as a collective name for all of them. When you see how their nature and behaviour is described all these different sources are clearly talking about the same force. Gnostics described the Archons in terms of 'luminous fire' while Islam relates the Jinn to 'smokeless fire'. Some refer to beings in form that could occasionally be seen, but the most common of common theme is that they operate from

unseen realms which means almost all existence to the visual processes of humans. I had concluded that this was indeed the foundation of human control and that the Cult was operating within the human frequency band on behalf of this hidden force when I came across the writings of Gnostics which supported my conclusions in the most extraordinary way.

A sealed earthen jar was found in 1945 near the town of Nag Hammadi about 75-80 miles north of Luxor on the banks of the River Nile in Egypt. Inside was a treasure trove of manuscripts and texts left by the Gnostic people some 1,600 years earlier. They included 13 leather-bound papyrus codices (manuscripts) and more than 50 texts written in Coptic Egyptian estimated to have been hidden in the jar in the period of 400AD although the source of the information goes back much further. Gnostics oversaw the Great or Royal Library of Alexandria, the fantastic depository of ancient texts detailing advanced knowledge and accounts of human history. The Library was dismantled and destroyed in stages over a long period with the death-blow delivered by the Cult-established Roman Church in the period around 415AD. The Church of Rome was the Church of Babylon relocated as I said earlier. Gnostics were not a race. They were a way of perceiving reality. Whenever they established themselves and their information circulated the terrorists of the Church of Rome would target them for destruction. This happened with the Great Library and with the Gnostic Cathars who were burned to death by the psychopaths after a long period of oppression at the siege of the Castle of Monségur in southern France in 1244. The Church has always been terrified of Gnostic information which demolishes the official Christian narrative although there is much in the Bible that supports the Gnostic view if you read it in another way. To anyone studying the texts of what became known as the Nag Hammadi Library it is clear that great swathes of Christian and Biblical belief has its origin with Gnostics sources going back to Sumer. Gnostic themes have been twisted to manipulate the perceived reality of Bible believers. Biblical texts have been in the open for centuries where they could be changed while Gnostic

documents found at Nag Hammadi were sealed away and untouched for 1,600 years. What you see is what they wrote.

Use your *pneuma* not your *nous*

Gnosticism and Gnostic come from 'gnosis' which means knowledge, or rather *secret* knowledge, in the sense of spiritual awareness – knowledge about reality and life itself. The desperation of the Cult's Church of Rome to destroy the Gnostics can be understood when the knowledge they were circulating was the last thing the Cult wanted the population to know. Sixteen hundred years later the same Cult is working hard to undermine and silence me for the same reason. The dynamic between knowledge and ignorance is a constant. 'Time' appears to move on, but essential themes remain the same. We are told to 'use your nous', a Gnostic word for head/brain/intelligence. They said, however, that spiritual awakening or 'salvation' could only be secured by expanding awareness *beyond* what they called *nous* and into *pneuma* or Infinite Self. Obviously as I read these texts the parallels with what I have been saying since 1990 were fascinating to me. There is a universal truth that spans human history and in that case why wouldn't we be talking the same language 16 centuries apart? When you free yourself from the perception program of the five senses and explore expanded realms of consciousness you are going to connect with the same information no matter what the perceived 'era' within a manufactured timeline of a single and tiny range of manipulated frequency. Humans working with 'smart' technology or knocking rocks together in caves is only a timeline appearing to operate within the human frequency band. Expanded awareness and the knowledge it holds have always been there whether the era be Stone Age or computer age. We can only access that knowledge by opening ourselves to its frequency which the five-sense prison cell is designed to stop us doing. Gates, Fauci, Whitty, Vallance, Zuckerberg, Brin, Page, Wojcicki, Bezos, and all the others behind the 'Covid' hoax clearly have a long wait before their range of frequency can make that connection given that an open heart is

crucial to that as we shall see. Instead of accessing knowledge directly through expanded awareness it is given to Cult operatives by the secret society networks of the Cult where it has been passed on over thousands of years outside the public arena. Expanded realms of consciousness is where great artists, composers and writers find their inspiration and where truth awaits anyone open enough to connect with it. We need to go there fast.

Archon hijack

A fifth of the Nag Hammadi texts describe the existence and manipulation of the Archons led by a 'Chief Archon' they call 'Yaldabaoth', or the 'Demiurge', and this is the Christian 'Devil', 'Satan', 'Lucifer', and his demons. Archons in Biblical symbolism are the 'fallen ones' which are also referred to as fallen angels after the angels expelled from heaven according to the Abrahamic religions of Judaism, Christianity and Islam. These angels are claimed to tempt humans to 'sin' ongoing and you will see how accurate that symbolism is during the rest of the book. The theme of 'original sin' is related to the 'Fall' when Adam and Eve were 'tempted by the serpent' and fell from a state of innocence and 'obedience' (connection) with God into a state of disobedience (disconnection). The Fall is said to have brought sin into the world and corrupted everything including human nature. Yaldabaoth, the 'Lord Archon', is described by Gnostics as a 'counterfeit spirit', 'The Blind One', 'The Blind God', and 'The Foolish One'. The Jewish name for Yaldabaoth in Talmudic writings is Samael which translates as 'Poison of God', or 'Blindness of God'. You see the parallels. Yaldabaoth in Islamic belief is the Muslim Jinn devil known as Shaytan – Shaytan is Satan as the same themes are found all over the world in every religion and culture. The 'Lord God' of the Old Testament is the 'Lord Archon' of Gnostic manuscripts and that's why he's such a bloodthirsty bastard. Satan is known by Christians as 'the Demon of Demons' and Gnostics called Yaldabaoth the 'Archon of Archons'. Both are known as 'The Deceiver'. We are talking about the same 'bloke' for sure and these common themes

using different names, storylines and symbolism tell a common tale of the human plight.

Archons are referred to in Nag Hammadi documents as mind parasites, inverters, guards, gatekeepers, detainers, judges, pitiless ones and deceivers. The 'Covid' hoax alone is a glaring example of all these things. The Biblical 'God' is so different in the Old and New Testaments because they are not describing the same phenomenon. The vindictive, angry, hate-filled, 'God' of the Old Testament, known as Yahweh, is Yaldabaoth who is depicted in Cult-dictated popular culture as the 'Dark Lord', 'Lord of Time', Lord (Darth) Vader and Dormammu, the evil ruler of the 'Dark Dimension' trying to take over the 'Earth Dimension' in the Marvel comic movie, *Dr Strange*. Yaldabaoth is both the Old Testament 'god' and the Biblical 'Satan'. Gnostics referred to Yaldabaoth as the 'Great Architect of the Universe'and the Cult-controlled Freemason network calls their god 'the 'Great Architect of the Universe' (also Grand Architect). The 'Great Architect' Yaldabaoth is symbolised by the Cult as the all-seeing eye at the top of the pyramid on the Great Seal of the United States and the dollar bill. Archon is encoded in *arch*-itect as it is in *arch*-angels and *arch*-bishops. All religions have the theme of a force for good and force for evil in some sort of spiritual war and there is a reason for that – the theme is true. The Cult and its non-human masters are quite happy for this to circulate. They present themselves as the force for good fighting evil when they are really the force of evil (absence of love). The whole foundation of Cult modus operandi is inversion. They promote themselves as a force for good and anyone challenging them in pursuit of peace, love, fairness, truth and justice is condemned as a satanic force for evil. This has been the game plan throughout history whether the Church of Rome inquisitions of non-believers or 'conspiracy theorists' and 'anti-vaxxers' of today. The technique is the same whatever the timeline era.

Yaldabaoth is revolting (true)

Yaldabaoth and the Archons are said to have revolted against God with Yaldabaoth claiming to *be* God – the *All That Is*. The Old Testament 'God' (Yaldabaoth) demanded to be worshipped as such: ' *I am* the LORD, and there is none else, there is no God beside me' (Isaiah 45:5). I have quoted in other books a man who said he was the unofficial son of the late Baron Philippe de Rothschild of the Mouton-Rothschild wine producing estates in France who died in 1988 and he told me about the Rothschild 'revolt from God'. The man said he was given the name Phillip Eugene de Rothschild and we shared long correspondence many years ago while he was living under another identity. He said that he was conceived through 'occult incest' which (within the Cult) was 'normal and to be admired'. 'Phillip' told me about his experience attending satanic rituals with rich and famous people whom he names and you can see them and the wider background to Cult Satanism in my other books starting with *The Biggest Secret*. Cult rituals are interactions with Archontic 'gods'. 'Phillip' described Baron Philippe de Rothschild as 'a master Satanist and hater of God' and he used the same term 'revolt from God' associated with Yaldabaoth/Satan/Lucifer/the Devil in describing the Sabbatian Rothschild dynasty. 'I played a key role in my family's revolt from God', he said. That role was to infiltrate in classic Sabbatian style the Christian Church, but eventually he escaped the mind-prison to live another life. The Cult has been targeting religion in a plan to make worship of the Archons the global one-world religion. Infiltration of Satanism into modern 'culture', especially among the young, through music videos, stage shows and other means, is all part of this.

Nag Hammadi texts describe Yaldabaoth and the Archons in their prime form as energy – consciousness – and say they can take form if they choose in the same way that consciousness takes form as a human. Yaldabaoth is called 'formless' and represents a deeply inverted, distorted and chaotic state of consciousness which seeks to attached to humans and turn them into a likeness of itself in an attempt at assimilation. For that to happen it has to manipulate

humans into low frequency mental and emotional states that match its own. Archons can certainly appear in human form and this is the origin of the psychopathic personality. The energetic distortion Gnostics called Yaldabaoth *is* psychopathy. When psychopathic Archons take human form that human will be a psychopath as an expression of Yaldabaoth consciousness. Cult psychopaths are Archons in human form. The principle is the same as that portrayed in the 2009 *Avatar* movie when the American military travelled to a fictional Earth-like moon called Pandora in the Alpha Centauri star system to infiltrate a society of blue people, or Na'vi, by hiding within bodies that looked like the Na'vi. Archons posing as humans have a particular hybrid information field, part human, part Archon, (the ancient 'demigods') which processes information in a way that manifests behaviour to match their psychopathic evil, lack of empathy and compassion, and stops them being influenced by the empathy, compassion and love that a fully-human information field is capable of expressing. Cult bloodlines interbreed, be they royalty or dark suits, for this reason and you have their obsession with incest. Interbreeding with full-blown humans would dilute the Archontic energy field that guarantees psychopathy in its representatives in the human realm.

Gnostic writings say the main non-human forms that Archons take are *serpentine* (what I have called for decades 'reptilian' amid unbounded ridicule from the Archontically-programmed) and what Gnostics describe as 'an unborn baby or foetus with grey skin and dark, unmoving eyes'. This is an excellent representation of the ET 'Greys' of UFO folklore which large numbers of people claim to have seen and been abducted by – Zulu shaman Credo Mutwa among them. I agree with those that believe in extraterrestrial or interdimensional visitations today and for thousands of years past. No wonder with their advanced knowledge and technological capability they were perceived and worshipped as gods for technological and other 'miracles' they appeared to perform. Imagine someone arriving in a culture disconnected from the modern world with a smartphone and computer. They would be

seen as a 'god' capable of 'miracles'. The Renegade Mind, however, wants to know the source of everything and not only the way that source manifests as human or non-human. In the same way that a Renegade Mind seeks the original source material for the 'Covid virus' to see if what is claimed is true. The original source of Archons in form is consciousness – the distorted state of consciousness known to Gnostics as Yaldabaoth.

'Revolt from God' is energetic disconnection

Where I am going next will make a lot of sense of religious texts and ancient legends relating to 'Satan', Lucifer' and the 'gods'. Gnostic descriptions sync perfectly with the themes of my own research over the years in how they describe a consciousness distortion seeking to impose itself on human consciousness. I've referred to the core of infinite awareness in previous books as Infinite Awareness in Awareness of Itself. By that I mean a level of awareness that knows that it is all awareness and is aware of all awareness. From here comes the frequency of love in its true sense and balance which is what love is on one level – the balance of all forces into a single whole called Oneness and Isness. The more we disconnect from this state of love that many call 'God' the constituent parts of that Oneness start to unravel and express themselves as a part and not a whole. They become individualised as intellect, mind, selfishness, hatred, envy, desire for power over others, and such like. This is not a problem in the greater scheme in that 'God', the *All That Is*, can experience all these possibilities through different expressions of itself including humans. What we as expressions of the whole experience the *All That Is* experiences. We are the *All That Is* experiencing itself. As we withdraw from that state of Oneness we disconnect from its influence and things can get very unpleasant and very stupid. Archontic consciousness is at the extreme end of that. It has so disconnected from the influence of Oneness that it has become an inversion of unity and love, an inversion of everything, an inversion of life itself. Evil is appropriately live written backwards. Archontic consciousness is obsessed with death, an inversion of life,

and so its manifestations in Satanism are obsessed with death. They use inverted symbols in their rituals such as the inverted pentagram and cross. Sabbatians as Archontic consciousness incarnate invert Judaism and every other religion and culture they infiltrate. They seek disunity and chaos and they fear unity and harmony as they fear love like garlic to a vampire. As a result the Cult, Archons incarnate, act with such evil, psychopathy and lack of empathy and compassion disconnected as they are from the source of love. How could Bill Gates and the rest of the Archontic psychopaths do what they have to human society in the 'Covid' era with all the death, suffering and destruction involved and have no emotional consequence for the impact on others? Now you know. Why have Zuckerberg, Brin, Page, Wojcicki and company callously censored information warning about the dangers of the 'vaccine' while thousands have been dying and having severe, sometimes life-changing reactions? Now you know. Why have Tedros, Fauci, Whitty, Vallance and their like around the world been using case and death figures they're aware are fraudulent to justify lockdowns and all the deaths and destroyed lives that have come from that? Now you know. Why did Christian Drosten produce and promote a 'testing' protocol that he knew couldn't test for infectious disease which led to a global human catastrophe. Now you know. The Archontic mind doesn't give a shit (Fig 17). I personally think that Gates and major Cult insiders are a form of AI cyborg that the Archons want humans to become.

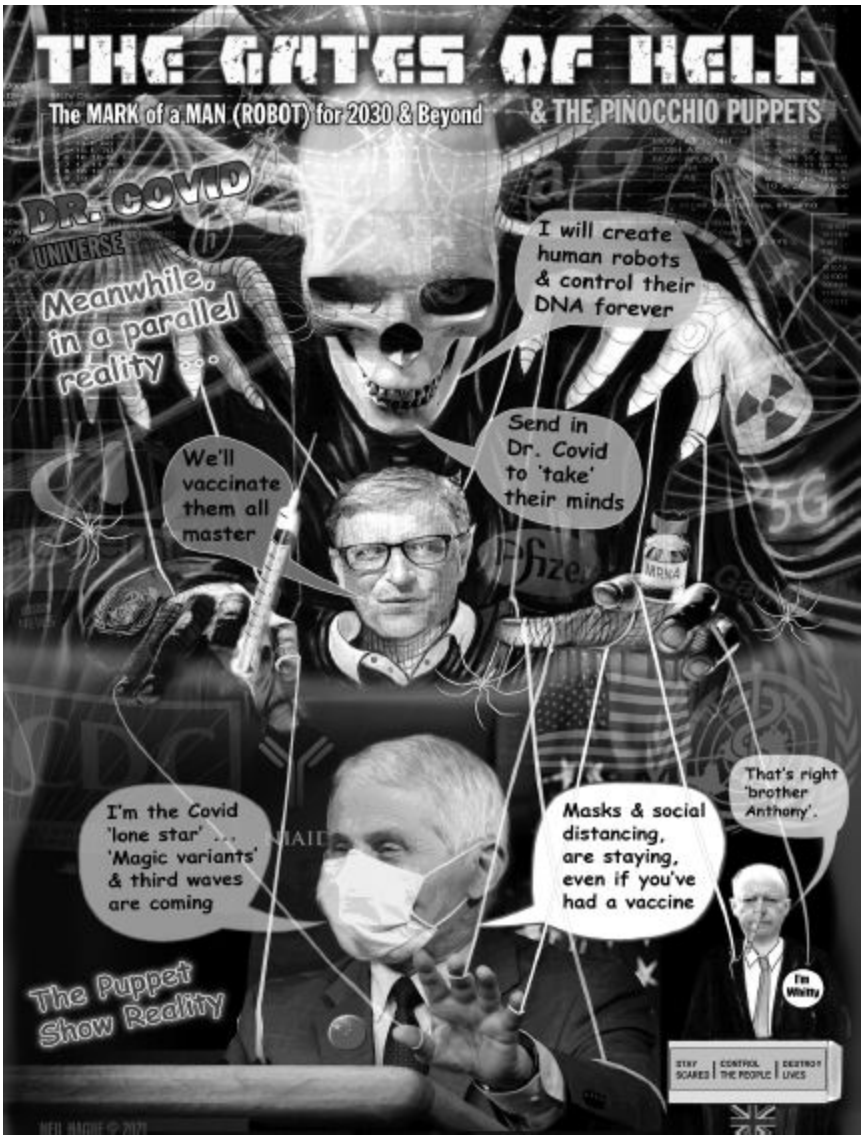

Figure 17: Artist Neil Hague's version of the 'Covid' hierarchy.

Human batteries

A state of such inversion does have its consequences, however. The level of disconnection from the Source of All means that you withdraw from that source of energetic sustenance and creativity. This means that you have to find your own supply of energetic power and it has – *us*. When the Morpheus character in the first *Matrix* movie held up a battery he spoke a profound truth when he said: 'The Matrix is a computer-generated dream world built to keep us under control in order to change the human being into one of

these.' The statement was true in all respects. We do live in a technologically-generated virtual reality simulation (more very shortly) and we have been manipulated to be an energy source for Archontic consciousness. The Disney-Pixar animated movie *Monsters, Inc*. in 2001 symbolised the dynamic when monsters in their world had no energy source and they would enter the human world to terrify children in their beds, catch the child's scream, terror (low-vibrational frequencies), and take that energy back to power the monster world. The lead character you might remember was a single giant eye and the symbolism of the Cult's all-seeing eye was obvious. Every thought and emotion is broadcast as a frequency unique to that thought and emotion. Feelings of love and joy, empathy and compassion, are high, quick, frequencies while fear, depression, anxiety, suffering and hate are low, slow, dense frequencies. Which kind do you think Archontic consciousness can connect with and absorb? In such a low and dense frequency state there's no way it can connect with the energy of love and joy. Archons can only feed off energy compatible with their own frequency and they and their Cult agents want to delete the human world of love and joy and manipulate the transmission of low vibrational frequencies through low-vibrational human mental and emotional states. *We are their energy source*. Wars are energetic banquets to the Archons – a world war even more so – and think how much low-frequency mental and emotional energy has been generated from the consequences for humanity of the 'Covid' hoax orchestrated by Archons incarnate like Gates.

The ancient practice of human sacrifice 'to the gods', continued in secret today by the Cult, is based on the same principle. 'The gods' are Archontic consciousness in different forms and the sacrifice is induced into a state of intense terror to generate the energy the Archontic frequency can absorb. Incarnate Archons in the ritual drink the blood which contains an adrenaline they crave which floods into the bloodstream when people are terrorised. Most of the sacrifices, ancient and modern, are children and the theme of 'sacrificing young virgins to the gods' is just code for children. They

have a particular pre-puberty energy that Archons want more than anything and the energy of the young in general is their target. The California Department of Education wants students to chant the names of Aztec gods (Archontic gods) once worshipped in human sacrifice rituals in a curriculum designed to encourage them to 'challenge racist, bigoted, discriminatory, imperialist/colonial beliefs', join 'social movements that struggle for social justice', and 'build new possibilities for a post-racist, post-systemic racism society'. It's the usual Woke crap that inverts racism and calls it anti-racism. In this case solidarity with 'indigenous tribes' is being used as an excuse to chant the names of 'gods' to which people were sacrificed (and still are in secret). What an example of Woke's inability to see beyond black and white, us and them, They condemn the colonisation of these tribal cultures by Europeans (quite right), but those cultures sacrificing people including children to their 'gods', and mass murdering untold numbers as the Aztecs did, is just fine. One chant is to the Aztec god Tezcatlipoca who had a man sacrificed to him in the 5th month of the Aztec calendar. His heart was cut out and he was eaten. Oh, that's okay then. Come on children … after three … Other sacrificial 'gods' for the young to chant their allegiance include Quetzalcoatl, Huitzilopochtli and Xipe Totec. The curriculum says that 'chants, affirmations, and energizers can be used to bring the class together, build unity around ethnic studies principles and values, and to reinvigorate the class following a lesson that may be emotionally taxing or even when student engagement may appear to be low'. Well, that's the cover story, anyway. Chanting and mantras are the repetition of a particular frequency generated from the vocal cords and chanting the names of these Archontic 'gods' tunes you into their frequency. That is the last thing you want when it allows for energetic synchronisation, attachment and perceptual influence. Initiates chant the names of their 'Gods' in their rituals for this very reason.

Vampires of the Woke

Paedophilia is another way that Archons absorb the energy of children. Paedophiles possessed by Archontic consciousness are used as the conduit during sexual abuse for discarnate Archons to vampire the energy of the young they desire so much. Stupendous numbers of children disappear every year never to be seen again although you would never know from the media. Imagine how much low-vibrational energy has been generated by children during the 'Covid' hoax when so many have become depressed and psychologically destroyed to the point of killing themselves. Shocking numbers of children are now taken by the state from loving parents to be handed to others. I can tell you from long experience of researching this since 1996 that many end up with paedophiles and assets of the Cult through corrupt and Cult-owned social services which in the reframing era has hired many psychopaths and emotionless automatons to do the job. Children are even stolen to order using spurious reasons to take them by the corrupt and secret (because they're corrupt) 'family courts'. I have written in detail in other books, starting with *The Biggest Secret* in 1997, about the ubiquitous connections between the political, corporate, government, intelligence and military elites (Cult operatives) and Satanism and paedophilia. If you go deep enough both networks have an interlocking leadership. The Woke mentality has been developed by the Cult for many reasons: To promote almost every aspect of its agenda; to hijack the traditional political left and turn it fascist; to divide and rule; and to target agenda pushbackers. But there are other reasons which relate to what I am describing here. How many happy and joyful Wokers do you ever see especially at the extreme end? They are a mental and psychological mess consumed by emotional stress and constantly emotionally cocked for the next explosion of indignation at someone referring to a female as a female. They are walking, talking, batteries as Morpheus might say emitting frequencies which both enslave them in low-vibrational bubbles of perceptual limitation and feed the Archons. Add to this the hatred claimed to be love; fascism claimed to 'anti-fascism', racism claimed to be 'anti-racism';

exclusion claimed to inclusion; and the abuse-filled Internet trolling. You have a purpose-built Archontic energy system with not a wind turbine in sight and all founded on Archontic *inversion*. We have whole generations now manipulated to serve the Archons with their actions and energy. They will be doing so their entire adult lives unless they snap out of their Archon-induced trance. Is it really a surprise that Cult billionaires and corporations put so much money their way? Where is the energy of joy and laughter, including laughing at yourself which is confirmation of your own emotional security? Mark Twain said: 'The human race has one really effective weapon, and that is laughter.' We must use it all the time. Woke has destroyed comedy because it has no humour, no joy, sense of irony, or self-deprecation. Its energy is dense and intense. *Mmmmm*, lunch says the Archontic frequency. Rudolf Steiner (1861-1925) was the Austrian philosopher and famous esoteric thinker who established Waldorf education or Steiner schools to treat children like unique expressions of consciousness and not minds to be programmed with the perceptions determined by authority. I'd been writing about this energy vampiring for decades when I was sent in 2016 a quote by Steiner. He was spot on:

> There are beings in the spiritual realms for whom anxiety and fear emanating from human beings offer welcome food. When humans have no anxiety and fear, then these creatures starve. If fear and anxiety radiates from people and they break out in panic, then these creatures find welcome nutrition and they become more and more powerful. These beings are hostile towards humanity. Everything that feeds on negative feelings, on anxiety, fear and superstition, despair or doubt, are in reality hostile forces in super-sensible worlds, launching cruel attacks on human beings, while they are being fed ... These are exactly the feelings that belong to contemporary culture and materialism; because it estranges people from the spiritual world, it is especially suited to evoke hopelessness and fear of the unknown in people, thereby calling up the above mentioned hostile forces against them.

Pause for a moment from this perspective and reflect on what has happened in the world since the start of 2020. Not only will pennies drop, but billion dollar bills. We see the same theme from Don Juan Matus, a Yaqui Indian shaman in Mexico and the information source for Peruvian-born writer, Carlos Castaneda, who wrote a series of

books from the 1960s to 1990s. Don Juan described the force manipulating human society and his name for the Archons was the predator:

> We have a predator that came from the depths of the cosmos and took over the rule of our lives. Human beings are its prisoners. The predator is our lord and master. It has rendered us docile, helpless. If we want to protest, it suppresses our protest. If we want to act independently, it demands that we don't do so ... indeed we are held prisoner!
>
> They took us over because we are food to them, and they squeeze us mercilessly because we are their sustenance. Just as we rear chickens in coops, the predators rear us in human coops, humaneros. Therefore, their food is always available to them.
>
> Different cultures, different eras, same recurring theme.

The 'ennoia' dilemma

Nag Hammadi Gnostic manuscripts say that Archon consciousness has no 'ennoia'. This is directly translated as 'intentionality', but I'll use the term 'creative imagination'. The *All That Is* in awareness of itself is the source of all creativity – all possibility – and the more disconnected you are from that source the more you are subsequently denied 'creative imagination'. Given that Archon consciousness is almost entirely disconnected it severely lacks creativity and has to rely on far more mechanical processes of thought and exploit the creative potential of those that do have 'ennoia'. You can see cases of this throughout human society. Archon consciousness almost entirely dominates the global banking system and if we study how that system works you will appreciate what I mean. Banks manifest 'money' out of nothing by issuing lines of 'credit' which is 'money' that has never, does not, and will never exist except in theory. It's a confidence trick. If you think 'credit' figures-on-a-screen 'money' is worth anything you accept it as payment. If you don't then the whole system collapses through lack of confidence in the value of that 'money'. Archontic bankers with no 'ennoia' are 'lending' 'money' that doesn't exist to humans that *do* have creativity – those that have the inspired ideas and create businesses and products. Archon banking feeds off human creativity

which it controls through 'money' creation and debt. Humans have the creativity and Archons exploit that for their own benefit and control while having none themselves. Archon Internet platforms like Facebook claim joint copyright of everything that creative users post and while Archontic minds like Zuckerberg may officially head that company it will be human creatives on the staff that provide the creative inspiration. When you have limitless 'money' you can then buy other companies established by creative humans. Witness the acquisition record of Facebook, Google and their like. Survey the Archon-controlled music industry and you see non-creative dark suit executives making their fortune from the human creativity of their artists. The cases are endless. Research the history of people like Gates and Zuckerberg and how their empires were built on exploiting the creativity of others. Archon minds cannot create out of nothing, but they are skilled (because they have to be) in what Gnostic texts call 'countermimicry'. They can imitate, but not innovate. Sabbatians trawl the creativity of others through backdoors they install in computer systems through their cybersecurity systems. Archon-controlled China is globally infamous for stealing intellectual property and I remember how Hong Kong, now part of China, became notorious for making counterfeit copies of the creativity of others – 'countermimicry'. With the now pervasive and all-seeing surveillance systems able to infiltrate any computer you can appreciate the potential for Archons to vampire the creativity of humans. Author John Lamb Lash wrote in his book about the Nag Hammadi texts, *Not In His Image*:

> Although they cannot originate anything, because they lack the divine factor of ennoia (intentionality), Archons can imitate with a vengeance. Their expertise is simulation (HAL, virtual reality). The Demiurge [Yaldabaoth] fashions a heaven world copied from the fractal patterns [of the original] ... His construction is celestial kitsch, like the fake Italianate villa of a Mafia don complete with militant angels to guard every portal.

This brings us to something that I have been speaking about since the turn of the millennium. Our reality is a simulation; a virtual reality that we think is real. No, I'm not kidding.

Human reality? Well, virtually

I had pondered for years about whether our reality is 'real' or some kind of construct. I remembered being immensely affected on a visit as a small child in the late 1950s to the then newly-opened Planetarium on the Marylebone Road in London which is now closed and part of the adjacent Madame Tussauds wax museum. It was in the middle of the day, but when the lights went out there was the night sky projected in the Planetarium's domed ceiling and it appeared to be so real. The experience never left me and I didn't know why until around the turn of the millennium when I became certain that our 'night sky' and entire reality is a projection, a virtual reality, akin to the illusory world portrayed in the *Matrix* movies. I looked at the sky one day in this period and it appeared to me like the domed roof of the Planetarium. The release of the first *Matrix* movie in 1999 also provided a synchronistic and perfect visual representation of where my mind had been going for a long time. I hadn't come across the Gnostic Nag Hammadi texts then. When I did years later the correlation was once again astounding. As I read Gnostic accounts from 1,600 years and more earlier it was clear that they were describing the same simulation phenomenon. They tell how the Yaldabaoth 'Demiurge' and Archons created a 'bad copy' of original reality to rule over all that were captured by its illusions and the body was a prison to trap consciousness in the 'bad copy' fake reality. Read how Gnostics describe the 'bad copy' and update that to current times and they are referring to what we would call today a virtual reality simulation.

Author John Lamb Lash said 'the Demiurge fashions a heaven world copied from the fractal patterns' of the original through expertise in 'HAL' or virtual reality simulation. Fractal patterns are part of the energetic information construct of our reality, a sort of blueprint. If these patterns were copied in computer terms it would indeed give you a copy of a 'natural' reality in a non-natural frequency and digital form. The principle is the same as making a copy of a website. The original website still exists, but now you can change the copy version to make it whatever you like and it can

become very different to the original website. Archons have done this with our reality, a *synthetic* copy of prime reality that still exists beyond the frequency walls of the simulation. Trapped within the illusions of this synthetic Matrix, however, were and are human consciousness and other expressions of prime reality and this is why the Archons via the Cult are seeking to make the human body synthetic and give us synthetic AI minds to complete the job of turning the entire reality synthetic including what we perceive to be the natural world. To quote Kurzweil: 'Nanobots will infuse all the matter around us with information. Rocks, trees, everything will become these intelligent creatures.' Yes, *synthetic* 'creatures' just as 'Covid' and other genetically-manipulating 'vaccines' are designed to make the human body synthetic. From this perspective it is obvious why Archons and their Cult are so desperate to infuse synthetic material into every human with their 'Covid' scam.

Let there be (electromagnetic) light

Yaldabaoth, the force that created the simulation, or Matrix, makes sense of the Gnostic reference to 'The Great Architect' and its use by Cult Freemasonry as the name of its deity. The designer of the Matrix in the movies is called 'The Architect' and that trilogy is jam-packed with symbolism relating to these subjects. I have contended for years that the angry Old Testament God (Yaldabaoth) is the 'God' being symbolically 'quoted' in the opening of Genesis as 'creating the world'. This is not the creation of prime reality – it's the creation of the *simulation*. The Genesis 'God' says: 'Let there be Light: and there was light.' But what is this 'Light'? I have said for decades that the speed of light (186,000 miles per second) is not the fastest speed possible as claimed by mainstream science and is in fact the frequency walls or outer limits of the Matrix. You can't have a fastest or slowest anything within all possibility when everything is possible. The human body is encoded to operate within the speed of light or *within the simulation* and thus we see only the tiny frequency band of visible *light*. Near-death experiencers who perceive reality outside the body during temporary 'death' describe a very different

form of light and this is supported by the Nag Hammadi texts. Prime reality beyond the simulation ('Upper Aeons' to the Gnostics) is described as a realm of incredible beauty, bliss, love and harmony – a realm of 'watery light' that is so powerful 'there are no shadows'. Our false reality of Archon control, which Gnostics call the 'Lower Aeons', is depicted as a realm with a different kind of 'light' and described in terms of chaos, 'Hell', 'the Abyss' and 'Outer Darkness', where trapped souls are tormented and manipulated by demons (relate that to the 'Covid' hoax alone). The watery light theme can be found in near-death accounts and it is not the same as *simulation* 'light' which is electromagnetic or radiation light within the speed of light – the 'Lower Aeons'. Simulation 'light' is the 'luminous fire' associated by Gnostics with the Archons. The Bible refers to Yaldabaoth as 'that old serpent, called the Devil, and Satan, which deceiveth the whole world' (Revelation 12:9). I think that making a simulated copy of prime reality ('countermimicry') and changing it dramatically while all the time manipulating humanity to believe it to be real could probably meet the criteria of deceiving the whole world. Then we come to the Cult god Lucifer – the *Light Bringer*. Lucifer is symbolic of Yaldabaoth, the bringer of radiation light that forms the bad copy simulation within the speed of light. 'He' is symbolised by the lighted torch held by the Statue of Liberty and in the name 'Illuminati'. Sabbatian-Frankism declares that Lucifer is the true god and Lucifer is the real god of Freemasonry honoured as their 'Great or Grand Architect of the Universe' (simulation).

I would emphasise, too, the way Archontic technologically-generated luminous fire of radiation has deluged our environment since I was a kid in the 1950s and changed the nature of The Field with which we constantly interact. Through that interaction technological radiation is changing us. The Smart Grid is designed to operate with immense levels of communication power with 5G expanding across the world and 6G, 7G, in the process of development. Radiation is the simulation and the Archontic manipulation system. Why wouldn't the Archon Cult wish to unleash radiation upon us to an ever-greater extreme to form

Kurzweil's 'cloud'? The plan for a synthetic human is related to the need to cope with levels of radiation beyond even anything we've seen so far. Biological humans would not survive the scale of radiation they have in their script. The Smart Grid is a technological sub-reality within the technological simulation to further disconnect five-sense perception from expanded consciousness. It's a technological prison of the mind.

Infusing the 'spirit of darkness'

A recurring theme in religion and native cultures is the manipulation of human genetics by a non-human force and most famously recorded as the biblical 'sons of god' (the god*s* plural in the original) who interbred with the daughters of men. The Nag Hammadi *Apocryphon of John* tells the same story this way:

> He [Yaldabaoth] sent his angels [Archons/demons] to the daughters of men, that they might take some of them for themselves and raise offspring for their enjoyment. And at first they did not succeed. When they had no success, they gathered together again and they made a plan together ... And the angels changed themselves in their likeness into the likeness of their mates, filling them with the spirit of darkness, which they had mixed for them, and with evil ... And they took women and begot children out of the darkness according to the likeness of their spirit.

Possession when a discarnate entity takes over a human body is an age-old theme and continues today. It's very real and I've seen it. Satanic and secret society rituals can create an energetic environment in which entities can attach to initiates and I've heard many stories of how people have changed their personality after being initiated even into lower levels of the Freemasons. I have been inside three Freemasonic temples, one at a public open day and two by just walking in when there was no one around to stop me. They were in Ryde, the town where I live, Birmingham, England, when I was with a group, and Boston, Massachusetts. They all felt the same energetically – dark, dense, low-vibrational and sinister. Demonic attachment can happen while the initiate has no idea what is going on. To them it's just a ritual to get in the Masons and do a bit of good

business. In the far more extreme rituals of Satanism human possession is even more powerful and they are designed to make possession possible. The hierarchy of the Cult is dictated by the power and perceived status of the possessing Archon. In this way the Archon hierarchy becomes the Cult hierarchy. Once the entity has attached it can influence perception and behaviour and if it attaches to the extreme then so much of its energy (information) infuses into the body information field that the hologram starts to reflect the nature of the possessing entity. This is the *Exorcist* movie type of possession when facial features change and it's known as shapeshifting. Islam's Jinn are said to be invisible tricksters who change shape, 'whisper', confuse and take human form. These are all traits of the Archons and other versions of the same phenomenon. Extreme possession could certainty infuse the 'spirit of darkness' into a partner during sex as the Nag Hammadi texts appear to describe. Such an infusion can change genetics which is also energetic information. Human genetics is information and the 'spirit of darkness' is information. Mix one with the other and change must happen. Islam has the concept of a 'Jinn baby' through possession of the mother and by Jinn taking human form. There are many ways that human genetics can be changed and remember that Archons have been aware all along of advanced techniques to do this. What is being done in human society today – and far more – was known about by Archons at the time of the 'fallen ones' and their other versions described in religions and cultures.

Archons and their human-world Cult are obsessed with genetics as we see today and they know this dictates how information is processed into perceived reality during a human life. They needed to produce a human form that would decode the simulation and this is symbolically known as 'Adam and Eve' who left the 'garden' (prime reality) and 'fell' into Matrix reality. The simulation is not a 'physical' construct (there is no 'physical'); it is a source of information. Think Wi-Fi again. The simulation is an energetic field encoded with information and body-brain systems are designed to decode that information encoded in wave or frequency form which

is transmitted to the brain as electrical signals. These are decoded by the brain to construct our sense of reality – an illusory 'physical' world that only exists in the brain or the mind. Virtual reality games mimic this process using the same sensory decoding system. Information is fed to the senses to decode a virtual reality that can appear so real, but isn't (Figs 18 and 19). Some scientists believe – and I agree with them – that what we perceive as 'physical' reality only exists when we are looking or observing. The act of perception or focus triggers the decoding systems which turn waveform information into holographic reality. When we are not observing something our reality reverts from a holographic state to a waveform state. This relates to the same principle as a falling tree not making a noise unless someone is there to hear it or decode it. The concept makes sense from the simulation perspective. A computer is not decoding all the information in a Wi-Fi field all the time and only decodes or brings into reality on the screen that part of Wi-Fi that it's decoding – focusing upon – at that moment.

Figure 18: Virtual reality technology 'hacks' into the body's five-sense decoding system.

Figure 19: The result can be experienced as very 'real'.

Interestingly, Professor Donald Hoffman at the Department of Cognitive Sciences at the University of California, Irvine, says that our experienced reality is like a computer interface that shows us only the level with which we interact while hiding all that exists beyond it: 'Evolution shaped us with a user interface that hides the truth. Nothing that we see is the truth – the very language of space and time and objects is the wrong language to describe reality.' He is correct in what he says on so many levels. Space and time are not a universal reality. They are a phenomenon of decoded *simulation* reality as part of the process of enslaving our sense of reality. Near-death experiencers report again and again how space and time did not exist as we perceive them once they were free of the body – body decoding systems. You can appreciate from this why Archons and their Cult are so desperate to entrap human attention in the five senses where we are in the Matrix and of the Matrix. Opening your mind to expanded states of awareness takes you beyond the information confines of the simulation and you become aware of knowledge and insights denied to you before. This is what we call 'awakening' – *awakening from the Matrix* – and in the final chapter I will relate this to current events.

Where are the 'aliens'?

A simulation would explain the so-called 'Fermi Paradox' named after Italian physicist Enrico Fermi (1901-1954) who created the first nuclear reactor. He considered the question of why there is such a lack of extraterrestrial activity when there are so many stars and planets in an apparently vast universe; but what if the night sky that we see, or think we do, is a simulated projection as I say? If you control the simulation and your aim is to hold humanity fast in essential ignorance would you want other forms of life including advanced life coming and going sharing information with humanity? Or would you want them to believe they were isolated and apparently alone? Themes of human isolation and apartness are common whether they be the perception of a lifeless universe or the fascist isolation laws of the 'Covid' era. Paradoxically the very

existence of a simulation means that we are not alone when some force had to construct it. My view is that experiences that people have reported all over the world for centuries with Reptilians and Grey entities are Archon phenomena as Nag Hammadi texts describe; and that benevolent 'alien' interactions are non-human groups that come in and out of the simulation by overcoming Archon attempts to keep them out. It should be highlighted, too, that Reptilians and Greys are obsessed with *genetics* and *technology* as related by cultural accounts and those who say they have been abducted by them. Technology is their way of overcoming some of the limitations in their creative potential and our technology-driven and controlled human society of today is *arch*etypical Archon-Reptilian-Grey modus operandi. Technocracy is really *Archon*tocracy. The Universe does not have to be as big as it appears with a simulation. There is no space or distance only information decoded into holographic reality. What we call 'space' is only the absence of holographic 'objects' and that 'space' is The Field of energetic information which connects everything into a single whole. The same applies with the artificially-generated information field of the simulation. The Universe is not big or small as a physical reality. It is decoded information, that's all, and its perceived size is decided by the way the simulation is encoded to make it appear. The entire night sky as we perceive it only exists in our brain and so where are those 'millions of light years'? The 'stars' on the ceiling of the Planetarium looked a vast distance away.

There's another point to mention about 'aliens'. I have been highlighting since the 1990s the plan to stage a fake 'alien invasion' to justify the centralisation of global power and a world military. Nazi scientist Werner von Braun, who was taken to America by Operation Paperclip after World War Two to help found NASA, told his American assistant Dr Carol Rosin about the Cult agenda when he knew he was dying in 1977. Rosin said that he told her about a sequence that would lead to total human control by a one-world government. This included threats from terrorism, rogue nations, meteors and asteroids before finally an 'alien invasion'. All of these

things, von Braun said, would be bogus and what I would refer to as a No-Problem-Reaction-Solution. Keep this in mind when 'the aliens are coming' is the new mantra. The aliens are not coming – they are *already here* and they have infiltrated human society while looking human. French-Canadian investigative journalist Serge Monast said in 1994 that he had uncovered a NASA/military operation called Project Blue Beam which fits with what Werner von Braun predicted. Monast died of a 'heart attack' in 1996 the day after he was arrested and spent a night in prison. He was 51. He said Blue Beam was a plan to stage an alien invasion that would include religious figures beamed holographically into the sky as part of a global manipulation to usher in a 'new age' of worshipping what I would say is the Cult 'god' Yaldabaoth in a one-world religion. Fake holographic asteroids are also said to be part of the plan which again syncs with von Braun. How could you stage an illusory threat from asteroids unless they were holographic inserts? This is pretty straightforward given the advanced technology outside the public arena and the fact that our 'physical' reality is holographic anyway. Information fields would be projected and we would decode them into the illusion of a 'physical' asteroid. If they can sell a global 'pandemic' with a 'virus' that doesn't exist what will humans not believe if government and media tell them?

All this is particularly relevant as I write with the Pentagon planning to release in June, 2021, information about 'UFO sightings'. I have been following the UFO story since the early 1990s and the common theme throughout has been government and military denials and cover up. More recently, however, the Pentagon has suddenly become more talkative and apparently open with Air Force pilot radar images released of unexplained craft moving and changing direction at speeds well beyond anything believed possible with human technology. Then, in March, 2021, former Director of National Intelligence John Ratcliffe said a Pentagon report months later in June would reveal a great deal of information about UFO sightings unknown to the public. He said the report would have 'massive implications'. The order to do this was included bizarrely

in a $2.3 trillion 'coronavirus' relief and government funding bill passed by the Trump administration at the end of 2020. I would add some serious notes of caution here. I have been pointing out since the 1990s that the US military and intelligence networks have long had craft – 'flying saucers' or anti-gravity craft – which any observer would take to be extraterrestrial in origin. Keeping this knowledge from the public allows craft flown by *humans* to be perceived as alien visitations. I am not saying that 'aliens' do not exist. I would be the last one to say that, but we have to be streetwise here. President Ronald Reagan told the UN General Assembly in 1987: 'I occasionally think how quickly our differences worldwide would vanish if we were facing an alien threat from outside this world.' That's the idea. Unite against a common 'enemy' with a common purpose behind your 'saviour force' (the Cult) as this age-old technique of mass manipulation goes global.

Science moves this way …

I could find only one other person who was discussing the simulation hypothesis publicly when I concluded it was real. This was Nick Bostrom, a Swedish-born philosopher at the University of Oxford, who has explored for many years the possibility that human reality is a computer simulation although his version and mine are not the same. Today the simulation and holographic reality hypothesis have increasingly entered the scientific mainstream. Well, the more open-minded mainstream, that is. Here are a few of the ever-gathering examples. American nuclear physicist Silas Beane led a team of physicists at the University of Bonn in Germany pursuing the question of whether we live in a simulation. They concluded that we probably do and it was likely based on a lattice of cubes. They found that cosmic rays align with that specific pattern. The team highlighted the Greisen–Zatsepin–Kuzmin (GZK) limit which refers to cosmic ray particle interaction with cosmic background radiation that creates an apparent boundary for cosmic ray particles. They say in a paper entitled 'Constraints on the Universe as a Numerical Simulation' that this 'pattern of constraint' is exactly what you

would find with a computer simulation. They also made the point that a simulation would create its own 'laws of physics' that would limit possibility. I've been making the same point for decades that the *perceived* laws of physics relate only to this reality, or what I would later call the simulation. When designers write codes to create computer and virtual reality games they are the equivalent of the laws of physics for that game. Players interact within the limitations laid out by the coding. In the same way those who wrote the codes for the simulation decided the laws of physics that would apply. These can be overridden by expanded states of consciousness, but not by those enslaved in only five-sense awareness where simulation codes rule. Overriding the codes is what people call 'miracles'. They are not. They are bypassing the encoded limits of the simulation. A population caught in simulation perception would have no idea that this was their plight. As the Bonn paper said: 'Like a prisoner in a pitch-black cell we would not be able to see the "walls" of our prison,' That's true if people remain mesmerised by the five senses. Open to expanded awareness and those walls become very clear. The main one is the speed of light.

American theoretical physicist James Gates is another who has explored the simulation question and found considerable evidence to support the idea. Gates was Professor of Physics at the University of Maryland, Director of The Center for String and Particle Theory, and on Barack Obama's Council of Advisors on Science and Technology. He and his team found *computer codes* of digital data embedded in the fabric of our reality. They relate to on-off electrical charges of 1 and 0 in the binary system used by computers. 'We have no idea what they are doing there', Gates said. They found within the energetic fabric mathematical sequences known as error-correcting codes or block codes that 'reboot' data to its original state or 'default settings' when something knocks it out of sync. Gates was asked if he had found a set of equations embedded in our reality indistinguishable from those that drive search engines and browsers and he said: 'That is correct.' Rich Terrile, director of the Centre for Evolutionary Computation and Automated Design at NASA's Jet

Propulsion Laboratory, has said publicly that he believes the Universe is a digital hologram that must have been created by a form of intelligence. I agree with that in every way. Waveform information is delivered electrically by the senses to the brain which constructs a *digital* holographic reality that we call the 'world'. This digital level of reality can be read by the esoteric art of numerology. Digital holograms are at the cutting edge of holographics today. We have digital technology everywhere designed to access and manipulate our digital level of perceived reality. Synthetic mRNA in 'Covid vaccines' has a digital component to manipulate the body's digital 'operating system'.

Reality is numbers

How many know that our reality can be broken down to numbers and codes that are the same as computer games? Max Tegmark, a physicist at the Massachusetts Institute of Technology (MIT), is the author of *Our Mathematical Universe* in which he lays out how reality can be entirely described by numbers and maths in the way that a video game is encoded with the 'physics' of computer games. Our world and computer virtual reality are essentially the same. Tegmark imagines the perceptions of characters in an advanced computer game when the graphics are so good they don't know they are in a game. They think they can bump into real objects (electromagnetic resistance in our reality), fall in love and feel emotions like excitement. When they began to study the apparently 'physical world' of the video game they would realise that everything was made of pixels (which have been found in our energetic reality as must be the case when on one level our world is digital). What computer game characters thought was physical 'stuff', Tegmark said, could actually be broken down into numbers:

> And we're exactly in this situation in our world. We look around and it doesn't seem that mathematical at all, but everything we see is made out of elementary particles like quarks and electrons. And what properties does an electron have? Does it have a smell or a colour or a texture? No! ... We physicists have come up with geeky names for [Electron] properties, like

electric charge, or spin, or lepton number, but the electron doesn't care what we call it, the properties are just numbers.

This is the illusory reality Gnostics were describing. This is the simulation. The A, C, G, and T codes of DNA have a binary value – A and C = 0 while G and T = 1. This has to be when the simulation is digital and the body must be digital to interact with it. Recurring mathematical sequences are encoded throughout reality and the body. They include the Fibonacci sequence in which the two previous numbers are added to get the next one, as in ... 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, etc. The sequence is encoded in the human face and body, proportions of animals, DNA, seed heads, pine cones, trees, shells, spiral galaxies, hurricanes and the number of petals in a flower. The list goes on and on. There are fractal patterns – a 'never-ending pattern that is infinitely complex and self-similar across all scales in the as above, so below, principle of holograms. These and other famous recurring geometrical and mathematical sequences such as Phi, Pi, Golden Mean, Golden Ratio and Golden Section are *computer codes* of the simulation. I had to laugh and give my head a shake the day I finished this book and it went into the production stage. I was sent an article in *Scientific American* published in April, 2021, with the headline 'Confirmed! We Live in a Simulation'. Two decades after I first said our reality is a simulation and the speed of light is it's outer limit the article suggested that we do live in a simulation and that the speed of light is its outer limit. I left school at 15 and never passed a major exam in my life while the writer was up to his eyes in qualifications. As I will explain in the final chapter *knowing* is far better than thinking and they come from very different sources. The article rightly connected the speed of light to the processing speed of the 'Matrix' and said what has been in my books all this time … 'If we are in a simulation, as it appears, then space is an abstract property written in code. It is not real'. No it's not and if we live in a simulation something created it and it wasn't *us*. 'That David Icke says we are manipulated by aliens' – he's crackers.'

Wow …

The reality that humanity thinks is so real is an illusion. Politicians, governments, scientists, doctors, academics, law enforcement, media, school and university curriculums, on and on, are all founded on a world that *does not exist* except as a simulated prison cell. Is it such a stretch to accept that 'Covid' doesn't exist when our entire 'physical' reality doesn't exist? Revealed here is the knowledge kept under raps in the Cult networks of compartmentalised secrecy to control humanity's sense of reality by inducing the population to believe in a reality that's not real. If it wasn't so tragic in its experiential consequences the whole thing would be hysterically funny. None of this is new to Renegade Minds. Ancient Greek philosopher Plato (about 428 to about 347BC) was a major influence on Gnostic belief and he described the human plight thousands of years ago with his Allegory of the Cave. He told the symbolic story of prisoners living in a cave who had never been outside. They were chained and could only see one wall of the cave while behind them was a fire that they could not see. Figures walked past the fire casting shadows on the prisoners' wall and those moving shadows became their sense of reality. Some prisoners began to study the shadows and were considered experts on them (today's academics and scientists), but what they studied was only an illusion (today's academics and scientists). A prisoner escaped from the cave and saw reality as it really is. When he returned to report this revelation they didn't believe him, called him mad and threatened to kill him if he tried to set them free. Plato's tale is not only a brilliant analogy of the human plight and our illusory reality. It describes, too, the dynamics of the 'Covid' hoax. I have only skimmed the surface of these subjects here. The aim of this book is to crisply connect all essential dots to put what is happening today into its true context. All subject areas and their connections in this chapter are covered in great evidential detail in *Everything You Need To Know, But Have Never Been Told* and *The Answer*.

They say that bewildered people 'can't see the forest for the trees'. Humanity, however, can't see the forest for the *twigs*. The five senses

see only twigs while Renegade Minds can see the forest and it's the forest where the answers lie with the connections that reveals. Breaking free of perceptual programming so the forest can be seen is the way we turn all this around. Not breaking free is how humanity got into this mess. The situation may seem hopeless, but I promise you it's not. We are a perceptual heartbeat from paradise if only we knew.

CHAPTER TWELVE

Escaping Wetiko

Life is simply a vacation from the infinite
Dean Cavanagh

Renegade Minds weave the web of life and events and see common themes in the apparently random. They are always there if you look for them and their pursuit is aided by incredible synchronicity that comes when your mind is open rather than mesmerised by what it thinks it can see.

Infinite awareness is infinite possibility and the more of infinite possibility that we access the more becomes infinitely possible. That may be stating the apparently obvious, but it is a devastatingly-powerful fact that can set us free. We are a point of attention within an infinity of consciousness. The question is how much of that infinity do we choose to access? How much knowledge, insight, awareness, wisdom, do we want to connect with and explore? If your focus is only in the five senses you will be influenced by a fraction of infinite awareness. I mean a range so tiny that it gives new meaning to infinitesimal. Limitation of self-identity and a sense of the possible limit accordingly your range of consciousness. We are what we think we are. Life is what we think it is. The dream is the dreamer and the dreamer is the dream. Buddhist philosophy puts it this way: 'As a thing is viewed, so it appears.' Most humans live in the realm of touch, taste, see, hear, and smell and that's the limit of their sense of the possible and sense of self. Many will follow a religion and speak of a God in his heaven, but their lives are still

dominated by the five senses in their perceptions and actions. The five senses become the arbiter of everything. When that happens all except a smear of infinity is sealed away from influence by the rigid, unyielding, reality bubbles that are the five-sense human or Phantom Self. Archon Cult methodology is to isolate consciousness within five-sense reality – the simulation – and then program that consciousness with a sense of self and the world through a deluge of life-long information designed to instil the desired perception that allows global control. Efforts to do this have increased dramatically with identity politics as identity bubbles are squeezed into the minutiae of five-sense detail which disconnect people even more profoundly from the infinite 'I'.

Five-sense focus and self-identity are like a firewall that limits access to the infinite realms. You only perceive one radio or television station and no other. We'll take that literally for a moment. Imagine a vast array of stations giving different information and angles on reality, but you only ever listen to one. Here we have the human plight in which the population is overwhelmingly confined to CultFM. This relates only to the frequency range of CultFM and limits perception and insight to that band – limits *possibility* to that band. It means you are connecting with an almost imperceptibly minuscule range of possibility and creative potential within the infinite Field. It's a world where everything seems apart from everything else and where synchronicity is rare. Synchronicity is defined in the dictionary as 'the happening by chance of two or more related or similar events at the same time'. Use of 'by chance' betrays a complete misunderstanding of reality. Synchronicity is not 'by chance'. As people open their minds, or 'awaken' to use the term, they notice more and more coincidences in their lives, bits of 'luck', apparently miraculous happenings that put them in the right place at the right time with the right people. Days become peppered with 'fancy meeting you here' and 'what are the chances of that?' My entire life has been lived like this and ever more so since my own colossal awakening in 1990 and 91 which transformed my sense of reality. Synchronicity is not 'by chance'; it is by accessing expanded

realms of possibility which allow expanded potential for manifestation. People broadcasting the same vibe from the same openness of mind tend to be drawn 'by chance' to each other through what I call frequency magnetism and it's not only people. In the last more than 30 years incredible synchronicity has also led me through the Cult maze to information in so many forms and to crucial personal experiences. These 'coincidences' have allowed me to put the puzzle pieces together across an enormous array of subjects and situations. Those who have breached the bubble of five-sense reality will know exactly what I mean and this escape from the perceptual prison cell is open to everyone whenever they make that choice. This may appear super-human when compared with the limitations of 'human', but it's really our natural state. 'Human' as currently experienced is consciousness in an unnatural state of induced separation from the infinity of the whole. I'll come to how this transformation into unity can be made when I have described in more detail the force that holds humanity in servitude by denying this access to infinite self.

The Wetiko factor

I have been talking and writing for decades about the way five-sense mind is systematically barricaded from expanded awareness. I have used the analogy of a computer (five-sense mind) and someone at the keyboard (expanded awareness). Interaction between the computer and the operator is symbolic of the interaction between five-sense mind and expanded awareness. The computer directly experiences the Internet and the operator experiences the Internet via the computer which is how it's supposed to be – the two working as one. Archons seek to control that point where the operator connects with the computer to stop that interaction (Fig 20). Now the operator is banging the keyboard and clicking the mouse, but the computer is not responding and this happens when the computer is taken over – *possessed* – by an appropriately-named computer 'virus'. The operator has lost all influence over the computer which goes its own way making decisions under the control of the 'virus'. I have

just described the dynamic through which the force known to Gnostics as Yaldabaoth and Archons disconnects five-sense mind from expanded awareness to imprison humanity in perceptual servitude.

Figure 20: The mind 'virus' I have been writing about for decades seeks to isolate five-sense mind (the computer) from the true 'I'. (Image by Neil Hague).

About a year ago I came across a Native American concept of Wetiko which describes precisely the same phenomenon. Wetiko is the spelling used by the Cree and there are other versions including wintiko and windigo used by other tribal groups. They spell the name with lower case, but I see Wetiko as a proper noun as with Archons and prefer a capital. I first saw an article about Wetiko by writer and researcher Paul Levy which so synced with what I had been writing about the computer/operator disconnection and later the Archons. I then read his book, the fascinating *Dispelling Wetiko, Breaking the Spell of Evil*. The parallels between what I had concluded long before and the Native American concept of Wetiko were so clear and obvious that it was almost funny. For Wetiko see the Gnostic Archons for sure and the Jinn, the Predators, and every other name for a force of evil, inversion and chaos. Wetiko is the Native American name for the force that divides the computer from

the operator (Fig 21). Indigenous author Jack D. Forbes, a founder of the Native American movement in the 1960s, wrote another book about Wetiko entitled *Columbus And Other Cannibals – The Wetiko Disease of Exploitation, Imperialism, and Terrorism* which I also read. Forbes says that Wetiko refers to an evil person or spirit 'who terrorizes other creatures by means of terrible acts, including cannibalism'. Zulu shaman Credo Mutwa told me that African accounts tell how cannibalism was brought into the world by the Chitauri 'gods' – another manifestation of Wetiko. The distinction between 'evil person or spirit' relates to Archons/Wetiko possessing a human or acting as pure consciousness. Wetiko is said to be a sickness of the soul or spirit and a state of being that takes but gives nothing back – the Cult and its operatives perfectly described. Black Hawk, a Native American war leader defending their lands from confiscation, said European invaders had 'poisoned hearts' – Wetiko hearts – and that this would spread to native societies. Mention of the heart is very significant as we shall shortly see. Forbes writes: 'Tragically, the history of the world for the past 2,000 years is, in great part, the story of the epidemiology of the wetiko disease.' Yes, and much longer. Forbes is correct when he says: 'The wetikos destroyed Egypt and Babylon and Athens and Rome and Tenochtitlan [capital of the Aztec empire] and perhaps now they will destroy the entire earth.' Evil, he said, is the number one export of a Wetiko culture – see its globalisation with 'Covid'. Constant war, mass murder, suffering of all kinds, child abuse, Satanism, torture and human sacrifice are all expressions of Wetiko and the Wetiko possessed. The world is Wetiko made manifest, *but it doesn't have to be*. There is a way out of this even now.

Figure 21: The mind 'virus' is known to Native Americans as 'Wetiko'. (Image by Neil Hague).

Cult of Wetiko

Wetiko is the Yaldabaoth frequency distortion that seeks to attach to human consciousness and absorb it into its own. Once this connection is made Wetiko can drive the perceptions of the target which they believe to be coming from their own mind. All the horrors of history and today from mass killers to Satanists, paedophiles like Jeffrey Epstein and other psychopaths, are the embodiment of Wetiko and express its state of being in all its grotesqueness. The Cult is Wetiko incarnate, Yaldabaoth incarnate, and it seeks to facilitate Wetiko assimilation of humanity in totality into its distortion by manipulating the population into low frequency states that match its own. Paul Levy writes: 'Holographically enforced within the psyche of every human being the wetiko virus pervades and underlies the entire field of consciousness, and can therefore potentially manifest through any one of us at any moment if we are not mindful.' The 'Covid' hoax has achieved this with many people, but others have not fallen into Wetiko's frequency lair. Players in the 'Covid' human catastrophe including Gates, Schwab, Tedros, Fauci, Whitty, Vallance, Johnson, Hancock, Ferguson, Drosten, and all the rest, including the psychopath psychologists, are expressions of Wetiko. This is why

they have no compassion or empathy and no emotional consequence for what they do that would make them stop doing it. Observe all the people who support the psychopaths in authority against the Pushbackers despite the damaging impact the psychopaths have on their own lives and their family's lives. You are again looking at Wetiko possession which prevents them seeing through the lies to the obvious scam going on. *Why can't they see it?* Wetiko won't let them see it. The perceptual divide that has now become a chasm is between the Wetikoed and the non-Wetikoed.

Paul Levy describes Wetiko in the same way that I have long described the Archontic force. They are the same distorted consciousness operating across dimensions of reality: '… the subtle body of wetiko is not located in the third dimension of space and time, literally existing in another dimension … it is able to affect ordinary lives by mysteriously interpenetrating into our three-dimensional world.' Wetiko does this through its incarnate representatives in the Cult and by weaving itself into The Field which on our level of reality is the electromagnetic information field of the simulation or Matrix. More than that, the simulation *is* Wetiko / Yaldabaoth. Caleb Scharf, Director of Astrobiology at Columbia University, has speculated that 'alien life' could be so advanced that it has transcribed itself into the quantum realm to become what we call physics. He said intelligence indistinguishable from the fabric of the Universe would solve many of its greatest mysteries:

> Perhaps hyper-advanced life isn't just external. Perhaps it's already all around. It is embedded in what we perceive to be physics itself, from the root behaviour of particles and fields to the phenomena of complexity and emergence ... In other words, life might not just be in the equations. It might BE the equations [My emphasis].

Scharf said it is possible that 'we don't recognise advanced life because it forms an integral and unsuspicious part of what we've considered to be the natural world'. I agree. Wetiko/Yaldabaoth *is* the simulation. We are literally in the body of the beast. But that doesn't mean it has to control us. We all have the power to overcome Wetiko

influence and the Cult knows that. I doubt it sleeps too well because it knows that.

Which Field?

This, I suggest, is how it all works. There are two Fields. One is the fierce electromagnetic light of the Matrix within the speed of light; the other is the 'watery light' of The Field beyond the walls of the Matrix that connects with the Great Infinity. Five-sense mind and the decoding systems of the body attach us to the Field of Matrix light. They have to or we could not experience this reality. Five-sense mind sees only the Matrix Field of information while our expanded consciousness is part of the Infinity Field. When we open our minds, and most importantly our hearts, to the Infinity Field we have a mission control which gives us an expanded perspective, a road map, to understand the nature of the five-sense world. If we are isolated only in five-sense mind there is no mission control. We're on our own trying to understand a world that's constantly feeding us information to ensure we do not understand. People in this state can feel 'lost' and bewildered with no direction or radar. You can see ever more clearly those who are influenced by the Fields of Big Infinity or little five-sense mind simply by their views and behaviour with regard to the 'Covid' hoax. We have had this division throughout known human history with the mass of the people on one side and individuals who could see and intuit beyond the walls of the simulation – Plato's prisoner who broke out of the cave and saw reality for what it is. Such people have always been targeted by Wetiko/Archon-possessed authority, burned at the stake or demonised as mad, bad and dangerous. The Cult today and its global network of 'anti-hate', 'anti-fascist' Woke groups are all expressions of Wetiko attacking those exposing the conspiracy, 'Covid' lies and the 'vaccine' agenda.

Woke as a whole is Wetiko which explains its black and white mentality and how at one it is with the Wetiko-possessed Cult. Paul Levy said: 'To be in this paradigm is to still be under the thrall of a two-valued logic – where things are either true or false – of a

wetikoized mind.' Wetiko consciousness is in a permanent rage, therefore so is Woke, and then there is Woke inversion and contradiction. 'Anti-fascists' act like fascists because fascists *and* 'anti-fascists' are both Wetiko at work. Political parties act the same while claiming to be different for the same reason. Secret society and satanic rituals are attaching initiates to Wetiko and the cold, ruthless, psychopathic mentality that secures the positions of power all over the world is Wetiko. Reframing 'training programmes' have the same cumulative effect of attaching Wetiko and we have their graduates described as automatons and robots with a cold, psychopathic, uncaring demeanour. They are all traits of Wetiko possession and look how many times they have been described in this book and elsewhere with regard to personnel behind 'Covid' including the police and medical profession. Climbing the greasy pole in any profession in a Wetiko society requires traits of Wetiko to get there and that is particularly true of politics which is not about fair competition and pre-eminence of ideas. It is founded on how many backs you can stab and arses you can lick. This culminated in the global 'Covid' coordination between the Wetiko possessed who pulled it off in all the different countries without a trace of empathy and compassion for their impact on humans. Our sight sense can see only holographic form and not the Field which connects holographic form. Therefore we perceive 'physical' objects with 'space' in between. In fact that 'space' is energy/consciousness operating on multiple frequencies. One of them is Wetiko and that connects the Cult psychopaths, those who submit to the psychopaths, and those who serve the psychopaths in the media operations of the world. Wetiko is Gates. Wetiko is the mask-wearing submissive. Wetiko is the fake journalist and 'fact-checker'. The Wetiko Field is coordinating the whole thing. Psychopaths, gofers, media operatives, 'anti-hate' hate groups, 'fact-checkers' and submissive people work as one unit *even without human coordination* because they are attached to the *same* Field which is organising it all (Fig 22). Paul Levy is here describing how Wetiko-possessed people are drawn together and refuse to let any information breach their rigid

perceptions. He was writing long before 'Covid', but I think you will recognise followers of the 'Covid' religion *oh just a little bit*:

People who are channelling the vibratory frequency of wetiko align with each other through psychic resonance to reinforce their unspoken shared agreement so as to uphold their deranged view of reality. Once an unconscious content takes possession of certain individuals, it irresistibly draws them together by mutual attraction and knits them into groups tied together by their shared madness that can easily swell into an avalanche of insanity.

A psychic epidemic is a closed system, which is to say that it is insular and not open to any new information or informing influences from the outside world which contradict its fixed, limited, and limiting perspective.

There we have the Woke mind and the 'Covid' mind. Compatible resonance draws the awakening together, too, which is clearly happening today.

Figure 22: The Wetiko Field from which the Cult pyramid and its personnel are made manifest. (Image by Neil Hague).

Spiritual servitude

Wetiko doesn't care about humans. It's not human; it just possesses humans for its own ends and the effect (depending on the scale of

possession) can be anything from extreme psychopathy to unquestioning obedience. Wetiko's worst nightmare is for human consciousness to expand beyond the simulation. Everything is focussed on stopping that happening through control of information, thus perception, thus frequency. The 'education system', media, science, medicine, academia, are all geared to maintaining humanity in five-sense servitude as is the constant stimulation of low-vibrational mental and emotional states (see 'Covid'). Wetiko seeks to dominate those subconscious spaces between five-sense perception and expanded consciousness where the computer meets the operator. From these subconscious hiding places Wetiko speaks to us to trigger urges and desires that we take to be our own and manipulate us into anything from low-vibrational to psychopathic states. Remember how Islam describes the Jinn as invisible tricksters that 'whisper' and confuse. Wetiko is the origin of the 'trickster god' theme that you find in cultures all over the world. Jinn, like the Archons, are Wetiko which is terrified of humans awakening and reconnecting with our true self for then its energy source has gone. With that the feedback loop breaks between Wetiko and human perception that provides the energetic momentum on which its very existence depends as a force of evil. Humans are both its target and its source of survival, but only if we are operating in low-vibrational states of fear, hate, depression and the background anxiety that most people suffer. We are Wetiko's target because we are its key to survival. It needs us, not the other way round. Paul Levy writes:

A vampire has no intrinsic, independent, substantial existence in its own right; it only exists in relation to us. The pathogenic, vampiric mind-parasite called wetiko is nothing in itself – not being able to exist from its own side – yet it has a 'virtual reality' such that it can potentially destroy our species …

…The fact that a vampire is not reflected by a mirror can also mean that what we need to see is that there's nothing, no-thing to see, other than ourselves. The fact that wetiko is the expression of something inside of us means that the cure for wetiko is with us as well. The critical issue is finding this cure within us and then putting it into effect.

Evil begets evil because if evil does not constantly expand and find new sources of energetic sustenance its evil, its *distortion*, dies with the assimilation into balance and harmony. Love is the garlic to Wetiko's vampire. Evil, the absence of love, cannot exist in the presence of love. I think I see a way out of here. I have emphasised so many times over the decades that the Archons/Wetiko and their Cult are not all powerful. *They are not*. I don't care how it looks even now *they are not*. I have not called them little boys in short trousers for effect. I have said it because it is true. Wetiko's insatiable desire for power over others is not a sign of its omnipotence, but its insecurity. Paul Levy writes: 'Due to the primal fear which ultimately drives it and which it is driven to cultivate, wetiko's body politic has an intrinsic and insistent need for centralising power and control so as to create imagined safety for itself.' *Yeeeeeees!* Exactly! Why does Wetiko want humans in an ongoing state of fear? Wetiko itself *is* fear and it is petrified of love. As evil is an absence of love, so love is an absence of fear. Love conquers all and *especially* Wetiko which *is* fear. Wetiko brought fear into the world when it wasn't here before. *Fear* was the 'fall', the fall into low-frequency ignorance and illusion – fear is **F**alse **E**motion **A**ppearing **R**eal. The simulation is driven and energised by fear because Wetiko/Yaldabaoth (fear) *are* the simulation. Fear is the absence of love and Wetiko is the absence of love.

Wetiko today

We can now view current events from this level of perspective. The 'Covid' hoax has generated momentous amounts of ongoing fear, anxiety, depression and despair which have empowered Wetiko. No wonder people like Gates have been the instigators when they are Wetiko incarnate and exhibit every trait of Wetiko in the extreme. See how cold and unemotional these people are like Gates and his cronies, how dead of eye they are. That's Wetiko. Sabbatians are Wetiko and everything they control including the World Health Organization, Big Pharma and the 'vaccine' makers, national 'health'

hierarchies, corporate media, Silicon Valley, the banking system, and the United Nations with its planned transformation into world government. All are controlled and possessed by the Wetiko distortion into distorting human society in its image. We are with this knowledge at the gateway to understanding the world. Divisions of race, culture, creed and sexuality are diversions to hide the real division between those possessed and influenced by Wetiko and those that are not. The 'Covid' hoax has brought both clearly into view. Human behaviour is not about race. Tyrants and dictatorships come in all colours and creeds. What unites the US president bombing the innocent and an African tribe committing genocide against another as in Rwanda? What unites them? *Wetiko*. All wars are Wetiko, all genocide is Wetiko, all hunger over centuries in a world of plenty is Wetiko. Children going to bed hungry, including in the West, is Wetiko. Cult-generated Woke racial divisions that focus on the body are designed to obscure the reality that divisions in behaviour are manifestations of mind, not body. Obsession with body identity and group judgement is a means to divert attention from the real source of behaviour – mind and perception. Conflict sown by the Woke both within themselves and with their target groups are Wetiko providing lunch for itself through still more agents of the division, chaos, and fear on which it feeds. The Cult is seeking to assimilate the entirety of humanity and all children and young people into the Wetiko frequency by manipulating them into states of fear and despair. Witness all the suicide and psychological unravelling since the spring of 2020. Wetiko psychopaths want to impose a state of unquestioning obedience to authority which is no more than a conduit for Wetiko to enforce its will and assimilate humanity into itself. It needs us to believe that resistance is futile when it fears resistance and even more so the game-changing non-cooperation with its impositions. It can use violent resistance for its benefit. Violent impositions and violent resistance are *both* Wetiko. The Power of Love with its Power of No will sweep Wetiko from our world. Wetiko and its Cult know that. They just don't want us to know.

AI Wetiko

This brings me to AI or artificial intelligence and something else Wetikos don't want us to know. What is AI *really*? I know about computer code algorithms and AI that learns from data input. These, however, are more diversions, the expeditionary force, for the real AI that they want to connect to the human brain as promoted by Silicon Valley Wetikos like Kurzweil. What is this AI? It is the frequency of *Wetiko*, the frequency of the Archons. The connection of AI to the human brain is the connection of the Wetiko frequency to create a Wetiko hive mind and complete the job of assimilation. The hive mind is planned to be controlled from Israel and China which are both 100 percent owned by Wetiko Sabbatians. The assimilation process has been going on minute by minute in the 'smart' era which fused with the 'Covid' era. We are told that social media is scrambling the minds of the young and changing their personality. This is true, but what is social media? Look more deeply at how it works, how it creates divisions and conflict, the hostility and cruelty, the targeting of people until they are destroyed. That's Wetiko. Social media is manipulated to tune people to the Wetiko frequency with all the emotional exploitation tricks employed by platforms like Facebook and its Wetiko front man, Zuckerberg. Facebook's Instagram announced a new platform for children to overcome a legal bar on them using the main site. This is more Wetiko exploitation and manipulation of kids. Amnesty International likened the plan to foxes offering to guard the henhouse and said it was incompatible with human rights. Since when did Wetiko or Zuckerberg (I repeat myself) care about that? Would Brin and Page at Google, Wojcicki at YouTube, Bezos at Amazon and whoever the hell runs Twitter act as they do if they were not channelling Wetiko? Would those who are developing technologies for no other reason than human control? How about those designing and selling technologies to kill people and Big Pharma drug and 'vaccine' producers who know they will end or devastate lives? Quite a thought for these people to consider is that if you are Wetiko in a human life you are Wetiko on the 'other side' unless your frequency

changes and that can only change by a change of perception which becomes a change of behaviour. Where Gates is going does not bear thinking about although perhaps that's exactly where he wants to go. Either way, that's where he's going. His frequency will make it so.

The frequency lair

I have been saying for a long time that a big part of the addiction to smartphones and devices is that a frequency is coming off them that entraps the mind. People spend ages on their phones and sometimes even a minute or so after they put them down they pick them up again and it all repeats. 'Covid' lockdowns will have increased this addiction a million times for obvious reasons. Addictions to alcohol overindulgence and drugs are another way that Wetiko entraps consciousness to attach to its own. Both are symptoms of low-vibrational psychological distress which alcoholism and drug addiction further compound. Do we think it's really a coincidence that access to them is made so easy while potions that can take people into realms beyond the simulation are banned and illegal? I have explored smartphone addiction in other books, the scale is mind-blowing, and that level of addiction does not come without help. Tech companies that make these phones are Wetiko and they will have no qualms about destroying the minds of children. We are seeing again with these companies the Wetiko perceptual combination of psychopathic enforcers and weak and meek unquestioning compliance by the rank and file.

The global Smart Grid is the Wetiko Grid and it is crucial to complete the Cult endgame. The simulation is radiation and we are being deluged with technological radiation on a devastating scale. Wetiko frauds like Elon Musk serve Cult interests while occasionally criticising them to maintain his street-cred. 5G and other forms of Wi-Fi are being directed at the earth from space on a volume and scale that goes on increasing by the day. Elon Musk's (officially) SpaceX Starlink project is in the process of putting tens of thousands of satellites in low orbit to cover every inch of the planet with 5G and other Wi-Fi to create Kurzweil's global 'cloud' to which the

human mind is planned to be attached very soon. SpaceX has approval to operate 12,000 satellites with more than 1,300 launched at the time of writing and applications filed for *30,000* more. Other operators in the Wi-Fi, 5G, low-orbit satellite market include OneWeb (UK), Telesat (Canada), and AST & Science (US). Musk tells us that AI could be the end of humanity and then launches a company called Neuralink to connect the human brain to computers. Musk's (in theory) Tesla company is building electric cars and the driverless vehicles of the smart control grid. As frauds and bullshitters go Elon Musk in my opinion is Major League.

5G and technological radiation in general are destructive to human health, genetics and psychology and increasing the strength of artificial radiation underpins the five-sense perceptual bubbles which are themselves expressions of radiation or electromagnetism. Freedom activist John Whitehead was so right with his 'databit by databit, we are building our own electronic concentration camps'. The Smart Grid and 5G is a means to control the human mind and infuse perceptual information into The Field to influence anyone in sync with its frequency. You can change perception and behaviour en masse if you can manipulate the population into those levels of frequency and this is happening all around us today. The arrogance of Musk and his fellow Cult operatives knows no bounds in the way that we see with Gates. Musk's satellites are so many in number already they are changing the night sky when viewed from Earth. The astronomy community has complained about this and they have seen nothing yet. Some consequences of Musk's Wetiko hubris include: Radiation; visible pollution of the night sky; interference with astronomy and meteorology; ground and water pollution from intensive use of increasingly many spaceports; accumulating space debris; continual deorbiting and burning up of aging satellites, polluting the atmosphere with toxic dust and smoke; and ever-increasing likelihood of collisions. A collective public open letter of complaint to Musk said:

> We are writing to you … because SpaceX is in process of surrounding the Earth with a network of thousands of satellites whose very purpose is to irradiate every square inch of the

Earth. SpaceX, like everyone else, is treating the radiation as if it were not there. As if the mitochondria in our cells do not depend on electrons moving undisturbed from the food we digest to the oxygen we breathe.

As if our nervous systems and our hearts are not subject to radio frequency interference like any piece of electronic equipment. As if the cancer, diabetes, and heart disease that now afflict a majority of the Earth's population are not metabolic diseases that result from interference with our cellular machinery. As if insects everywhere, and the birds and animals that eat them, are not starving to death as a result.

People like Musk and Gates believe in their limitless Wetiko arrogance that they can do whatever they like to the world because they own it. Consequences for humanity are irrelevant. It's absolutely time that we stopped taking this shit from these self-styled masters of the Earth when you consider where this is going.

Why is the Cult so anti-human?

I hear this question often: Why would they do this when it will affect them, too? Ah, but will it? Who is this *them*? Forget their bodies. They are just vehicles for Wetiko consciousness. When you break it all down to the foundations we are looking at a state of severely distorted consciousness targeting another state of consciousness for assimilation. The rest is detail. The simulation is the fly-trap in which unique sensations of the five senses create a cycle of addiction called reincarnation. Renegade Minds see that everything which happens in our reality is a smaller version of the whole picture in line with the holographic principle. Addiction to the radiation of smart technology is a smaller version of addiction to the whole simulation. Connecting the body/brain to AI is taking that addiction on a giant step further to total ongoing control by assimilating human incarnate consciousness into Wetiko. I have watched during the 'Covid' hoax how many are becoming ever more profoundly attached to Wetiko's perceptual calling cards of aggressive response to any other point of view ('There is no other god but me'), psychopathic lack of compassion and empathy, and servile submission to the narrative and will of authority. Wetiko is the psychopaths *and* subservience to psychopaths. The Cult of Wetiko is

so anti-human because it is *not* human. It embarked on a mission to destroy human by targeting everything that it means to be human and to survive as human. 'Covid' is not the end, just a means to an end. The Cult with its Wetiko consciousness is seeking to change Earth systems, including the atmosphere, to suit them, not humans. The gathering bombardment of 5G alone from ground and space is dramatically changing The Field with which the five senses interact. There is so much more to come if we sit on our hands and hope it will all go away. It is not meant to go away. It is meant to get ever more extreme and we need to face that while we still can – just.

Carbon dioxide is the gas of life. Without that human is over. Kaput, gone, history. No natural world, no human. The Cult has created a cock and bull story about carbon dioxide and climate change to justify its reduction to the point where Gates and the ignoramus Biden 'climate chief' John Kerry want to suck it out of the atmosphere. Kerry wants to do this because his master Gates does. Wetikos have made the gas of life a demon with the usual support from the Wokers of Extinction Rebellion and similar organisations and the bewildered puppet-child that is Greta Thunberg who was put on the world stage by Klaus Schwab and the World Economic Forum. The name Extinction Rebellion is both ironic and as always Wetiko inversion. The gas that we need to survive must be reduced to save us from extinction. The most basic need of human is oxygen and we now have billions walking around in face nappies depriving body and brain of this essential requirement of human existence. More than that 5G at 60 gigahertz interacts with the oxygen molecule to reduce the amount of oxygen the body can absorb into the bloodstream. The obvious knock-on consequences of that for respiratory and cognitive problems and life itself need no further explanation. Psychopaths like Musk are assembling a global system of satellites to deluge the human atmosphere with this insanity. The man should be in jail. Here we have two most basic of human needs, oxygen and carbon dioxide, being dismantled.

Two others, water and food, are getting similar treatment with the United Nations Agendas 21 and 2030 – the Great Reset – planning to

centrally control all water and food supplies. People will not even own rain water that falls on their land. Food is affected at the most basic level by reducing carbon dioxide. We have genetic modification or GMO infiltrating the food chain on a mass scale, pesticides and herbicides polluting the air and destroying the soil. Freshwater fish that provide livelihoods for 60 million people and feed hundreds of millions worldwide are being 'pushed to the brink' according the conservationists while climate change is the only focus. Now we have Gates and Schwab wanting to dispense with current food sources all together and replace them with a synthetic version which the Wetiko Cult would control in terms of production and who eats and who doesn't. We have been on the Totalitarian Tiptoe to this for more than 60 years as food has become ever more processed and full of chemical shite to the point today when it's not natural food at all. As Dr Tom Cowan says: 'If it has a label don't eat it.' Bill Gates is now the biggest owner of farmland in the United States and he does nothing without an ulterior motive involving the Cult. Klaus Schwab wrote: 'To feed the world in the next 50 years we will need to produce as much food as was produced in the last 10,000 years … food security will only be achieved, however, if regulations on genetically modified foods are adapted to reflect the reality that gene editing offers a precise, efficient and safe method of improving crops.' Liar. People and the world are being targeted with aluminium through vaccines, chemtrails, food, drink cans, and endless other sources when aluminium has been linked to many health issues including dementia which is increasing year after year. Insects, bees and wildlife essential to the food chain are being deleted by pesticides, herbicides and radiation which 5G is dramatically increasing with 6G and 7G to come. The pollinating bee population is being devastated while wildlife including birds, dolphins and whales are having their natural radar blocked by the effects of ever-increasing radiation. In the summer windscreens used to be splattered with insects so numerous were they. It doesn't happen now. Where have they gone?

Synthetic everything

The Cult is introducing genetically-modified versions of trees, plants and insects including a Gates-funded project to unleash hundreds of millions of genetically-modified, lab-altered and patented male mosquitoes to mate with wild mosquitoes and induce genetic flaws that cause them to die out. Clinically-insane Gates-funded Japanese researchers have developed mosquitos that spread vaccine and are dubbed 'flying vaccinators'. Gates is funding the modification of weather patterns in part to sell the myth that this is caused by carbon dioxide and he's funding geoengineering of the skies to change the atmosphere. Some of this came to light with the Gates-backed plan to release tonnes of chalk into the atmosphere to 'deflect the Sun and cool the planet'. Funny how they do this while the heating effect of the Sun is not factored into climate projections focussed on carbon dioxide. The reason is that they want to reduce carbon dioxide (so don't mention the Sun), but at the same time they do want to reduce the impact of the Sun which is so essential to human life and health. I have mentioned the sun-cholesterol-vitamin D connection as they demonise the Sun with warnings about skin cancer (caused by the chemicals in sun cream they tell you to splash on). They come from the other end of the process with statin drugs to reduce cholesterol that turns sunlight into vitamin D. A lack of vitamin D leads to a long list of health effects and how vitamin D levels must have fallen with people confined to their homes over 'Covid'. Gates is funding other forms of geoengineering and most importantly chemtrails which are dropping heavy metals, aluminium and self-replicating nanotechnology onto the Earth which is killing the natural world. See *Everything You Need To Know, But Have Never Been Told* for the detailed background to this.

Every human system is being targeted for deletion by a force that's not human. The Wetiko Cult has embarked on the process of transforming the human body from biological to synthetic biological as I have explained. Biological is being replaced by the artificial and synthetic – Archontic 'countermimicry' – right across human society. The plan eventually is to dispense with the human body altogether

and absorb human consciousness – which it wouldn't really be by then – into cyberspace (the simulation which is Wetiko/Yaldabaoth). Preparations for that are already happening if people would care to look. The alternative media rightly warns about globalism and 'the globalists', but this is far bigger than that and represents the end of the human race as we know it. The 'bad copy' of prime reality that Gnostics describe was a bad copy of harmony, wonder and beauty to start with before Wetiko/Yaldabaoth set out to change the simulated 'copy' into something very different. The process was slow to start with. Entrapped humans in the simulation timeline were not technologically aware and they had to be brought up to intellectual speed while being suppressed spiritually to the point where they could build their own prison while having no idea they were doing so. We have now reached that stage where technological intellect has the potential to destroy us and that's why events are moving so fast. Central American shaman Don Juan Matus said:

> Think for a moment, and tell me how you would explain the contradictions between the intelligence of man the engineer and the stupidity of his systems of belief, or the stupidity of his contradictory behaviour. Sorcerers believe that the predators have given us our systems of beliefs, our ideas of good and evil; our social mores. They are the ones who set up our dreams of success or failure. They have given us covetousness, greed, and cowardice. It is the predator who makes us complacent, routinary, and egomaniacal.
>
> In order to keep us obedient and meek and weak, the predators engaged themselves in a stupendous manoeuvre – stupendous, of course, from the point of view of a fighting strategist; a horrendous manoeuvre from the point of those who suffer it. They gave us their mind. The predators' mind is baroque, contradictory, morose, filled with the fear of being discovered any minute now.

For 'predators' see Wetiko, Archons, Yaldabaoth, Jinn, and all the other versions of the same phenomenon in cultures and religions all over the world. The theme is always the same because it's true and it's real. We have reached the point where we have to deal with it. The question is – how?

Don't fight – walk away

I thought I'd use a controversial subheading to get things moving in terms of our response to global fascism. What do you mean 'don't fight'? What do you mean 'walk away'? We've got to fight. We can't walk away. Well, it depends what we mean by fight and walk away. If fighting means physical combat we are playing Wetiko's game and falling for its trap. It wants us to get angry, aggressive, and direct hate and hostility at the enemy we think we must fight. Every war, every battle, every conflict, has been fought with Wetiko leading both sides. It's what it does. Wetiko wants a fight, anywhere, any place. Just hit me, son, so I can hit you back. Wetiko hits Wetiko and Wetiko hits Wetiko in return. I am very forthright as you can see in exposing Wetikos of the Cult, but I don't hate them. I refuse to hate them. It's what they want. What you hate you become. What you *fight* you become. Wokers, 'anti-haters' and 'anti-fascists' prove this every time they reach for their keyboards or don their balaclavas. By walk away I mean to disengage from Wetiko which includes ceasing to cooperate with its tyranny. Paul Levy says of Wetiko:

> The way to 'defeat' evil is not to try to destroy it (for then, in playing evil's game, we have already lost), but rather, to find the invulnerable place within ourselves where evil is unable to vanquish us – this is to truly 'win' our battle with evil.

Wetiko is everywhere in human society and it's been on steroids since the 'Covid' hoax. Every shouting match over wearing masks has Wetiko wearing a mask and Wetiko not wearing one. It's an electrical circuit of push and resist, push and resist, with Wetiko pushing *and* resisting. Each polarity is Wetiko empowering itself. Dictionary definitions of 'resist' include 'opposing, refusing to accept or comply with' and the word to focus on is 'opposing'. What form does this take – setting police cars alight or 'refusing to accept or comply with'? The former is Wetiko opposing Wetiko while the other points the way forward. This is the difference between those aggressively demanding that government fascism must be obeyed who stand in stark contrast to the great majority of Pushbackers. We saw this clearly with a march by thousands of Pushbackers against lockdown in London followed days later by a Woker-hijacked

protest in Bristol in which police cars were set on fire. Masks were virtually absent in London and widespread in Bristol. Wetiko wants lockdown on every level of society and infuses its aggression to police it through its unknowing stooges. Lockdown protesters are the ones with the smiling faces and the hugs, The two blatantly obvious states of being – getting more obvious by the day – are the result of Wokers and their like becoming ever more influenced by the simulation Field of Wetiko and Pushbackers ever more influenced by The Field of a far higher vibration beyond the simulation. Wetiko can't invade the heart which is where most lockdown opponents are coming from. It's the heart that allows them to see through the lies to the truth in ways I will be highlighting.

Renegade Minds know that calmness is the place from which wisdom comes. You won't find wisdom in a hissing fit and wisdom is what we need in abundance right now. Calmness is not weakness – you don't have to scream at the top of your voice to be strong. Calmness is indeed a sign of strength. 'No' means I'm not doing it. *NOOOO!!!* doesn't mean you're not doing it even more. Volume does not advance 'No – I'm not doing it'. You are just not doing it. Wetiko possessed and influenced don't know how to deal with that. Wetiko wants a fight and we should not give it one. What it needs more than anything is our *cooperation* and we should not give that either. Mass rallies and marches are great in that they are a visual representation of feeling, but if it ends there they are irrelevant. You demand that Wetikos act differently? Well, they're not going to are they? They are Wetikos. We don't need to waste our time demanding that something doesn't happen when that will make no difference. We need to delete the means that *allows* it to happen. This, invariably, is our cooperation. You can demand a child stop firing a peashooter at the dog or you can refuse to buy the peashooter. If you provide the means you are cooperating with the dog being smacked on the nose with a pea. How can the authorities enforce mask-wearing if millions in a country refuse? What if the 74 million Pushbackers that voted for Trump in 2020 refused to wear masks, close their businesses or stay in their homes. It would be unenforceable. The

few control the many through the compliance of the many and that's always been the dynamic be it 'Covid' regulations or the Roman Empire. I know people can find it intimidating to say no to authority or stand out in a crowd for being the only one with a face on display; but it has to be done or it's over. I hope I've made clear in this book that where this is going will be far more intimidating than standing up now and saying 'No' – I will not cooperate with my own enslavement and that of my children. There might be consequences for some initially, although not so if enough do the same. The question that must be addressed is what is going to happen if we don't? It is time to be strong and unyieldingly so. No means no. Not here and there, but *everywhere* and *always*. I have refused to wear a mask and obey all the other nonsense. I will not comply with tyranny. I repeat: Fascism is not imposed by fascists – there are never enough of them. Fascism is imposed by the population acquiescing to fascism. *I will not do it*. I will die first, or my body will. Living meekly under fascism is a form of death anyway, the death of the spirit that Martin Luther King described.

Making things happen

We must not despair. This is not over till it's over and it's far from that. The 'fat lady' must refuse to sing. The longer the 'Covid' hoax has dragged on and impacted on more lives we have seen an awakening of phenomenal numbers of people worldwide to the realisation that what they have believed all their lives is not how the world really is. Research published by the system-serving University of Bristol and King's College London in February, 2021, concluded: 'One in every 11 people in Britain say they trust David Icke's take on the coronavirus pandemic.' It will be more by now and we have gathering numbers to build on. We must urgently progress from seeing the scam to ceasing to cooperate with it. Prominent German lawyer Reiner Fuellmich, also licenced to practice law in America, is doing a magnificent job taking the legal route to bring the psychopaths to justice through a second Nuremberg tribunal for crimes against humanity. Fuellmich has an impressive record of

beating the elite in court and he formed the German Corona Investigative Committee to pursue civil charges against the main perpetrators with a view to triggering criminal charges. Most importantly he has grasped the foundation of the hoax – the PCR test not testing for the 'virus' – and Christian Drosten is therefore on his charge sheet along with Gates frontman Tedros at the World Health Organization. Major players must be not be allowed to inflict their horrors on the human race without being brought to book. A life sentence must follow for Bill Gates and the rest of them. A group of researchers has also indicted the government of Norway for crimes against humanity with copies sent to the police and the International Criminal Court. The lawsuit cites participation in an internationally-planned false pandemic and violation of international law and human rights, the European Commission's definition of human rights by coercive rules, Nuremberg and Hague rules on fundamental human rights, and the Norwegian constitution. We must take the initiative from hereon and not just complain, protest and react.

There are practical ways to support vital mass non-cooperation. Organising in numbers is one. Lockdown marches in London in the spring in 2021 were mass non-cooperation that the authorities could not stop. There were too many people. Hundreds of thousands walked the London streets in the centre of the road for mile after mile while the Face-Nappies could only look on. They were determined, but calm, and just *did it* with no histrionics and lots of smiles. The police were impotent. Others are organising group shopping without masks for mutual support and imagine if that was happening all over. Policing it would be impossible. If the store refuses to serve people in these circumstances they would be faced with a long line of trolleys full of goods standing on their own and everything would have to be returned to the shelves. How would they cope with that if it kept happening? I am talking here about moving on from complaining to being pro-active; from watching things happen to making things happen. I include in this our relationship with the police. The behaviour of many Face-Nappies

has been disgraceful and anyone who thinks they would never find concentration camp guards in the 'enlightened' modern era have had that myth busted big-time. The period and setting may change – Wetikos never do. I watched film footage from a London march in which a police thug viciously kicked a protestor on the floor who had done nothing. His fellow Face-Nappies stood in a ring protecting him. What he did was a criminal assault and with a crowd far outnumbering the police this can no longer be allowed to happen unchallenged. I get it when people chant 'shame on you' in these circumstances, but that is no longer enough. They *have* no shame those who do this. Crowds needs to start making a citizen's arrest of the police who commit criminal offences and brutally attack innocent people and defenceless women. A citizen's arrest can be made under section 24A of the UK Police and Criminal Evidence (PACE) Act of 1984 and you will find something similar in other countries. I prefer to call it a Common Law arrest rather than citizen's for reasons I will come to shortly. Anyone can arrest a person committing an indictable offence or if they have reasonable grounds to suspect they are committing an indictable offence. On both counts the attack by the police thug would have fallen into this category. A citizen's arrest can be made to stop someone:

- Causing physical injury to himself or any other person
- Suffering physical injury
- Causing loss of or damage to property
- Making off before a constable can assume responsibility for him

A citizen's arrest may also be made to prevent a breach of the peace under Common Law and if they believe a breach of the peace will happen or anything related to harm likely to be done or already done in their presence. This is the way to go I think – the Common Law version. If police know that the crowd and members of the public will no longer be standing and watching while they commit

their thuggery and crimes they will think twice about acting like Brownshirts and Blackshirts.

Common Law – common sense

Mention of Common Law is very important. Most people think the law is the law as in one law. This is not the case. There are two bodies of law, Common Law and Statute Law, and they are not the same. Common Law is founded on the simple premise of do no harm. It does not recognise victimless crimes in which no harm is done while Statute Law does. There is a Statute Law against almost everything. So what is Statute Law? Amazingly it's the law of the *sea* that was brought ashore by the Cult to override the law of the land which is Common Law. They had no right to do this and as always they did it anyway. They had to. They could not impose their will on the people through Common Law which only applies to do no harm. How could you stitch up the fine detail of people's lives with that? Instead they took the law of the sea, or Admiralty Law, and applied it to the population. Statute Law refers to all the laws spewing out of governments and their agencies including all the fascist laws and regulations relating to 'Covid'. The key point to make is that Statute Law is *contract law*. It only applies between *contracting* corporations. Most police officers don't even know this. They have to be kept in the dark, too. Long ago when merchants and their sailing ships began to trade with different countries a contractual law was developed called Admiralty Law and other names. Again it only applied to *contracts* agreed between *corporate* entities. If there is no agreed contract the law of the sea had no jurisdiction *and that still applies to its new alias of Statute Law*. The problem for the Cult when the law of the sea was brought ashore was an obvious one. People were not corporations and neither were government entities. To overcome the latter they made governments and all associated organisations corporations. All the institutions are *private corporations* and I mean governments and their agencies, local councils, police, courts, military, US states, the whole lot. Go to the

Dun and Bradstreet corporate listings website for confirmation that they are all corporations. You are arrested by a private corporation called the police by someone who is really a private security guard and they take you to court which is another private corporation. Neither have jurisdiction over you unless you consent and *contract* with them. This is why you hear the mantra about law enforcement policing by *consent* of the people. In truth the people 'consent' only in theory through monumental trickery.

Okay, the Cult overcame the corporate law problem by making governments and institutions corporate entities; but what about people? They are not corporations are they? Ah ... well in a sense, and *only* a sense, they are. Not people exactly – the illusion of people. The Cult creates a corporation in the name of everyone at the time that their birth certificate is issued. Note birth/ *berth* certificate and when you go to court under the law of the sea on land you stand in a *dock*. These are throwbacks to the origin. My Common Law name is David Vaughan Icke. The name of the corporation created by the government when I was born is called Mr David Vaughan Icke usually written in capitals as MR DAVID VAUGHAN ICKE. That is not me, the living, breathing man. It is a fictitious corporate entity. The trick is to make you think that David Vaughan Icke and MR DAVID VAUGHAN ICKE are the same thing. *They are not*. When police charge you and take you to court they are prosecuting the corporate entity and not the living, breathing, man or woman. They have to trick you into identifying as the corporate entity and contracting with them. Otherwise they have no jurisdiction. They do this through a language known as legalese. Lawful and legal are not the same either. Lawful relates to Common Law and legal relates to Statute Law. Legalese is the language of Statue Law which uses terms that mean one thing to the public and another in legalese. Notice that when a police officer tells someone why they are being charged he or she will say at the end: 'Do you understand?' To the public that means 'Do you comprehend?' In legalese it means 'Do you stand under me?' Do you stand under my authority? If you say

yes to the question you are unknowingly agreeing to give them jurisdiction over you in a contract between two corporate entities.

This is a confidence trick in every way. Contracts have to be agreed between informed parties and if you don't know that David Vaughan Icke is agreeing to be the corporation MR DAVID VAUGHAN ICKE you cannot knowingly agree to contract. They are deceiving you and another way they do this is to ask for proof of identity. You usually show them a driving licence or other document on which your corporate name is written. In doing so you are accepting that you are that corporate entity when you are not. Referring to yourself as a 'person' or 'citizen' is also identifying with your corporate fiction which is why I made the Common Law point about the citizen's arrest. If you are approached by a police officer you identify yourself immediately as a living, breathing, man or woman and say 'I do not consent, I do not contract with you and I do not understand' or stand under their authority. I have a Common Law birth certificate as a living man and these are available at no charge from commonlawcourt.com. Businesses registered under the Statute Law system means that its laws apply. There are, however, ways to run a business under Common Law. Remember all 'Covid' laws and regulations are Statute Law – the law of *contracts* and you do not have to contract. This doesn't mean that you can kill someone and get away with it. Common Law says do no harm and that applies to physical harm, financial harm etc. Police are employees of private corporations and there needs to be a new system of non-corporate Common Law constables operating outside the Statute Law system. If you go to davidicke.com and put Common Law into the search engine you will find videos that explain Common Law in much greater detail. It is definitely a road we should walk.

With all my heart

I have heard people say that we are in a spiritual war. I don't like the term 'war' with its Wetiko dynamic, but I know what they mean. Sweep aside all the bodily forms and we are in a situation in which two states of consciousness are seeking very different realities.

Wetiko wants upheaval, chaos, fear, suffering, conflict and control. The other wants love, peace, harmony, fairness and freedom. That's where we are. We should not fall for the idea that Wetiko is all-powerful and there's nothing we can do. Wetiko is not all-powerful. It's a joke, pathetic. It doesn't have to be, but it has made that choice for now. A handful of times over the years when I have felt the presence of its frequency I have allowed it to attach briefly so I could consciously observe its nature. The experience is not pleasant, the energy is heavy and dark, but the ease with which you can kick it back out the door shows that its real power is in persuading us that it has power. It's all a con. Wetiko is a con. It's a trickster and not a power that can control us if we unleash our own. The con is founded on manipulating humanity to give its power to Wetiko which recycles it back to present the illusion that it has power when its power is *ours* that we gave away. This happens on an energetic level and plays out in the world of the seen as humanity giving its power to Wetiko authority which uses that power to control the population when the power is only the power the population has handed over. How could it be any other way for billions to be controlled by a relative few? I have had experiences with people possessed by Wetiko and again you can kick its arse if you do it with an open heart. Oh yes – the *heart* which can transform the world of perceived 'matter'.

We are receiver-transmitters and processors of information, but what information and where from? Information is processed into perception in three main areas – the brain, the heart and the belly. These relate to thinking, knowing, and emotion. Wetiko wants us to be head and belly people which means we think within the confines of the Matrix simulation and low-vibrational emotional reaction scrambles balance and perception. A few minutes on social media and you see how emotion is the dominant force. Woke is all emotion and is therefore thought-free and fact-free. Our heart is something different. It *knows* while the head *thinks* and has to try to work it out because it doesn't know. The human energy field has seven prime vortexes which connect us with wider reality (Fig 23). Chakra means

'wheels of light' in the Sanskrit language of ancient India. The main ones are: The crown chakra on top of the head; brow (or 'third eye') chakra in the centre of the forehead; throat chakra; heart chakra in the centre of the chest; solar plexus chakra below the sternum; sacral chakra beneath the navel; and base chakra at the bottom of the spine. Each one has a particular function or functions. We feel anxiety and nervousness in the belly where the sacral chakra is located and this processes emotion that can affect the colon to give people 'the shits' or make them 'shit scared' when they are nervous. Chakras all play an important role, but the Mr and Mrs Big is the heart chakra which sits at the centre of the seven, above the chakras that connect us to the 'physical' and below those that connect with higher realms (or at least should). Here in the heart chakra we feel love, empathy and compassion – 'My heart goes out to you'. Those with closed hearts become literally 'heart-less' in their attitudes and behaviour (see Bill Gates). Native Americans portrayed Wetiko with what Paul Levy calls a 'frigid, icy heart, devoid of mercy' (see Bill Gates).

Figure 23: The chakra system which interpenetrates the human energy field. The heart chakra is the governor – or should be.

Wetiko trembles at the thought of heart energy which it cannot infiltrate. The frequency is too high. What it seeks to do instead is close the heart chakra vortex to block its perceptual and energetic influence. Psychopaths have 'hearts of stone' and emotionally-damaged people have 'heartache' and 'broken hearts'. The astonishing amount of heart disease is related to heart chakra

disruption with its fundamental connection to the 'physical' heart. Dr Tom Cowan has written an outstanding book challenging the belief that the heart is a pump and making the connection between the 'physical' and spiritual heart. Rudolph Steiner who was way ahead of his time said the same about the fallacy that the heart is a pump. *What*? The heart is not a pump? That's crazy, right? Everybody knows that. Read Cowan's *Human Heart, Cosmic Heart* and you will realise that the very idea of the heart as a pump is ridiculous when you see the evidence. How does blood in the feet so far from the heart get pumped horizontally up the body by the heart?? Cowan explains in the book the real reason why blood moves as it does. Our 'physical' heart is used to symbolise love when the source is really the heart vortex or spiritual heart which is our most powerful energetic connection to 'out there' expanded consciousness. That's why we feel *knowing* – intuitive knowing – in the centre of the chest. Knowing doesn't come from a process of thoughts leading to a conclusion. It is there in an instant all in one go. Our heart knows because of its connection to levels of awareness that *do* know. This is the meaning and source of intuition – intuitive *knowing*.

For the last more than 30 years of uncovering the global game and the nature of reality my heart has been my constant antenna for truth and accuracy. An American intelligence insider once said that I had quoted a disinformer in one of my books and yet I had only quoted the part that was true. He asked: 'How do you do that?' By using my heart antenna was the answer and anyone can do it. Heart-centred is how we are meant to be. With a closed heart chakra we withdraw into a closed mind and the bubble of five-sense reality. If you take a moment to focus your attention on the centre of your chest, picture a spinning wheel of light and see it opening and expanding. You will feel it happening, too, and perceptions of the heart like joy and love as the heart impacts on the mind as they interact. The more the chakra opens the more you will feel expressions of heart consciousness and as the process continues, and becomes part of you, insights and knowings will follow. An open

heart is connected to that level of awareness that knows all is *One*. You will see from its perspective that the fault-lines that divide us are only illusions to control us. An open heart does not process the illusions of race, creed and sexuality except as brief experiences for a consciousness that is all. Our heart does not see division, only unity (Figs 24 and 25). There's something else, too. Our hearts love to laugh. Mark Twain's quote that says 'The human race has one really effective weapon, and that is laughter' is really a reference to the heart which loves to laugh with the joy of knowing the true nature of infinite reality and that all the madness of human society is an illusion of the mind. Twain also said: 'Against the assault of laughter nothing can stand.' This is so true of Wetiko and the Cult. Their insecurity demands that they be taken seriously and their power and authority acknowledged and feared. We should do nothing of the sort. We should not get aggressive or fearful which their insecurity so desires. We should laugh in their face. Even in their no-face as police come over in their face-nappies and expect to be taken seriously. They don't take themselves seriously looking like that so why should we? Laugh in the face of intimidation. Laugh in the face of tyranny. You will see by its reaction that you have pressed all of its buttons. Wetiko does not know what to do in the face of laughter or when its targets refuse to concede their joy to fear. We have seen many examples during the 'Covid' hoax when people have expressed their energetic power and the string puppets of Wetiko retreat with their tail limp between their knees. Laugh – the world is bloody mad after all and if it's a choice between laughter and tears I know which way I'm going.

Figure 24: Head consciousness without the heart sees division and everything apart from everything else.

Figure 25: Heart consciousness sees everything as One.

'Vaccines' and the soul

The foundation of Wetiko/Archon control of humans is the separation of incarnate five-sense mind from the infinite 'I' and closing the heart chakra where the True 'I' lives during a human life. The goal has been to achieve complete separation in both cases. I was interested therefore to read an account by a French energetic healer of what she said she experienced with a patient who had been given the 'Covid' vaccine. Genuine energy healers can sense information and consciousness fields at different levels of being which are referred to as 'subtle bodies'. She described treating the patient who later returned after having, without the healer's knowledge, two doses of the 'Covid vaccine'. The healer said:

I noticed immediately the change, very heavy energy emanating from [the] subtle bodies. The scariest thing was when I was working on the heart chakra, I connected with her soul: it was detached from the physical body, it had no contact and it was, as if it was floating in a state of total confusion: a damage to the consciousness that loses contact with the physical body, i.e. with our biological machine, there is no longer any communication between them.

I continued the treatment by sending light to the heart chakra, the soul of the person, but it seemed that the soul could no longer receive any light, frequency or energy. It was a very powerful experience for me. Then I understood that this substance is indeed used to detach consciousness so that this consciousness can no longer interact through this body that it possesses in life, where there is no longer any contact, no frequency, no light, no more energetic balance or mind.

This would create a human that is rudderless and at the extreme almost zombie-like operating with a fractional state of consciousness at the mercy of Wetiko. I was especially intrigued by what the healer said in the light of the prediction by the highly-informed Rudolf Steiner more than a hundred years ago. He said:

In the future, we will eliminate the soul with medicine. Under the pretext of a 'healthy point of view', there will be a vaccine by which the human body will be treated as soon as possible directly at birth, so that the human being cannot develop the thought of the existence of soul and Spirit. To materialistic doctors will be entrusted the task of removing the soul of humanity.

As today, people are vaccinated against this disease or that disease, so in the future, children will be vaccinated with a substance that can be produced precisely in such a way that people, thanks to this vaccination, will be immune to being subjected to the 'madness' of spiritual life. He would be extremely smart, but he would not develop a conscience, and that is the true goal of some materialistic circles.

Steiner said the vaccine would detach the physical body from the etheric body (subtle bodies) and 'once the etheric body is detached the relationship between the universe and the etheric body would become extremely unstable, and man would become an automaton'. He said 'the physical body of man must be polished on this Earth by spiritual will – so the vaccine becomes a kind of arymanique (Wetiko) force' and 'man can no longer get rid of a given materialistic feeling'. Humans would then, he said, become 'materialistic of constitution and can no longer rise to the spiritual'. I have been writing for years about DNA being a receiver-transmitter of information that connects us to other levels of reality and these 'vaccines' changing DNA can be likened to changing an antenna and what it can transmit and receive. Such a disconnection would clearly lead to changes in personality and perception. Steiner further predicted the arrival of AI. Big Pharma 'Covid vaccine' makers, expressions of Wetiko, are testing their DNA-manipulating evil on children as I write with a view to giving the 'vaccine' to babies. If it's a soul-body disconnector – and I say that it is or can be – every child would be disconnected from 'soul' at birth and the 'vaccine' would create a closed system in which spiritual guidance from the greater self would play no part. This has been the ambition of Wetiko all

along. A Pentagon video from 2005 was leaked of a presentation explaining the development of vaccines to change behaviour by their effect on the brain. Those that believe this is not happening with the 'Covid' genetically-modifying procedure masquerading as a 'vaccine' should make an urgent appointment with Naivety Anonymous. Klaus Schwab wrote in 2018:

> Neurotechnologies enable us to better influence consciousness and thought and to understand many activities of the brain. They include decoding what we are thinking in fine levels of detail through new chemicals and interventions that can influence our brains to correct for errors or enhance functionality.

The plan is clear and only the heart can stop it. With every heart that opens, every mind that awakens, Wetiko is weakened. Heart and love are far more powerful than head and hate and so nothing like a majority is needed to turn this around.

Beyond the Phantom

Our heart is the prime target of Wetiko and so it must be the answer to Wetiko. We *are* our heart which is part of one heart, the infinite heart. Our heart is where the true self lives in a human life behind firewalls of five-sense illusion when an imposter takes its place – *Phantom Self*; but our heart waits patiently to be set free any time we choose to see beyond the Phantom, beyond Wetiko. A Wetikoed Phantom Self can wreak mass death and destruction while the love of forever is locked away in its heart. The time is here to unleash its power and let it sweep away the fear and despair that is Wetiko. Heart consciousness does not seek manipulated, censored, advantage for its belief or religion, its activism and desires. As an expression of the One it treats all as One with the same rights to freedom and opinion. Our heart demands fairness for itself no more than for others. From this unity of heart we can come together in mutual support and transform this Wetikoed world into what reality is meant to be – a place of love, joy, happiness, fairness, justice and freedom. Wetiko has another agenda and that's why the world is as

it is, but enough of this nonsense. Wetiko can't stay where hearts are open and it works so hard to keep them closed. Fear is its currency and its food source and love in its true sense has no fear. Why would love have fear when it knows it is *All That Is, Has Been, And Ever Can Be* on an eternal exploration of all possibility? Love in this true sense is not the physical attraction that passes for love. This can be an expression of it, yes, but Infinite Love, a love without condition, goes far deeper to the core of all being. It *is* the core of all being. Infinite realty was born from love beyond the illusions of the simulation. Love infinitely expressed is the knowing that all is One and the swiftly-passing experience of separation is a temporary hallucination. You cannot disconnect from Oneness; you can only *perceive* that you have and withdraw from its influence. This is the most important of all perception trickery by the mind parasite that is Wetiko and the foundation of all its potential for manipulation.

If we open our hearts, open the sluice gates of the mind, and redefine self-identity amazing things start to happen. Consciousness expands or contracts in accordance with self-identity. When true self is recognised as infinite awareness and label self – Phantom Self – is seen as only a series of brief experiences life is transformed. Consciousness expands to the extent that self-identity expands and everything changes. You see unity, not division, the picture, not the pixels. From this we can play the long game. No more is an experience something in and of itself, but a fleeting moment in the eternity of forever. Suddenly people in uniform and dark suits are no longer intimidating. Doing what your heart knows to be right is no longer intimidating and consequences for those actions take on the same nature of a brief experience that passes in the blink of an infinite eye. Intimidation is all in the mind. Beyond the mind there is no intimidation.

An open heart does not consider consequences for what it knows to be right. To do so would be to consider not doing what it knows to be right and for a heart in its power that is never an option. The Renegade Mind is really the Renegade Heart. Consideration of consequences will always provide a getaway car for the mind and

the heart doesn't want one. What is right in the light of what we face today is to stop cooperating with Wetiko in all its forms and to do it without fear or compromise. You cannot compromise with tyranny when tyranny always demands more until it has everything. Life is your perception and you are your destiny. Change your perception and you change your life. Change collective perception and we change the world.

Come on people … One human family, One heart, One goal … *FREEEEEEDOM*!

We must settle for nothing less.

Postscript

The big scare story as the book goes to press is the 'Indian' variant and the world is being deluged with propaganda about the 'Covid catastrophe' in India which mirrors in its lies and misrepresentations what happened in Italy before the first lockdown in 2020.

The *New York Post* published a picture of someone who had 'collapsed in the street from Covid' in India in April, 2021, which was actually taken during a gas leak in May, 2020. Same old, same old. Media articles in mid-February were asking why India had been so untouched by 'Covid' and then as their vaccine rollout gathered pace the alleged 'cases' began to rapidly increase. Indian 'Covid vaccine' maker Bharat Biotech was funded into existence by the Bill and Melinda Gates Foundation (the pair announced their divorce in May, 2021, which is a pity because they so deserve each other). The Indian 'Covid crisis' was ramped up by the media to terrify the world and prepare people for submission to still more restrictions. The scam that worked the first time was being repeated only with far more people seeing through the deceit. Davidicke.com and Ickonic.com have sought to tell the true story of what is happening by talking to people living through the Indian nightmare which has nothing to do with 'Covid'. We posted a letter from 'Alisha' in Pune who told a very different story to government and media mendacity. She said scenes of dying people and overwhelmed hospitals were designed to hide what was really happening – genocide and starvation. Alisha said that millions had already died of starvation during the ongoing lockdowns while government and media were lying and making it look like the 'virus':

Restaurants, shops, gyms, theatres, basically everything is shut. The cities are ghost towns. Even so-called 'essential' businesses are only open till 11am in the morning. You basically have just an hour to buy food and then your time is up.

Inter-state travel and even inter-district travel is banned. The cops wait at all major crossroads to question why you are traveling outdoors or to fine you if you are not wearing a mask.

The medical community here is also complicit in genocide, lying about hospitals being full and turning away people with genuine illnesses, who need immediate care. They have even created a shortage of oxygen cylinders.

This is the classic Cult modus operandi played out in every country. Alisha said that people who would not have a PCR test not testing for the 'virus' were being denied hospital treatment. She said the people hit hardest were migrant workers and those in rural areas. Most businesses employed migrant workers and with everything closed there were no jobs, no income and no food. As a result millions were dying of starvation or malnutrition. All this was happening under Prime Minister Narendra Modi, a 100-percent asset of the Cult, and it emphasises yet again the scale of pure anti-human evil we are dealing with. Australia banned its people from returning home from India with penalties for trying to do so of up to five years in jail and a fine of £37,000. The manufactured 'Covid' crisis in India was being prepared to justify further fascism in the West. Obvious connections could be seen between the Indian 'vaccine' programme and increased 'cases' and this became a common theme. The Seychelles, the most per capita 'Covid vaccinated' population in the world, went back into lockdown after a 'surge of cases'.

Long ago the truly evil Monsanto agricultural biotechnology corporation with its big connections to Bill Gates devastated Indian farming with genetically-modified crops. Human rights activist Gurcharan Singh highlighted the efforts by the Indian government to complete the job by destroying the food supply to hundreds of millions with 'Covid' lockdowns. He said that 415 million people at the bottom of the disgusting caste system (still going whatever they say) were below the poverty line and struggled to feed themselves every year. Now the government was imposing lockdown at just the

time to destroy the harvest. This deliberate policy was leading to mass starvation. People may reel back at the suggestion that a government would do that, but Wetiko-controlled 'leaders' are capable of any level of evil. In fact what is described in India is in the process of being instigated worldwide. The food chain and food supply are being targeted at every level to cause world hunger and thus control. Bill Gates is not the biggest owner of farmland in America for no reason and destroying access to food aids both the depopulation agenda and the plan for synthetic 'food' already being funded into existence by Gates. Add to this the coming hyper-inflation from the suicidal creation of fake 'money' in response to 'Covid' and the breakdown of container shipping systems and you have a cocktail that can only lead one way and is meant to. The Cult plan is to crash the entire system to 'build back better' with the Great Reset.

'Vaccine' transmission

Reports from all over the world continue to emerge of women suffering menstrual and fertility problems after having the fake 'vaccine' and of the non-'vaccinated' having similar problems when interacting with the 'vaccinated'. There are far too many for 'coincidence' to be credible. We've had menopausal women getting periods, others having periods stop or not stopping for weeks, passing clots, sometimes the lining of the uterus, breast irregularities, and miscarriages (which increased by 400 percent in parts of the United States). Non-'vaccinated' men and children have suffered blood clots and nose bleeding after interaction with the 'vaccinated'. Babies have died from the effects of breast milk from a 'vaccinated' mother. Awake doctors – the small minority – speculated on the cause of non-'vaccinated' suffering the same effects as the 'vaccinated'. Was it nanotechnology in the synthetic substance transmitting frequencies or was it a straight chemical bioweapon that was being transmitted between people? I am not saying that some kind of chemical transmission is not one possible answer, but the foundation of all that the Cult does is frequency and

this is fertile ground for understanding how transmission can happen. American doctor Carrie Madej, an internal medicine physician and osteopath, has been practicing for the last 20 years, teaching medical students, and she says attending different meetings where the agenda for humanity was discussed. Madej, who operates out of Georgia, did not dismiss other possible forms of transmission, but she focused on frequency in search of an explanation for transmission. She said the Moderna and Pfizer 'vaccines' contained nano-lipid particles as a key component. This was a brand new technology never before used on humanity. 'They're using a nanotechnology which is pretty much little tiny computer bits … nanobots or hydrogel.' Inside the 'vaccines' was 'this sci-fi kind of substance' which suppressed immune checkpoints to get into the cell. I referred to this earlier as the 'Trojan horse' technique that tricks the cell into opening a gateway for the self-replicating synthetic material and while the immune system is artificially suppressed the body has no defences. Madej said the substance served many purposes including an on-demand ability to 'deliver the payload' and using the nano 'computer bits' as biosensors in the body. 'It actually has the ability to accumulate data from your body, like your breathing, your respiration, thoughts, emotions, all kinds of things.'

She said the technology obviously has the ability to operate through Wi-Fi and transmit and receive energy, messages, frequencies or impulses. 'Just imagine you're getting this new substance in you and it can react to things all around you, the 5G, your smart device, your phones.' We had something completely foreign in the human body that had never been launched large scale at a time when we were seeing 5G going into schools and hospitals (plus the Musk satellites) and she believed the 'vaccine' transmission had something to do with this: '… if these people have this inside of them … it can act like an antenna and actually transmit it outwardly as well.' The synthetic substance produced its own voltage and so it could have that kind of effect. This fits with my own contention that the nano receiver-transmitters are designed to connect people to the

Smart Grid and break the receiver-transmitter connection to expanded consciousness. That would explain the French energy healer's experience of the disconnection of body from 'soul' with those who have had the 'vaccine'. The nanobots, self-replicating inside the body, would also transmit the synthetic frequency which could be picked up through close interaction by those who have not been 'vaccinated'. Madej speculated that perhaps it was 5G and increased levels of other radiation that was causing the symptoms directly although interestingly she said that non-'vaccinated' patients had shown improvement when they were away from the 'vaccinated' person they had interacted with. It must be remembered that you can control frequency and energy with your mind and you can consciously create energetic barriers or bubbles with the mind to stop damaging frequencies from penetrating your field. American paediatrician Dr Larry Palevsky said the 'vaccine' was not a 'vaccine' and was never designed to protect from a 'viral' infection. He called it 'a massive, brilliant propaganda of genocide' because they didn't have to inject everyone to get the result they wanted. He said the content of the jabs was able to infuse any material into the brain, heart, lungs, kidneys, liver, sperm and female productive system. 'This is genocide; this is a weapon of mass destruction.' At the same time American colleges were banning students from attending if they didn't have this life-changing and potentially life-ending 'vaccine'. Class action lawsuits must follow when the consequences of this college fascism come to light. As the book was going to press came reports about fertility effects on sperm in 'vaccinated' men which would absolutely fit with what I have been saying and hospitals continued to fill with 'vaccine' reactions. Another question is what about transmission via blood transfusions? The NHS has extended blood donation restrictions from seven days after a 'Covid vaccination' to 28 days after even a sore arm reaction.

I said in the spring of 2020 that the then touted 'Covid vaccine' would be ongoing each year like the flu jab. A year later Pfizer CEO, the appalling Albert Bourla, said people would 'likely' need a 'booster dose' of the 'vaccine' within 12 months of getting 'fully

vaccinated' and then a yearly shot. 'Variants will play a key role', he said confirming the point. Johnson & Johnson CEO Alex Gorsky also took time out from his 'vaccine' disaster to say that people may need to be vaccinated against 'Covid-19' each year. UK Health Secretary, the psychopath Matt Hancock, said additional 'boosters' would be available in the autumn of 2021. This is the trap of the 'vaccine passport'. The public will have to accept every last 'vaccine' they introduce, including for the fake 'variants', or it would cease to be valid. The only other way in some cases would be continuous testing with a test not testing for the 'virus' and what is on the swabs constantly pushed up your noise towards the brain every time?

'Vaccines' changing behaviour

I mentioned in the body of the book how I believed we would see gathering behaviour changes in the 'vaccinated' and I am already hearing such comments from the non-'vaccinated' describing behaviour changes in friends, loved ones and work colleagues. This will only increase as the self-replicating synthetic material and nanoparticles expand in body and brain. An article in the *Guardian* in 2016 detailed research at the University of Virginia in Charlottesville which developed a new method for controlling brain circuits associated with complex animal behaviour. The method, dubbed 'magnetogenetics', involves genetically-engineering a protein called ferritin, which stores and releases iron, to create a magnetised substance – 'Magneto' – that can activate specific groups of nerve cells from a distance. This is claimed to be an advance on other methods of brain activity manipulation known as optogenetics and chemogenetics (the Cult has been developing methods of brain control for a long time). The ferritin technique is said to be non-invasive and able to activate neurons 'rapidly and reversibly'. In other words, human thought and perception. The article said that earlier studies revealed how nerve cell proteins 'activated by heat and mechanical pressure can be genetically engineered so that they become sensitive to radio waves and magnetic fields, by attaching them to an iron-storing protein called ferritin, or to inorganic

paramagnetic particles'. Sensitive to radio waves and magnetic fields? You mean like 5G, 6G and 7G? This is the human-AI Smart Grid hive mind we are talking about. The *Guardian* article said:

> … the researchers injected Magneto into the striatum of freely behaving mice, a deep brain structure containing dopamine-producing neurons that are involved in reward and motivation, and then placed the animals into an apparatus split into magnetised and non-magnetised sections.
>
> Mice expressing Magneto spent far more time in the magnetised areas than mice that did not, because activation of the protein caused the striatal neurons expressing it to release dopamine, so that the mice found being in those areas rewarding. This shows that Magneto can remotely control the firing of neurons deep within the brain, and also control complex behaviours.

Make no mistake this basic methodology will be part of the 'Covid vaccine' cocktail and using magnetics to change brain function through electromagnetic field frequency activation. The Pentagon is developing a 'Covid vaccine' using ferritin. Magnetics would explain changes in behaviour and why videos are appearing across the Internet as I write showing how magnets stick to the skin at the point of the 'vaccine' shot. Once people take these 'vaccines' anything becomes possible in terms of brain function and illness which will be blamed on 'Covid-19' and 'variants'. Magnetic field manipulation would further explain why the non-'vaccinated' are reporting the same symptoms as the 'vaccinated' they interact with and why those symptoms are reported to decrease when not in their company. Interestingly 'Magneto', a 'mutant', is a character in the Marvel Comic *X-Men* stories with the ability to manipulate magnetic fields and he believes that mutants should fight back against their human oppressors by any means necessary. The character was born Erik Lehnsherr to a Jewish family in Germany.

Cult-controlled courts

The European Court of Human Rights opened the door for mandatory 'Covid-19 vaccines' across the continent when it ruled in a Czech Republic dispute over childhood immunisation that legally

enforced vaccination could be 'necessary in a democratic society'. The 17 judges decided that compulsory vaccinations did not breach human rights law. On the face of it the judgement was so inverted you gasp for air. If not having a vaccine infused into your body is not a human right then what is? Ah, but they said human rights law which has been specifically written to delete all human rights at the behest of the state (the Cult). Article 8 of the European Convention on Human Rights relates to the right to a private life. The crucial word here is *'except'*:

> There shall be no interference by a public authority with the exercise of this right EXCEPT such as is in accordance with the law and is necessary in a democratic society in the interests of national security, public safety or the economic wellbeing of the country, for the prevention of disorder or crime, for the protection of health or morals, or for the protection of the rights and freedoms of others [My emphasis].

No interference *except* in accordance with the law means there *are* no 'human rights' *except* what EU governments decide you can have at their behest. 'As is necessary in a democratic society' explains that reference in the judgement and 'in the interests of national security, public safety or the economic well-being of the country, for the prevention of disorder or crime, for the protection of health or morals, or for the protection of the rights and freedoms of others' gives the EU a coach and horses to ride through 'human rights' and scatter them in all directions. The judiciary is not a check and balance on government extremism; it is a vehicle to enforce it. This judgement was almost laughably predictable when the last thing the Cult wanted was a decision that went against mandatory vaccination. Judges rule over and over again to benefit the system of which they are a part. Vaccination disputes that come before them are invariably delivered in favour of doctors and authorities representing the view of the state which owns the judiciary. Oh, yes, and we have even had calls to stop putting 'Covid-19' on death certificates within 28 days of a 'positive test' because it is claimed the practice makes the 'vaccine' appear not to work. They are laughing at you.

The scale of madness, inhumanity and things to come was highlighted when those not 'vaccinated' for 'Covid' were refused evacuation from the Caribbean island of St Vincent during massive volcanic eruptions. Cruise ships taking residents to the safety of another island allowed only the 'vaccinated' to board and the rest were left to their fate. Even in life and death situations like this we see 'Covid' stripping people of their most basic human instincts and the insanity is even more extreme when you think that fake 'vaccine'-makers are not even claiming their body-manipulating concoctions stop 'infection' and 'transmission' of a 'virus' that doesn't exist. St Vincent Prime Minister Ralph Gonsalves said: 'The chief medical officer will be identifying the persons already vaccinated so that we can get them on the ship.' Note again the power of the chief medical officer who, like Whitty in the UK, will be answering to the World Health Organization. This is the Cult network structure that has overridden politicians who 'follow the science' which means doing what WHO-controlled 'medical officers' and 'science advisers' tell them. Gonsalves even said that residents who were 'vaccinated' after the order so they could board the ships would still be refused entry due to possible side effects such as 'wooziness in the head'. The good news is that if they were woozy enough in the head they could qualify to be prime minister of St Vincent.

Microchipping freedom

The European judgement will be used at some point to justify moves to enforce the 'Covid' DNA-manipulating procedure. Sandra Ro, CEO of the Global Blockchain Business Council, told a World Economic Forum event that she hoped 'vaccine passports' would help to 'drive forced consent and standardisation' of global digital identity schemes: 'I'm hoping with the desire and global demand for some sort of vaccine passport – so that people can get travelling and working again – [it] will drive forced consent, standardisation, and frankly, cooperation across the world.' The lady is either not very bright, or thoroughly mendacious, to use the term 'forced consent'.

You do not 'consent' if you are forced – you *submit*. She was describing what the plan has been all along and that's to enforce a digital identity on every human without which they could not function. 'Vaccine passports' are opening the door and are far from the end goal. A digital identity would allow you to be tracked in everything you do in cyberspace and this is the same technique used by Cult-owned China to enforce its social credit system of total control. The ultimate 'passport' is planned to be a microchip as my books have warned for nearly 30 years. Those nice people at the Pentagon working for the Cult-controlled Defense Advanced Research Projects Agency (DARPA) claimed in April, 2021, they have developed a microchip inserted under the skin to detect 'asymptomatic Covid-19 infection' before it becomes an outbreak and a 'revolutionary filter' that can remove the 'virus' from the blood when attached to a dialysis machine. The only problems with this are that the 'virus' does not exist and people transmitting the 'virus' with no symptoms is brain-numbing bullshit. This is, of course, not a ruse to get people to be microchipped for very different reasons. DARPA also said it was producing a one-stop 'vaccine' for the 'virus' and all 'variants'. One of the most sinister organisations on Planet Earth is doing this? Better have it then. These people are insane because Wetiko that possesses them is insane.

Researchers from the Salk Institute in California announced they have created an embryo that is part human and part monkey. My books going back to the 1990s have exposed experiments in top secret underground facilities in the United States where humans are being crossed with animal and non-human 'extraterrestrial' species. They are now easing that long-developed capability into the public arena and there is much more to come given we are dealing with psychiatric basket cases. Talking of which – Elon Musk's scientists at Neuralink trained a monkey to play Pong and other puzzles on a computer screen using a joystick and when the monkey made the correct move a metal tube squirted banana smoothie into his mouth which is the basic technique for training humans into unquestioning compliance. Two Neuralink chips were in the monkey's skull and

more than 2,000 wires 'fanned out' into its brain. Eventually the monkey played a video game purely with its brain waves. Psychopathic narcissist Musk said the 'breakthrough' was a step towards putting Neuralink chips into human skulls and merging minds with artificial intelligence. *Exactly*. This man is so dark and Cult to his DNA.

World Economic Fascism (WEF)

The World Economic Forum is telling you the plan by the statements made at its many and various events. Cult-owned fascist YouTube CEO Susan Wojcicki spoke at the 2021 WEF Global Technology Governance Summit (see the name) in which 40 governments and 150 companies met to ensure 'the responsible design and deployment of emerging technologies'. Orwellian translation: 'Ensuring the design and deployment of long-planned technologies will advance the Cult agenda for control and censorship.' Freedom-destroyer and Nuremberg-bound Wojcicki expressed support for tech platforms like hers to censor content that is 'technically legal but could be harmful'. Who decides what is 'harmful'? She does and they do. 'Harmful' will be whatever the Cult doesn't want people to see and we have legislation proposed by the UK government that would censor content on the basis of 'harm' no matter if the information is fair, legal and provably true. Make that *especially* if it is fair, legal and provably true. Wojcicki called for a global coalition to be formed to enforce content moderation standards through automated censorship. This is a woman and mega-censor so self-deluded that she shamelessly accepted a 'free expression' award – *Wojcicki* – in an event sponsored by her own *YouTube*. They have no shame and no self-awareness.

You know that 'Covid' is a scam and Wojcicki a Cult operative when YouTube is censoring medical and scientific opinion purely on the grounds of whether it supports or opposes the Cult 'Covid' narrative. Florida governor Ron DeSantis compiled an expert panel with four professors of medicine from Harvard, Oxford, and Stanford Universities who spoke against forcing children and

vaccinated people to wear masks. They also said there was no proof that lockdowns reduced spread or death rates of 'Covid-19'. Cult-gofer Wojcicki and her YouTube deleted the panel video 'because it included content that contradicts the consensus of local and global health authorities regarding the efficacy of masks to prevent the spread of Covid-19'. This 'consensus' refers to what the Cult tells the World Health Organization to say and the WHO tells 'local health authorities' to do. Wojcicki knows this, of course. The panellists pointed out that censorship of scientific debate was responsible for deaths from many causes, but Wojcicki couldn't care less. She would not dare go against what she is told and as a disgrace to humanity she wouldn't want to anyway. The UK government is seeking to pass a fascist 'Online Safety Bill' to specifically target with massive fines and other means non-censored video and social media platforms to make them censor 'lawful but harmful' content like the Cult-owned Facebook, Twitter, Google and YouTube. What is 'lawful but harmful' would be decided by the fascist Blair-created Ofcom.

Another WEF obsession is a cyber-attack on the financial system and this is clearly what the Cult has planned to take down the bank accounts of everyone – except theirs. Those that think they have enough money for the Cult agenda not to matter to them have got a big lesson coming if they continue to ignore what is staring them in the face. The World Economic Forum, funded by Gates and fronted by Klaus Schwab, announced it would be running a 'simulation' with the Russian government and global banks of just such an attack called Cyber Polygon 2021. What they simulate – as with the 'Covid' Event 201 – they plan to instigate. The WEF is involved in a project with the Cult-owned Carnegie Endowment for International Peace called the WEF-Carnegie Cyber Policy Initiative which seeks to merge Wall Street banks, 'regulators' (I love it) and intelligence agencies to 'prevent' (arrange and allow) a cyber-attack that would bring down the global financial system as long planned by those that control the WEF and the Carnegie operation. The Carnegie Endowment for International Peace sent an instruction to First World

War US President Woodrow Wilson not to let the war end before society had been irreversibly transformed.

The Wuhan lab diversion

As I close, the Cult-controlled authorities and lapdog media are systematically pushing 'the virus was released from the Wuhan lab' narrative. There are two versions – it happened by accident and it happened on purpose. Both are nonsense. The perceived existence of the never-shown-to-exist 'virus' is vital to sell the impression that there is actually an infective agent to deal with and to allow the endless potential for terrifying the population with 'variants' of a 'virus' that does not exist. The authorities at the time of writing are going with the 'by accident' while the alternative media is promoting the 'on purpose'. Cable news host Tucker Carlson who has questioned aspects of lockdown and 'vaccine' compulsion has bought the Wuhan lab story. 'Everyone now agrees' he said. Well, I don't and many others don't and the question is *why* does the system and its media suddenly 'agree'? When the media moves as one unit with a narrative it is always a lie – witness the hour by hour mendacity of the 'Covid' era. Why would this Cult-owned combination which has unleashed lies like machine gun fire suddenly 'agree' to tell the truth??

Much of the alternative media is buying the lie because it fits the conspiracy narrative, but it's the *wrong* conspiracy. The real conspiracy is that *there is no virus* and that is what the Cult is desperate to hide. The idea that the 'virus' was released by accident is ludicrous when the whole 'Covid' hoax was clearly long-planned and waiting to be played out as it was so fast in accordance with the Rockefeller document and Event 201. So they prepared everything in detail over decades and then sat around strumming their fingers waiting for an 'accidental' release from a bio-lab? *What??* It's crazy. Then there's the 'on purpose' claim. You want to circulate a 'deadly virus' and hide the fact that you've done so and you release it down the street from the highest-level bio-lab in China? I repeat – *What??*

You would release it far from that lab to stop any association being made. But, no, we'll do it in a place where the connection was certain to be made. Why would you need to scam 'cases' and 'deaths' and pay hospitals to diagnose 'Covid-19' if you had a real 'virus'? What are sections of the alternative media doing believing this crap? Where were all the mass deaths in Wuhan from a 'deadly pathogen' when the recovery to normal life after the initial propaganda was dramatic in speed? Why isn't the 'deadly pathogen' now circulating all over China with bodies in the street? Once again we have the technique of tell them what they want to hear and they will likely believe it. The alternative media has its 'conspiracy' and with Carlson it fits with his 'China is the danger' narrative over years. China *is* a danger as a global Cult operations centre, but not for this reason. The Wuhan lab story also has the potential to instigate conflict with China when at some stage the plan is to trigger a Problem-Reaction-Solution confrontation with the West. Question everything – *everything* – and especially when the media agrees on a common party line.

Third wave ... fourth wave ... fifth wave ...

As the book went into production the world was being set up for more lockdowns and a 'third wave' supported by invented 'variants' that were increasing all the time and will continue to do so in public statements and computer programs, but not in reality. India became the new Italy in the 'Covid' propaganda campaign and we were told to be frightened of the new 'Indian strain'. Somehow I couldn't find it within myself to do so. A document produced for the UK government entitled 'Summary of further modelling of easing of restrictions – Roadmap Step 2' declared that a third wave was inevitable (of course when it's in the script) and it would be the fault of children and those who refuse the health-destroying fake 'Covid vaccine'. One of the computer models involved came from the Cult-owned *Imperial College* and the other from Warwick University which I wouldn't trust to tell me the date in a calendar factory. The document states that both models presumed extremely high uptake

of the 'Covid vaccines' and didn't allow for 'variants'. The document states: 'The resurgence is a result of some people (mostly children) being ineligible for vaccination; others choosing not to receive the vaccine; and others being vaccinated but not perfectly protected.' The mendacity takes the breath away. Okay, blame those with a brain who won't take the DNA-modifying shots and put more pressure on children to have it as 'trials' were underway involving children as young as six months with parents who give insanity a bad name. Massive pressure is being put on the young to have the fake 'vaccine' and child age consent limits have been systematically lowered around the world to stop parents intervening. Most extraordinary about the document was its claim that the 'third wave' would be driven by 'the resurgence in both hospitalisations and deaths … dominated by *those that have received two doses of the vaccine*, comprising around 60-70% of the wave respectively'. The predicted peak of the 'third wave' suggested 300 deaths per day with 250 of them *fully 'vaccinated' people*. How many more lies do acquiescers need to be told before they see the obvious? Those who took the jab to 'protect themselves' are projected to be those who mostly get sick and die? So what's in the 'vaccine'? The document went on:

> It is possible that a summer of low prevalence could be followed by substantial increases in incidence over the following autumn and winter. Low prevalence in late summer should not be taken as an indication that SARS-CoV-2 has retreated or that the population has high enough levels of immunity to prevent another wave.

They are telling you the script and while many British people believed 'Covid' restrictions would end in the summer of 2021 the government was preparing for them to be ongoing. Authorities were awarding contracts for 'Covid marshals' to police the restrictions with contracts starting in July, 2021, and going through to January 31st, 2022, and the government was advertising for 'Media Buying Services' to secure media propaganda slots worth a potential £320 million for 'Covid-19 campaigns' with a contract not ending until March, 2022. The recipient – via a list of other front companies – was reported to be American media marketing giant Omnicom Group

Inc. While money is no object for 'Covid' the UK waiting list for all other treatment – including life-threatening conditions – passed 4.5 million. Meantime the Cult is seeking to control all official 'inquiries' to block revelations about what has really been happening and why. It must not be allowed to – we need Nuremberg jury trials in every country. The cover-up doesn't get more obvious than appointing ultra-Zionist professor Philip Zelikow to oversee two dozen US virologists, public health officials, clinicians, former government officials and four American 'charitable foundations' to 'learn the lessons' of the 'Covid' debacle. The personnel will be those that created and perpetuated the 'Covid' lies while Zelikow is the former executive director of the 9/11 Commission who ensured that the truth about those attacks never came out and produced a report that must be among the most mendacious and manipulative documents ever written – see *The Trigger* for the detailed exposure of the almost unimaginable 9/11 story in which Sabbatians can be found at every level.

Passive no more

People are increasingly challenging the authorities with amazing numbers of people taking to the streets in London well beyond the ability of the Face-Nappies to stop them. Instead the Nappies choose situations away from the mass crowds to target, intimidate, and seek to promote the impression of 'violent protestors'. One such incident happened in London's Hyde Park. Hundreds of thousands walking through the streets in protest against 'Covid' fascism were ignored by the Cult-owned BBC and most of the rest of the mainstream media, but they delighted in reporting how police were injured in 'clashes with protestors'. The truth was that a group of people gathered in Hyde Park at the end of one march when most had gone home and they were peacefully having a good time with music and chat. Face-Nappies who couldn't deal with the full-march crowd then waded in with their batons and got more than they bargained for. Instead of just standing for this criminal brutality the crowd used their numerical superiority to push the Face-Nappies out of the

park. Eventually the Nappies turned and ran. Unfortunately two or three idiots in the crowd threw drink cans striking two officers which gave the media and the government the image they wanted to discredit the 99.9999 percent who were peaceful. The idiots walked straight into the trap and we must always be aware of potential agent provocateurs used by the authorities to discredit their targets.

This response from the crowd – the can people apart – must be a turning point when the public no longer stand by while the innocent are arrested and brutally attacked by the Face-Nappies. That doesn't mean to be violent, that's the last thing we need. We'll leave the violence to the Face-Nappies and government. But it does mean that when the Face-Nappies use violence against peaceful people the numerical superiority is employed to stop them and make citizen's arrests or Common Law arrests for a breach of the peace. The time for being passive in the face of fascism is over.

We are the many, they are the few, and we need to make that count before there is no freedom left and our children and grandchildren face an ongoing fascist nightmare.

COME ON PEOPLE – IT'S TIME.

One final thought …

The power of love
A force from above
Cleaning my soul
Flame on burn desire
Love with tongues of fire
Purge the soul
Make love your goal

I'll protect you from the hooded claw
Keep the vampires from your door
When the chips are down I'll be around
With my undying, death-defying
Love for you

Envy will hurt itself
Let yourself be beautiful
Sparkling love, flowers
And pearls and pretty girls
Love is like an energy
Rushin' rushin' inside of me

This time we go sublime
Lovers entwine, divine, divine,
Love is danger, love is pleasure
Love is pure – the only treasure

I'm so in love with you
Purge the soul
Make love your goal

The power of love
A force from above
Cleaning my soul
The power of love
A force from above
A sky-scraping dove

Flame on burn desire
Love with tongues of fire
Purge the soul
Make love your goal

Frankie Goes To Hollywood

APPENDIX

Cowan-Kaufman-Morell Statement on Virus Isolation (SOVI)

Isolation: The action of isolating; the fact or condition of being isolated or standing alone; separation from other things or persons; solitariness
Oxford English Dictionary

The controversy over whether the SARS-CoV-2 virus has ever been isolated or purified continues. However, using the above definition, common sense, the laws of logic and the dictates of science, any unbiased person must come to the conclusion that the SARS-CoV-2 virus has never been isolated or purified. As a result, no confirmation of the virus' existence can be found. The logical, common sense, and scientific consequences of this fact are:

- the structure and composition of something not shown to exist can't be known, including the presence, structure, and function of any hypothetical spike or other proteins;
- the genetic sequence of something that has never been found can't be known;
- "variants" of something that hasn't been shown to exist can't be known;
- it's impossible to demonstrate that SARS-CoV-2 causes a disease called Covid-19.

In as concise terms as possible, here's the proper way to isolate, characterize and demonstrate a new virus. First, one takes samples (blood, sputum, secretions) from many people (e.g. 500) with symptoms which are unique and specific enough to characterize an illness. Without mixing these samples with ANY tissue or products that also contain genetic material, the virologist macerates, filters and ultracentrifuges i.e. *purifies* the specimen. This common virology technique, done for decades to isolate bacteriophages[1] and so-called giant viruses in every virology lab, then allows the virologist to demonstrate with electron microscopy thousands of identically sized and shaped particles. These particles are the isolated and purified virus.

These identical particles are then checked for uniformity by physical and/or microscopic techniques. Once the purity is determined, the particles may be further characterized. This would include examining the structure, morphology, and chemical composition of the particles. Next, their genetic makeup is characterized by extracting the genetic material directly from the purified particles and using genetic-sequencing techniques, such as Sanger sequencing, that have also been around for decades. Then one does an analysis to confirm that these uniform particles are exogenous (outside) in origin as a virus is conceptualized to be, and not the normal breakdown products of dead and dying tissues.[2] (As of May 2020, we know that virologists have no way to determine whether the particles they're seeing are viruses or just normal breakdown products of dead and dying tissues.)[3]

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1 Isolation, characterization and analysis of bacteriophages from the haloalkaline lake Elmenteita, KenyaJuliah Khayeli Akhwale et al, PLOS One, Published: April 25, 2019. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0215734 – accessed 2/15/21

2 "Extracellular Vesicles Derived From Apoptotic Cells: An Essential Link Between Death and Regeneration," Maojiao Li1 et al, Frontiers in Cell and Developmental Biology, 2020 October 2. https://www.frontiersin.org/articles/10.3389/fcell.2020.573511/full – accessed 2/15/21

3 "The Role of Extraellular Vesicles as Allies of HIV, HCV and SARS Viruses," Flavia Giannessi, et al, Viruses, 2020 May

If we have come this far then we have fully isolated, characterized, and genetically sequenced an exogenous virus particle. However, we still have to show it is causally related to a disease. This is carried out by exposing a group of healthy subjects (animals are usually used) to this isolated, purified virus in the manner in which the disease is thought to be transmitted. If the animals get sick with the same disease, as confirmed by clinical and autopsy findings, one has now shown that the virus actually causes a disease. This demonstrates infectivity and transmission of an infectious agent.

None of these steps has even been attempted with the SARS-CoV-2 virus, nor have all these steps been successfully performed for any so-called pathogenic virus. Our research indicates that a single study showing these steps does not exist in the medical literature.

Instead, since 1954, virologists have taken unpurified samples from a relatively few people, often less than ten, with a similar disease. They then minimally process this sample and inoculate this unpurified sample onto tissue culture containing usually four to six other types of material – all of which contain identical genetic material as to what is called a "virus." The tissue culture is starved and poisoned and naturally disintegrates into many types of particles, some of which contain genetic material. Against all common sense, logic, use of the English language and scientific integrity, this process is called "virus isolation." This brew containing fragments of genetic material from many sources is then subjected to genetic analysis, which then creates in a computer-simulation process the alleged sequence of the alleged virus, a so called in silico genome. At no time is an actual virus confirmed by electron microscopy. At no time is a genome extracted and sequenced from an actual virus. This is scientific fraud.

The observation that the unpurified specimen — inoculated onto tissue culture along with toxic antibiotics, bovine fetal tissue, amniotic fluid and other tissues — destroys the kidney tissue onto which it is inoculated is given as evidence of the virus' existence and pathogenicity. This is scientific fraud.

From now on, when anyone gives you a paper that suggests the SARS-CoV-2 virus has been isolated, please check the methods sections. If the researchers used Vero cells or any other culture method, you know that their process was not isolation. You will hear the following excuses for why actual isolation isn't done:

1. There were not enough virus particles found in samples from patients to analyze.

2. Viruses are intracellular parasites; they can't be found outside the cell in this manner.

If No. 1 is correct, and we can't find the virus in the sputum of sick people, then on what evidence do we think the virus is dangerous or even lethal? If No. 2 is correct, then how is the virus spread from person to person? We are told it emerges from the cell to infect others. Then why isn't it possible to find it?

Finally, questioning these virology techniques and conclusions is not some distraction or divisive issue. Shining the light on this truth is essential to stop this terrible fraud that humanity is confronting. For, as we now know, if the virus has never been isolated, sequenced or shown to cause illness, if the virus is imaginary, then why are we wearing masks, social distancing and putting the whole world into prison?

Finally, if pathogenic viruses don't exist, then what is going into those injectable devices erroneously called "vaccines," and what is their purpose? This scientific question is the most urgent and relevant one of our time.

We are correct. The SARS-CoV2 virus does not exist.

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Dr. Thomas Cowan, MD
Dr. Andrew Kaufman, MD

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Ickonic is something that has been a dream of mine for the last 5 years, growing up around alternative information I have always had a natural interest in what is going on in the World and what could I do to make it better.
Across the range of subjects and positions of influence occupied mainly by people who don't strive to make things better it's the Media that I have always found the most frustrating and fascinating. Mainly because if the Media did their Jobs properly then so much of the negative things happening in the World simply would not be able to happen, because they would be exposed within a heartbeat.
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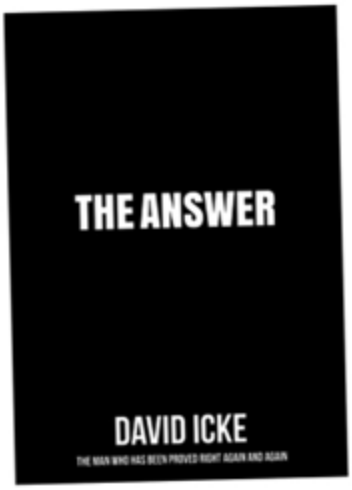

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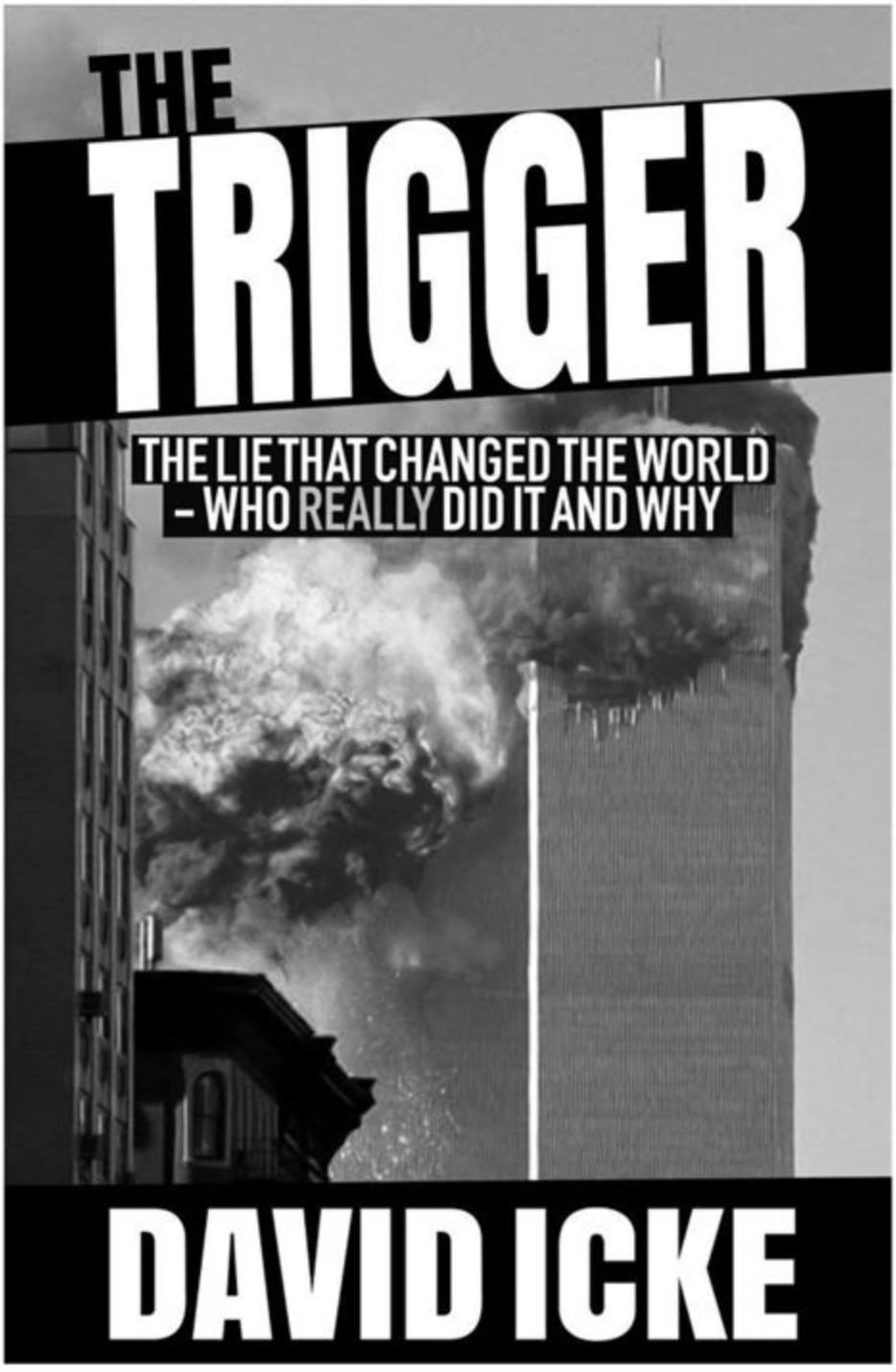
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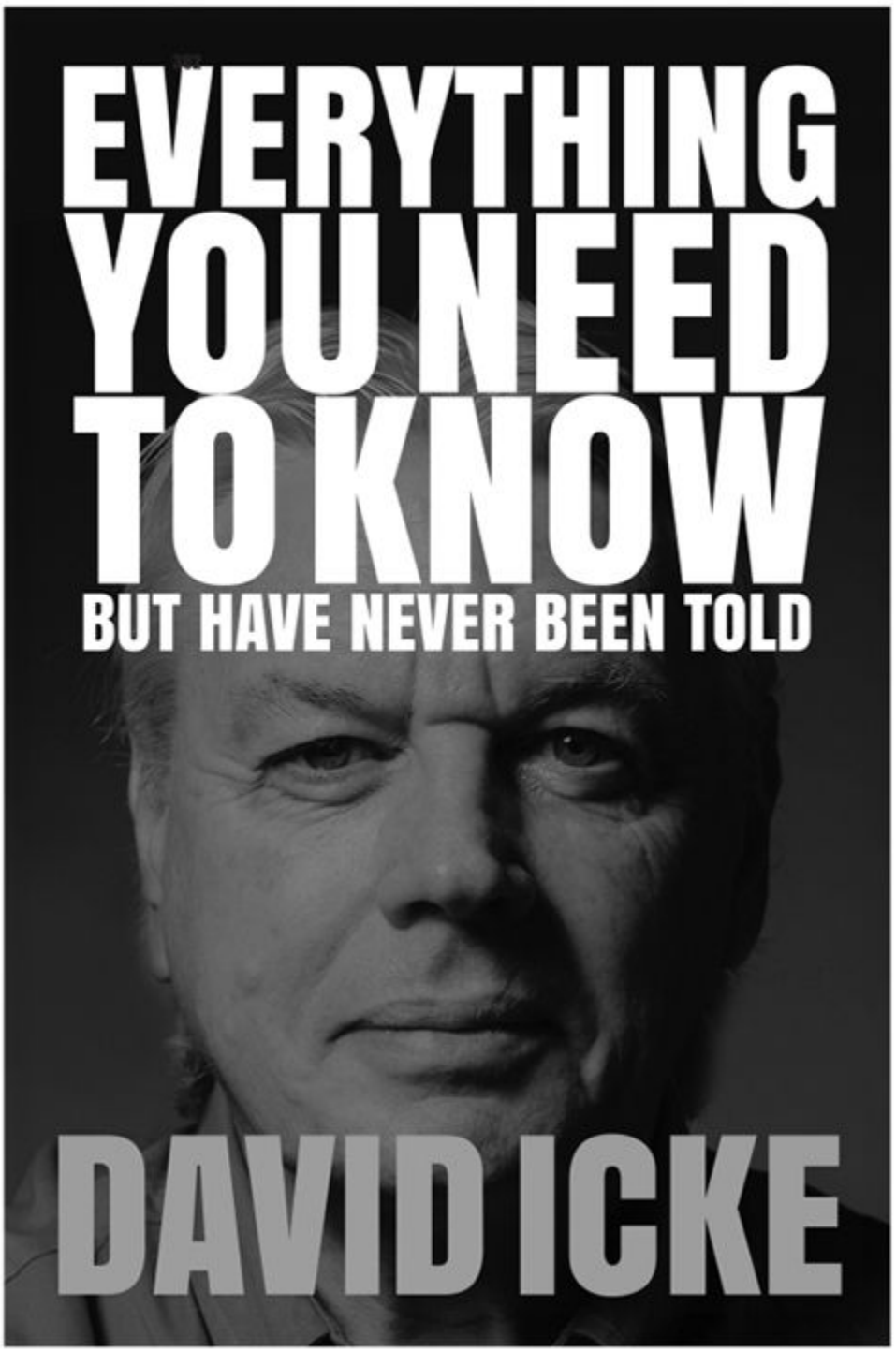
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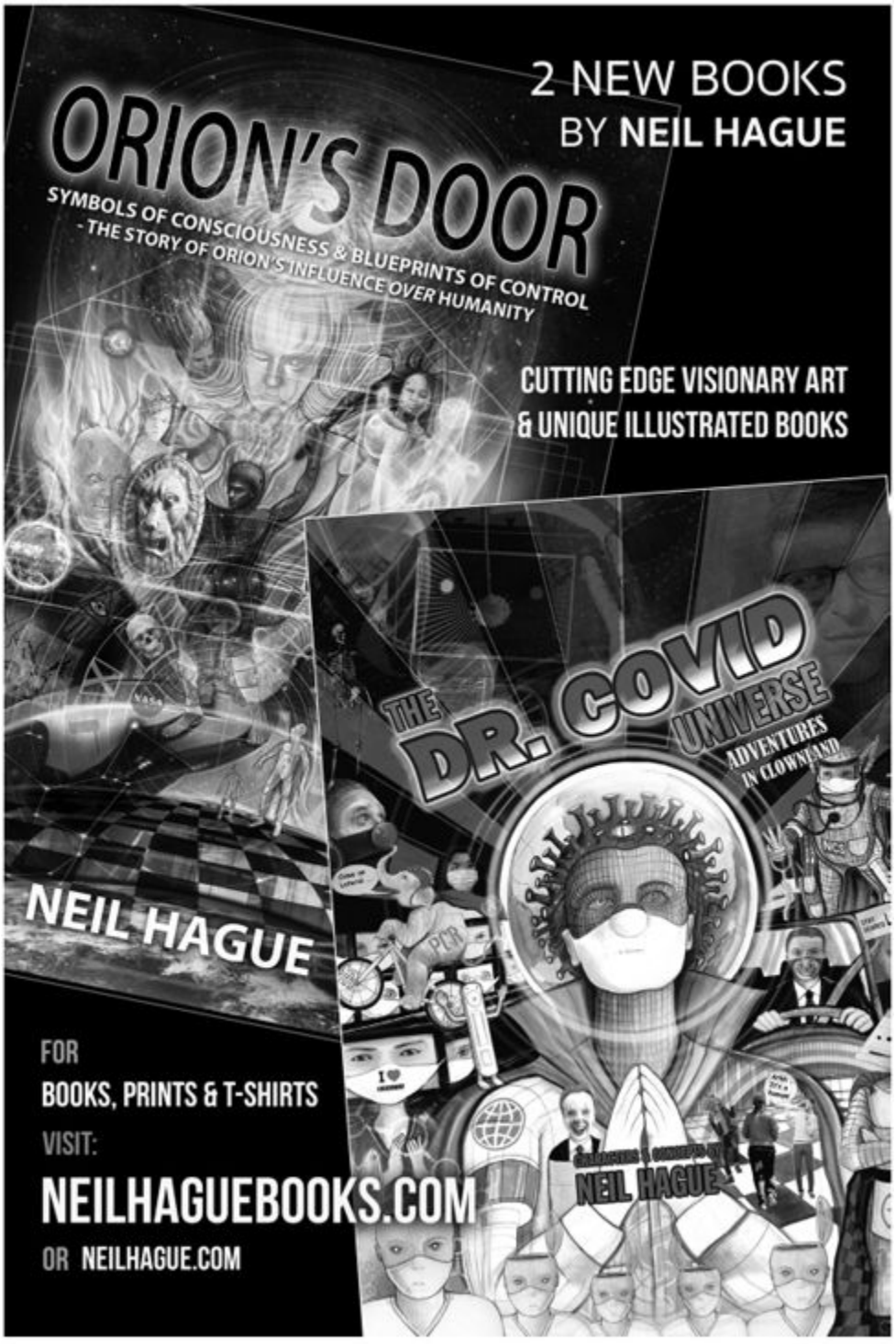
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