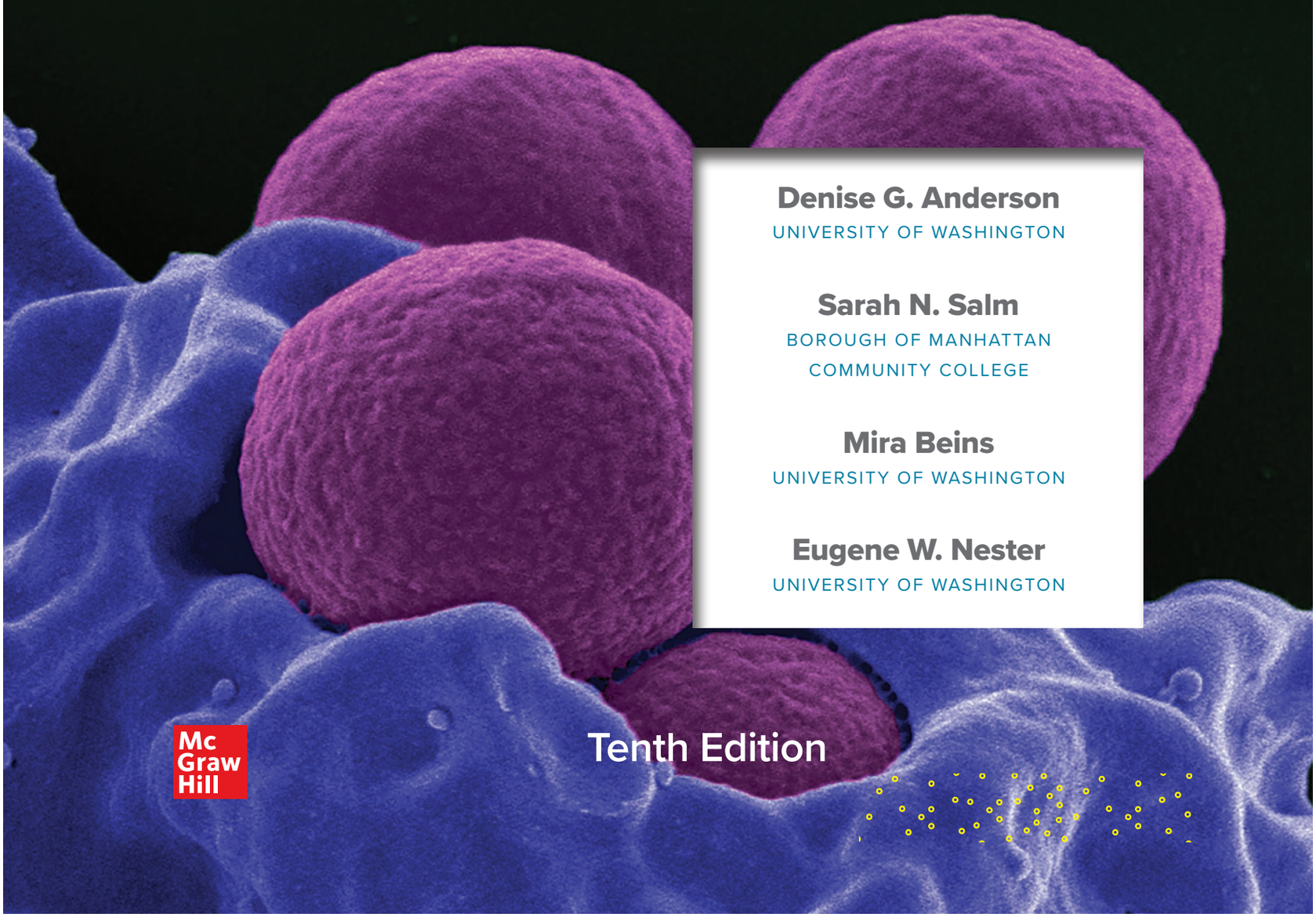
Nester's Microbiology

A HUMAN PERSPECTIVE

Denise Anderson | Sarah Salm | Mira Beins



Denise G. Anderson
UNIVERSITY OF WASHINGTON

Sarah N. Salm
BOROUGH OF MANHATTAN
COMMUNITY COLLEGE

Mira Beins
UNIVERSITY OF WASHINGTON

Eugene W. Nester
UNIVERSITY OF WASHINGTON

Mc Graw Hill

Tenth Edition

NESTER'S MICROBIOLOGY: A HUMAN PERSPECTIVE, TENTH EDITION

This book is printed on acid-free paper.

1 2 3 4 5 6 7 8 9 LWI 23 22 21

ISBN 978-1-260-73550-5 (bound edition)
MHID 1-260-73550-8 (bound edition)
ISBN 978-1-264-34198-6 (loose-leaf edition)
MHID 1-264-34198-9 (loose-leaf edition)

Portfolio Manager: *Lauren Vondra*
Product Developer: *Erin DeHeck*
Marketing Manager: *Tami Hodge*
Content Project Managers: *Laura Bies / Rachael Hillebrand*
Senior Buyer: *Laura Fuller*
Lead Designer: *David Hash*
Content Licensing Specialist: *Beth Cray*
Cover Image: ©*National Institute of Allergy and Infectious Diseases (NIAID)*
Compositor: *MPS Limited*

All credits appearing on page or at the end of the book are considered to be an extension of the copyright page.

Library of Congress Cataloging-in-Publication Data

Names: Anderson, Denise G. (Denise Gayle), author. | Salm, Sarah, author. | Beins, Mira, author. | Allen, Deborah (Deborah Patricia), author.
Title: Nester's microbiology : a human perspective / Denise G. Anderson, Sarah N. Salm, Mira Beins, [Deborah Allen].
Other titles: Microbiology
Description: Tenth edition. | New York, NY : McGraw Hill Education, [2022] | Includes index.
Identifiers: LCCN 2020041282 | ISBN 9781260735505 (hardcover; alk. paper)
Subjects: MESH: Microbiological Phenomena | Microbiological Techniques | Communicable Diseases–microbiology
Classification: LCC QR41.2 | NLM QW 4 | DDC 579–dc23
LC record available at https://lccn.loc.gov/2020041282

mheducation.com/highered

Brief Contents

PART I

Life and Death of Microorganisms

1. Humans and the Microbial World 1
2. The Molecules of Life 20
3. Cells and Methods to Observe Them 44
4. Dynamics of Microbial Growth 90
5. Control of Microbial Growth 117
6. Microbial Metabolism: Fueling Cell Growth 137
7. The Blueprint of Life, from DNA to Protein 175
8. Bacterial Genetics 203
9. Biotechnology 232

PART II

The Microbial World

10. Identifying and Classifying Microorganisms 255
11. The Diversity of Bacteria and Archaea 274
12. The Eukaryotic Members of the Microbial World 305
13. Viruses, Viroids, and Prions 329

PART III

Microorganisms and Humans

14. The Innate Immune Response 360
15. The Adaptive Immune Response 385
16. Host-Microbe Interactions 415
17. Applications of Immune Responses 439
18. Immunological Disorders 464
19. Epidemiology 481
20. Antimicrobial Medications 504

PART IV

Infectious Diseases

21. Respiratory System Infections 536
22. Skin Infections 582
23. Wound Infections 609
24. Digestive System Infections 630
25. Blood and Lymphatic Infections 672
26. Nervous System Infections 703
27. Genitourinary Tract Infections 738

PART V

Applied Microbiology

28. Microbial Ecology 777
29. Environmental Microbiology: Treatment of Water, Wastes, and Polluted Habitats 796
30. Food Microbiology 810

APPENDICES A–1

GLOSSARY/INDEX GI–1

About the Authors

The Nester Team:

Different Perspectives, One Vision, One Voice

The authors of this edition may be a set of individuals with different insights and unique experiences, but their cooperative relationship defines the word "team." What drives them is a single shared goal: to create the most learning-friendly introductory microbiology textbook available. Each chapter was edited with students in mind, using simpler words where appropriate while maintaining the scientific rigor so important for today's healthcare professionals.

Richard Moore

Denise Anderson

Denise Anderson is a Senior Lecturer Emeritus in the Department of Microbiology at the University of Washington, where she taught a variety of courses including general microbiology, medical bacteriology laboratory, recombinant DNA techniques, and medical mycology/parasitology laboratory for over 30 years. Equipped with a diverse educational background, including undergraduate work in nutrition and graduate work in food science and in microbiology, she first discovered a passion for teaching when she taught microbiology laboratory courses as part of her graduate training. Her enthusiastic teaching style, fueled by regular doses of Seattle's famous coffee, received high reviews from her students.

Denise now relaxes in the Yorkshire Dales of England, where she lives with her husband, Richard Moore. When not editing textbook chapters, she can usually be found walking scenic footpaths, chatting with friends, fighting weeds in her garden, or enjoying a fermented beverage at the local pub.

Sandy Coetzee

Sarah Salm

Sarah Salm is a Professor at the Borough of Manhattan Community College (BMCC) of the City University of New York, where she teaches microbiology, anatomy and physiology, and general biology. She earned her undergraduate and doctoral degrees at the University of the Witwatersrand in Johannesburg, South Africa. She later moved to New York, where she did postdoctoral work at the NYU School of Medicine. Her research background is diverse and includes plant virology, prostate cancer, and bacteria in contaminated water sources.

Mira Beins

Mira Beins

Mira Beins is an Associate Teaching Professor in the Department of Microbiology at the University of Washington, where she teaches general microbiology, medical bacteriology, and medical mycology/parasitology. She completed her undergraduate studies in Molecular Biology and Biotechnology at the University of the Philippines before moving to Wisconsin for graduate work in Microbiology. Her graduate and postdoctoral research both focused on virology, which solidified her belief that viruses are amazing—although she now begrudgingly admits that bacteria, fungi, and eukaryotic parasites are pretty cool, too.

Mira lives in Seattle with her husband Mike and two kids, Maya and Noah. When she's not busy teaching or driving the kids to their many activities, she enjoys reading books, watching movies, hanging out with friends and family, and planning the next family trip (which Denise hopes will be to the Yorkshire Dales!).

Mike Bohrer

Deborah Allen

Deborah Allen is a Professor at Jefferson College in Missouri, where she teaches microbiology as well as several other courses for students entering allied health careers. Her graduate work was in zoology at the University of Oklahoma and in neurobiology and behavior at Cornell University. She participated in cancer research at the University of Arkansas Medical Center before embarking on a career in publishing, working in acquisitions and development for books in the life sciences. She is now thrilled to be working on the other end of the desk with the Nester team. Away from campus, Deborah reads or listens to her favorite Eve Dallas novels, floats the rivers and listens to folk music in the Ozarks, and fully appreciates the local microbes while visiting Missouri wineries.

Courtesy Eugene Nester

Eugene Nester

Gene (Eugene) Nester was instrumental in establishing the text's reputation for excellence over the decades. Although no longer an active member of the author team, he wrote the original version of the present text with Evans Roberts and Nancy Pearsall more than 30 years ago. That text, *Microbiology: Molecules, Microbes and Man,* pioneered the organ system approach to the study of infectious disease and was developed specifically for allied health sciences.

Presentation Tools Allow Instructors to Customize Lecture

Everything you need, in one location

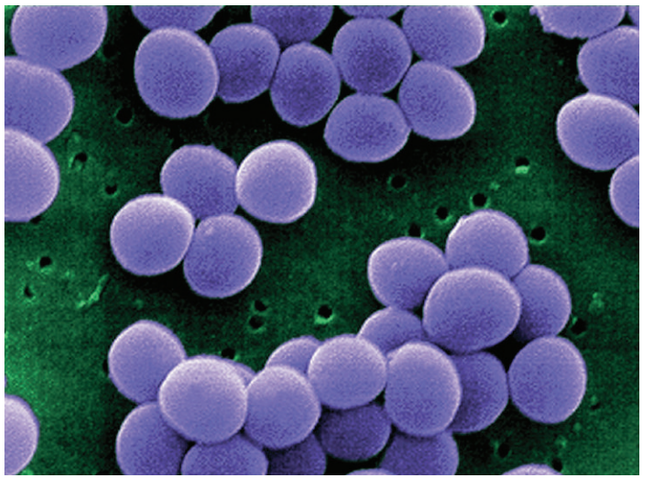
Enhanced Lecture Presentations contain lecture outlines, art, photos, tables, and animations embedded where appropriate. Fully customizable, but complete and ready to use, these presentations will enable you to spend less time preparing for lecture!

Animations—More than 100 animations bring key concepts to life; available for instructors and students.

Accessible PPTs—Our lecture presentations are formatted per the latest accessibility guidelines. Alternative text, written by our textbook author team, is included for all images and static tables.

Take your course online—*easily*—with one-click Digital Lecture Capture

McGraw-Hill Tegrity® records and distributes your lecture with just a click of a button. Students can view them anytime/anywhere via computer, tablet, or mobile device. Tegrity Campus indexes as it records your slideshow presentations and anything shown on your computer so **students can use keywords to find exactly what they want to study**.



Source: Janice Haney Carr/CDC

Remote Proctoring & Browser-Locking Capabilities

New remote proctoring and browser-locking capabilities, hosted by Proctorio within Connect, provide control of the assessment environment by enabling security options and verifying the identity of the student.

Seamlessly integrated within Connect, these services allow instructors to control students' assessment experience by restricting browser activity, recording students' activity, and verifying students are doing their own work.

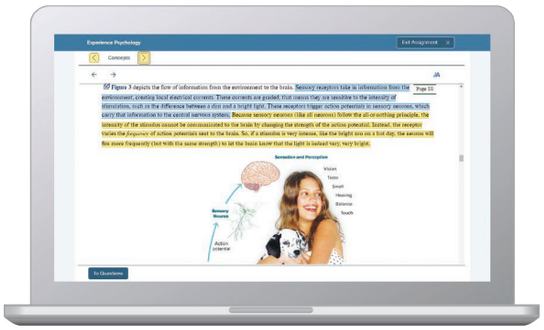
Instant and detailed reporting gives instructors an at-a-glance view of potential academic integrity concerns, thereby avoiding personal bias and supporting evidence-based claims.

Instructors: Student Success Starts with You

Tools to enhance your unique voice

Want to build your own course? No problem. Prefer to use our turnkey, prebuilt course? Easy. Want to make changes throughout the semester? Sure. And you'll save time with Connect's auto-grading too.

65%
Less Time Grading



Laptop: McGraw Hill; Woman/dog: George Doyle/Getty Images

Study made personal

Incorporate adaptive study resources like SmartBook® 2.0 into your course and help your students be better prepared in less time. Learn more about the powerful personalized learning experience available in SmartBook 2.0 at **www.mheducation.com/highered/connect/smartbook**

Affordable solutions, added value

Make technology work for you with LMS integration for single sign-on access, mobile access to the digital textbook, and reports to quickly show you how each of your students is doing. And with our Inclusive Access program you can provide all these tools at a discount to your students. Ask your McGraw Hill representative for more information.

Padlock: Jobalou/Getty Images

Solutions for your challenges

A product isn't a solution. Real solutions are affordable, reliable, and come with training and ongoing support when you need it and how you want it. Visit **www.supportateverystep.com** for videos and resources both you and your students can use throughout the semester.

Checkmark: Jobalou/Getty Images

SUPPORT AT every step

Students: Get Learning that Fits You

Effective tools for efficient studying

Connect is designed to make you more productive with simple, flexible, intuitive tools that maximize your study time and meet your individual learning needs. Get learning that works for you with Connect.

Study anytime, anywhere

Download the free ReadAnywhere app and access your online eBook or SmartBook 2.0 assignments when it's convenient, even if you're offline. And since the app automatically syncs with your eBook and SmartBook 2.0 assignments in Connect, all of your work is available every time you open it. Find out more at **www.mheducation.com/readanywhere**

> ***"I really liked this app—it made it easy to study when you don't have your textbook in front of you."***
>
> - Jordan Cunningham, Eastern Washington University

Calendar: owattaphotos/Getty Images

Everything you need in one place

Your Connect course has everything you need—whether reading on your digital eBook or completing assignments for class, Connect makes it easy to get your work done.

Learning for everyone

McGraw Hill works directly with Accessibility Services Departments and faculty to meet the learning needs of all students. Please contact your Accessibility Services Office and ask them to email accessibility@mheducation.com, or visit **www.mheducation.com/about/accessibility** for more information.

Top: Jenner Images/Getty Images, Left: Hero Images/Getty Images, Right: Hero Images/Getty Images

FOCUS ON UNDERSTANDING . . .

Student-Friendly Illustrations

Introduce the "big picture"

Focus figures provide an overview or highlight a key concept.

Keep the big picture in focus

A highlighted mini-version of the overview figure is often incorporated into the upper left corner of subsequent figures, helping students see how those figures fit into the big picture.

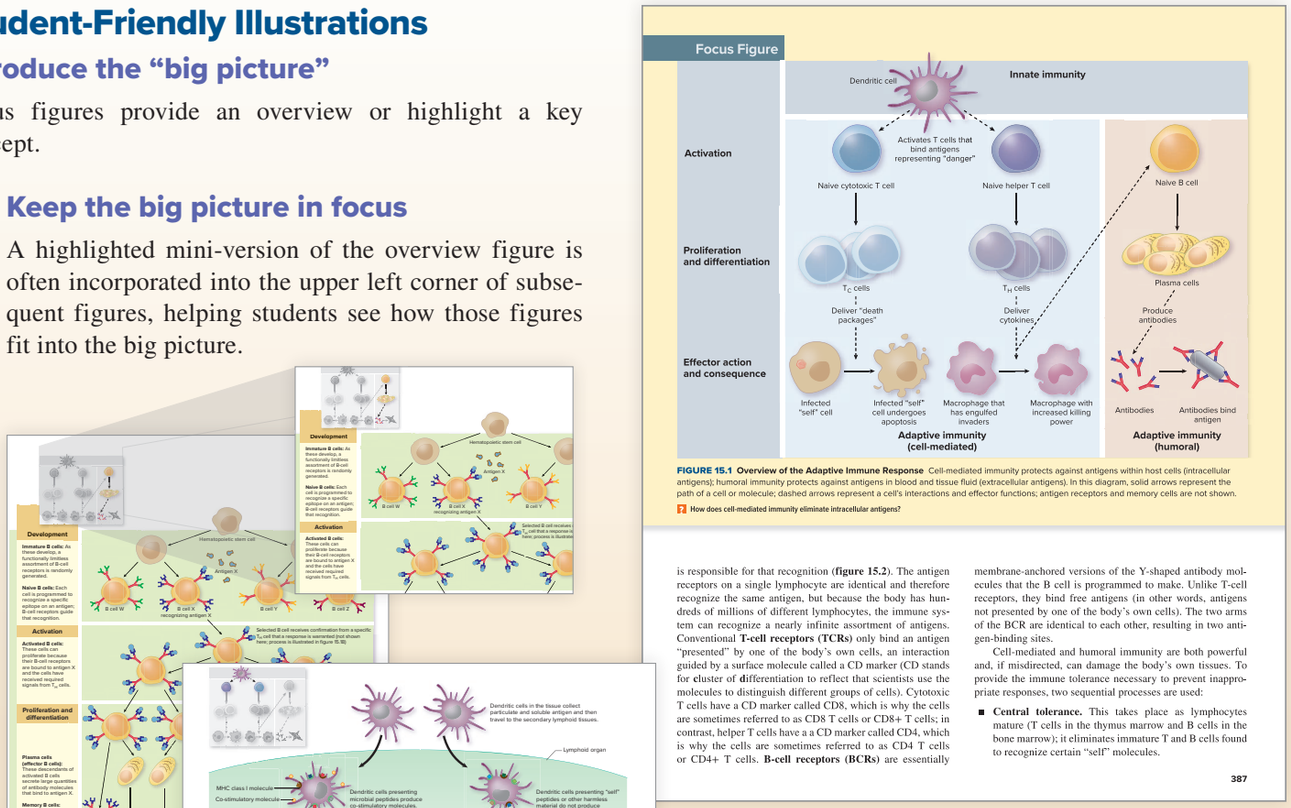


FIGURE 15.1 Overview of the Adaptive Immune Response Cell-mediated immunity protects against antigens within host cells (intracellular antigens); humoral immunity protects against antigens in blood and tissue fluid (extracellular antigens). In this diagram, solid arrows represent the path of a cell or molecule; dashed arrows represent a cell's interactions and effector functions; antigen receptors and memory cells are not shown.

? How does cell-mediated immunity eliminate intracellular antigens?

is responsible for that recognition (**figure 15.2**). The antigen receptors on a single lymphocyte are identical and therefore recognize the same antigen, but because the body has hundreds of millions of different lymphocytes, the immune system can recognize a nearly infinite assortment of antigens. Conventional **T-cell receptors (TCRs)** only bind an antigen "presented" by one of the body's own cells, an interaction guided by a surface molecule called a CD marker (CD stands for **c**luster of **d**ifferentiation to reflect that scientists use the molecules to distinguish different groups of cells). Cytotoxic T cells have a CD marker called CD8, which is why the cells are sometimes referred to as CD8 T cells or CD8+ T cells; in contrast, helper T cells have a a CD marker called CD4, which is why the cells are sometimes referred to as CD4 T cells or CD4+ T cells. **B-cell receptors (BCRs)** are essentially membrane-anchored versions of the Y-shaped antibody molecules that the B cell is programmed to make. Unlike T-cell receptors, they bind free antigens (in other words, antigens not presented by one of the body's own cells). The two arms of the BCR are identical to each other, resulting in two antigen-binding sites.

Cell-mediated and humoral immunity are both powerful and, if misdirected, can damage the body's own tissues. To provide the immune tolerance necessary to prevent inappropriate responses, two sequential processes are used:

- **Central tolerance.** This takes place as lymphocytes mature (T cells in the thymus marrow and B cells in the bone marrow); it eliminates immature T and B cells found to recognize certain "self" molecules.

387

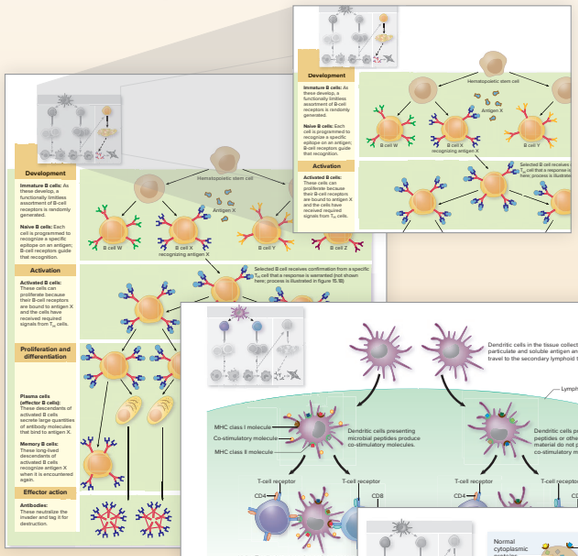


FIGURE 15.10

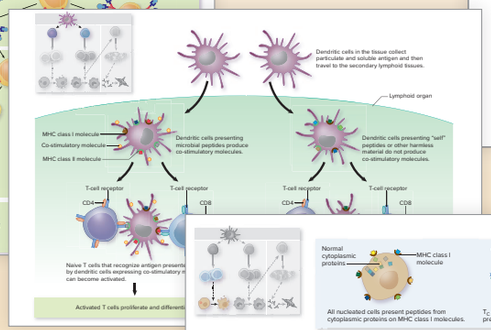


FIGURE 15.13

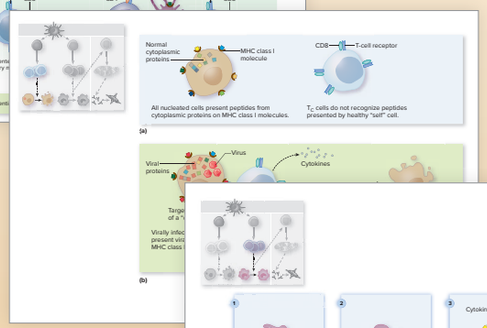


FIGURE 15.14

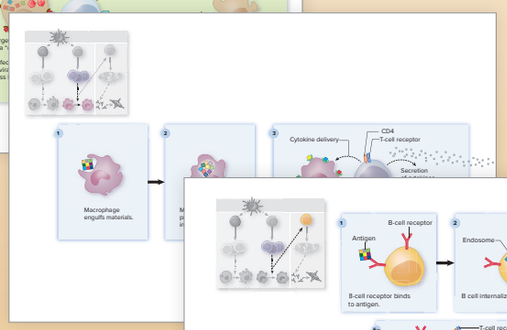


FIGURE 15.16

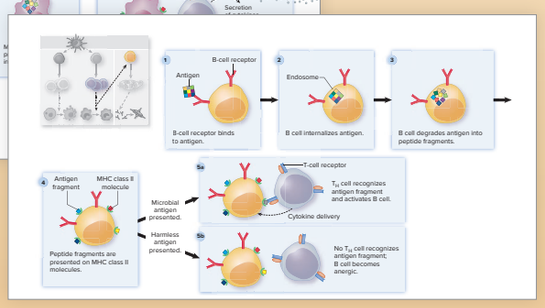


FIGURE 15.18

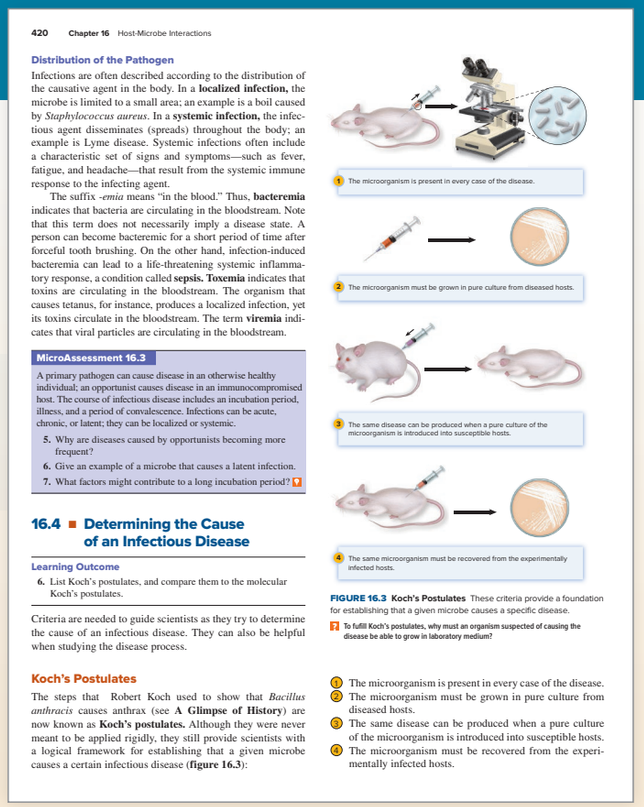
"Provides a logical unfolding conceptual framework that fosters better understanding."

—Jamal Bittar, University of Toledo

Walk through the processes

Step-by-step figures direct the student using numbered icons, often with corresponding icons in the text.

> *"The text and illustrations are 'tight' and give each other good support."*
>
> —Richard Shipee, Vincennes University



420 Chapter 16 Host-Microbe Interactions

Distribution of the Pathogen

Infections are often described according to the distribution of the causative agent in the body. In a **localized infection,** the microbe is limited to a small area; an example is a boil caused by *Staphylococcus aureus*. In a **systemic infection,** the infectious agent disseminates (spreads) throughout the body; an example is Lyme disease. Systemic infections often include a characteristic set of signs and symptoms—such as fever, fatigue, and headache—that result from the systemic immune response to the infecting agent.

The suffix *-emia* means "in the blood." Thus, **bacteremia** indicates that bacteria are circulating in the bloodstream. Note that this term does not necessarily imply a disease state. A person can become bacteremic for a short period of time after forceful tooth brushing. On the other hand, infection-induced bacteremia can lead to a life-threatening systemic inflammatory response, a condition called **sepsis. Toxemia** indicates that toxins are circulating in the bloodstream. The organism that causes tetanus, for instance, produces a localized infection, yet its toxins circulate in the bloodstream. The term **viremia** indicates that viral particles are circulating in the bloodstream.

MicroAssessment 16.3

A primary pathogen can cause disease in an otherwise healthy individual; an opportunist causes disease in an immunocompromised host. The course of infectious disease includes an incubation period, illness, and a period of convalescence. Infections can be acute, chronic, or latent; they can be localized or systemic.

5. Why are diseases caused by opportunists becoming more frequent?
6. Give an example of a microbe that causes a latent infection.
7. What factors might contribute to a long incubation period?

16.4 ■ Determining the Cause of an Infectious Disease

Learning Outcome

6. List Koch's postulates, and compare them to the molecular Koch's postulates.

Criteria are needed to guide scientists as they try to determine the cause of an infectious disease. They can also be helpful when studying the disease process.

Koch's Postulates

The steps that Robert Koch used to show that *Bacillus anthracis* causes anthrax (see **A Glimpse of History**) are now known as **Koch's postulates.** Although they were never meant to be applied rigidly, they still provide scientists with a logical framework for establishing that a given microbe causes a certain infectious disease (**figure 16.3**):

1. The microorganism is present in every case of the disease.
2. The microorganism must be grown in pure culture from diseased hosts.
3. The same disease can be produced when a pure culture of the microorganism is introduced into susceptible hosts.
4. The microorganism must be recovered from the experimentally infected hosts.

FIGURE 16.3 Koch's Postulates These criteria provide a foundation for establishing that a given microbe causes a specific disease.

? To fulfill Koch's postulates, why must an organism suspected of causing the disease be able to grow in laboratory medium?

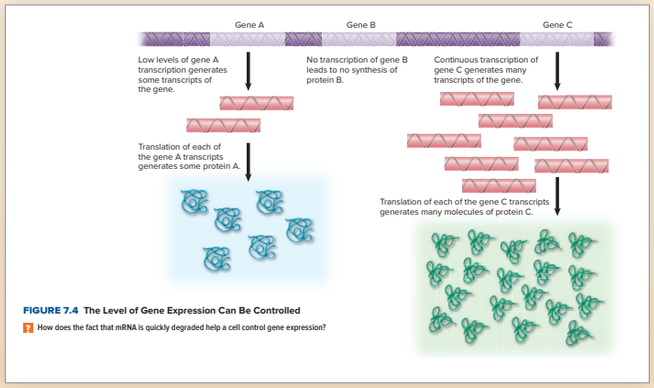


FIGURE 7.4 The Level of Gene Expression Can Be Controlled

? How does the fact that mRNA is quickly degraded help a cell control gene expression?

Encourage deeper understanding

Figures have accompanying questions that encourage students to think more carefully about the concept illustrated in a figure.

Introduce the body systems

Each disease chapter includes a stunning figure that introduces the students to the anatomy of the body system.

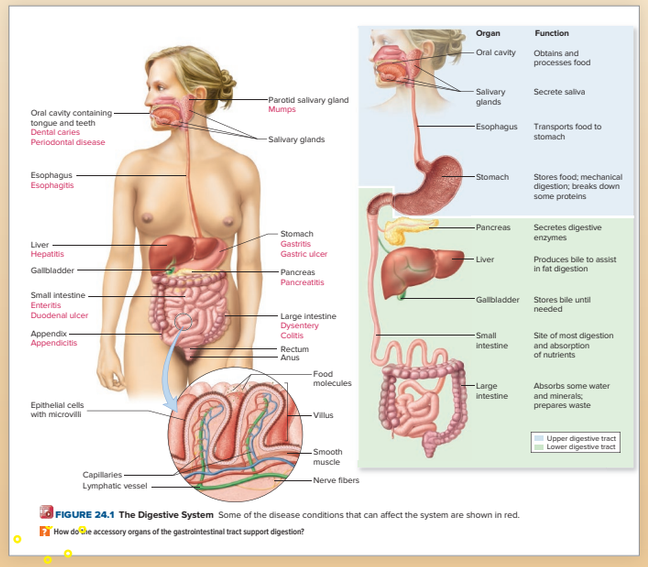


FIGURE 24.1 The Digestive System Some of the disease conditions that can affect the system are shown in red.

? How do the accessory organs of the gastrointestinal tract support digestion?

FOCUS ON UNDERSTANDING . . .

Student-Friendly Chapter Features

Provide the tools for understanding

Key Terms for each chapter are defined on the opening page.

Share the history

A **Glimpse of History** opens each chapter, featuring engaging stories about the men and women who pioneered the field of microbiology.

Define the expectations

Learning outcomes are found at the beginning of each numbered section, allowing organization, evaluation, and assessment of instruction.

Assess understanding

A **MicroAssessment** at the end of each numbered section summarizes the concepts and includes review questions, usually featuring one that stimulates critical thinking (indicated by a light bulb icon).

Engage the reader

MicroBytes found throughout the chapter provide small "bytes" of information, capturing the reader's attention.

26 Nervous System Infections

Structure of West Nile virus particles. ©Science Picture Co/Getty Images

A Glimpse of History

Today it is hard to appreciate the fear and loathing once attached to leprosy (*lepros,* meaning "scaly"). Many historical and religious texts refer to several disfiguring skin diseases, including leprosy, and portray those suffering from the diseases as unclean and sinful. Lepers were regularly segregated from mainstream society.

Gerhard Henrik Armauer Hansen (1841–1912) was a Norwegian physician with many interests, ranging from science to religion to polar exploration. After graduating from medical school, he went to work with Dr. Daniel C. Danielson, a leading authority on leprosy. Danielson believed that leprosy was a hereditary disease and considered the idea that it was contagious to be a "peasant superstition." Hansen, however, disproved Danielson's hypothesis in careful studies conducted over a number of years. He found a unique bacterium associated with the disease in every leprosy patient he studied. His 1873 report of the findings marked the first time that a specific bacterium was linked to a disease—almost a decade before Koch's proof of the cause of tuberculosis.

In the United States, even during the first half of the twentieth century, people diagnosed with leprosy risked having their houses burned to destroy the source of infection. Their names were changed to avoid embarrassing their families, and they were sent to a leprosarium such as the one at Carville, Louisiana, which was surrounded by a 12-foot fence topped with barbed wire. Sufferers were separated from spouses and children and were denied the right to marry or vote. Those who tried to escape were captured and brought back in handcuffs. The Carville leprosarium was finally closed and converted to a military-style academy in 1999.

KEY TERMS

Blood-Brain Barrier Cells that function together to create a protective semipermeable border that separates the CNS from the bloodstream.

Central Nervous System (CNS) Brain and spinal cord.

Cerebrospinal Fluid (CSF) Fluid produced in the brain that flows within and around the CNS.

Encephalitis Inflammation of the brain.

Meninges Membranes covering the brain and spinal cord.

Meningitis Inflammation of the meninges.

Peripheral Nervous System (PNS) Division of the nervous system that carries information to and from the CNS.

Transmissible Spongiform Encephalopathy (TSE) Chronic degenerative brain disease caused by prions; characterized by spongy appearance of brain tissue.

Because the word *leprosy* carries centuries of grim overtones, many people prefer to use the term *Hansen's disease,* a name that honors the discoverer of the causative bacterium. Today, the disease can be treated.

Nervous system infections are frightening. They threaten a person's ability to move, feel, or even think. Consider poliomyelitis, which can result in a paralyzed limb or the inability to breathe without mechanical assistance. Hansen's disease (leprosy) can result in loss of fingers or toes or deformity of the face. Infections of the brain or its covering membranes can render a child deaf or intellectually disabled. Before the discovery of antibiotics, bacterial infections of the nervous system were often fatal. Fortunately, these infections are uncommon.

26.1 ■ Anatomy, Physiology, and Ecology of the Nervous System

Learning Outcomes

1. Describe how information flows through and between neurons.
2. Differentiate between the central nervous system and the peripheral nervous system.
3. Explain how bone, cerebrospinal fluid, meninges, and the blood-brain barrier protect the central nervous system.

Nerve cells work together, transmitting electrical impulses throughout the body like a highly sophisticated circuit board. Each nerve cell, or **neuron,** has three functionally distinct regions: (1) branching projections called dendrites, (2) the cell

703

MicroAssessment 3.2

Peptidoglycan is a molecule unique to bacteria that provides strength to the cell wall. The Gram-positive cell wall is composed of a relatively thick layer of peptidoglycan as well as teichoic acids. Gram-negative cell walls have a thin layer of peptidoglycan and a lipopolysaccharide-containing outer membrane. Penicillin and lysozyme interfere with the structural integrity of peptidoglycan. *Mycoplasma* species lack a cell wall. Archaea have a variety of cell wall types.

4. What is the significance of lipid A?
5. How does the action of penicillin differ from that of lysozyme?
6. Explain why penicillin kills only actively multiplying cells, whereas lysozyme kills cells in any stage of growth.

MicroByte

There are more bacteria in just one person's mouth than there are people in the world!

Highlight the relevance

Focus on a Case boxes describe realistic clinical, veterinary, or environmental situations, along with questions and discussions designed to highlight the relevance of the information.

Provide perspective

Focus Your Perspective boxes show how microorganisms and their products influence our lives in many different ways.

Introduce the concepts

Focus on a Disease boxes introduce a general category of disease (pneumonia, diarrheal disease, meningitis, sexually transmitted infections), giving students a framework for understanding specific diseases.

Inspire the learner

Focus on the Future boxes describe pending challenges facing current and future microbiologists.

- **Summary** briefly reviews the key points.
- **Short Answer** questions review major chapter concepts.
- **Multiple Choice** questions allow self-testing; answers are provided in Appendix IV.
- **Application** questions provide an opportunity to use knowledge of microbiology to solve real-world problems.
- **Critical Thinking** questions encourage practice in analysis and problem solving that can be used by the student in any subject.

FOCUS ON A CASE 14.1

A 9-year-old boy with cystic fibrosis—a genetic disease that causes a number of problems, including the buildup of thick, sticky mucus in the lungs—complained of feeling tired, out of breath, and always coughing. When his mother took him to the doctor, she mentioned that his cough was productive, meaning that it contained sputum (pronounced *spew-tum*). She was particularly concerned that the sputum was a blue-green color. His doctor immediately suspected a lung infection by *Pseudomonas aeruginosa*—a common complication of cystic fibrosis. A sputum sample was collected and sent to the clinical laboratory.

In the clinical laboratory, the sample was plated onto MacConkey agar and blood agar and incubated. Mucoid colonies surrounded by a bluish-green color grew on both types of agar media. The colonies on MacConkey had no pink coloration, so the medical technologist concluded that the cells did not ferment lactose. She noted the blue-green color on the

The patient was treated with antibiotics, with only limited success. Like most cystic fibrosis patients, he developed a chronic lung infection that continued to require repeated treatment.

1. What role did cystic fibrosis play in the disease process?
2. What is the significance of the mucoid phenotype of the colonies?
3. How would the siderophore (the iron-binding compound) benefit the bacterium?
4. Why would the boy's lung infection make his pre-existing respiratory problems even worse?

Discussion

1. Cystic fibrosis patients often have an accumulation of thick mucus in their lungs, which interferes with the mucociliary escalator and other first-line defenses. With a compromised (weakened) mucociliary escalator,

aeruginosa cells to form biofilms. The biofilm protects the bacterial cells from various components of the immune system, including antimicrobial peptides and phagocytes. Bacteria growing within a biofilm are much more difficult for the immune system to destroy.

3. Siderophores help the bacterium obtain iron from the host. Recall that the body produces iron-binding proteins, including lactoferrin and transferrin; this prevents microbes from using the host's iron and thereby limits their growth. Microorganisms that make siderophores essentially engage in a "tug-of-war" with the body over iron. This tug-of-war is especially important for *P. aeruginosa* because iron levels influence biofilm formation. When iron is limiting, *P. aeruginosa* cells are motile and do not initiate biofilm formation.
4. In response to a bacterial infection in the lungs, an inflammatory response devel-

FOCUS YOUR PERSPECTIVE 9.1

The COVID-19 Response—The Power of Biotechnology

The COVID-19 response serves as an excellent illustration of the power of biotechnology. Because of several of the technologies described in this chapter, the pandemic's global outcome—although devastating—resulted in fewer deaths than many feared or predicted.

SARS-CoV-2, the virus that causes COVID-19, has an RNA genome. If a researcher needs a DNA copy of that genome, the enzyme reverse transcriptase is used to make cDNA. When the virus was first discovered in China, a cDNA copy of its genome was cloned and then sequenced. That sequence was then shared with scientists around the world, initiating what became a global effort to control the disease.

technologies; not only do CRISPR-Cas-based tests give results in about an hour or less, they do not compete with PCR-based tests with respect to the required reagents. The first CRISPR-Cas-based diagnostic test was approved for use only in certified laboratories, but researchers also worked toward developing similar but instrument-free versions that can be completed on-site (comparable to home pregnancy tests).

Data obtained via high-throughput sequencing were used to track the global spread of SARS-CoV-2. The tracking methods rely on detecting spontaneous mutations that inevitably occur as the virus replicates; these mutations serve as

facilitated research aimed at developing targeted antiviral therapies, as described in Focus on the Future 20.1. By analyzing the viral genome, scientists determined the amino acid sequence of key proteins essential for replication of the virus. Relatively soon thereafter, the 3-dimensional structure of two of those proteins was determined—one that the virus uses to attach to and then enter host cells and one it uses to replicate its genome. Knowing those protein structures allows scientists to focus their efforts on developing compounds that specifically target the parts essential for the structure's function—for example, the exact site on the attachment protein that contacts a host cell. The structures of other SARS-CoV-2

FOCUS ON PNEUMONIA

Pneumonia is a disease of the lower respiratory tract caused by bacterial, viral, or fungal infection of the lungs. An inflammatory response to the infection generally results in the alveoli (air sacs) of the lungs filling with fluids such as pus and blood. Pneumonia is the leading cause of death due to infectious disease in the United States.

Signs and Symptoms

The signs and symptoms of pneumonia generally include cough, chills, shortness of breath, fever, and chest pain. In severe cases, the patient may develop cyanosis (bluish skin color) due to poor blood oxygenation. Pneumonia ranges from mild to life-threatening, depending largely on the causative agent but also on any underlying health problems of the patient. It is often accompanied by a productive cough, meaning that a pus- and mucus-containing fluid called **sputum** comes up from the lungs.

Some pathogens cause what are referred to as atypical pneu-

destroy invading microbes cannot effectively eliminate the pathogen initially. Once opsonizing antibodies are produced during a B-cell response, however, phagocytes can remove the microbes.

The damage from pneumonia is largely a result of the inflammatory response. As the capillaries become leaky during inflammation, fluids collect in the alveoli and interfere with O_2 and CO_2 exchange. In addition, phagocytes and other leukocytes are recruited to the site of infection, and mucus production increases. Accumulating leukocytes and mucus create a thick substance that may clog the alveoli, a condition called consolidation. Consolidation is most common in severe bacterial pneumonia. The inflammatory response seen in severe pneumonia often affects nerve endings in the pleura, causing pain.

Epidemiology

Pneumonias are often categorized as either community-acquired, meaning that they develop in members of the general public, or

FOCUS ON THE FUTURE 20.1

The Race to Develop COVID-19 Treatments

Almost immediately after the emergence of the disease now called COVID-19, scientists raced to find effective treatments. An early focus was on drug repurposing—the use of approved or investigational drugs for new therapeutic uses. Approved drugs are those that have undergone the testing required for the U.S. Food and Drug Administration (FDA) to authorize marketing of the drug; investigational drugs are experimental drugs that the FDA has authorized for testing in humans. The repurposing options considered for COVID-19 treatments included not just antiviral drugs, but also medications to control the infection-induced cytokine storm and other damaging immune responses. An enormous advantage of a repurposed drug is that it has already gone through clinical trials to

so scientists from around the globe rushed to identify the functions and 3-dimensional structures of various SARS-CoV-2 proteins (the process was aided by earlier studies of the related virus, SARS-CoV). Armed with that information, other scientists then worked towards designing small molecules that specifically block a given protein's function. The virus can potentially mutate to develop resistance to a single medication, however, so a variety of drugs, each interfering with a different target, will likely be required. The SARS-CoV-2-specific medications are still early in the development stages at the time of this writing, but their targets are in some of the same categories as those of other antiviral medications:

its interaction with other viral proteins, various inhibitors that target the viral replication machinery are being developed. Some are nucleoside and nucleotide analogs, but finding effective versions of those is complicated by the fact that the replicase of SARS-CoV-2 has proofreading ability, which is unusual among RNA viruses. Thus, if the SARS-CoV-2 replicase incorporates an analog during RNA synthesis, the proofreading function might recognize and remove that analog, thereby avoiding production of a defective RNA molecule. Another potential SARS-CoV-2 target is a protein complex that adds a 5′ cap to viral RNA to make it

Review the information

End-of-chapter review encourages students to revisit the information.

Build the story

Logical chapter order helps students understand and connect the concepts.

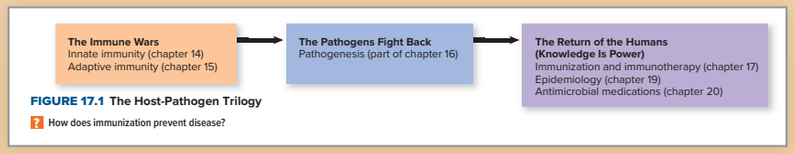


FIGURE 17.1 The Host-Pathogen Trilogy

? How does immunization prevent disease?

FOCUS ON UNDERSTANDING . . .

Student-Friendly Descriptions

Include analogies

WHY? Analogies provide students a comfortable framework for making sense of difficult topics. Here's an example from chapter 14.

> **Innate Immunity** *The innate immune system has three general components: first-line defenses, sensor systems, and innate effector actions. As a useful analogy, think of the defense systems of a high-security building or compound: The first-line defenses are the security walls surrounding the property; the sensor systems are the security cameras scattered throughout the property, monitoring the environment for signs of invasion; and the effector actions are the security teams sent to remove any invaders that have been detected, thereby eliminating the threat* ***(figure 14.1a).***

Steve Cole/E+/Getty Images

© Image Source, all rights reserved.

Moodboard/Brand X Pictures/Getty Images

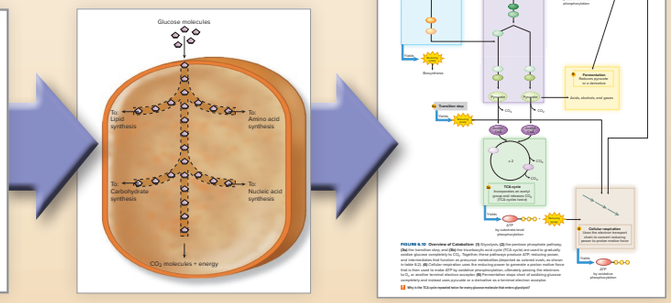
Emphasize the logic

WHY? Descriptions that emphasize the logic of processes make it easier for students to understand and retain the information. Here's an example from chapter 6.

TABLE 6.2 Precursor Metabolites

Precursor Metabolite	Biosynthetic Role	Pathway (or Step) Generated
Glucose-6-phosphate	Lipopolysaccharide	Glycolysis
Fructose-6-phosphate	Peptidoglycan	Glycolysis
Dihydroxyacetone phosphate	Lipids (glycerol component)	Glycolysis
3-Phosphoglycerate	Proteins (the amino acids cysteine, glycine, and serine)	Glycolysis
Phosphoenolpyruvate	Proteins (the amino acids phenylalanine, tryptophan, and tyrosine)	Glycolysis
Pyruvate	Proteins (the amino acids alanine, leucine, and valine)	Glycolysis
Ribose-5-phosphate	Nucleic acids and proteins (the amino acid histidine)	Pentose phosphate cycle
Erythrose-4-phosphate	Proteins (the amino acids phenylalanine, tryptophan, and tyrosine)	Pentose phosphate cycle
Acetyl-CoA	Lipids (fatty acids)	Transition step
α-Ketoglutarate	Proteins (the amino acids arginine, glutamate, glutamine, and proline)	TCA cycle
Oxaloacetate	Proteins (the amino acids aspartate, asparagine, isoleucine, lysine, methionine, and threonine)	TCA cycle

Note: The colored icons in the table are used in figures throughout the chapter to represent the respective precursor metabolites.



Introduce the players *Certain intermediates of catabolic pathways can be used in anabolic pathways; therefore they link these two types of pathways. These intermediates—* ***precursor metabolites****—serve as carbon skeletons from which subunits of macromolecules can be made* ***(table 6.2).***

Reinforce the concept *A cell's metabolic pathways make it easy for that cell to use glucose for multiple purposes. Think of the cells as extensive biological recycling centers that routinely process millions of glucose molecules* ***(figure 6.9).*** *Molecules that remain on the central deconstruction line are oxidized completely to CO_2, releasing the maximum amount of energy. Some breakdown intermediates, however, can exit that line to be used in biosynthesis.*

Put the pieces together *Three key metabolic pathways—****the central metabolic pathways****—gradually oxidize glucose to CO_2, as described by the following general reaction* ***(figure 6.10):***

$C_6H_{12}O_6 + 6\ O_2 \dashrightarrow 6\ CO_2 + 6\ H_2O$

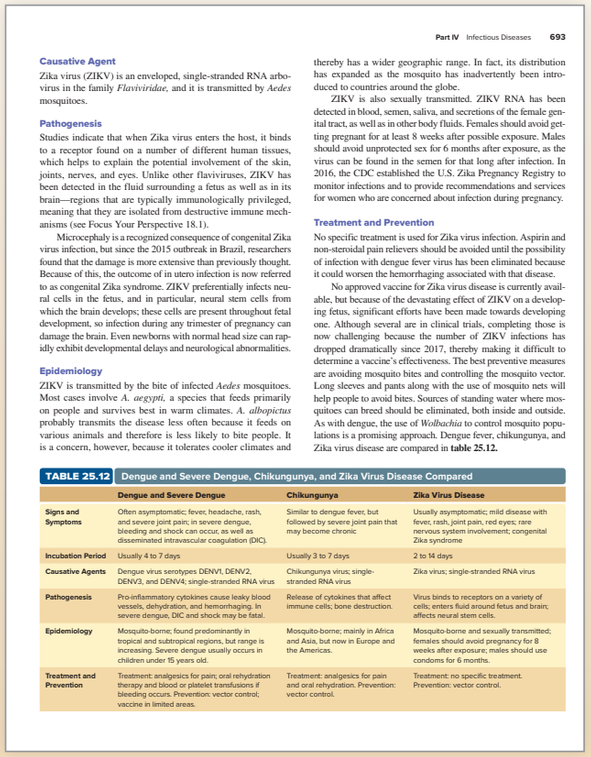
(glucose) (oxygen) (carbon dioxide) (water)

The pathways are catabolic, but the precursor metabolites and reducing power they generate can also be diverted for use in biosynthesis.

Student-Friendly Disease Presentations

Help students think like experts

Within each body system chapter, diseases are separated by major taxonomic category (bacteria, viruses, fungi, protozoa). This organization reflects a major consideration with respect to treatment options, an important consideration for students going into healthcare-related fields.



Causative Agent

Zika virus (ZIKV) is an enveloped, single-stranded RNA arbovirus in the family *Flaviviridae,* and it is transmitted by *Aedes* mosquitoes.

Pathogenesis

Studies indicate that when Zika virus enters the host, it binds to a receptor found on a number of different human tissues, which helps to explain the potential involvement of the skin, joints, nerves, and eyes. Unlike other flaviviruses, ZIKV has been detected in the fluid surrounding a fetus as well as in its brain—regions that are typically immunologically privileged, meaning that they are isolated from destructive immune mechanisms (see Focus Your Perspective 18.1).

Microcephaly is a recognized consequence of congenital Zika virus infection, but since the 2015 outbreak in Brazil, researchers found that the damage is more extensive than previously thought. Because of this, the outcome of in utero infection is now referred to as congenital Zika syndrome. ZIKV preferentially infects neural cells in the fetus, and in particular, neural stem cells from which the brain develops; these cells are present throughout fetal development, so infection during any trimester of pregnancy can damage the brain. Even newborns with normal head size can rapidly exhibit developmental delays and neurological abnormalities.

Epidemiology

ZIKV is transmitted by the bite of infected *Aedes* mosquitoes. Most cases involve *A. aegypti,* a species that feeds primarily on people and survives best in warm climates. *A. albopictus* probably transmits the disease less often because it feeds on various animals and therefore is less likely to bite people. It is a concern, however, because it tolerates cooler climates and thereby has a wider geographic range. In fact, its distribution has expanded as the mosquito has inadvertently been introduced to countries around the globe.

ZIKV is also sexually transmitted. ZIKV RNA has been detected in blood, semen, saliva, and secretions of the female genital tract, as well as in other body fluids. Females should avoid getting pregnant for at least 8 weeks after possible exposure. Males should avoid unprotected sex for 6 months after exposure, as the virus can be found in the semen for that long after infection. In 2016, the CDC established the U.S. Zika Pregnancy Registry to monitor infections and to provide recommendations and services for women who are concerned about infection during pregnancy.

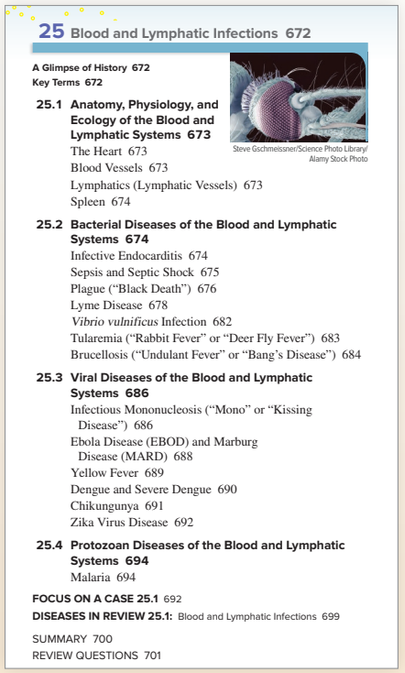
Treatment and Prevention

No specific treatment is used for Zika virus infection. Aspirin and non-steroidal pain relievers should be avoided until the possibility of infection with dengue fever virus has been eliminated because it could worsen the hemorrhaging associated with that disease.

No approved vaccine for Zika virus disease is currently available, but because of the devastating effect of ZIKV on a developing fetus, significant efforts have been made towards developing one. Although several are in clinical trials, completing those is now challenging because the number of ZIKV infections has dropped dramatically since 2017, thereby making it difficult to determine a vaccine's effectiveness. The best preventive measures are avoiding mosquito bites and controlling the mosquito vector. Long sleeves and pants along with the use of mosquito nets will help people to avoid bites. Sources of standing water where mosquitoes can breed should be eliminated, both inside and outside. As with dengue, the use of *Wolbachia* to control mosquito populations is a promising approach. Dengue fever, chikungunya, and Zika virus disease are compared in **table 25.12.**

TABLE 25.12 Dengue and Severe Dengue, Chikungunya, and Zika Virus Disease Compared

	Dengue and Severe Dengue	Chikungunya	Zika Virus Disease
Signs and Symptoms	Often asymptomatic; fever, headache, rash, and severe joint pain; in severe dengue, bleeding and shock can occur, as well as disseminated intravascular coagulation (DIC).	Similar to dengue fever, but followed by severe joint pain that may become chronic	Usually asymptomatic; mild disease with fever, rash, joint pain, red eyes; rare nervous system involvement; congenital Zika syndrome
Incubation Period	Usually 4 to 7 days	Usually 3 to 7 days	2 to 14 days
Causative Agents	Dengue virus serotypes DENV1, DENV2, DENV3, and DENV4; single-stranded RNA virus	Chikungunya virus; single-stranded RNA virus	Zika virus; single-stranded RNA virus
Pathogenesis	Pro-inflammatory cytokines cause leaky blood vessels, dehydration, and hemorrhaging. In severe dengue, DIC and shock may be fatal.	Release of cytokines that affect immune cells; bone destruction.	Virus binds to receptors on a variety of cells; enters fluid around fetus and brain; affects neural stem cells.
Epidemiology	Mosquito-borne; found predominantly in tropical and subtropical regions, but range is increasing. Severe dengue usually occurs in children under 15 years old.	Mosquito-borne; mainly in Africa and Asia, but now in Europe and the Americas.	Mosquito-borne and sexually transmitted; females should avoid pregnancy for 8 weeks after exposure; males should use condoms for 6 months.
Treatment and Prevention	Treatment: analgesics for pain; oral rehydration therapy and blood or platelet transfusions if bleeding occurs. Prevention: vector control; vaccine in limited areas.	Treatment: analgesics for pain and oral rehydration. Prevention: vector control.	Treatment: no specific treatment. Prevention: vector control.



25 Blood and Lymphatic Infections 672

A Glimpse of History 672
Key Terms 672

25.1 Anatomy, Physiology, and Ecology of the Blood and Lymphatic Systems 673
The Heart 673
Blood Vessels 673
Lymphatics (Lymphatic Vessels) 673
Spleen 674

25.2 Bacterial Diseases of the Blood and Lymphatic Systems 674
Infective Endocarditis 674
Sepsis and Septic Shock 675
Plague ("Black Death") 676
Lyme Disease 678
Vibrio vulnificus Infection 682
Tularemia ("Rabbit Fever" or "Deer Fly Fever") 683
Brucellosis ("Undulant Fever" or "Bang's Disease") 684

25.3 Viral Diseases of the Blood and Lymphatic Systems 686
Infectious Mononucleosis ("Mono" or "Kissing Disease") 686
Ebola Disease (EBOD) and Marburg Disease (MARD) 688
Yellow Fever 689
Dengue and Severe Dengue 690
Chikungunya 691
Zika Virus Disease 692

25.4 Protozoan Diseases of the Blood and Lymphatic Systems 694
Malaria 694

FOCUS ON A CASE 25.1 692
DISEASES IN REVIEW 25.1: Blood and Lymphatic Infections 699
SUMMARY 700
REVIEW QUESTIONS 701

Steve Gschmeissner/Science Photo Library/ Alamy Stock Photo

Provide a consistent conceptual framework

Disease discussions are separated into consistent subsections, providing a conceptual framework and breaking the material into "bite-sized" pieces.

Summarize each disease's characteristics

Summary tables serve as brief reminders of the important features of each disease. Major diseases are represented with an enhanced summary table that includes an outline of the disease process keyed to a human figure, showing the entry and exit of the pathogen.

Review the diseases as a group

Each disease chapter ends with a table that summarizes the key features of the diseases discussed in that chapter.

Diseases in Review 21.1

Respiratory System Diseases

Disease	Causative Agent	Comment	Summary Table
BACTERIAL INFECTIONS OF THE UPPER RESPIRATORY TRACT			
Conjunctivitis (pink eye), otitis media (earache), sinus infection	Usually *Haemophilus influenzae* or *Streptococcus pneumoniae*	Often occur together; factors involved in the transmission are unknown.	
Streptococcal pharyngitis ("strep throat")	*Streptococcus pyogenes* (group A streptococcus)	Treated with antibiotics, partly to avoid sequelae; must be distinguished from viral pharyngitis, which cannot be treated with antibiotics.	Table 21.3
Diphtheria	*Corynebacterium diphtheriae*	Toxin-mediated disease characterized by pseudomembrane in the upper respiratory tract. Preventable by vaccination.	Table 21.4
VIRAL INFECTIONS OF THE UPPER RESPIRATORY TRACT			
Common cold	Rhinoviruses and other viruses	Runny nose, sore throat, and cough are due to the inflammatory response and cell destruction.	Table 21.5
Adenovirus pharyngitis	Adenoviruses	Similar to the common cold but with fever; spread to the lower respiratory tract can result in severe disease.	Table 21.6
BACTERIAL INFECTIONS OF THE LOWER RESPIRATORY TRACT			
Pneumococcal pneumonia	*Streptococcus pneumoniae*	Organism common in the throat of healthy people; causes disease when mucociliary escalator is impaired or with underlying conditions. Vaccine that protects against multiple strains is available.	Table 21.7
***Klebsiella* pneumonia**	*Klebsiella species, commonly K. pneumoniae*	Common hospital-acquired bacterium; characterized by thick, bloody, jelly-like sputum. Drug resistance is a major problem.	Table 21.7
Mycoplasmal pneumonia ("walking pneumonia")	*Mycoplasma pneumoniae*	Relatively mild pneumonia; common among college students and military recruits. Cannot be treated with medications that inhibit cell wall synthesis.	Table 21.7
Pertussis ("whooping cough")	*Bordetella pertussis*	Characterized by frequent violent coughing. Preventable by vaccination.	Table 21.8
Tuberculosis ("TB")	*Mycobacterium tuberculosis*	Most infections result in latent tuberculosis infection (LTBI), but these can reactivate to cause tuberculosis disease (TB disease). Treated using combination drug therapy, but drug resistance is an increasing problem.	Table 21.9
Legionnaires' disease	*Legionella pneumophila*	Transmitted via aerosolized water drops; smokers and those with impaired defenses are most at risk of developing disease.	Table 21.10
Inhalation anthrax	*Bacillus anthracis*	Rare zoonotic disease; may be associated with bioterrorism; high case-fatality rate.	Table 21.11
VIRAL INFECTIONS OF THE LOWER RESPIRATORY TRACT			
Influenza ("flu")	Influenza viruses	New vaccine developed yearly; viruses change seasonally due to antigenic drift; antigenic shifts cause pandemics.	Table 21.12
Respiratory syncytial virus infections	RSV	Serious disease in infants, young children, and the elderly.	Table 21.13
COVID-19, SARS and MERS	Coronaviruses	Emerging infectious diseases characterized by severe lower respiratory symptoms; zoonotic	Table 21.14
Hantavirus pulmonary syndrome	Hantaviruses	Acquired via inhaled dust contaminated with rodent saliva, urine, or feces. Frequently fatal.	Table 21.15
FUNGAL INFECTIONS OF THE RESPIRATORY TRACT			
Coccidioidomycosis ("valley fever")	*Coccidioides immitis* and *C. posadasii*	Environmental reservoir (soil in semi-arid desert areas); most infections are asymptomatic.	Table 21.16
Histoplasmosis ("spelunker's disease")	*Histoplasma capsulatum*	Environmental reservoir (bat droppings and soil enriched with bird droppings); most infections are asymptomatic.	Table 21.17
Pneumocystis pneumonia (PCP)	*Pneumocystis jirovecii* (formerly *carinii*)	Organism is an opportunistic fungus that causes serious lung disease in immunocompromised people, such as those with HIV/AIDS.	Table 21.18

UPDATES—Maintaining the Cutting Edge

Global Changes

- Added information about COVID-19 and SARS-CoV-2, including the following boxes:
  - Focus Your Perspective 9.1 *(The COVID-19 Response—The Power of Biotechnology)*
  - Focus on a Case 13.1
  - Focus on the Future 20.1 *(The Race to Develop COVID-19 Treatments)*
  - Focus Your Perspective 21.1 *(A Global Lesson in Microbiology: The COVID-19 Pandemic)*
- Updated disease statistics, vaccine recommendations, treatments, and terminology
- Rearranged some content to improve flow in the digital text (the information most relevant to a particular figure is now in the paragraph immediately preceding the figure, and summary tables have been moved to the end of the coverage)
- Converted many of the descriptions that support multi-step figures to bullet lists that correspond to the steps
- Continued "wordsmithing" to improve the clarity and readability of the descriptions

Key Changes in Individual Chapters

Chapter 1 – Humans and the Microbial World

- Added SARS-CoV-2 and *Candida auris* to the section on emerging pathogens
- Added the African swine fever to the list of epidemics in non-human populations
- Expanded the coverage of the human microbiome
- Defined the term *strain*
- Moved the information about bacterial cell shape from chapter 3 to section 1.3
- Added a MicroByte about the Microbiome Conservancy collecting/storing fecal samples from populations around the world

Chapter 2 – The Molecules of Life

- Consolidated and expanded the information on water's characteristics
- Added a subsection on short-chain fatty acids, to allow a description of butyrate
- Added a description of waxes
- Described the distinction between a Lewis symbol and a Lewis structure
- Rearranged the three-part figure showing DNA
- Added a MicroByte on the use of artificial intelligence and a video game to determine protein folding

Chapter 3 – Cells and Methods to Observe Them

- Rearranged the chapter sections so that cell structure and function is discussed before microscopy and staining methods; revised the chapter title to reflect the change
- Revised the coverage of active transport systems to place more emphasis on the concept rather than the different types
- Updated the section on gas vesicles to include information about other protein-based compartments (bacterial microcompartments and encapsulin nanocompartments)
- Introduced the term *archaellum*
- Described periplasm in Gram-positive cells
- Moved the information about bacterial cell shape to chapter 1

Chapter 4 – Dynamics of Microbial Growth

- Introduced the term *contact-dependent growth inhibition*

Chapter 5 – Control of Microbial Growth

- Combined the physical methods of microbial control into one section
- Expanded the discussion of biosafety levels
- Added the recent FDA rulings that limit the use of many previously allowed ingredients in antiseptic lotions until they are shown to be safe and effective

Chapter 6 – Microbial Metabolism: Fueling Cell Growth

- Rearranged the information about energy sources and terminal electron acceptors so that the more conceptually simple information comes first.
- Revised tables 6.2 (Precursor Metabolites) and 6.4 (Some Vitamins and Their Use in Coenzymes)
- Added new figure (6.11) to emphasize the difference in energy yield between aerobic respiration and fermentation

- Simplified the detailed discussion of the central metabolic pathways
- Simplified the discussion of photosynthesis

Chapter 7 – The Blueprint of Life, from DNA to Protein

- Combined the subsections that describe DNA replication
- Added a MicroByte about the target of the new influenza medication (baloxavir marboxil)
- Added a MicroByte about the first approved RNAi-based medication
- Split the figure that illustrates the process of translation to emphasize its three phases (initiation, elongation, and termination; now figures 7.5–7.17)

Chapter 8 – Bacterial Genetics

- Changed the term *silent mutation* to *synonymous mutation*, and explained that this type of mutation is not always silent
- Changed the term *DNA-mediated transformation* to *bacterial transformation*
- Broadened the coverage of section 8.10 (now "Genome Variability") and added the term *pan-genome*
- Simplified the format of the end-of-chapter multiple choice questions

Chapter 9 – Biotechnology

- Added a new Focus Your Perspective Box: *The COVID-19 Response—The Power of Biotechnology*
- Emphasized the importance of CRISPR-Cas technologies by creating a numbered section (section 9.3); the expanded coverage includes a description of a rapid COVID-19 diagnostic test that relies on the technologies
- Expanded the chapter introduction to emphasize the applications of biotechnology
- Added a MicroByte about a bacterial enzyme engineered to efficiently break down a common type of plastic
- Changed the title of section 9.2 to "Molecular Cloning" (was "Genetic Engineering") to reflect a more narrow focus
- Added a simplified view of the cloning process (in a bullet list format) that matches figure 9.4
- Converted the description of vectors to a bullet list that matches figure 9.6 (was 9.8)
- Converted the description of how a PCR product is generated to a bullet list that matches figure 9.13 (was 9.17)
- Deleted the section on the dideoxy chain termination method of DNA sequencing
- Updated the Focus On the Future box by changing the name of the initiative described to *All of Us*

Chapter 10 – Identifying and Classifying Microorganisms

- Updated information about the new online *Bergey's Manual of Systematics of Archaea and Bacteria*
- Changed the example of nomenclature change to *Cutibacterium acnes*

Chapter 11 – The Diversity of Bacteria and Archaea

- Added information about the release of *Wolbachia*-infected mosquitoes as a means to prevent mosquito-borne diseases

Chapter 12 – The Eukaryotic Members of the Microbial World

- Extensive revision, including new photographs throughout; moved the section on protozoa forward, and increased the medical emphasis throughout
- Expanded the discussion of the difficulties of classification
- Added a disease-based grouping of fungi
- Added information about the spread of a fungal disease that destroys banana plants
- Expanded the discussion of medically important protozoa
- Added a figure that illustrates the origin of chloroplasts through primary endosymbiosis
- Simplified the figure that illustrates phylogenetic groups of eukaryotes (now figure 12.18)

Chapter 13 – Viruses, Viroids, and Prions

- Changed the topic of the Focus on a Case box to COVID-19
- Updated viral taxonomy
- Added *Pneumoviridae* to table 13.1
- Bulleted the steps of the lytic bacteriophage life cycle to match figure 13.5
- Bulleted the steps of specialized transduction to match figure 13.9
- Split the figure showing replication strategies of animal viruses into three separate figures for clarity (now figures 13.12–13.14)
- Updated information on viruses and human tumors to include oncogenic and oncolytic viruses
- Added Focus on the Future 13.1: *The Potential of Phage Therapy*

Chapter 14 – The Innate Immune Response

- Modified and updated the descriptions of granulocytes, particularly neutrophils

- Expanded the information on cell types to increase the emphasis on mast cells
- Updated the information on macrophages to indicate that tissue-resident macrophages can self-renew
- Separated the description of inflammation into vascular changes and cellular changes
- Expanded the discussion on damaging effects of inflammation
- Added necroptosis to the paragraph that describes pyroptosis

Chapter 15 – The Adaptive Immune Response

- Extensive revision; reorganized the chapter to create a more linear flow (T cells and their activation are now described before B cells)
- Expanded and rearranged the overview to reflect the new chapter organization
- Expanded the discussion of immune tolerance to distinguish between central tolerance and peripheral tolerance

Chapter 16 – Host-Microbe Interactions

- Increased the emphasis on the importance of butyrate on intestinal barrier functions
- Revised the discussion of Koch's postulates

Chapter 17 – Applications of Immune Responses

- Moved the chapter forward (was chapter 18) so that monoclonal antibodies could be described before the chapter that mentions their use in allergy therapies.
- Added a section on immunotherapies (section 17.3), particularly focusing on the new cancer therapies (checkpoint inhibitors and CAR T cells)
- Added the new the dengue disease vaccine to table 17.5
- Added information about the new combination vaccine that includes HepB

Chapter 18 – Immunological Disorders

- Bulleted the steps involved in type I hypersensitivities to match the accompanying figure
- Updated information on type II hypersensitivities
- Updated the information on immune disorder treatments, including adding information on immunotherapy
- Eliminated the section on treatment of autoimmune diseases, and instead describe the treatments in the context of the respective conditions
- Added a MicroByte on the Neurological Conditions Surveillance System (NNCSS)

Chapter 19 – Epidemiology

- Added COVID-19 as an example of the significance of asymptomatic infections in the spread of a disease
- Changed the MicroByte in section 19.1 to mention the secondary attack rate of measles
- Added measles to the factors that contribute to disease emergence
- Updated table of notifiable infectious diseases
- Updated the description of the *Morbidity and Mortality Weekly Report*
- Added the URL for the CDC's National Notifiable Diseases Surveillance System (NNDSS)
- Added COVID-19 and *Candida auris* infection to the section on emerging diseases

Chapter 20 – Antimicrobial Medications

- Added a Focus on the Future Box: *The Race to Develop COVID-19 Treatments*
- Explained that oral administration of poorly absorbed medications is useful for treating intestinal infections
- Added information about the new rifamycin for treating some types of travelers' diarrhea
- Updated the section on *Mycobacterium tuberculosis* resistance by adding information about the new combination treatment specifically for XDR-TB
- Updated the table that describes the microorganisms on the CDC's list of antibiotic resistance threats (table 20.2)
- Mentioned the resistance of *Candida auris* in the section on antifungal medications
- Updated the section on antiviral medications by adding a subsection on cap-snatching inhibitors
- Added moxidectin for treating river blindness and triclabendazole for treating liver flukes to table 20.5

Chapter 21 – Respiratory System Infections

- Added a Focus Your Perspective Box: *A Global Lesson in Microbiology: The COVID-19 Pandemic*
- Expanded the discussion of coronavirus lower respiratory tract infections to include not only SARS and MERS, but also COVID-19
- Updated the information on Group A *Streptococcus* virulence factors to include only those clearly associated with pathogenesis
- Updated the discussion of mycoplasmal pneumonia pathogenesis to include the CARDS toxin, which has been shown to be a key virulence factor
- Changed Legionellosis to Legionnaires' disease to more specifically refer to *Legionella* pneumonia

- Bulleted the discussion of TB pathogenesis to match figure 21.19
- Updated the discussion on the WHO's program to combat TB; also introduced the newly FDA-approved drug trial program for XDR-TB called Nix-TB
- Updated the pathogenesis discussion on several viral diseases, including the common cold, adenovirus respiratory infections, hantavirus pulmonary syndrome
- Updated the classification of influenza viruses to include influenza D; updated the influenza strain nomenclature to be more in line with the CDC and WHO; introduced the new anti-influenza medication baloxavir
- Updated the information on RSV classification, pathogenesis, and treatment

Chapter 22 – Skin Infections

- Added new bullet list of characteristic skin lesions and rashes, including descriptions and disease examples
- Expanded the section on acne
- Added disease summary tables for acne and hair follicle infections
- Expanded the information on impetigo
- Added information about hand-foot-and-mouth disease (HFMD)

Chapter 23 – Wound Infections

- Added a new part to figure 23.9 to illustrate the mechanism of tetanospasmin
- Reduced the coverage of streptobacillary rat bite fever, assigning it to a new section called *Other Bacterial Bite Wound Infections*

Chapter 24 – Digestive System Infections

- Added a MicroByte on the Global Microbiome Conservancy to section 24.1
- Updated the information on dental caries and modified the accompanying figure
- Updated Focus on a Case 24.1 to reflect diagnosis of *H. pylori* infections by the urea breath test
- Changed the heading *Typhoid and Paratyphoid Fevers* to *Enteric Fever (Typhoid and Paratyphoid)*

Chapter 25 – Blood and Lymphatic Infections

- Revised the section on sepsis and simplified the accompanying figure
- Updated the information on different forms of tularemia
- Updated and explained the evolving terminology of Ebola disease and Marburg disease
- Updated the terminology by changing *dengue fever* to *dengue* and *severe dengue*
- Added a description of how *Wolbachia*-infected mosquitoes can be used to control dengue and other mosquito-borne diseases

Chapter 26 – Nervous System Infections

- Changed the heading "Viral Encephalitis" to "West Nile and Other Types of Viral Encephalitis," and put the focus on West Nile encephalitis
- Changed the MicroByte topic in section 26.3 to acute flaccid myelitis (AFM)
- Updated the information on African trypanosomiasis (African sleeping sickness)

Chapter 27 – Genitourinary Tract Infections

- Updated the coverage of leptospirosis
- Updated Focus Your Perspective 27.1 and changed the title to "Conquering Syphilis"
- Added information about a new monoclonal antibody approved for use as a component of antiretroviral therapy (ART)
- Updated the information on HIV disease
- Removed tables 27.16 (People at Increased Risk for HIV Disease) and 27.18 (Behaviors that Help Control an AIDS Epidemic)

Chapter 28 – Microbial Ecology

- Added the definition of oligotroph
- Revised the section on mycorrhiza; added the terms *arbuscular mycorrhiza* and *Hartig net*, as well as information about fungal networks
- Add a MicroByte to section 28.6 about corn that produces syrup-coated aerial roots to nourish nitrogen-fixing bacteria

Chapter 29 – Environmental Microbiology: Treatment of Water, Wastes, and Polluted Habitats

- Expanded the description of MUG/ONPG

Chapter 30 – Food Microbiology

- Bulleted the descriptions that support figures 30.4 and 30.5

Acknowledgments

First and foremost, special thanks goes to Gene Nester, the leader of the team that wrote the first version of what became *Microbiology, A Human Perspective*. His efforts helped pioneer a new type of introductory microbiology textbook, designed specifically for students entering healthcare-related fields. This edition proudly builds on that original vision.

We would also like to thank the reviewers and other instructors who guided us as we developed this edition, as well as those whose input has helped the text evolve over the years. Deciding what to eliminate, what to add, and what to rearrange is always difficult, so we appreciate your suggestions.

Past students have been incredibly helpful as well. Every question helps us decide which parts of the textbook need more clarification, and every compliment lets us know when we're on the right track.

Special thanks also go to our friends, families, and colleagues for picking up the many hairs we tore out while working on the textbook. Revising a textbook is an all-consuming task—from the initial development stage to proofing the pages during production—and numerous people have acted as advisors and cheerleaders throughout. This text would not exist without the contributions of our strong group of supporters.

A list of acknowledgments is not complete without thanking our fearless leaders and friends from McGraw-Hill. Our product developer Erin DeHeck and portfolio manager Lauren Vondra not only gave inspiration and sound advice, but they also laughed at our jokes and barely rolled their eyes at our idiosyncrasies. Lauren Vondra and Tami Hodge helped ensure that word got out about this new edition, allowing it to find its way into your hands. It was wonderful to have Laura Bies as our content project manager to guide us through some rocky waters on the way to publication. Additionally, we would like to thank digital content project manager Rachael Hillebrand for helping produce our digital resources that support the text and Lisa Burgess, who provided many wonderful photographs.

We hope that this text will be interesting and educational for students and helpful to instructors. Our goal is excellence, so with that in mind we would appreciate any comments and suggestions from our readers.

Denise Anderson
Sarah Salm
Mira Beins

Reviewers for the Tenth Edition

Andrea R. Beyer, *Virginia State University*
Bruce Bleakley, *South Dakota State University*
Anar A. Brahmbhatt, *San Diego Mesa College*
Linda D. Bruslind, *Oregon State University*
Carron Bryant, *East Mississippi Community College*
Matthew B. Crook, *Weber State University*
Jeremiah Davie, *D'Youville College*
Karim Dawkins, *Broward College*
Matthew Dodge, *Olympic College*
Robert A. Holmes, *University of Missouri–Kansas City*
Joshua Hughes, *Dakota State University*
Ilko B. Iliev, *Southern University at Shreveport*
Karen Kowalski, *Tidewater Community College*
Ruhul Kuddus, *Utah Valley University*
Lorie Lana, *Stevenson University*
Eddystone C. Nebel, *Delgado Community College*
Olabisi Ojo, *Albany State University*
Jennifer L. B. Roshek, *Stevenson University*
Dan Smith, *Seattle University*
Renato V. Tameta, *Schenectady County Community College*
Krystal Taylor, *Beaufort County Community College*
Roger Wainwright, *University of Central Arkansas*

Contents

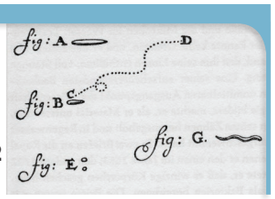
About the Authors iv

PART I

Life and Death of Microorganisms

1 Humans and the Microbial World 1

A Glimpse of History 1
Key Terms 1



INTERFOTO/Alamy, Stock Photo

1.1 The Dispute over Spontaneous Generation 2
Early Experiments 2
Experiments of Pasteur 2
Experiments of Tyndall 2
The Golden Age of Microbiology 3
The Scientific Method 3

1.2 Microbiology: A Human Perspective 5
The Human Microbiome 5
Microorganisms in the Environment 6
Commercial Benefits of Microorganisms 6
Microbes as Research Tools 7
Microbes and Disease 7

1.3 Members of the Microbial World 10
Scientific Names 11
Bacteria 13
Archaea 14
Eukarya 14
Acellular Infectious Agents 15

FOCUS ON A CASE 1.1 9
FOCUS YOUR PERSPECTIVE 1.1: Every Rule Has an Exception 12
FOCUS ON THE FUTURE 1.1: Meet the Microbiomes! 17

SUMMARY 17
REVIEW QUESTIONS 18

2 The Molecules of Life 20

A Glimpse of History 20
Key Terms 20


Lisa Burgess/McGraw-Hill Education

2.1 Elements and Atoms 21
Atomic Structure 21
The Role of Electrons 21
Isotopes 22

2.2 Chemical Bonds and Reactions 23
Ions and Ionic Bonds 23
Covalent Bonds 23
Hydrogen Bonds 24
Molarity 24
Chemical Reactions 25

2.3 Water, pH, and Buffers 26
Water 26
pH of Aqueous Solutions 27
Buffers 27

2.4 Organic Molecules 28
Carbohydrates 29
Lipids 30
Proteins 33
Nucleic Acids 38

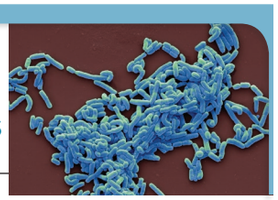
FOCUS ON A CASE 2.1 32
FOCUS YOUR PERSPECTIVE 2.1: Right-Handed and Left-Handed Molecules 36

SUMMARY 41
REVIEW QUESTIONS 42

3 Cells and Methods to Observe Them 44

A Glimpse of History 44
Key Terms 44

PROKARYOTIC CELL STRUCTURES AND THEIR FUNCTIONS


Steve Gschmeissner/Getty Images

3.1 The Cytoplasmic Membrane of Prokaryotic Cells 46
Structure of the Cytoplasmic Membrane 46
Permeability of the Cytoplasmic Membrane 46
The Role of the Cytoplasmic Membrane in Energy Transformation 47
Transport of Small Molecules Across the Cytoplasmic Membrane 48
Protein Secretion 49

3.2 The Cell Wall of Prokaryotic Cells 50
Peptidoglycan 50
The Gram-Positive Cell Wall 50
The Gram-Negative Cell Wall 52
Antibacterial Substances That Target Peptidoglycan 54
Bacteria That Lack a Cell Wall 54
Cell Walls of Archaea 55

3.3 Structures Outside the Cell Wall of Prokaryotic Cells 56
Capsules and Slime Layers 56
Flagella 56
Pili 58

3.4 Internal Components of Prokaryotic Cells 59
Chromosome and Plasmids 59
Ribosomes 59
Cytoskeleton 60
Storage Granules 60
Protein-Based Compartments 60
Endospores 60

EUKARYOTIC CELL STRUCTURES AND THEIR FUNCTIONS

3.5 Cytoplasmic Membrane of Eukaryotic Cells 64
Structure and Function of the Cytoplasmic Membrane 64
Transfer of Molecules Across the Cytoplasmic Membrane 64

3.6 Protein Structures Within Eukaryotic Cells 66
Ribosomes 66
Cytoskeleton 66
Flagella and Cilia 67

3.7 Membrane-Bound Organelles of Eukaryotic Cells 68
Nucleus 68
Mitochondria 68
Chloroplasts 70
Endoplasmic Reticulum (ER) 70
Golgi Apparatus 71
Lysosomes and Peroxisomes 71

METHODS TO OBSERVE CELLS

3.8 Microscopes 72
Principles of Light Microscopy: Bright-Field Microscopes 73
Light Microscopes That Increase Contrast 74
Light Microscopes That Detect Fluorescence 76
Electron Microscopes 77
Scanning Probe Microscopes 78

3.9 Preparing Specimens for Light Microscopy 81
Simple Staining 81
Differential Staining 82
Special Stains to Observe Cell Structures 84
Fluorescent Dyes and Tags 85

FOCUS ON A CASE 3.1 55
FOCUS YOUR PERSPECTIVE 3.1: Pathogens Hijacking Actin 67

SUMMARY 86
REVIEW QUESTIONS 88

4 Dynamics of Microbial Growth 90

A Glimpse of History 90
Key Terms 90


Lisa Burgess/McGraw-Hill Education

4.1 Principles of Microbial Growth 90

4.2 Microbial Growth in Nature 91
Biofilms 92
Interactions of Mixed Microbial Communities 93

4.3 Microbial Growth in Laboratory Conditions 93
Obtaining a Pure Culture 94
The Growth Curve 95
Colony Growth 96
Continuous Culture 96

4.4 Environmental Factors That Influence Microbial Growth 97
Temperature Requirements 97
Oxygen (O_2) Requirements 98
pH 99
Water Availability 99

4.5 Nutritional Factors That Influence Microbial Growth 100
Required Elements 100
Growth Factors 101
Energy Sources 101
Nutritional Diversity 101

4.6 Cultivating Microorganisms in the Laboratory 103
General Categories of Culture Media 103
Special Types of Culture Media 104
Providing Appropriate Atmospheric Conditions 105
Enrichment Cultures 106

4.7 Methods to Detect and Measure Microbial Growth 107
Direct Cell Counts 107
Viable Cell Counts 108
Measuring Biomass 110
Detecting Cell Products 112

FOCUS ON A CASE 4.1 102
FOCUS YOUR PERSPECTIVE 4.1: Can Microorganisms Live on Only Rocks and Water? 103
FOCUS ON THE FUTURE 4.1: Seeing How the Other 99% Lives 113

SUMMARY 114
REVIEW QUESTIONS 115

5 Control of Microbial Growth 117

A Glimpse of History 117
Key Terms 117


Arthur Tilley/Getty Images

5.1 Approaches to Control 117
Principles of Control 118
Situational Considerations 118

5.2 Selecting an Antimicrobial Procedure 121
Types of Microbes 121
Number of Microbes 121
Environmental Conditions 122
Risk for Infection 122
Composition of the Item 122

5.3 Physical Methods Used to Destroy or Remove Microorganisms and Viruses 122
Moist Heat 122
Dry Heat 124
Filtration 124
Irradiation 125
High Pressure 126

5.4 Chemical Methods Used to Destroy Microorganisms and Viruses 127
Selecting the Appropriate Germicidal Chemical 127
Categories of Germicidal Potency 128
Classes of Germicidal Chemicals 128

5.5 Preservation of Perishable Products 132
Chemical Preservatives 132
Low-Temperature Storage 132
Reducing the Available Water 132

FOCUS ON A CASE 5.1 120
FOCUS ON THE FUTURE 5.1: Too Much of a Good Thing? 133

SUMMARY 134
REVIEW QUESTIONS 135

6 Microbial Metabolism: Fueling Cell Growth 137

A Glimpse of History 137
Key Terms 137


©Comstock/PunchStock

6.1 Overview of Microbial Metabolism 138
Energy 138
Components of Metabolic Pathways 140
Precursor Metabolites 143
Catabolism 144

6.2 Enzymes 147
Mechanisms and Consequences of Enzyme Action 147
Cofactors 147
Environmental Factors That Influence Enzyme Activity 148
Allosteric Regulation 149
Enzyme Inhibition 150

6.3 The Central Metabolic Pathways 151
Glycolysis 152
Pentose Phosphate Pathway 152
Transition Step and Tricarboxylic Acid (TCA) Cycle 152

6.4 Cellular Respiration 155
The Electron Transport Chain (ETC)—Generating a Proton Motive Force 155
ATP Synthase—Using the Proton Motive Force to Synthesize ATP 157
ATP Yield of Aerobic Respiration in Prokaryotes 159

6.5 Fermentation 160

6.6 Catabolism of Organic Compounds Other Than Glucose 162
Polysaccharides and Disaccharides 162
Lipids 163
Proteins 164

6.7 Chemolithotrophs 164

6.8 Photosynthesis 165
Light-Dependent Reactions 165
Light-Independent Reactions 167

6.9 Carbon Fixation 168
Calvin Cycle 168

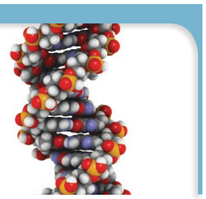
6.10 Anabolic Pathways—Synthesizing Subunits from Precursor Molecules 169
Lipid Synthesis 170
Amino Acid Synthesis 170
Nucleotide Synthesis 171

FOCUS ON A CASE 6.1 162
FOCUS YOUR PERSPECTIVE 6.1: Mining with Microbes 165
FOCUS ON THE FUTURE 6.1: Fueling the Future 171

SUMMARY 172
REVIEW QUESTIONS 173

7 The Blueprint of Life, from DNA to Protein 175

A Glimpse of History 175
Key Terms 175


Molekuul/SPL/age fotostock

7.1 Overview 176
Characteristics of DNA 176
Characteristics of RNA 177
Regulating Gene Expression 178

7.2 DNA Replication 179

7.3 Gene Expression in Bacteria 182

Transcription 182
Translation 184

7.4 Differences Between Eukaryotic and Prokaryotic Gene Expression 189

7.5 Sensing and Responding to Environmental Fluctuations 191
Signal Transduction 191
Natural Selection 192

7.6 Bacterial Gene Regulation 193
Mechanisms to Control Transcription 194
The *lac* Operon as a Model 196

7.7 Eukaryotic Gene Regulation 198

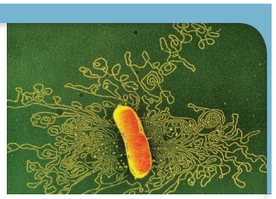
7.8 Genomics 199
Analyzing a Prokaryotic DNA Sequence 199
Metagenomics 200

FOCUS ON A CASE 7.1 192
FOCUS YOUR PERSPECTIVE 7.1: RNA: The First Macromolecule? 190
FOCUS ON THE FUTURE 7.1: Gems in the Genomes? 200

SUMMARY 200
REVIEW QUESTIONS 201

8 Bacterial Genetics 203

A Glimpse of History 203
Key Terms 203


Dr. Gopal Murti/Science Source

8.1 Genetic Change in Bacteria 203

MUTATION AS A MECHANISM OF GENETIC CHANGE

8.2 Spontaneous Mutations 205
Base Substitution 205
Deletion or Addition of Nucleotides 206
Transposons (Jumping Genes) 206

8.3 Induced Mutations 207
Chemical Mutagens 207
Transposition 208
Radiation 208

8.4 Repair of Damaged DNA 209
Repair of Errors in Nucleotide Incorporation 210
Repair of Damaged Nucleobases 210
Repair of Thymine Dimers 210
SOS Repair 211

8.5 Mutant Selection 212
Direct Selection 212
Indirect Selection 212
Screening for Possible Carcinogens 214

HORIZONTAL GENE TRANSFER AS A MECHANISM OF GENETIC CHANGE

8.6 Overview of Horizontal Gene Transfer 215

8.7 Bacterial Transformation 216
Competence 217
The Process of Natural Transformation 218

8.8 Transduction 220

8.9 Conjugation 221
Plasmid Transfer 221
Chromosome Transfer 222
F′ Donors 223

8.10 Genome Variability 225
Mobile Genetic Elements (MGEs) 225

8.11 Bacterial Defenses Against Invading DNA 228
Restriction-Modification Systems 228
CRISPR Systems 228

FOCUS ON A CASE 8.1 227
FOCUS YOUR PERSPECTIVE 8.1: The Biological Function of DNA: A Discovery Ahead of Its Time 219
FOCUS YOUR PERSPECTIVE 8.2: Bacteria Can Conjugate with Plants: A Natural Case of Genetic Engineering 224

SUMMARY 229
REVIEW QUESTIONS 231

9 Biotechnology 232

A Glimpse of History 232
Key Terms 232


atic12/123RF

9.1 Fundamental Tools Used in Biotechnology 234
Restriction Enzymes 234
Reverse Transcriptase 235
DNA Gel Electrophoresis 236

9.2 Molecular Cloning 237
The Cloning Process—A Simplified View 237
Applications of Molecular Cloning 237
Creating a DNA Library—A Detailed View of the Cloning Process 237

9.3 CRISPR-Cas Technologies 240
Applications of CRISPR-Cas Technologies 240

9.4 DNA Sequencing 241
Applications of DNA Sequencing 242
High-Throughput Sequencing Methods 242
RNA-Seq (RNA Sequencing) 243

9.5 Polymerase Chain Reaction (PCR) 243
Applications of PCR 243
The PCR Method 244

9.6 Probe Technologies 249
Colony Blotting 249
Fluorescence In Situ Hybridization (FISH) 250
DNA Microarrays 250

9.7 Concerns Regarding Biotechnology 251

FOCUS ON A CASE 9.1 248
FOCUS YOUR PERSPECTIVE 9.1: The COVID-19 Response—The Power of Biotechnology 244
FOCUS ON THE FUTURE 9.1: Precision Medicine 252

SUMMARY 252
REVIEW QUESTIONS 253

PART II
The Microbial World

10 Identifying and Classifying Microorganisms 255

A Glimpse of History 255
Key Terms 255


Diane Keough/Moment/Getty Images

10.1 Principles of Taxonomy 256
Strategies Used to Identify Microorganisms 256
Strategies Used to Classify Microorganisms 256
Nomenclature 257

10.2 Identification Methods Based on Phenotype 259
Microscopic Morphology 259
Culture Characteristics 260
Metabolic Capabilities 260
Serological Characteristics 262
Protein Profile 262

10.3 Identification Methods Based on Genotype 264
Detecting Specific Nucleotide Sequences 264
Sequencing Ribosomal RNA Genes 264
Whole Genome Sequencing 265

10.4 Characterizing Strain Differences 266
Biochemical Typing 266
Serological Typing 266
Whole Genome Sequencing 266
Phage Typing 267
Antibiograms 267

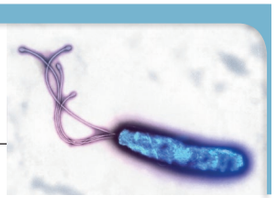
10.5 Classifying Microorganisms 269
Sequence Analysis of Ribosomal Components 269
DNA-DNA Hybridization (DDH) 270
Sequence Analysis of Genomes 270
G + C Content 270
Phenotypic Methods 271

FOCUS ON A CASE 10.1 268
FOCUS ON THE FUTURE 10.1: Pushing the Limits of MALDI-TOF MS 271

SUMMARY 272
REVIEW QUESTIONS 273

11 The Diversity of Bacteria and Archaea 274

A Glimpse of History 274
Key Terms 274


Heather Davies/Science Photo Library/Getty Images

METABOLIC DIVERSITY

11.1 Anaerobic Chemotrophs 275
Anaerobic Chemolithotrophs 275
Anaerobic Chemoorganotrophs—Anaerobic Respiration 276
Anaerobic Chemoorganotrophs—Fermentation 276

11.2 Anoxygenic Phototrophs 277
Purple Bacteria 278
Green Bacteria 278
Other Anoxygenic Phototrophs 279

11.3 Oxygenic Phototrophs 279
Cyanobacteria 279

11.4 Aerobic Chemolithotrophs 280
Sulfur-Oxidizing Bacteria 281
Nitrifiers 281
Hydrogen-Oxidizing Bacteria 282

11.5 Aerobic Chemoorganotrophs 282
Obligate Aerobes 282
Facultative Anaerobes 284

ECOPHYSIOLOGICAL DIVERSITY

11.6 Thriving in Terrestrial Environments 286
Bacteria That Form a Resting Stage 286
Bacteria That Associate with Plants 287

11.7 Thriving in Aquatic Environments 289
Sheathed Bacteria 289
Prosthecate Bacteria 289
Bacteria That Derive Nutrients from Other Organisms 290
Bacteria That Move by Unusual Mechanisms 291
Bacteria That Form Storage Granules 292

11.8 Animals as Habitats 293
Bacteria that Inhabit the Skin 293
Bacteria That Inhabit Mucous Membranes 294
Obligate Intracellular Parasites 296

11.9 Archaea That Thrive in Extreme Conditions 299
Extreme Halophiles 299
Extreme Thermophiles 300

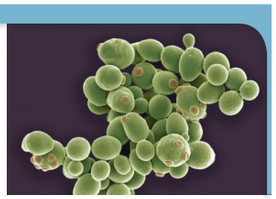
FOCUS ON A CASE 11.1 294

FOCUS ON THE FUTURE 11.1: Astrobiology: Searching for Life Beyond Earth 301

SUMMARY 301
REVIEW QUESTIONS 303

12 The Eukaryotic Members of the Microbial World 305

A Glimpse of History 305
Key Terms 305


Steve Gschmeissner/ Science Photo Library/Getty Images

12.1 Fungi 306
Characteristics of Fungi 307
Classification of Fungi 309
Groups of Medically Important Fungi 310
Economic Importance of Fungi 311
Symbiotic Relationships of Fungi 312

12.2 Protozoa 313
Characteristics of Protozoa 313
Groups of Medically Significant Protozoa 314
Other Protozoan Groups 315

12.3 Algae 318
Characteristics of Algae 318
Types of Algae 319
Exceptions to the Rule 320

12.4 Multicellular Parasites: Helminths 321
Life Cycles and Transmission of Helminths 321
Roundworms (Nematodes) 322
Tapeworms (Cestodes) 322
Flukes (Trematodes) 324

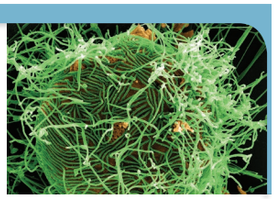
12.5 Arthropods 325

FOCUS ON A CASE 12.1 317
FOCUS YOUR PERSPECTIVE 12.1: What Causes River Blindness? 322

SUMMARY 326
REVIEW QUESTIONS 327

13 Viruses, Viroids, and Prions 329

A Glimpse of History 329
Key Terms 329


Source: National Institute of Allergy and Infectious Diseases (NIAID)/CDC

13.1 General Characteristics of Viruses 330
Viral Structure 330
Viral Taxonomy 330

13.2 Bacteriophages 335
Lytic Phage Infections: T4 Phage as a Model 335
Temperate Phage Infections: Lambda Phage as a Model 337
Filamentous Phage Infections: M13 Phage as a Model 338

13.3 The Roles of Bacteriophages in Horizontal Gene Transfer 339
Generalized Transduction 339
Specialized Transduction 339

13.4 Methods Used to Study Bacteriophages 340

13.5 Animal Virus Replication 341
Attachment 341
Entry and Uncoating 341
Synthesis of Viral Proteins and Replication of the Genome 342
Assembly (Maturation) 345
Release 346

13.6 Categories of Animal Virus Infections 347
Acute Infections 347
Persistent Infections 347

13.7 Viruses and Human Tumors 349
Cancer-Causing Viruses 349
Cancer-Fighting Viruses 350

13.8 Cultivating and Quantitating Animal Viruses 351
Cultivating Animal Viruses 351
Quantitating Animal Viruses 352

13.9 Plant Viruses 353

13.10 Other Infectious Agents: Viroids and Prions 354
Viroids 354
Prions 354

FOCUS ON A CASE 13.1 346
FOCUS YOUR PERSPECTIVE 13.1: Microbe Mimicker 335
FOCUS ON THE FUTURE 13.1: The Potential of Phage Therapy 356

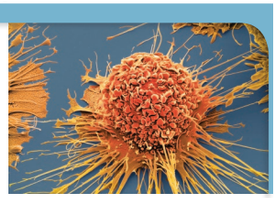
SUMMARY 357
REVIEW QUESTIONS 358

PART III

Microorganisms and Humans

14 The Innate Immune Response 360

A Glimpse of History 360
Key Terms 360


Science Photo Library/Alamy Stock Photo

14.1 Overview of the Innate Immune Defenses 361

14.2 First-Line Defenses 362
Physical Barriers 363
Antimicrobial Substances 363
Normal Microbiota (Flora) 364

14.3 The Cells of the Immune System 364
Granulocytes 365
Mononuclear Phagocytes 366
Dendritic Cells 367
Lymphocytes 367

14.4 Cell Communication 368
Surface Receptors 368
Cytokines 368
Adhesion Molecules 368

14.5 Pattern Recognition Receptors (PRRs) 369
Pattern Recognition Receptors (PRRs) That Monitor a Cell's Surroundings 370
Pattern Recognition Receptors (PRRs) That Monitor Material Ingested by a Cell 370
Pattern Recognition Receptors (PRRs) That Monitor a Cell's Cytoplasm 371
An Outcome of Cytoplasmic Pattern Recognition: The Interferon Response 371

14.6 The Complement System 372
Complement System Activation 373
Effector Functions of the Complement System 374
Regulation of the Complement System 374

14.7 Phagocytosis 375
The Process of Phagocytosis 375
Characteristics of Macrophages 376
Characteristics of Neutrophils 377

14.8 The Inflammatory Response 377
The Inflammatory Process 378
Damaging Effects of Inflammation 378
Cell Death and the Inflammatory Response 380

14.9 Fever 380

FOCUS ON A CASE 14.1 381

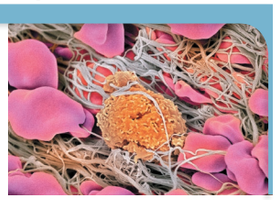
FOCUS YOUR PERSPECTIVE 14.1: For *Schistosoma*, the Inflammatory Response Delivers 380

SUMMARY 382

REVIEW QUESTIONS 383

15 The Adaptive Immune Response 385

A Glimpse of History 385
Key Terms 385



Science Photo Library/Getty Images

15.1 Overview of the Adaptive Immune Response 386
Cell-Mediated Immunity 388
Humoral Immunity 389
The Nature of Antigens 389
The Lymphatic System 391
The Big Picture Summary 392

15.2 Clonal Selection and Expansion of Lymphocytes 394

15.3 The T-Cell Response: Cell-Mediated Immunity 396
General Characteristics of T Cells 396
Activation of T Cells 397
Effector Functions of T_C (CD8) Cells 398
Effector Functions of T_H (CD4) Cells 399

15.4 The B-Cell Response: Humoral Immunity 402
General Characteristics of B cells 402
B-Cell Activation 402
Characteristics of Antibodies 402
Evolution of the Humoral Response to T-Dependent Antigens 404
The Response to T-Independent Antigens 407

15.5 Lymphocyte Development 408
Generation of Diversity 408
Negative Selection of Self-Reactive B Cells 408
Positive and Negative Selection of Self-Reactive T Cells 408

15.6 Natural Killer (NK) Cells 409

FOCUS ON A CASE 15.1 410

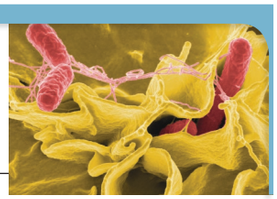
FOCUS YOUR PERSPECTIVE 15.1: What Flavors Are Your Major Histocompatibility Complex (MHC) Molecules? 401

SUMMARY 411

REVIEW QUESTIONS 413

16 Host-Microbe Interactions 415

A Glimpse of History 415
Key Terms 415



NIAID, NIH, Rocky Mountain Laboratories

MICROBES, HEALTH, AND DISEASE

16.1 The Anatomical Barriers as Ecosystems 416

16.2 The Human Microbiome 416
Composition of the Microbiome 417
Beneficial Roles of the Human Microbiome 417

16.3 Principles of Infectious Disease 418
Pathogenicity 418
Characteristics of Infectious Disease 419

16.4 Determining the Cause of an Infectious Disease 420
Koch's Postulates 420
Molecular Koch's Postulates 421

MECHANISMS OF PATHOGENESIS

16.5 Establishing Infection 422
Adherence 422
Colonization 422
Delivering Effector Proteins to Host Cells 423

16.6 Invasion—Breaching the Anatomical Barriers 423
Penetrating the Skin 423
Penetrating Mucous Membranes 423

16.7 Avoiding the Host Defenses 424
Hiding Within a Host Cell 424
Avoiding Destruction by Phagocytes 425
Avoiding Killing by Complement System Proteins 426
Avoiding Recognition by Antibodies 427

16.8 Damage to the Host 427
Exotoxins 427
Endotoxin and Other Bacterial Cell Wall Components 430
Damaging Effects of the Immune Response 432

16.9 Mechanisms of Viral Pathogenesis 433
Binding to Host Cells and Invasion 433
Avoiding Immune Responses 433
Damage to the Host 434

16.10 Mechanisms of Eukaryotic Pathogenesis 434
Fungi 434
Protozoa and Helminths 435

FOCUS ON A CASE 16.1 429
FOCUS ON THE FUTURE 16.1: The Potential of Probiotics 435

SUMMARY 436
REVIEW QUESTIONS 437

17 Applications of Immune Responses 439

A Glimpse of History 439
Key Terms 439


©Kevin Horan/The Image Bank/Getty Images

IMMUNIZATION AND IMMUNOTHERAPY

17.1 Principles of Immunization 440
Active Immunity 440
Passive Immunity 440

17.2 Vaccines and Immunization Procedures 441
Attenuated Vaccines 441
Inactivated Vaccines 442
The Importance of Vaccines 443
An Example of Vaccination Strategy—The Campaign to Eliminate Poliomyelitis 444

17.3 Immunotherapies 446
Immunotherapies for Cancer 446
Immunotherapies for Immunological Disorders 449
Immunotherapies for Infectious Diseases 450

IMMUNOLOGICAL TESTING

17.4 Principles of Immunoassays 451
Quantifying Antigen-Antibody Reactions 452
Obtaining Known Antibodies 452

17.5 Common Types of Immunoassays 453
Immunoassays That Use Labeled Antibodies 454
Immunoassays That Involve Visible Antigen-Antibody Aggregates 457

FOCUS ON A CASE 17.1 453
FOCUS YOUR PERSPECTIVE 17.1: Obtaining Monoclonal Antibodies 449

SUMMARY 461
REVIEW QUESTIONS 462

18 Immunological Disorders 464

A Glimpse of History 464
Key Terms 464


©bubutu/Shutterstock

18.1 Hypersensitivities 464
Type I Hypersensitivities: Immediate IgE-Mediated 465
Type II Hypersensitivities: Cytotoxic 468
Type III Hypersensitivities: Immune Complex–Mediated 470
Type IV Hypersensitivities: Delayed-Type Cell-Mediated 471

18.2 Autoimmune Disease 473
Systemic Autoimmune Diseases 474
Organ-Specific Autoimmune Diseases 474

18.3 Immunodeficiency Disorders 476
Primary Immunodeficiencies 476
Secondary Immunodeficiencies 477

FOCUS ON A CASE 18.1 476
FOCUS YOUR PERSPECTIVE 18.1: The Fetus as an Allograft 473

SUMMARY 478
REVIEW QUESTIONS 479

19 Epidemiology 481

A Glimpse of History 481
Key Terms 481


Source: CDC/James Gathany

19.1 Basic Concepts of Epidemiology 482

19.2 Chain of Infection 483
Reservoirs of Infection 483
Portals of Exit 484
Disease Transmission 484
Portals of Entry 487

19.3 Factors That Influence the Epidemiology of Disease 487
The Dose 488
The Incubation Period 488
The Host Population 488
The Environment 488

19.4 Epidemiological Studies 489
Descriptive Studies 489
Analytical Studies 490
Experimental Studies 491

19.5 Infectious Disease Surveillance 491
National Disease Surveillance Network 493
Worldwide Disease Surveillance 494
Reduction and Eradication of Disease 494

19.6 Emerging Infectious Diseases 495

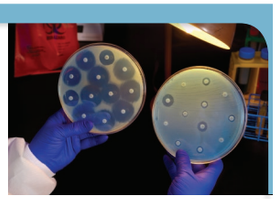
19.7 Healthcare-Associated Infections 496
Reservoirs of Infectious Agents in Healthcare Settings 496
Transmission of Infectious Agents in Healthcare Settings 498
Preventing Healthcare-Associated Infections 498

FOCUS ON A CASE 19.1 492
FOCUS YOUR PERSPECTIVE 19.1: Standard Precautions—Protecting Patients and Healthcare Personnel 499
FOCUS ON THE FUTURE 19.1: Maintaining Vigilance Against Bioterrorism 500

SUMMARY 501
REVIEW QUESTIONS 502

20 Antimicrobial Medications 504

A Glimpse of History 504
Key Terms 504


Source: James Gathany/CDC

20.1 History and Development of Antimicrobial Medications 504
Discovery of Antimicrobial Medications 505
Discovery of Antibiotics 505
Development of New Antimicrobial Medications 505

20.2 Characteristics of Antimicrobial Medications 507
Selective Toxicity 507
Antimicrobial Action 507
Spectrum of Activity 507
Effects of Antimicrobial Combinations 507
Tissue Distribution, Metabolism, and Excretion of the Medication 507
Adverse Effects 508
Resistance to Antimicrobials 508

20.3 Mechanisms of Action of Antibacterial Medications 508
Inhibit Cell Wall Synthesis 509
Inhibit Protein Synthesis 512
Inhibit Nucleic Acid Synthesis 513
Interfere with Metabolic Pathways 514
Interfere with Cell Membrane Integrity 514
Effective Against *Mycobacterium tuberculosis* 514

20.4 Antimicrobial Susceptibility Testing 516
Conventional Disc Diffusion Method 516
Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC and MBC) 517
Commercial Modifications of Antimicrobial Susceptibility Testing 518

20.5 Resistance to Antimicrobial Medications 519
Mechanisms of Acquired Resistance 519
Acquisition of Resistance 521
Examples of Emerging Resistance 521
Preventing Resistance 523

20.6 Mechanisms of Action of Antiviral Medications 526
Prevent Viral Entry 526
Interfere with Viral Uncoating 526
Interfere with Nucleic Acid Synthesis 527
Prevent Genome Integration 527
Prevent Assembly and Release of Viral Particles 527

20.7 Mechanisms of Action of Antifungal Medications 529
Interfere with Cytoplasmic Membrane Synthesis and Function 529
Interfere with Cell Wall Synthesis 529
Interfere with Cell Division 530
Interfere with Nucleic Acid Synthesis 530
Interfere with Protein Synthesis 530

20.8 Mechanisms of Action of Antiprotozoan and Antihelminthic Medications 531

FOCUS ON A CASE 20.1 525
FOCUS YOUR PERSPECTIVE 20.1: Using Diffusion Tests to Measure the Concentration of an Antimicrobial Medication in Blood or Other Body Fluids 519
FOCUS ON THE FUTURE 20.1: The Race to Develop COVID-19 Treatments 532

SUMMARY 533
REVIEW QUESTIONS 534

PART IV
Infectious Diseases

21 Respiratory System Infections 536

A Glimpse of History 536
Key Terms 536


Centers for Disease Control and Prevention

21.1 Anatomy, Physiology, and Ecology of the Respiratory System 536
The Upper Respiratory Tract 537
The Lower Respiratory Tract 539

UPPER RESPIRATORY TRACT INFECTIONS

21.2 Bacterial Infections of the Upper Respiratory System 540
Pink Eye, Earache, and Sinus Infections 540
Streptococcal Pharyngitis ("Strep Throat") 541
Post-Streptococcal Sequelae 544
Diphtheria 545

21.3 Viral Infections of the Upper Respiratory System 548
The Common Cold 548
Adenovirus Respiratory Tract Infections 549

LOWER RESPIRATORY TRACT INFECTIONS

FOCUS ON PNEUMONIA 551

21.4 Bacterial Infections of the Lower Respiratory System 552
Pneumococcal Pneumonia 552
Klebsiella Pneumonia 553
Mycoplasmal Pneumonia ("Walking Pneumonia") 554
Pertussis ("Whooping Cough") 555
Tuberculosis ("TB") 557
Legionnaires' Disease (*Legionella* Pneumonia) 561
Inhalation Anthrax 563

21.5 Viral Infections of the Lower Respiratory System 565
Influenza ("Flu") 565
Respiratory Syncytial Virus (RSV) Infections 568
Coronavirus Infections: COVID-19, SARS, and MERS 569
Hantavirus Pulmonary Syndrome 572

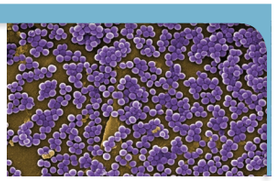
21.6 Fungal Infections of the Lower Respiratory System 573
Coccidioidomycosis ("Valley Fever") 574
Histoplasmosis ("Spelunker's Disease") 575
Pneumocystis Pneumonia (PCP) 576

FOCUS ON A CASE 21.1 546
FOCUS YOUR PERSPECTIVE 22.1: A Global Lesson in Microbiology: The COVID-19 Pandemic 571
DISEASES IN REVIEW 21.1: Respiratory System Diseases 578

SUMMARY 579
REVIEW QUESTIONS 580

22 Skin Infections 582

A Glimpse of History 582
Key Terms 582



Source: Janice Carr/CDC

22.1 Anatomy, Physiology, and Ecology of the Skin 582

22.2 Bacterial Diseases of the Skin 584
Acne Vulgaris 584
Hair Follicle Infections 586
Staphylococcal Scalded Skin Syndrome 589
Impetigo 590
Rocky Mountain Spotted Fever 591
Cutaneous Anthrax 593

22.3 Viral Diseases of the Skin 594
Varicella (Chickenpox) 595
Rubeola (Measles) 597
Rubella (German Measles) 600
Other Viral Rashes of Childhood 601
Warts 603

22.4 Fungal Diseases of the Skin 603
Superficial Cutaneous Mycoses 603
Other Fungal Diseases 604

FOCUS ON A CASE 22.1 600
FOCUS YOUR PERSPECTIVE 22.1: The Ghost of Smallpox: An Evil Shade 598
DISEASES IN REVIEW 22.1: Common Bacterial, Viral, and Fungal Skin Diseases 606

SUMMARY 607
REVIEW QUESTIONS 607

23 Wound Infections 609

A Glimpse of History 609
Key Terms 609

©Garry Watson/Science Source

23.1 Anatomy, Physiology, and Ecology of Wounds 609
Wound Abscesses 611

23.2 Common Bacterial Infections of Wounds 612
Staphylococcal Wound Infections 612
Group A Streptococcal "Flesh-Eating Disease" 613
Pseudomonas aeruginosa Infections 614

23.3 Diseases Due to Anaerobic Bacterial Wound Infections 617
Tetanus ("Lockjaw") 617
Clostridial Myonecrosis ("Gas Gangrene") 619

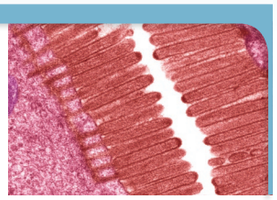
23.4 Bacterial Infections of Bite Wounds 622
Human Bites 622
Pasteurella multocida Bite Wound Infections 623
Bartonellosis ("Cat Scratch Disease") 624
Other Bacterial Bite Wound Infections 625

23.5 Fungal Wound Infections 625
Sporotrichosis ("Rose Gardener's Disease") 625

FOCUS ON A CASE 23.1 622
FOCUS YOUR PERSPECTIVE 23.1: Infection Caused by a Human "Bite" 624
DISEASES IN REVIEW 23.1: Wound Infections 627

SUMMARY 628
REVIEW QUESTIONS 628

24 Digestive System Infections 630


Steve Gschmeissner/SPL/Science Source

A Glimpse of History 630
Key Terms 630

24.1 Anatomy, Physiology, and Ecology of the Digestive System 631
The Upper Digestive System 632
The Lower Digestive System 633

UPPER DIGESTIVE SYSTEM INFECTIONS

24.2 Bacterial Diseases of the Upper Digestive System 634
Dental Caries 635
Periodontal Disease 636
Acute Necrotizing Ulcerative Gingivitis 637
Helicobacter pylori Gastritis 639

24.3 Viral Diseases of the Upper Digestive System 641
Oral Herpes (Cold Sores) 641
Mumps 642

LOWER DIGESTIVE SYSTEM INFECTIONS

FOCUS ON DIARRHEAL DISEASES 644

24.4 Bacterial Diseases of the Lower Digestive System 644
Cholera 645
Shigellosis 647
Escherichia coli Gastroenteritis 648
Salmonella Gastroenteritis 650
Enteric Fever (Typhoid and Paratyphoid) 652
Campylobacteriosis 653
Clostridioides (*Clostridium*) *difficile* Infection (CDI) 654

24.5 Viral Diseases of the Lower Digestive System—Intestinal Tract 656
Rotavirus Gastroenteritis 656
Norovirus Gastroenteritis 656

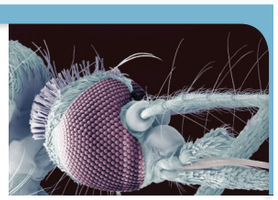
24.6 Viral Diseases of the Lower Digestive System—Liver 658
Hepatitis A 658
Hepatitis B 659
Hepatitis C 661

24.7 Protozoan Diseases of the Lower Digestive System 662
Giardiasis 662
Cryptosporidiosis ("Crypto") 663
Cyclosporiasis 665
Amebiasis 666

FOCUS ON A CASE 24.1 640
DISEASES IN REVIEW 24.1: Digestive System Diseases 668

SUMMARY 669
REVIEW QUESTIONS 670

25 Blood and Lymphatic Infections 672


Steve Gschmeissner/Science Photo Library/ Alamy Stock Photo

A Glimpse of History 672
Key Terms 672

25.1 Anatomy, Physiology, and Ecology of the Blood and Lymphatic Systems 673
The Heart 673
Blood Vessels 673
Lymphatics (Lymphatic Vessels) 673
Spleen 674

25.2 Bacterial Diseases of the Blood and Lymphatic Systems 674
Infective Endocarditis 674
Sepsis and Septic Shock 675
Plague ("Black Death") 676
Lyme Disease 678
Vibrio vulnificus Infection 682
Tularemia ("Rabbit Fever" or "Deer Fly Fever") 683
Brucellosis ("Undulant Fever" or "Bang's Disease") 684

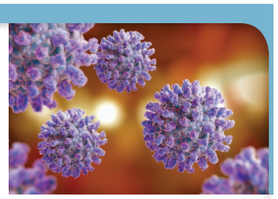
25.3 Viral Diseases of the Blood and Lymphatic Systems 686
Infectious Mononucleosis ("Mono" or "Kissing Disease") 686
Ebola Disease (EBOD) and Marburg Disease (MARD) 688
Yellow Fever 689
Dengue and Severe Dengue 690
Chikungunya 691
Zika Virus Disease 692

25.4 Protozoan Diseases of the Blood and Lymphatic Systems 694
Malaria 694

FOCUS ON A CASE 25.1 692
DISEASES IN REVIEW 25.1: Blood and Lymphatic Infections 699

SUMMARY 700
REVIEW QUESTIONS 701

26 Nervous System Infections 703


Science Picture Co/Getty Images

A Glimpse of History 703
Key Terms 703

26.1 Anatomy, Physiology, and Ecology of the Nervous System 703

CENTRAL NERVOUS SYSTEM INFECTIONS

FOCUS ON MENINGITIS 706

26.2 Bacterial Diseases of the Central Nervous System 706
Pneumococcal Meningitis 707

Meningococcal Meningitis 708
Haemophilus influenzae Meningitis 709
Neonatal Meningitis 710
Listeriosis 711

26.3 Viral Diseases of the Central Nervous System 713
Viral Meningitis 713
West Nile and Other Types of Viral Encephalitis 714
Poliomyelitis 715
Rabies 718

26.4 Fungal Diseases of the Central Nervous System 721
Cryptococcal Meningoencephalitis 721

26.5 Protozoan Diseases of the Central Nervous System 723
African Trypanosomiasis ("African Sleeping Sickness") 723
Toxoplasmosis 724
Primary Amebic Meningoencephalitis (PAM) 726

26.6 Diseases Caused by Prions 727
Transmissible Spongiform Encephalopathies in Humans 728

PERIPHERAL NERVOUS SYSTEM INFECTIONS

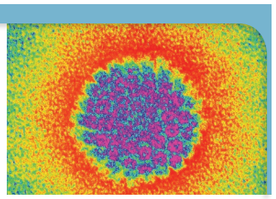
26.7 Bacterial Diseases of the Peripheral Nervous System 729
Hansen's Disease (Leprosy) 729
Botulism 731

FOCUS ON A CASE 26.1 711
FOCUS YOUR PERSPECTIVE 26.1: Rabies Survivors! 721
DISEASES IN REVIEW 26.1: Nervous System Diseases 734

SUMMARY 735
REVIEW QUESTIONS 736

27 Genitourinary Tract Infections 738

A Glimpse of History 738
Key Terms 738


Science Photo Library - PASIEKA/Getty Images

27.1 Anatomy, Physiology, and Ecology of the Genitourinary System 738
The Urinary System 738
The Genital System 739

27.2 Urinary Tract Infections 740
Bacterial Cystitis ("Bladder Infection") 740
Leptospirosis 741

27.3 Genital System Diseases 744
Bacterial Vaginosis (BV) 745
Vulvovaginal Candidiasis (VVC) 746
Staphylococcal Toxic Shock Syndrome 746

SEXUALLY TRANSMITTED INFECTIONS

FOCUS ON SEXUALLY TRANSMITTED INFECTIONS 748

27.4 Bacterial STIs 748
Chlamydial Infections 748
Gonorrhea 751
Mycoplasma genitalium Infections 753
Syphilis 755
Chancroid 758

27.5 Viral STIs 760
Genital Herpes 760
Human Papillomavirus STIs: Genital Warts and Cervical Cancer 761
HIV/AIDS 763

27.6 Protozoan STIs 771
Trichomoniasis ("Trich") 771

FOCUS ON A CASE 27.1 742
FOCUS YOUR PERSPECTIVE 27.1: Conquering Syphilis 759
FOCUS ON THE FUTURE 27.1: Getting Control of Sexually Transmitted Infections 772
DISEASES IN REVIEW 27.1: Genitourinary Infections 773

SUMMARY 774
REVIEW QUESTIONS 775

PART V

Applied Microbiology

28 Microbial Ecology 777

A Glimpse of History 777
Key Terms 777


Photo by Tim McCabe, USDA Natural Resource Conservation Service

28.1 Principles of Microbial Ecology 778
Nutrient Acquisition 778
Microbes in Low-Nutrient Environments 778
Microbial Competition 779
Microorganisms and Environmental Changes 779
Microbial Communities 780

28.2 Studying Microbial Ecology 781

28.3 Aquatic Habitats 782
Marine Environments 782
Freshwater Environments 783
Specialized Aquatic Environments 783

28.4 Terrestrial Habitats 783
Characteristics of Soil 784
Microorganisms in Soil 784

28.5 Biogeochemical Cycling and Energy Flow 785
Carbon Cycle 785

Nitrogen Cycle 786
Sulfur Cycle 788
Phosphorus Cycle and Other Cycles 788
Energy Sources for Ecosystems 789

28.6 Mutualistic Relationships Between Microorganisms and Eukaryotes 790
Mycorrhizas 790
Symbiotic Nitrogen-Fixers and Plants 790
Microorganisms and Herbivores 792

FOCUS ON A CASE 28.1 792

SUMMARY 793
REVIEW QUESTIONS 794

29 Environmental Microbiology: Treatment of Water, Wastes, and Polluted Habitats 796

A Glimpse of History 796
Key Terms 796


Robert Glusic/Getty Images

29.1 Microbiology of Wastewater Treatment 797
Biochemical Oxygen Demand (BOD) 797
Municipal Wastewater Treatment Methods 797
Individual Wastewater Treatment Systems 800

29.2 Drinking Water Treatment and Testing 801
Water Treatment Processes 801
Water Testing 802

29.3 Microbiology of Solid Waste Treatment 805
Sanitary Landfills for Solid Waste Disposal 805
Municipal and Backyard Composting—Alternative to Landfills 805

29.4 Microbiology of Bioremediation 806
Pollutants 806
Strategies of Bioremediation 807

FOCUS ON A CASE 29.1 804

SUMMARY 808
REVIEW QUESTIONS 808

30 Food Microbiology 810

A Glimpse of History 810
Key Terms 810


Images by Tang Ming Tung/Getty Images

30.1 Factors Influencing the Growth of Microorganisms in Foods 811
Intrinsic Factors 811
Extrinsic Factors 812

30.2 Microorganisms in Food and Beverage Production 812
Lactic Acid Fermentations by the Lactic Acid Bacteria 813
Alcoholic Fermentations by Yeast 815
Changes Due to Mold Growth 817

30.3 Food Spoilage 818
Common Spoilage Bacteria 818
Common Spoilage Fungi 818

30.4 Foodborne Illness 819
Foodborne Intoxication 819
Foodborne Infection 819

30.5 Food Preservation 822

FOCUS ON A CASE 30.1 821
FOCUS YOUR PERSPECTIVE 30.1: Botox for Beauty and Pain Relief 821

SUMMARY 823
REVIEW QUESTIONS 824

APPENDIX I Microbial Mathematics A–1

APPENDIX II Pronunciation Key for Bacterial, Fungal, Protozoan, and Viral Names A–2

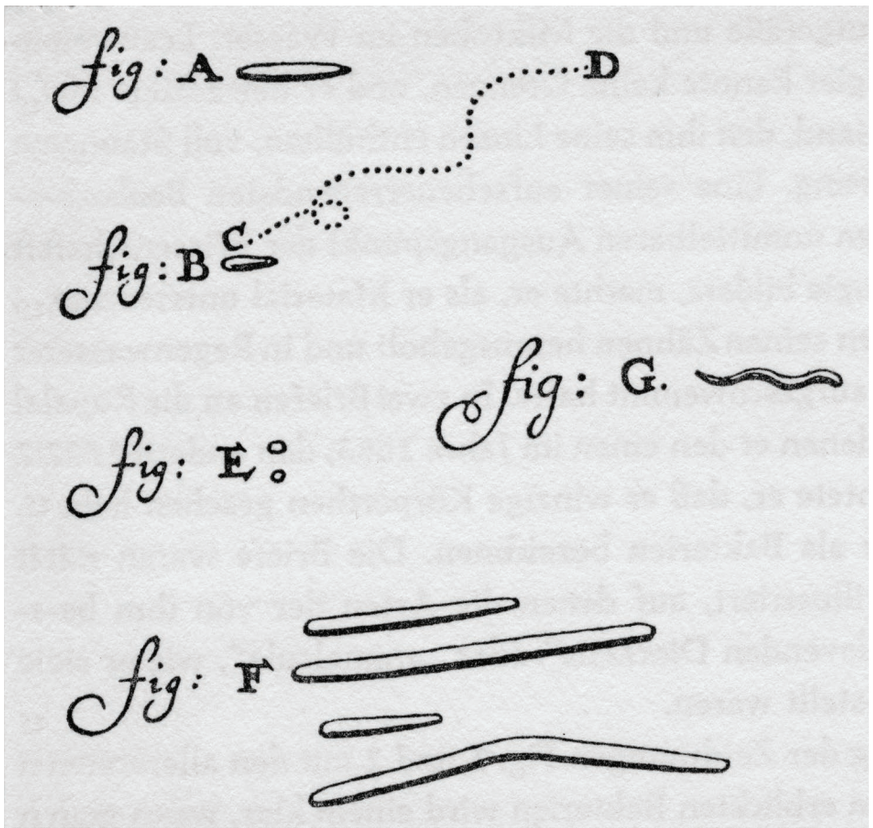
APPENDIX III Metabolic Pathways A–4

APPENDIX IV Answers to Multiple Choice Questions A–7

APPENDIX V Microbial Terminology A–8

GLOSSARY/INDEX GI–1

1 | Humans and the Microbial World



Drawings that van Leeuwenhoek made in 1683 of microorganisms he saw through his single-lens microscope. He also observed organism B moving from position C to D. *(INTERFOTO/Alamy, Stock Photo)*

KEY TERMS

Domain The highest level in biological classification. There are three domains: Bacteria, Archaea, and Eukarya.

Eukaryote Organism composed of one or more eukaryotic cells; members of the domain Eukarya are eukaryotes.

Eukaryotic Cell Cell type characterized by a membrane-bound nucleus.

Prion An acellular infectious agent consisting only of protein.

Prokaryote Single-celled organism consisting of a prokaryotic cell; members of the domains Bacteria and Archaea are prokaryotes.

Prokaryotic Cell Cell type characterized by the lack of a membrane-bound nucleus.

Viroid An acellular infectious agent consisting only of RNA.

Virus An acellular infectious agent consisting of nucleic acid surrounded by a protein coat.

A Glimpse of History

Microbiology as a science was born in 1674 when Antonie van Leeuwenhoek, an inquisitive Dutch fabric merchant, looked at a drop of lake water through a glass lens he had carefully made. Although many people before him had used curved glass to magnify objects, Leeuwenhoek's skilled hands made a lens that uncovered a startling and amazing sight—the world of microbes. As van Leeuwenhoek wrote in a letter to the Royal Society of London, he saw

> Very many little animalcules, whereof some were roundish, while others a bit bigger consisted of an oval. On these last, I saw two little legs near the head, and two little fins at the hind most end of the body. Others were somewhat longer than an oval, and these were very slow a-moving, and few in number. These animalcules had diverse colours, some being whitish and transparent; others with green and very glittering little scales, others again were green in the middle, and before and behind white; others yet were ashed grey. And the motion of most of these animalcules in the water was so swift, and so various, upwards, downwards, and round about, that 'twas wonderful to see.

Before van Leeuwenhoek made these observations, Robert Hooke, an English microscopist, saw another kind of microorganism. In 1665, he described what he called a "microscopical mushroom." His drawing was so accurate that his specimen could later be identified as a common bread mold. Hooke also described how to make the kind of microscope that van Leeuwenhoek constructed almost 10 years later. Both men deserve equal credit for revealing the world of microbes—a world you are about to study.

Microbiology is the study of an amazing world made up of members too small to be seen without the aid of a microscope. Antonie van Leeuwenhoek described this world when he observed what he called "animalcules" through his simple microscope (**figure 1.1**). What he saw were **microorganisms** (organisms too small to see with the naked eye), including bacteria, protozoa, and some fungi and algae. The microbial world also includes viruses and other infectious agents that are not considered organisms because they are not composed of cells; they are acellular. When referring to general members of the microbial world, the term **microbe** is often used.

Microorganisms are the foundation for all life on Earth. They have existed on this planet for about 3.5 billion years, and over this time, plants, animals, and modern microorganisms have evolved from them. Even today, they continue to be a driving force in the evolution of all living things. Microorganisms may be small, but as you are about to learn, our life depends on their activities.

FIGURE 1.1 Model of van Leeuwenhoek's Microscope The original made in 1673 could magnify an object almost 300 times. The object is brought into focus with the adjusting screws. Tetra Images/Alamy Stock Photo

What kinds of organisms did van Leeuwenhoek observe through his microscope?

1.1 ■ The Dispute over Spontaneous Generation

Learning Outcomes

1. Describe the key experiments of scientists who disproved spontaneous generation.
2. Explain how the successful challenge to the idea of spontaneous generation led to the Golden Age of Microbiology.
3. Describe the scientific method, using Pasteur's swan-necked flask experiment as an example.

The discovery of microorganisms in various specimens raised an interesting question: "Where did these microscopic forms originate?" Some people believed that worms and other life-forms arise from non-living material in a process known as **spontaneous generation.** This was challenged by an Italian biologist and physician, Francesco Redi. In 1668, he used a simple experiment to show that worms found on rotting meat originated from fly eggs, not from the decaying meat as supporters of spontaneous generation believed. In his experiment, Redi covered the meat with fine gauze that prevented flies from depositing their eggs; when he did this, no worms appeared. Despite Redi's work, it took more than 200 years and many experiments to amass conclusive evidence that microorganisms did not arise by spontaneous generation.

Early Experiments

In 1749, John Needham, a scientist and Catholic priest, showed that flasks containing various broths (made by soaking a nutrient source such as hay or chicken in water) gave rise to microorganisms even when the flasks were boiled and sealed with a cork. At that time, brief boiling was thought to kill all organisms, so this suggested that microorganisms did indeed arise spontaneously.

In 1776, the animal physiologist and priest Lazzaro Spallanzani obtained results that contradicted Needham's experiments; no bacteria appeared in Spallanzani's broths after boiling. His experiments differed from Needham's in two significant ways: Spallanzani boiled the broths for longer periods, and he sealed the flasks by melting their glass necks closed. Using these techniques, he repeatedly demonstrated that broths remained sterile (free of microorganisms). However, if the neck of the flask cracked, the broth rapidly became cloudy due to the growth of organisms. Spallanzani concluded that microorganisms had entered the broth with the air, and the corks used by Needham and other investigators did not keep them out.

Spallanzani's experiments did not stop the controversy. Some people argued that the heating process destroyed a "vital force" in the air that was necessary for spontaneous generation, and so the debate continued.

Experiments of Pasteur

One giant in science who helped disprove spontaneous generation was Louis Pasteur, the French chemist considered by many to be the father of modern microbiology. In 1861, he did a series of clever experiments. First, he demonstrated that air contains microorganisms. He did this by filtering air through a cotton plug, trapping microorganisms. He then examined the trapped microorganisms with a microscope and found that many looked identical to those described by others who had been studying broths. When Pasteur dropped the cotton plug into a sterilized broth, the broth became cloudy from the growth of these microorganisms.

Most important, Pasteur demonstrated that sterile broths in specially constructed swan-necked flasks remained sterile even when left open to air (**figure 1.2**). Microorganisms from the air settled in the bends of the flask necks, never reaching the broth. Only when the flasks were tipped would microorganisms enter the broth and grow. Pasteur's simple and elegant experiments ended the arguments that unheated air or the broths themselves contained a "vital force" necessary for spontaneous generation. They led to the theory of **biogenesis,** the production of living things from other living things (*bio* means "life"; *genesis* means "to create").

Experiments of Tyndall

Although most scientists were convinced by Pasteur's experiments, some remained skeptical because they could not reproduce his results. An English physicist, John Tyndall, finally explained the conflicting data and, in turn,

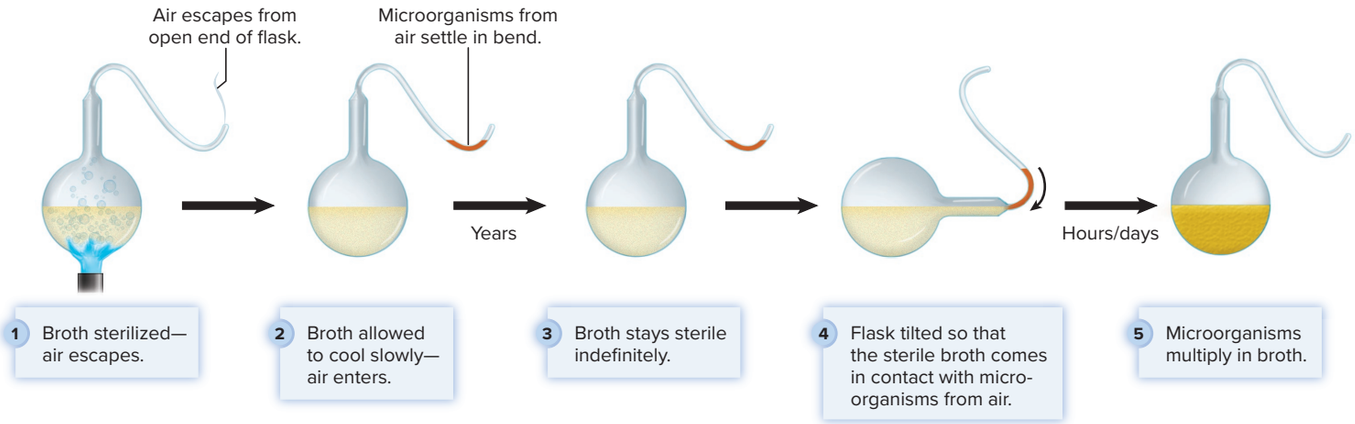


FIGURE 1.2 Pasteur's Experiment with the Swan-Necked Flask

? How did this experiment end arguments that a "vital force" in the air was necessary for spontaneous generation?

showed that Pasteur was correct. Tyndall found that various types of broths required different boiling times to be sterilized. Some were sterilized by boiling for 5 minutes, whereas others, most notably broths made from hay, still contained living microorganisms even after boiling for 5 hours! Even when hay was merely present in the laboratory, broths that had previously been sterilized by boiling for 5 minutes could not be sterilized by boiling for several hours. What was going on? Tyndall finally realized that the hay contained heat-resistant forms of microorganisms. When hay was brought into the laboratory, dust particles must have transferred these heat-resistant forms to the broths. Tyndall concluded that some microorganisms exist in two forms: a cell easily killed by boiling, and one that is heat resistant. In the same year (1876), a German botanist, Ferdinand Cohn, discovered **endospores,** the heat-resistant forms of some bacteria.

The extreme heat resistance of endospores explains the differences between Pasteur's results and those of other investigators. Organisms that produce endospores are commonly found in the soil and were likely present in broths made from hay. Pasteur used only broths made with sugar or yeast extract, so his experiments probably did not have endospores. Scientists at the time did not appreciate the importance of the source of the broth, but in hindsight, the source was critical. This points out an important lesson for all scientists: When repeating an experiment, all conditions must be reproduced as closely as possible. What may seem like a trivial difference might be extremely important.

The Golden Age of Microbiology

The work of Pasteur and others in disproving spontaneous generation started an era called the Golden Age of Microbiology, during which time the field of microbiology blossomed. Many important advances were made during this period, including discoveries that led to the acceptance of the suggestion that microorganisms cause certain diseases, a principle now called the Germ Theory of Disease.

Figure 1.3 lists some of the important advances in microbiology made over the years in the context of other historical events. Rather than cover more history now, we will return to many of these milestones in brief stories called "A Glimpse of History" that open each chapter.

The Scientific Method

The dispute over spontaneous generation offers an excellent example of the process of science. This process, called the **scientific method,** separates science from intuition and beliefs. The scientific method involves a series of steps, including:

- **Making an observation and asking a question about that situation.** An example from this chapter was the observation that microorganisms were present in various examined specimens. This observation led to the question, "Where did the microorganisms originate?"
- **Developing an explanation and then devising an experiment that tests the explanation.** A testable explanation of an observation is called a **hypothesis,** and experiments are done to test the hypothesis. The dispute over spontaneous generation led to two opposing hypotheses: biogenesis and spontaneous generation. Various people designed different experiments to test the hypotheses.
- **Doing the experiment, collecting the data, and drawing a conclusion.** Experiments such as the one illustrated in figure 1.2 provided data about the growth of

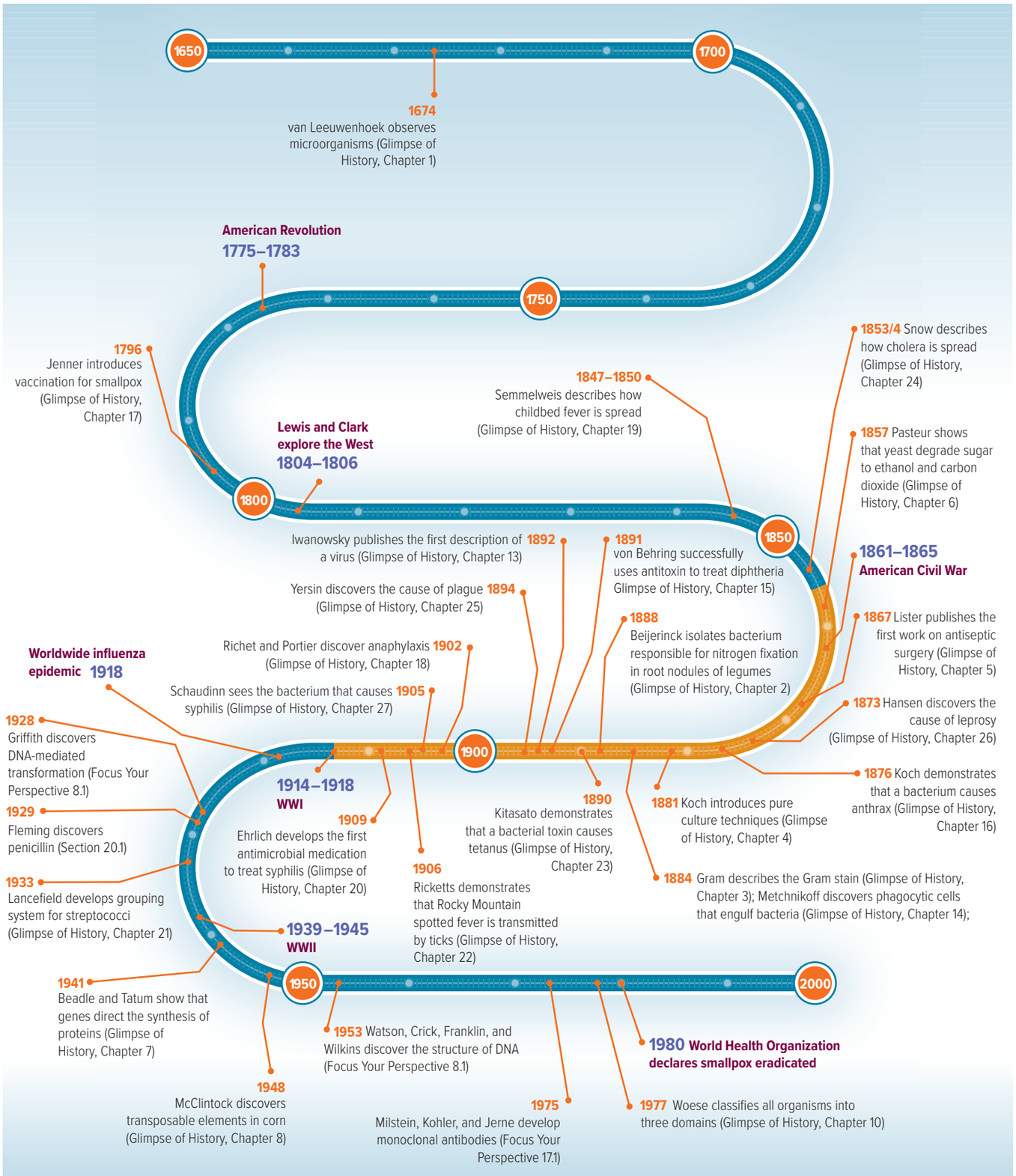


FIGURE 1.3 Historical Events in Microbiology Some major milestones in microbiology—and their timeline in relation to other historical events. The gold band indicates the Golden Age of Microbiology.

? What is the Golden Age of Microbiology?

microorganisms in previously sterile broths. In doing a scientific experiment, a critical component is a **control.** A control helps rule out alternative explanations of the results by showing that the only feature that varied in the experiment was the characteristic being tested. Pasteur's swan-necked flask experiment was brilliantly designed because it provided the following control: After showing that the fluid in the swan-necked flasks remained sterile even when opened to air, he tipped the flasks so that bacteria could enter the fluid. By doing this, he showed that nothing in his original set-up would have prevented bacteria from growing in the broth.

- **Communicating the methods, results, and conclusions.** Scientists share their work by publishing it in scientific journals. This step is particularly important because it allows other scientists to repeat the experiment to ensure the validity of the findings. Today, the respected scientific journals use a review process in which other experts in the field read communications before they are published. If deficiencies or flaws are noticed, the reviewers give suggestions for improving the experiments.

When an extensive amount of experimental evidence supports a hypothesis, that explanation may become a scientific **theory,** such as the Germ Theory of Disease. Note that the scientific meaning of the word *theory* is far different from the meaning of the word in common language, which is "a speculation or guess."

As you read the information in this textbook, continually challenge yourself by asking questions about what you have learned. If you find yourself asking a question such as "How does that happen?" try to develop a hypothesis and then devise an experiment. As you do this, consider the controls you could use. Start learning to think like a scientist!

MicroAssessment 1.1

Experiments of Pasteur and Tyndall helped disprove spontaneous generation by showing that life arises from life. Many important discoveries were made during the Golden Age of Microbiology, including ones that led to the acceptance of the Germ Theory of Disease. The scientific method uses experimental evidence, including proper controls, to support or refute hypotheses.

1. Describe Pasteur's experiment that disproved the idea that a "vital force" in air was responsible for spontaneous generation.
2. How is the meaning of the word "theory" in science different from its meaning in everyday conversation?
3. Why is it important for scientists to repeat the experiments of others?

1.2 ■ Microbiology: A Human Perspective

Learning Outcomes

4. Explain the importance of microorganisms in the health of humans and the surrounding environment.
5. List three commercial benefits of microorganisms.
6. Describe why microorganisms are useful research tools.
7. Describe the role of microbes in disease, including examples of past triumphs and remaining challenges.

Microorganisms have a tremendous impact on all living things. We could not survive without them, and they also make our lives much more comfortable. At the same time, microbes can be harmful, and they have killed far more people than have ever been killed in war.

The Human Microbiome

Your body carries an enormous population of microorganisms—tens of trillions of bacterial cells alone. Many sources claim that the body carries 10 times as many microbial cells as human cells, but recent and probably more accurate estimates indicate that the ratio is likely closer to 1:1. Regardless, scientists have known for years that these microorganisms, collectively referred to as the **normal microbiota** or normal flora, play an essential role in human health. For example, they prevent disease by competing with disease-causing microbes, help to degrade foods that the body otherwise could not digest, and promote the development of the immune system. In fact, studies indicate that early exposure to certain common microorganisms lessens the likelihood that an individual will develop allergies, asthma, and some other diseases. According to what is sometimes referred to as the "Old Friends" hypothesis, this early exposure helps the immune system learn to distinguish "friendly" microbes from those that can cause severe disease. In addition, animal studies suggest that the composition of the normal microbiota can affect brain chemistry and behavior, as well as the tendency to gain or lose weight.

The important role of the normal microbiota became even more obvious in recent years, thanks in part to the **Human Microbiome Project.** This coordinated set of studies, started in 2007, used DNA sequencing technologies to characterize the microbial communities that inhabit the human body. The term **microbiome** has two overlapping meanings: (1) the total genetic content of a microbial community and (2) the microbial community itself. While the different meanings might seem confusing, they are actually quite similar because at this point the communities must be examined by studying their genetic material. The reason for this is that less than 1% of microorganisms can currently be grown in the laboratory, so for every microbe that had been studied in the laboratory, more than 99 others can only be characterized using DNA sequencing technologies.

The Human Microbiome Project changed the way scientists view the human body and also revealed how much more there is to discover about our microbial partners. To understand their significance, think of Earth's ecosystems (the environments and their interacting inhabitants). Over time, an interacting assortment of organisms has evolved to live in a given environment, resulting in a relatively stable community. Sudden changes can alter individual populations, often with negative consequences to the community as a whole. In turn, a disturbance in one ecosystem can affect the overall health of the planet. The human body, like a planet, is composed of various ecosystems—for example, the desert-like dry areas of the skin, and the nutrient-rich environment of the intestinal tract. An important part of these ecosystems is a population of interacting microbes. Disturbances in a microbial population can create an imbalance that may have negative consequences to that community, which, in turn, can harm a person's health. Observations such as these have led some scientists to suggest that the human body be considered a superorganism, meaning that our own cells interact with the body's normal microbiota to form a single cooperative unit.

The human microbiome's effect on health and disease is an exciting area of active research, but it is more difficult to understand than it might seem. For example, researchers have found that the intestinal microbiome of people diagnosed with depression differs from those who report a good quality of life, but this correlation could be an effect of mood—perhaps even dietary changes associated with certain moods—rather than a cause. Likewise, bacterial species associated with gum disease have been found in the brains of people with Alzheimer's disease, but again, this could be effect rather than cause. Continuing studies aim to clarify the situation.

MicroByte

The Global Microbiome Conservancy is collecting fecal samples from people around the world in an effort to study and preserve the diversity of intestinal bacteria.

Microorganisms in the Environment

Microorganisms are the masters of recycling, and without them we would run out of certain nutrients. For instance, humans and other animals all require nitrogen, an essential part of nucleic acids and proteins. A plentiful source of nitrogen is N_2—the most common gas in the atmosphere—yet neither plants nor animals can use it. Instead, we depend on certain microbes that convert N_2 into a form of nitrogen that other organisms can use, a process called nitrogen fixation. Without nitrogen-fixing microbes, life as we know it would not exist.

Microorganisms are also important because they can degrade certain materials that other organisms cannot. Cellulose (an important component of plants) is an excellent example. Although humans and other animals cannot digest cellulose, certain microorganisms can, which is why leaves and fallen trees do not pile up in the environment. Cellulose-degrading microorganisms in the specialized stomach of ruminants (a group of plant-eating animals that includes cattle, sheep, and deer) help those animals digest plant material. Without the assistance of microbes, the ruminants would starve.

In recognition of the important role that microorganisms play in all aspects of life, additional programs promise to expand the scope of existing DNA-based studies. In 2016, the National Microbiome Initiative (NMI) was started to support research on the microbiomes of humans as well as the surrounding environment. Perhaps the most ambitious DNA sequencing program so far is the Earth BioGenome Project, an international effort launched in 2018 to sequence all the known animal, plant, protozoan, and fungal species.

Commercial Benefits of Microorganisms

In addition to the crucial roles microorganisms play in our very existence, they also have made life more comfortable for humans over the centuries.

Food Production

Microorganisms have been used in food production since ancient times. In fact, Egyptians used yeast to make bread and beer. Virtually every population that raised milk-producing animals such as cows and goats also developed procedures to ferment milk. This allowed them to make foods such as yogurt, cheeses, and buttermilk. Today, the bacteria added to some fermented milk products are advertised as probiotics (live microorganisms that provide a health benefit), protecting against digestive disruptions.

Biodegradation

Microorganisms play essential roles in degrading various environmental pollutants. These include materials in wastewater, as well as toxic chemicals in contaminated soil and water. Bacteria also lessen the damage from oil spills. In some cases, microorganisms are added to pollutants to hasten their decay, a process called **bioremediation.**

Commercially Valuable Products from Microorganisms

Microorganisms synthesize a wide variety of commercially valuable products. Examples include: antibiotics used to treat infectious diseases, ethanol used as a biofuel, hydrogen gas and certain oils potentially used as biofuels, amino acids used as dietary supplements, insect toxins used in insecticides, cellulose used in headphones, and polyhydroxybutyrate used in the manufacture of disposable diapers and plastics.

Biotechnology

Biotechnology—the use of microbiological and biochemical techniques to solve practical problems—depends on members of the microbial world. Information learned by studying microorganisms led to easier production of many medications, including the insulin used to treat diabetes. In the past, insulin was isolated from the pancreatic glands of cattle and pigs,

but now certain microorganisms have been genetically engineered to make human insulin. The microbe-produced insulin is easier to obtain, and patients who use it have fewer allergic reactions than occurred with the animal-derived product. Biotechnology also allows scientists to genetically engineer plants to give them desirable qualities.

Microbes as Research Tools

Microorganisms are wonderful model organisms to study because they have the same fundamental metabolic and genetic properties as higher life-forms. All cells are composed of the same chemical elements, and they synthesize their cell structures by similar mechanisms. They all duplicate their DNA, and when they degrade foods to harvest energy, they do so via the same metabolic pathways. To paraphrase a Nobel Prize–winning microbiologist, Dr. Jacques Monod: What is true of elephants is also true of bacteria, and bacteria are much easier to study! In addition, bacteria can be used to obtain results very quickly because they grow rapidly and form billions of cells per milliliter on simple, inexpensive growth media. In fact, most major advances made in the last century toward understanding life have come through the study of microbes.

Microbes and Disease

Although most microbes are beneficial or not harmful, some are **pathogens,** meaning they can cause disease (a noticeable impairment in body function). The disease symptoms can result from damage caused by the pathogen's growth and products or by the body's defense mechanisms inadvertently damaging host tissues during the attempt to control the infection.

To appreciate the effect an infectious disease can have on a population, consider that more Americans died of influenza in 1918–1919 than were killed in World Wars I and II and the Korean, Vietnam, and Iraq wars combined. The COVID-19 pandemic has resulted in the death of more than 1,000,000 people worldwide, including over 200,000 Americans.

Epidemics are not limited to human populations. The great famine in Ireland in the 1800s was due, in part, to a microbial disease of potatoes. A bacterial disease that kills olive trees was found in southern Italy in 2013, and it has since spread to Spain and France, contributing to a recent worldwide drop in olive oil production. A fungal disease called "wheat blast" that devastated wheat crops in South America spread to Bangladesh in 2016, resulting in the loss of over 35,000 acres of crops that year. In 2001, a catastrophic outbreak of foot-and-mouth disease of livestock occurred in parts of England. To contain this viral disease, one of the most contagious known, almost 4 million pigs, sheep, and cattle were destroyed. More recently, over a million pigs in China either died from African swine fever or were killed to contain the disease, and officials in other countries are trying to limit its spread. Meanwhile, frog populations around the world have been decimated by chytridiomycosis, a fungal disease.

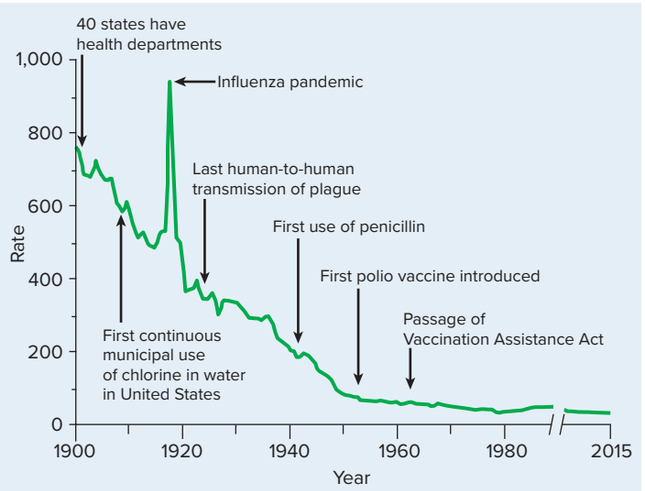


FIGURE 1.4 Trend in Death Rates Due to Infectious Diseases
Crude death rate for infectious disease, United States, per 100,000 population per year.

? **Why would the creation of health departments lower the disease rate?**

Past Triumphs

The Golden Age of Microbiology included an important period when scientists learned a great deal about pathogens. Between 1876 and 1918, most pathogenic bacteria were identified, and early work on viruses had begun. Once people realized that microbes could cause disease, they tried to prevent their spread. As illustrated in **figure 1.4,** the death rate due to infectious diseases has decreased dramatically over the last 100 years or so, due largely to preventing the spread of pathogens, developing vaccines to provide immunity, and using antibiotics to treat bacterial diseases when they do occur. To maintain this success, we must continue to develop new medications, vaccines, and disease-prevention strategies.

Perhaps the most significant triumph with respect to disease control was the eradication (elimination) of smallpox. This viral disease was one of the most devastating the world has ever known, killing about one-third of those infected. Survivors were sometimes blinded and often left with disfiguring scars. When Europeans carried the disease to the Americas, the effect on the populations of native inhabitants who had not been exposed before was catastrophic. A worldwide vaccination program eliminated the disease in nature, with no cases being reported since 1977. Laboratory stocks of the smallpox virus remain, however, raising the possibility that the virus could be used in bioterrorist attacks.

Polio, a disease that can cause paralysis and sometimes death, was once relatively common, but it has been nearly eliminated because of vaccination. In fact, the disease now occurs in only a few countries, and the goal is to eradicate it globally.

Plague is another major killer that has largely been brought under control. In the fourteenth century, one-third of the population of Europe, or approximately 25 million people, died of this bacterial disease in only 4 years (1347–1351).

We now know that rodents can carry the bacterium, and their fleas can transmit the disease, so we take measures to control the rodent populations. We have also learned that the pneumonic form of the disease (meaning that it is in the lungs) can spread from human to human through respiratory secretions, so special precautions are taken when a patient has pneumonic plague. In addition, the discovery of antibiotics in the twentieth century made treatment possible. As a result, fewer than 100 people worldwide die from plague in a typical year.

Remaining Challenges

Although progress has been impressive against infectious diseases, much more still needs to be done. On a worldwide basis, infectious diseases remain too common, particularly in developing countries. Even in developed countries with sophisticated healthcare systems, infectious diseases remain a serious threat, costing lives and money.

Emerging Infectious Diseases An **emerging infectious disease (EID)** is an infectious disease that has become more common in the last several decades. The EID that everyone is now likely familiar with is COVID-19 (for coronavirus disease 2019), the disease that emerged in late 2019 and then spread rapidly around the globe. COVID-19 is caused by a virus officially called SARS-CoV-2 (for severe acute respiratory syndrome coronavirus 2) but commonly referred to as the COVID-19 virus. Like COVID-19, many EIDs are new or newly recognized; examples include Ebola disease (EBOD), congenital Zika syndrome, *Candida auris* infection, hepatitis C, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), certain types of influenza, Lyme disease, acquired immunodeficiency syndrome (AIDS), mad cow disease (bovine spongiform encephalopathy), and hantavirus pulmonary syndrome (**figure 1.5**). Others such as malaria and tuberculosis have been present for years but have spread or become more common recently.

Some diseases arise as infectious agents evolve to infect new hosts, cause different types of damage, or become more difficult to treat because of antibiotic resistance. Genetic analysis indicates that the virus that causes COVID-19 arose from a strain that infects bats. HIV-1 (**h**uman **i**mmunodeficiency **v**irus type 1), the most common type of HIV to cause AIDS, arose from a virus that infects chimpanzees. A bacterium called *E. coli* O104:H4, which caused a severe foodborne diarrheal outbreak in Europe, appears to have gained the ability to make a specific toxin by acquiring genes from a related organism. Tuberculosis and malaria have increased in

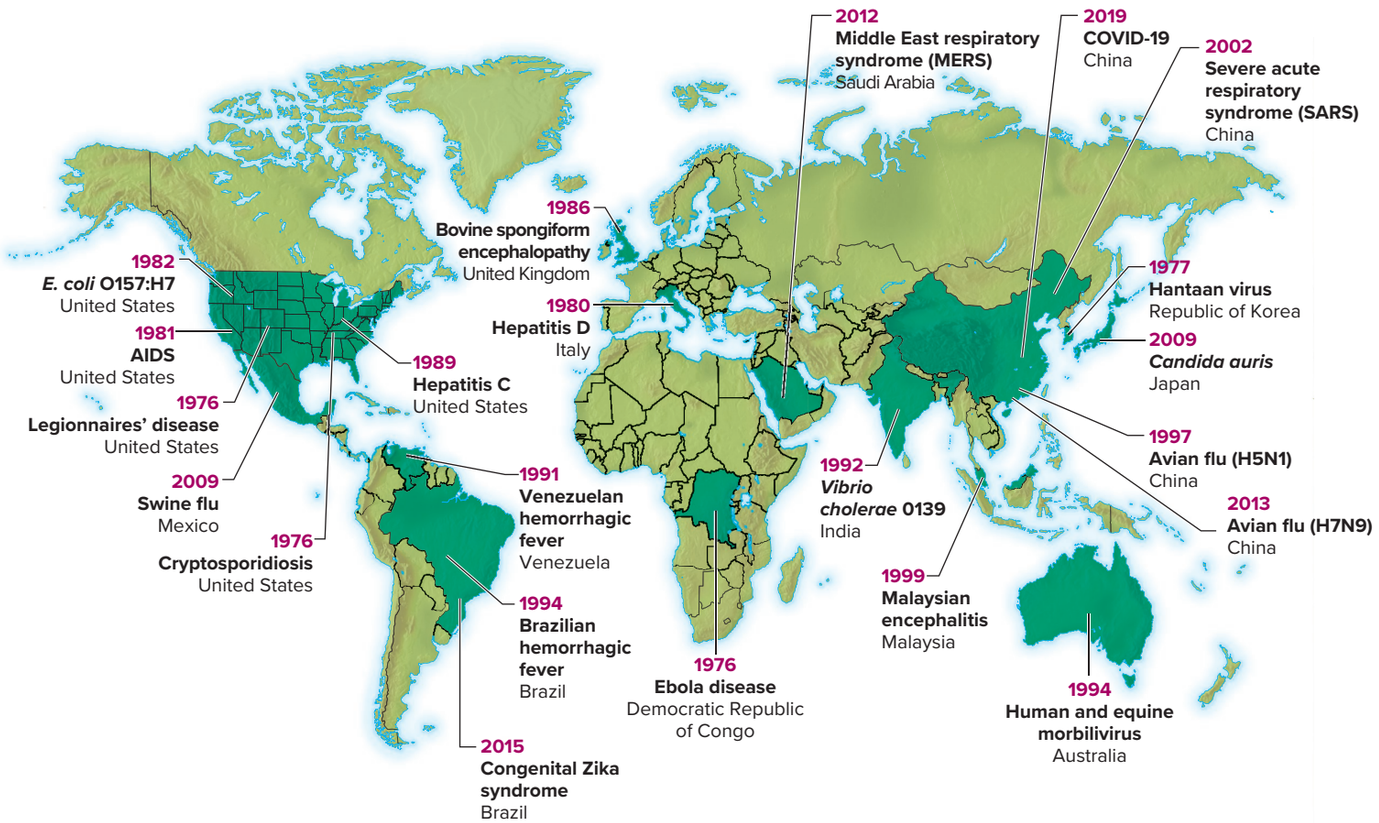


FIGURE 1.5 New and Newly Recognized Infectious Diseases or Disease Agents in Humans and Animals Since 1976 Countries where cases first appeared or were identified appear in a darker shade.

? Why might so many of the diseases first appear or be identified in the United States and Western European countries?

FOCUS ON A CASE 1.1

A 24-year-old woman had suffered from recurrent severe episodes of an intestinal disorder called *Clostridioides (*formerly *Clostridium) difficile* infection (CDI) for the past 13 months. She routinely experienced profuse watery diarrhea, abdominal pain, and fever. In addition, she was feeling tired and hopeless because she did not seem to be getting well, despite long attempts at multiple different treatments.

As with most patients who develop CDI, the woman had been taking an oral antibiotic shortly before her symptoms began—in this case, to treat a tooth infection. The antibiotic had successfully killed the bacteria that caused her tooth infection, but it also killed some members of her normal intestinal microbiota. As a result, the bacterium *C. difficile*—often referred to simply as "*C. diff*"—thrived in her intestinal tract, growing to much higher numbers than it could before. The strain that caused her infection was able to make a toxin that damaged the lining of her intestinal tract.

When the patient first started experiencing CDI, her doctor told her to stop taking the antibiotic prescribed for her tooth infection, hoping that her CDI would resolve on its own. When that did not help, the doctor prescribed a different antibiotic that is often effective in treating CDI. The patient started feeling better, but the symptoms quickly returned when she stopped taking the medication. She also tried oral supplements containing *Lactobacillus* GG, a bacterium that sometimes appears to be effective in preventing antibiotic-associated diarrhea.

Because the patient's health was declining, doctors suggested a fecal transplant, an experimental procedure that involves inserting feces from a healthy person into the patient's intestinal tract in order to repopulate that environment with appropriate microbes. They chose to use her sister as a fecal donor, screening both the donor and the patient to ensure that neither was infected with certain microbes, including various intestinal pathogens and HIV. Approximately ¼ cup of fresh feces was mixed with 1 quart of water and delivered to her intestinal tract via a colonoscope. Within days after the transplant, the patient began feeling better, and she soon recovered completely.

1. Why would certain oral antibiotics allow *C. difficile* to thrive in the intestinal tract?
2. Why would the doctors screen both the patient and the fecal donor for certain infectious agents?
3. Why would the doctors transplant feces rather than introducing isolated bacteria from feces to repopulate the colon?

Discussion

1. Antibiotics kill or inhibit not just pathogens, but also beneficial members of the normal microbiota, a group that protects against infection in at least two general ways. First, the normal microbiota quickly uses nutrients that would otherwise be available to *C. difficile* and other pathogens. Also, some members of the normal microbiota make compounds that are toxic or inhibitory to other organisms. The environment of the intestinal tract is quite complex, however, so other factors might also be playing a role.
2. Physicians screen the fecal donor to decrease the likelihood that disease-causing microbes could be transferred to the patient by the procedure. The doctors screen the patients to ensure that they are not already infected with the pathogens. For example, if this patient developed symptoms of a *Salmonella* infection after the procedure, how would the physicians know that she acquired the infection as a result of the procedure if they had not checked beforehand?
3. Feces contain many types of bacteria that cannot yet be grown in the laboratory. In addition, scientists do not yet know which types of fecal bacteria protect against CDI.

incidence in recent years, in part because the causative organisms became resistant to many of the available medications.

As the rapid spread of COVID-19 around the globe certainly demonstrated, mobile populations can contribute to disease emergence as people may inadvertently carry pathogens to different regions. Even diseases such as malaria, cholera, plague, and yellow fever that have largely been eliminated from developed countries can be carried to other places if travelers to regions where they still exist become infected and then move on before recovering. Meanwhile, as city suburbs expand into rural areas, human populations come into closer contact with animals as well as the mosquitoes and other arthropods that normally feed on those animals. Consequently, people are exposed to pathogens they might not have encountered previously.

The preventive measures used to control certain infectious diseases can become victims of their own success, a situation that can also lead to disease emergence. Decades of vaccination have nearly eliminated measles, mumps, and whooping cough in developed countries, so most people no longer have first-hand knowledge of the dangers of these diseases. Couple this with misinformation about vaccines, and some people develop irrational fears, falsely believing that vaccines are more harmful than the diseases they prevent. When this happens, parents often refuse to vaccinate their children appropriately, leading to situations where the diseases become more common again. Measles had been declared eliminated in the United States in 2000, but outbreaks in 2019 resulted in the highest number of cases in 25 years. Outbreaks generally start with unvaccinated travelers who bring the disease into the country, where it then spreads among others who are not vaccinated.

Chronic Diseases Some chronic illnesses once attributed to other causes may be due to microorganisms. Perhaps the best-known example is stomach ulcers, once thought to be due to stress. We now know that stomach ulcers are often caused by a bacterium *(Helicobacter pylori)* and are treatable with antibiotics. Chronic indigestion may be caused by the same bacterium. Another example is cervical cancer, which we now know is caused by human papillomavirus (HPV) infection; a vaccine against HPV prevents that cancer. Infectious microbes may play important roles in other chronic diseases as well.

Focus Figure

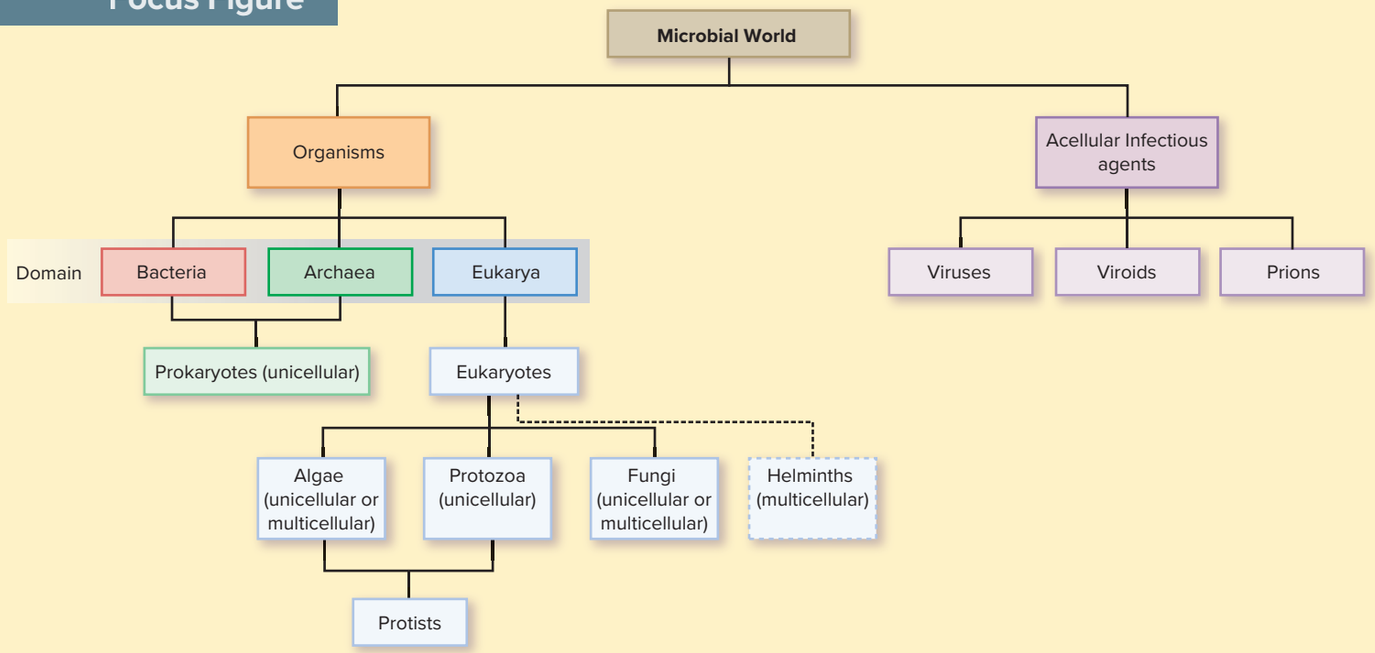


FIGURE 1.6 The Microbial World Although adult helminths (worms) can often be seen with the naked eye, some stages in the life cycle of helminths are microscopic.

Members of which two domains are prokaryotes?

MicroAssessment 1.2

Microbial activities are essential to human life as well as being commercially valuable. Microbes are important research tools. Although most microbes are beneficial or not harmful, some cause disease. Enormous progress has been made in preventing and curing infectious diseases, but some diseases are becoming more common.

4. Describe two microbial activities essential to life and three that make our lives more comfortable.
5. Describe three factors that cause certain infectious diseases to become more common.
6. Why would it seem logical, even inevitable, that at least some bacteria would attack the human body and cause disease?

1.3 ■ Members of the Microbial World

Learning Outcomes

8. Compare and contrast characteristics of members of the Bacteria, Archaea, and Eukarya.
9. Explain the features of an organism's scientific name.
10. Compare and contrast the algae, fungi, and protozoa.
11. Compare and contrast viruses, viroids, and prions.

Considering that small size is the only shared feature of all microbes, the group is tremendously diverse. If you look at the macroscopic world around you—the plants and animals—you should be impressed by the assortment of what you see. That range of types, however, is dwarfed by the huge variety of microbes! The extent of that diversity makes sense considering that microbes have inhabited this planet for billions of years and have evolved to thrive in every conceivable environment—from the hydrothermal vents at the bottom of the ocean to the icy tops of the highest mountains. Many people associate microbes with disease, but their contributions to our world go far beyond that. In fact, as section 1.2 described, we could not survive without them.

Living organisms are all composed of cells with one of two basic structures—prokaryotic (*pro* means "prior to" and *karyote* means "nucleus") and eukaryotic (*eu* means "true"). **Prokaryotic cells** do not have a membrane-bound nucleus. Instead, the genetic material is located in a region called the nucleoid. In contrast, the genetic material in eukaryotic cells is contained within a membrane-bound nucleus. **Eukaryotic cells** often have a variety of other membrane-bound organelles as well, and they are typically much larger and more complex than prokaryotic cells. Organisms that consist of one or more eukaryotic cells are called **eukaryotes,** whereas those composed of a prokaryotic cell are called **prokaryotes.** Prokaryotes fall into two very different groups—bacteria and archaea—as different from each other as they are from eukaryotes.

Because of the fundamental differences between bacteria, archaea, and eukaryotes, all living organisms are now classified into three different **domains:** Bacteria, Archaea, and Eukarya (sometimes spelled Eucarya) (**figure 1.6**).

TABLE 1.1 Characteristics of Members of the Three Domains

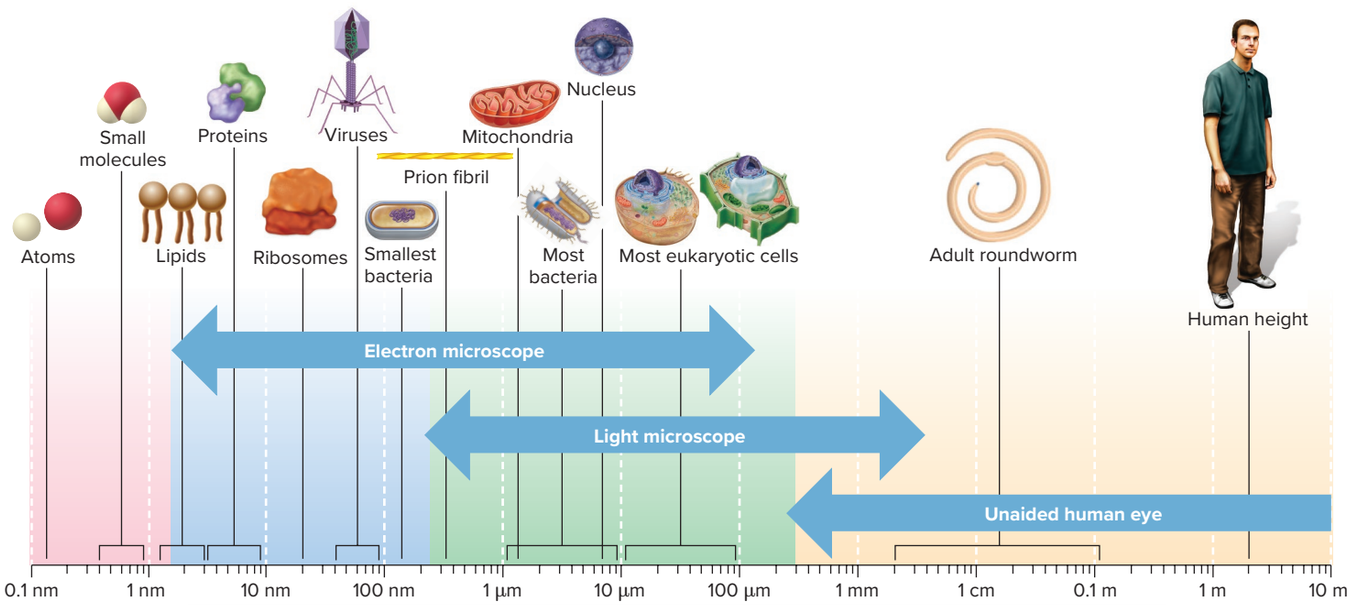
Characteristic	Bacteria	Archaea	Eukarya
Cell type	Prokaryotic	Prokaryotic	Eukaryotic
Number of cells	Unicellular	Unicellular	Unicellular or multicellular
Membrane-bound organelles	No	No	Yes
Ribosomal RNA sequences unique to the group	Yes	Yes	Yes
Peptidoglycan in cell wall	Yes	No	No
Typical size range	0.3–2 μm	0.3–2 μm	5–50 μm

Members of the Bacteria and Archaea are prokaryotes, whereas members of the Eukarya are eukaryotes. The names of the domains have the first letter capitalized and the members of the domains are referred to as bacteria, archaea, and eukarya, respectively. **Table 1.1** compares some features of members of the three domains, but you will learn other important differences in later chapters.

The small size of microbes requires measurements not commonly used in everyday life (**figure 1.7**). Logarithms are extremely helpful in this regard, so you will find a brief discussion of them in Appendix I.

Scientific Names

When referring to microbes, we use their scientific names, which are written and pronounced in a Latin style. A pronunciation guide for these names is in Appendix II, and an audio version is available on your Connect class site. The scientific names are assigned according to the binomial (two-part name) system of nomenclature developed by Carl Linnaeus in the 1700s. The first part of the name indicates the **genus,** with the first letter always capitalized; the second part indicates the specific epithet, or **species,** and is not capitalized. Both are usually italicized or underlined—for example, *Escherichia coli.* The



The basic unit of length is the meter (m), and all other units are fractions of a meter.	These units of measurement correspond to units in an older but still widely used convention.
nanometer (nm) = 10^{-9} meter = 0.000000001 meter micrometer (μm) = 10^{-6} meter = 0.000001 meter millimeter (mm) = 10^{-3} meter = 0.001 meter 1 meter = 39.4 inches	1 angstrom (Å) = 10^{-10} meter 1 micron (μ) = 10^{-6} meter

FIGURE 1.7 Sizes of Molecules, Non-Living Agents, and Organisms Note that the scale here is logarithmic (rather than linear), and each labeled increment increases by a factor of 10.

? Why is a logarithmic scale useful when comparing sizes of members of the microbial world?

FOCUS YOUR PERSPECTIVE 1.1

Every Rule Has an Exception

We might assume that because microorganisms have been so intensively studied over the past hundred years, no major surprises are left to be discovered. This, however, is far from the truth. In the mid-1990s, a large, peculiar-looking organism was found in the intestinal tract of certain fish from both the Red Sea in the Middle East and the Great Barrier Reef in Australia. This organism, named *Epulopiscium fishelsoni*, cannot be cultured in the laboratory. Its large size (600 µm long and 80 µm wide) make it clearly visible without any magnification and suggested that this organism was a eukaryote. However, it does not have a membrane-bound nucleus. A chemical analysis of the cell confirmed that it is a prokaryote and a member of the domain Bacteria.

In 1999, an even larger prokaryote was isolated from the muck of the ocean floor off the coast of Namibia in Africa. It is a spherical organism 70 times greater in volume than *E. fishelsoni.* Since it grows on sulfur compounds and contains glistening globules of sulfur, it was named *Thiomargarita namibiensis,* meaning "sulfur pearl of Namibia."

In contrast to the examples of large bacteria, a unicellular alga found in the Mediterranean Sea is 1 µm in width. It is a eukaryote even though it is about the size of a typical bacterium.

How small can an organism be? A microbe discovered off the coast of Iceland is only about 400 nm (nanometers) in diameter and has one-tenth the amount of genetic information (DNA) compared to the common intestinal bacterium *Escherichia coli.* The tiny organism was found attached to a much larger microbe, a member of the Archaea growing in an ocean vent where the temperature was close to the boiling point of water. The larger organism is an *Ignicoccus* species (*igni* means "fire" and *coccus* means "sphere"). The tiny one, also a member of the Archaea, has been named *Nanoarchaeum equitans* (meaning "tiny archaea" and "rider"). *N. equitans* cannot be grown in the laboratory by itself, but *Ignicoccus* grows well without its *Nanoarchaeum* "rider."

These exceptions to long-standing rules point out the need to keep an open mind and not jump to conclusions! They also serve as excellent reminders that in a subject as complex as microbiology, there will almost always be exceptions!

part indicating the genus is commonly abbreviated, with the first letter capitalized—as in *E. coli.*

The origin of one or both parts of the name often reflects a characteristic of the organism or honors a particular scientist (**table 1.2**). In the case of *Escherichia coli,* the name of the genus honors Theodor Escherich, who discovered the bacterium; the species designation indicates the site where *E. coli* typically lives: the colon (large intestine). Within a given genus, there may be a number of different species. For example, the genus *Escherichia* includes species other than *E. coli*, such as *E. vulneris,* which was first isolated from human wounds (*vulneris* means "of a wound"). *E. vulneris* is genetically related to *E. coli,* but not closely enough to consider it in the same species.

Members of the same species may vary from one another in minor ways, but not enough to separate the organisms into different species. A genetic variant within a species is called a **strain.** In situations where genetic differences are important, such as in research, a particular microbe and its progeny may be indicated with a strain designation—for example, *E. coli* B or *E. coli* K12.

Groups of microbes are often referred to informally by names that resemble genus names but are not italicized. For instance, members of the genus *Staphylococcus* are often called staphylococci.

MicroByte

Bacterial species outnumber mammalian species by more than 10,000-fold!

TABLE 1.2 Origin of Various Scientific Names

Name	Genus Derivation	Species Derivation
***Escherichia coli* (bacterium)**	Honors Theodor Escherich, the scientist who discovered the bacterium.	Derived from the word "colon," the body site inhabited by the bacterium.
***Haemophilus influenzae* (bacterium)**	Derived from *haemo* (blood) and *phil* (loving), reflecting that the bacterium requires certain components of blood for growth.	Derived from the word "influenza," the disease mistakenly thought to be caused by the bacterium; we now know that influenza is caused by a virus.
***Saccharomyces cerevisiae* (fungus)**	Derived from *saccharo* (sugar) and *myces* (fungus).	Derived from *cerevisia* (beer), reflecting that the fungus (a yeast) is used to make beer.
***Shigella dysenteriae* (bacterium)**	Honors Kiyoshi Shiga, the scientist who discovered the bacterium.	Derived from the word "dysentery," the disease caused by the bacterium.
***Staphylococcus aureus* (bacterium)**	Derived from *staphylo* (bunch of grapes) and *kokkus* (berry), reflecting the grouping and shape of the cells.	The term *aureus* (golden) indicates the common color of visible masses of the cells.

Bacteria

Bacteria (singular: bacterium) are single-celled prokaryotes. They typically have rigid cell walls that contain peptidoglycan, a compound unique to bacteria. Many of the bacteria can move using flagella (singular: flagellum), filamentous appendages that extend from the cell.

Bacteria as a whole come in a variety of shapes, but cells of a given species are usually only one shape. Bacterial shapes include (**figure 1.8**):

- **Coccus (plural: cocci).** A spherical cell that may be flattened on one end or slightly oval.
- **Rod or bacillus (plural bacilli).** A cylindrical cell. One short enough to be confused with a coccus is called a coccobacillus. Note that the descriptive term "bacillus" should not be confused with *Bacillus,* the name of a genus. Although members of the genus *Bacillus* are rod-shaped, so are many other bacteria, including *Escherichia coli.*
- **Vibrio (plural: vibrios).** A short, curved rod.
- **Spirillum (plural: spirilla).** A curved rod long enough to form spirals.
- **Spirochete (plural: spirochetes).** A long, spiral-shaped cell with a flexible cell wall and a unique mechanism of motility.
- **Pleomorphic.** This is not an actual shape, but refers to bacteria that characteristically vary in their shape (pleo meaning "many" and morphic referring to shape).

Bacteria typically multiply by binary fission, a process in which one cell enlarges and then divides. This forms two cells, each equivalent to the original. The dividing cells often stick to each other, forming characteristic arrangements such as pairs, chains, or clusters, depending on the planes of division (**figure 1.9**). Cocci that typically remain as pairs are called diplococci; an important clue in the identification of *Neisseria gonorrhoeae* is its characteristic diplococcus arrangement. Division in one plane can also form long chains, a characteristic typical of some members of the genus *Streptococcus* (*strepto* means "twisted chain"). Cocci that divide in perpendicular planes form cubical packets; members of the genus *Sarcina* form such packets. Cocci that divide in several planes at random may form clusters; *Staphylococcus* species, which typically form grapelike clusters, are an example (*staphylo* means "bunch of grapes").

Some prokaryotes characteristically live as multicellular associations. Members of a group of bacteria called myxobacteria form swarms of cells that move as a pack, gliding over moist surfaces. The cells release enzymes, and, as a pack, they degrade organic material, including other bacterial cells. When water or nutrients become limiting, the cells come together to form a structure called a fruiting body, which is visible to the naked eye (see figure 11.14).

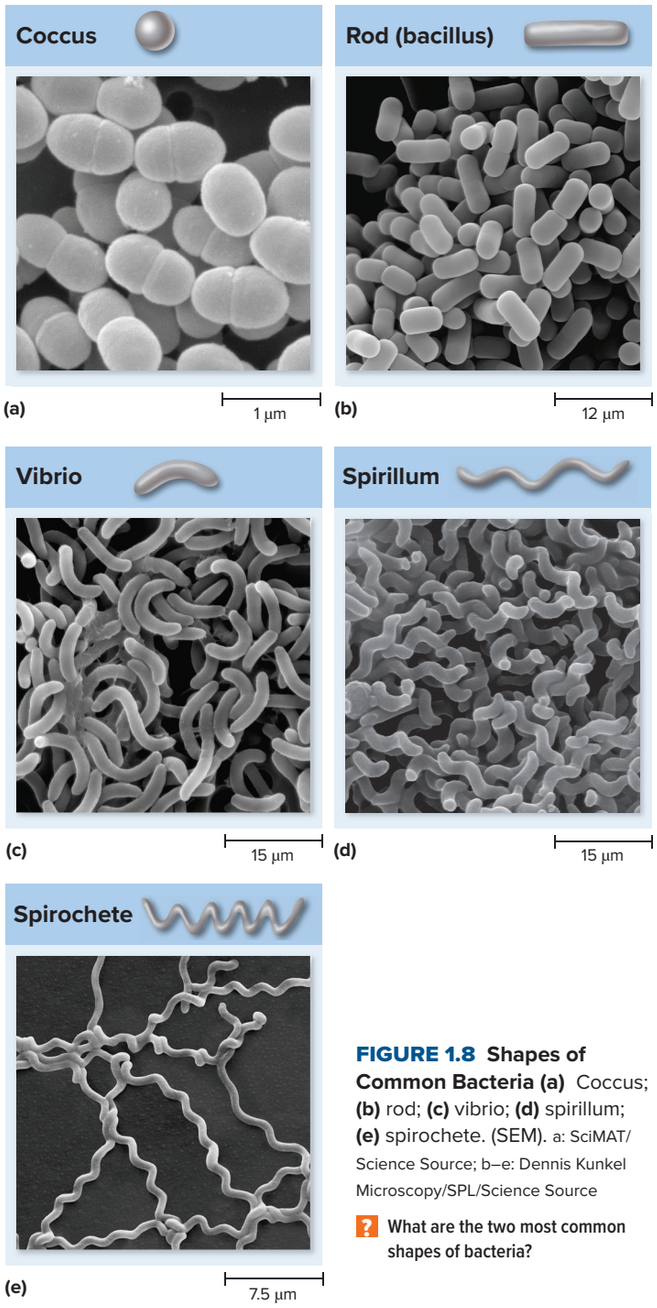


FIGURE 1.8 Shapes of Common Bacteria (a) Coccus; **(b)** rod; **(c)** vibrio; **(d)** spirillum; **(e)** spirochete. (SEM). a: SciMAT/Science Source; b–e: Dennis Kunkel Microscopy/SPL/Science Source

? What are the two most common shapes of bacteria?

Many bacteria obtain energy from foods similar to those humans eat, but others can gain energy from seemingly unlikely sources such as hydrogen sulfide (a gas that smells like rotten eggs). Still others are photosynthetic, meaning they make cellular material using the radiant energy of sunlight.

Although most bacteria are beneficial, some cause serious diseases, and these will be a focus in the "disease chapters" of this textbook. Many of the early chapters will focus on bacteria in general, often with the aim of providing the necessary background for understanding infectious diseases.

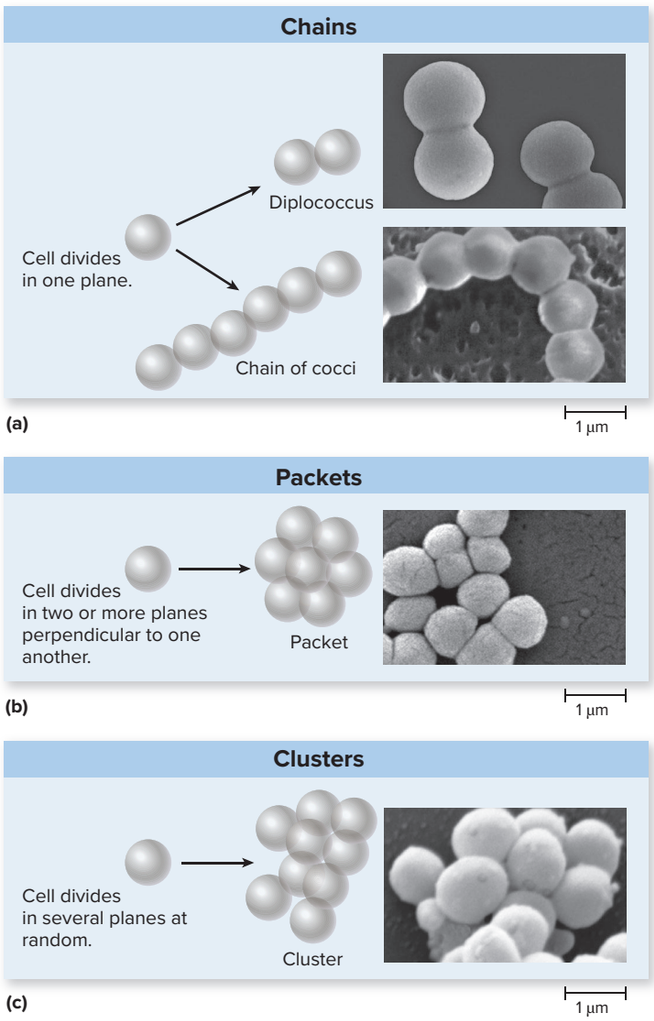


FIGURE 1.9 Common Cell Arrangements (a) Chains; **(b)** packets; **(c)** clusters. (SEM). a (top): Dennis Kunkel Microscopy/SPL/Science Source; a (bottom): BSIP SA/Alamy; b: Source: Betsy Crane/CDC; c: Eye of Science/Science Source

How does bacterial cell division determine the characteristic cell arrangements?

Archaea

Like bacteria, archaea (singular: archaeon) are single-celled prokaryotes. They have similar shapes, sizes, and appearances to bacteria. In addition, they also multiply by binary fission, move primarily by means of filamentous appendages, and have rigid cell walls. Like bacteria, different groups of archaea use different energy sources; some are photosynthetic, harvesting the energy of sunlight to make cellular material. Considering how much archaea look like bacteria, scientists initially believed they were closely related. We now know, however, that there are major differences between the two groups, and the groups are only distantly related to each other. In fact, you are more closely related to plants than archaea are to bacteria!

Archaea differ from bacteria in several of their structural and functional components. For example, the archaeal cell wall does not contain peptidoglycan, whereas the bacterial wall does. In addition, the composition and structural organization of the appendages the archaeal cells use for motility are different from bacterial flagella. Archaea also have characteristic nucleotide sequences in their ribosomal RNA (a molecule involved in protein synthesis) that differ significantly from those of bacteria. The discovery of the differences in ribosomal RNA sequences helped provide the basis for separating the two groups of prokaryotes into different domains.

An interesting feature of many archaea is their ability to grow in extreme environments in which most other organisms cannot survive. Some, for example, can grow in salt concentrations 10 times higher than that of seawater. These organisms grow in such habitats as the Great Salt Lake and the Dead Sea. Others grow best at extremely high temperatures. One archaeon can grow at a temperature of 122°C! (100°C is the temperature at which water boils at sea level.)

Although the archaea that grow in extreme environments are the most intensively studied, many others are common in moderate environments. They are widely distributed in soils, the oceans, and marshes, as well as in the intestinal tracts of animals. Some archaea are part of microbial communities associated with severe periodontitis, a destructive inflammation of the gums.

Eukarya

Eukarya are eukaryotes; those studied by microbiologists include fungi, algae, protozoa, and helminths (worms). Algae and protozoa are also referred to as **protists.**

Fungi

Fungi (singular: fungus) are a diverse group of eukaryotes, ranging from single-celled yeasts that reproduce by budding to multicellular filamentous molds (**figure 1.10**). The microscopic filaments of molds, called hyphae (singular: hypha), can branch as well as twist and turn to form a visible mat called a mycelium. When you see moldy foods, you are looking at the mycelium, sometimes along with structures that give rise to a reproductive form called conidia (also referred to as spores). The conidia easily become airborne, allowing the fungus to spread. Some fungi make macroscopic reproductive structures that we call mushrooms.

Fungi harvest energy from organic materials. To do this, they secrete enzymes that degrade the organic material and then take in the nutrients that are released. Fungi are found in most places where organic materials, including dead plants and animals, are present.

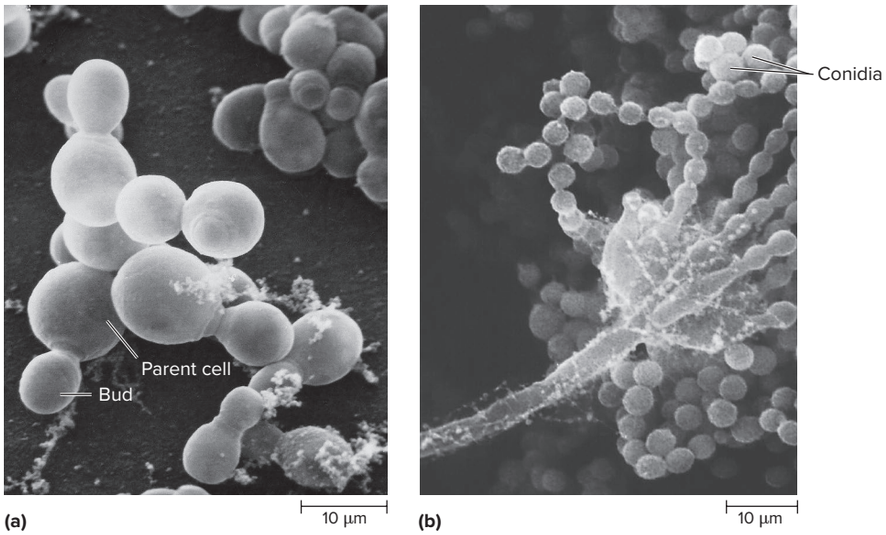


FIGURE 1.10 Fungi (a) Yeast, *Malassezia furfur.* **(b)** *Aspergillus,* a typical mold form whose reproductive structures rise above the mat of hyphae. a: Centers for Disease Control/Janice Haney Carr; b: Source: Janice Haney Carr/CDC

? **Molds and yeasts are made up of what type of cells?**

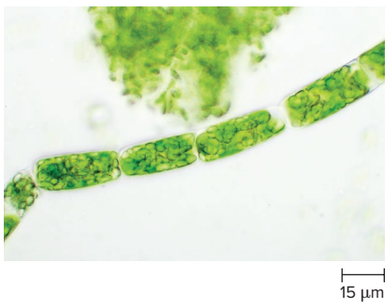


FIGURE 1.11 Alga *Ulothrix,* a filamentous green alga. Lisa Burgess/McGraw-Hill Education

? **What general features of algae distinguish them from other eukaryotic microorganisms?**

Algae

Algae (singular: alga) are a diverse group of photosynthetic eukaryotes. Some are single-celled, whereas others are multicellular, such as seaweed (**figure 1.11**). Photosynthesis in algae occurs in chloroplasts, which have chlorophyll, a green pigment. Some algae also have other pigments as well, giving them characteristic colors. The pigments absorb the energy of light, which is used in photosynthesis.

Algae are usually found near the surface of either salt or fresh water or in moist terrestrial habitats. Their cell walls are rigid, but the chemical composition of the wall is quite distinct from that of bacteria and archaea. Many algae move by means of flagella, which are structurally far more complex and unrelated to flagella of prokaryotes.

Protozoa

Protozoa (singular: protozoan) are a diverse group of microscopic, single-celled eukaryotes that live in both aquatic and terrestrial environments. Although microscopic, they are very complex organisms and generally much larger than prokaryotes (**figure 1.12**). Unlike algae and fungi, protozoa do not have a rigid cell wall. Most protozoa are motile and ingest organic material as food sources.

Helminths

Parasitic **helminths** are worms that live at the expense of a host. They are an important cause of disease, particularly in developing countries. The adult worms are generally macroscopic, meaning they can be seen with the naked eye, and some are quite large, so technically they are not microorganisms. Microbiologists study them, however, because diagnosis of the diseases they cause often involves identifying their eggs and larval forms, which are microscopic. Helminths include roundworms, tapeworms, and flukes.

Table 1.3 summarizes the characteristics of eukaryotic organisms studied by microbiologists.

Acellular Infectious Agents

Viruses, viroids, and prions are acellular infectious agents, meaning that they are not composed of cells. They cannot reproduce independently and are considered

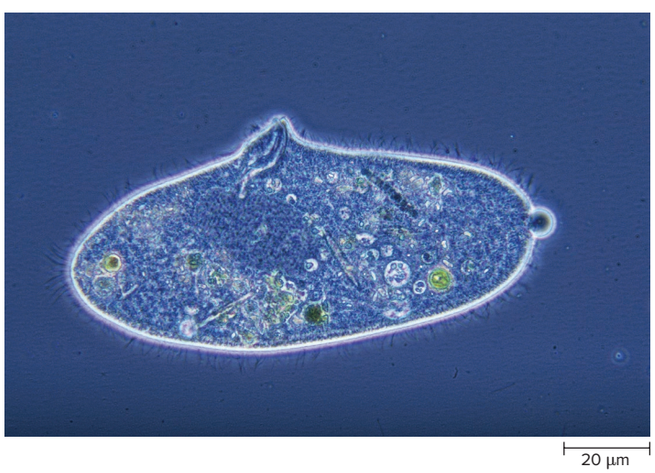


FIGURE 1.12 Protozoan A paramecium moves with the aid of hair-like appendages (called cilia) on the cell surface. Melba Photo Agency/Alamy

? **How do protozoa differ from both fungi and algae?**

TABLE 1.3 **Eukaryotic Organisms Studied by Microbiologists**

Organism	Characteristics
Fungi	Use organic material for energy. Size range from microscopic (yeasts) to macroscopic (molds); mushrooms are the reproductive structures of some fungi.
Algae	Use sunlight for energy. Size range from microscopic (single-celled algae) to macroscopic (multicellular algae).
Protozoa	Use organic material for energy. Single-celled microscopic organisms.
Helminths	Use organic material for energy. Adult worms are typically macroscopic and often quite large, but their eggs and larval forms are microscopic.

non-living. By definition, an organism must be composed of one or more cells, so these acellular infectious agents are not microorganisms.

Viruses

Viruses consist of nucleic acid packaged within a protein coat (**figure 1.13**). To multiply, viruses infect living cells—referred to as **hosts**—and then use the machinery and nutrients of those cells to replicate. Outside the hosts, however, viruses are inactive. Thus, viruses are obligate intracellular agents, meaning that they cannot replicate outside a host.

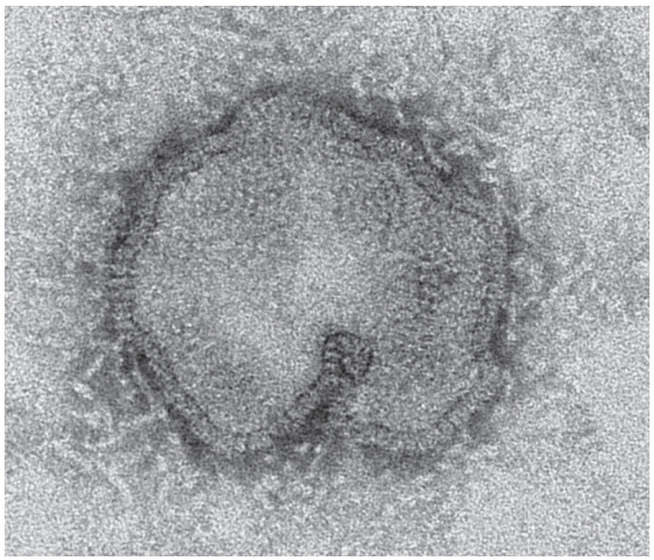


FIGURE 1.13 Virus Influenza virus, the cause of flu.
Source: Cynthia S. Goldsmith and Thomas Rowe/CDC

? Why can viruses be so much smaller than cells and still replicate?

All forms of life, including bacteria, archaea, and eukarya, can be infected by viruses but of different types. Although viruses frequently kill the cells in which they replicate, some types can also remain within the host cell without causing obvious ill effects. As the host cells multiply, they copy the viral genetic information, passing it along to their progeny.

Viroids

Viroids are simpler than viruses, consisting of only a single, short piece of ribonucleic acid (RNA). Like viruses, they are obligate intracellular agents. Viroids cause a number of plant diseases.

Prions

Prions are infectious proteins that cause diseases called spongiform encephalopathies, a name that reflects the sponge-like appearance of the affected brain tissue (*encephalo* means "brain" and *patho* means "disease"). Perhaps the most widely recognized example is bovine spongiform encephalopathy (BSE), commonly called mad cow disease. Prions are simply misfolded versions of normal cellular proteins found in the brain. When the misfolded version comes into contact with the normal cellular protein, it causes the normal protein to also misfold. These misfolded versions bind together within the cell to form thread-like structures called fibrils (**figure 1.14**). The fibril-filled cells are not able to function and eventually die, forming spaces in the brain that lead to the characteristic sponge-like appearance. Prions are more resistant to degradation by cellular enzymes than are their normal counterparts. They are also resistant to the usual sterilization procedures that destroy viruses and bacteria.

Characteristics of acellular infectious agents are listed in **table 1.4.**

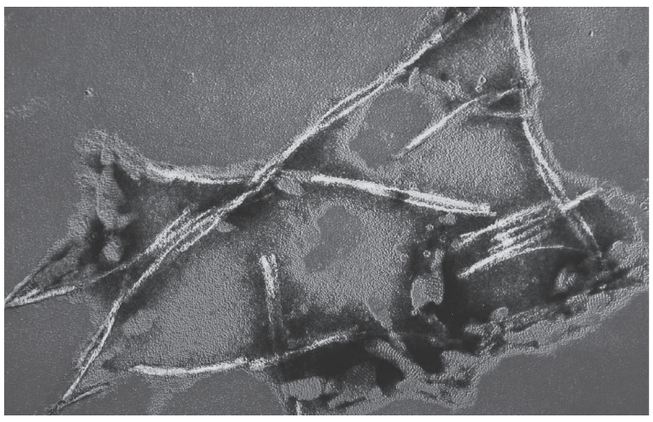


FIGURE 1.14 Prion Prion fibrils isolated from the brain of an infected cow. EM Unit, VLA/Science Source

? How are prions different from the normal versions of the related proteins made by cells?

MicroAssessment 1.3

Microbes are given a two-part name that indicates the genus and species. Three domains of life exist: Bacteria, Archaea, and Eukarya. Members of the Bacteria and Archaea are prokaryotes, but the two groups have significant differences. Members of the Eukarya are eukaryotes; within this group, microbiologists study algae, fungi, protozoa, and parasitic helminths. Viruses, viroids, and prions are acellular infectious agents.

7. List two features that distinguish prokaryotes from eukaryotes.
8. Describe the chemical composition of viruses, viroids, and prions.
9. Based on the genus name, what is the shape and arrangement of *Streptobacillus monoliformis* cells?

TABLE 1.4 Acellular Infectious Agents

Agent	Characteristic
Viruses	Consist of either DNA or RNA, surrounded by a protein coat. Obligate intracellular agents that use the machinery and nutrients of host cells to replicate.
Viroids	Consist only of RNA; no protein coat. Obligate intracellular agents that use the machinery and nutrients of host cells to replicate.
Prions	Consist only of protein; no DNA or RNA. Misfolded versions of normal cellular proteins that cause misfolding of the normal versions.

FOCUS ON THE FUTURE 1.1

Meet the Microbiomes!

As you study this textbook, you will probably be amazed by how much we know about the microbial world. You have already read, for example, that the human microbiome affects our well-being, and that life on this planet could not exist without microbes. But the more you learn, the more you will realize how little we actually know! Although scientists have studied microorganisms for hundreds of years, most of the advances occurred after the start of the Golden Age of Microbiology. Studies focused primarily on microorganisms that could be grown in the laboratory, and we now know that those examples represent less than 1% of all microbes. Complicating the matter even more is the fact that a microorganism's behavior in the laboratory can be quite different from that in a natural situation. So, yes, we know a great deal, but it really represents only the tip of the iceberg.

The depth of our understanding about microbial communities is rapidly increasing due to what could be considered the Golden Age of Microbiomes. As was already discussed, the Human Microbiome Project led to greater insight into the role of microorganisms in health and disease. It also opened up many new areas for research. For example, how many disease states are due to imbalances in our normal microbiota? Can we treat any of those by packaging certain microbes into a pill that can be taken orally? Can we track a person's microbial profiles to predict changes in health? Ongoing studies now promise to provide additional insights but will also lead to many new questions.

Summary

1.1 ■ The Dispute over Spontaneous Generation

The belief in **spontaneous generation** was challenged by Francesco Redi in the seventeenth century.

Early Experiments

The experiments of John Needham supported the idea of spontaneous generation, while those of Lazzaro Spallanzani did not.

Experiments of Pasteur

The experiments of Louis Pasteur disproved spontaneous generation and supported what is now known as the theory of **biogenesis** (figure 1.2).

Experiments of Tyndall

John Tyndall showed that some microbial forms are not killed by boiling. He and Ferdinand Cohn discovered **endospores,** the heat-resistant forms of some bacteria.

The Golden Age of Microbiology

The field of microbiology blossomed after Pasteur and others disproved spontaneous generation, leading to the Golden Age of Microbiology. Discoveries during this time led to the acceptance of the Germ Theory of Disease.

The Scientific Method

The **scientific method** includes (1) observing an occurrence and asking a question about that situation; (2) developing a **hypothesis** that explains the occurrence and devising an experiment that tests the hypothesis; (3) doing the experiment, collecting the data, and drawing conclusions; and (4) communicating the results, methods, and conclusions. A scientific **theory** is an explanation supported by a vast body of experimental evidence.

1.2 ■ Microbiology: A Human Perspective

The Human Microbiome

The **normal microbiota** is essential to human health. The **microbiome** is an interacting community of microorganisms as well as their genetic information.

Microorganisms in the Environment

Microorganisms replenish the O_2 that humans and other animals require to breathe, and they convert the nitrogen gas in the air into a form that other organisms can use.

Commercial Benefits of Microorganisms

Microorganisms are used in the production of bread, wine, beer, and cheeses. They are also used to degrade toxic pollutants and to synthesize a variety of different useful products. **Biotechnology** depends on members of the microbial world.

Microbes as Research Tools

Microorganisms are wonderful model organisms to study because they have the same fundamental metabolic and genetic properties as higher life-forms, and they grow rapidly on simple, inexpensive growth media.

Microbes and Disease

Pathogens cause disease, but the death rate from infectious diseases has declined over the past 100 years or so as a result of disease-prevention efforts, including vaccination (figure 1.4). More needs to be done to prevent **emerging infectious diseases,** some of which are new or newly recognized (figure 1.5). Some chronic diseases are caused by microorganisms.

1.3 ■ Members of the Microbial World

Considering that small size is the only shared feature of all microbes, the group is tremendously diverse (figure 1.6). All living organisms are classified into three **domains:** Bacteria, Archaea, and Eukarya (table 1.1). The small size of microbes requires measurements not commonly used in everyday life (figure 1.7).

Scientific Names

The first part of a scientific name indicates the **genus,** and the second part the **species;** these are written in italics or underlined (table 1.2). Members of the same species can vary, so strain designations are sometimes used.

Bacteria

Bacteria are single-celled **prokaryotes** that have peptidoglycan in their cell walls. Most bacteria are **cocci** or **rods;** other common shapes are **vibrios, spirilla,** and **spirochetes** (figure 1.8). Cells adhering to one another following division form characteristic arrangements such as chains, packets, and clusters (figure 1.9).

Archaea

Archaea are single-celled prokaryotes. Although they look like bacteria, there are significant differences. They do not contain peptidoglycan. Many archaea grow in extreme environments.

Eukarya

Eukarya are **eukaryotes** (table 1.3). **Fungi** include single-celled yeasts and multicellular molds and mushrooms; they use organic compounds as food (figure 1.10). **Algae** can be single-celled or multicellular, and they use sunlight as an energy source (figure 1.11). **Protozoa** are typically motile single-celled organisms that use organic compounds as food (figure 1.12). Parasitic **helminths** are worms that live at the expense of a host.

Acellular Infectious Agents

The non-living members of the microbial world are not composed of cells (table 1.4). **Viruses** consist of nucleic acid within a protein coat (figure 1.13). **Viroids** consist of a single, short RNA molecule. **Prions** consist only of protein; they are misfolded versions of normal cellular protein (figure 1.14).

Review Questions

Short Answer

1. How did Louis Pasteur help disprove spontaneous generation?
2. Describe the scientific method.
3. Explain why life could not exist without the activities of microorganisms.
4. How is the normal microbiota important to human health?
5. List four commercially important benefits of microorganisms.
6. What characteristics of microorganisms make them important research tools?
7. List three factors that contribute to the emergence of infectious diseases.
8. In the designation *Escherichia coli* B, which part of the name indicates the genus? Which part indicates the species? Which part indicates the strain?
9. Why are viruses not microorganisms?
10. Name three non-living groups in the microbial world, and describe their major properties.

Multiple Choice

1. The property of endospores that led to confusion in the experiments on spontaneous generation is their
   a) small size.
   b) ability to pass through cork stoppers.
   c) heat resistance.
   d) presence in all infusions.
   e) presence on cotton plugs.
2. The Golden Age of Microbiology was the time when
   a) microorganisms were first used to make bread.
   b) microorganisms were first used to make cheese.
   c) most pathogenic bacteria were identified.
   d) a vaccine against influenza was developed.
   e) antibiotics became available.
3. If all prokaryotes were eliminated from the planet,
   a) animals would thrive because there would be no disease.
   b) archaea would thrive because there would be no competition for nutrients.
   c) all animals would die.
   d) animals and archaea would thrive.
4. All of the following are emerging infectious diseases except
   a) smallpox.
   b) hepatitis C.
   c) Lyme disease.
   d) COVID-19.
   e) mad cow disease.
5. All of the following are biological domains except
   a) Bacteria.
   b) Archaea.

c) Prokaryota.
d) Eukarya.

6. Which name is written correctly?
   a) *staphylococcus aureus*
   b) *escherichia Coli*
   c) *Staphylococcus epidermidis*
   d) bacillus Anthracis
   e) Clostridium Botulinum
7. Members of which pairing are most similar in appearance to each other?
   a) fungi and algae
   b) algae and archaea
   c) archaea and bacteria
   d) bacteria and viruses
   e) viruses and algae
8. If you wanted to increase your chances of obtaining a member of the Archaea (rather than a member of another domain), which would be the best site to obtain a sample?
   a) intestine of an elephant
   b) skin of an elephant
   c) a 95°C hot spring in Yellowstone
   d) a 45°C hot spring in Hawaii
   e) a raw hamburger patty
9. Viruses
   a) contain both protein and nucleic acid.
   b) infect only eukaryotic cells.
   c) can grow in the absence of living cells.
   d) are generally the same size as prokaryotes.
   e) always kill the cells they infect.
10. Antonie van Leeuwenhoek could not have observed
    a) roundworms.
    b) *Escherichia coli.*
    c) yeasts.
    d) viruses.

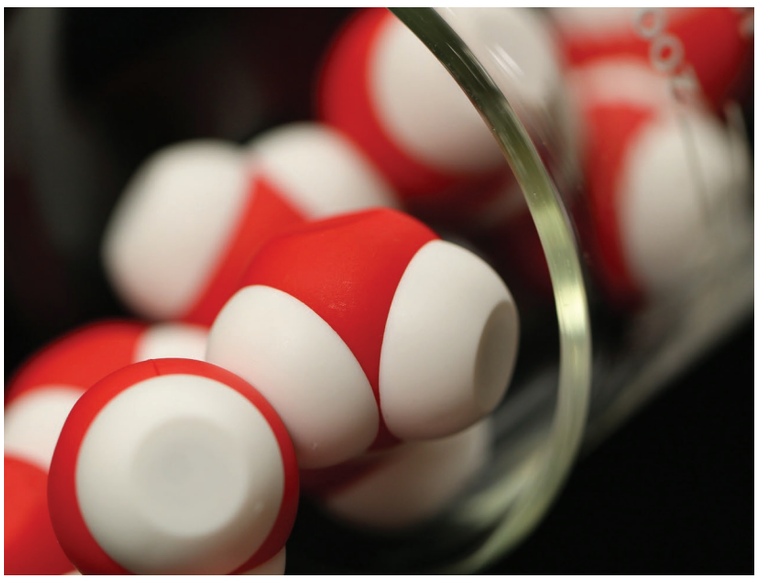
Applications

1. The American Society for Microbiology is preparing a "Microbe-Free" banquet to emphasize the importance of microorganisms in food production. What foods could not be on the menu?
2. If you were asked to nominate one of the individuals mentioned in this chapter for the Nobel Prize, who would it be? Make a statement supporting your choice.

Critical Thinking

1. A microbiologist obtained two pure biological samples: one of a virus, and the other of a viroid. Unfortunately, the labels had been lost. The microbiologist felt she could distinguish the two by analyzing for the presence or absence of a single molecule. What molecule would she search for and why?
2. Why would archaea that grow in extreme environments be more intensively studied than those that do not?

2 The Molecules of Life



Space-filling models of water molecules. *(Lisa Burgess/McGraw-Hill Education)*

KEY TERMS

Atom The basic unit of matter.

Buffer A chemical that stabilizes the pH of a solution.

Carbohydrate An organic compound composed of one or more simple sugars.

Covalent Bond A chemical bond formed when two atoms share electrons.

Hydrogen Bond The attraction between a hydrogen atom in a polar molecule and an electronegative atom in the same or another polar molecule.

Ion An atom or molecule that has gained or lost one or more electrons.

Ionic Bond A chemical bond resulting from the attraction between positively and negatively charged ions.

Lipid An organic molecule that is not soluble in water.

Molecule Two or more atoms held together by covalent bonds.

Nucleic Acid A macromolecule consisting of one or two nucleotide chains; DNA or RNA.

Organic Compound A compound that has a carbon atom covalently bonded to a hydrogen atom.

pH A measure of the hydrogen ion concentration or acidity of a solution on a scale of 0 to 14.

Protein A macromolecule consisting of one or more chains of amino acids.

A Glimpse of History

Farmers have understood for centuries that growing the same crop on the same piece of land year after year reduces the crop yield. Allowing a field to remain unplanted for one or more seasons lets wild plants grow, and these appear to improve the soil. During the late 1880s, Martinus Beijerinck, a microbiologist from the Netherlands, helped to explain the science behind what the farmers already knew.

Beijerinck was described as a "keen observer" who was able "to fuse results of remarkable observations with a profound and extensive knowledge of biology and the underlying sciences." Fortunately for farmers, he disliked medical microbiology, preferring to study the agricultural applications of microbiology instead. Based on the work of other scientists, Beijerinck knew that certain plants improve the soil because microorganisms help them accumulate nitrogen from the air. All forms of life require nitrogen because it is an essential component of cellular material, but soils have only limited amounts. Almost 80% of Earth's atmosphere is nitrogen gas (N_2), so using that atmospheric nitrogen source might seem like an easy task, but it is far from it. N_2 is very stable, with strong chemical bonds holding the two nitrogen atoms together. Plants, animals, and most other organisms lack the ability to break those bonds, so it was unclear how microorganisms were helping plants use atmospheric nitrogen.

Beijerinck and other scientists studied nitrogen accumulation in a group of plants called legumes. Members of this group bear seeds in pods and include peas, beans, and clover. Legume roots often have nodules (small growths that look like tumors). It had been reported that the root nodules contained microorganisms, and that these allowed the plant to accumulate nitrogen. Beijerinck made a significant breakthrough by isolating a bacterium from inside a root nodule and then showing that it could convert atmospheric nitrogen into a form that could be incorporated into cellular material. This process is called nitrogen fixation, and the nitrogen is said to be "fixed." Then he showed that root nodules form when the nitrogen-fixing bacterium—a member of a group now called rhizobia—is incubated with seedlings of legumes. As those plants, or even parts of the plants, die and decay, the cellular material is released, enriching the soil with nitrogen-containing nutrients.

When farmers plant crops like soybeans or allow wild clover to grow in a field, rhizobia in the nodules fix nitrogen, which can then be used by the host plant. We now know that there are many types of rhizobia, and they are specific with respect to the type of host plant with which they form a relationship. To honor Beijerinck for his achievements, a genus of nitrogen-fixing bacteria (Beijerinckia) is named for him.

Simply stated, chemistry is the study of matter, or the "stuff" of which the universe is composed. As you learn about microbiology, you will find that cells are masters at converting one set of chemicals to another and, in doing so, producing materials required to make new cells. This amazing ability to transform material is just one reason that microorganisms are crucial to life and that the principles described in this chapter are fundamental to information throughout the text.

2.1 ■ Elements and Atoms

Learning Outcomes

1. Describe the general structure of an atom and its isotopes.
2. Describe the importance of valence electrons.

Matter is categorized into **elements,** substances that have unique chemical properties and cannot be broken down by ordinary chemical means. An **atom** is the basic unit of all matter, and each element is composed of only one type of atom. Each element is indicated by a one- or two-letter symbol, with the first letter capitalized; for example, C represents carbon and Ca represents calcium. Not all chemical symbols are derived from English, as seen with the symbol Na, which stands for *natrium* in Latin, but which we know as sodium.

Of the 92 or so naturally occurring elements, biological systems are primarily composed of only six. As a way to remember these, think of the acronym CHONPS: carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), and sulfur (S). Other elements are also important in biological systems, including some referred to as trace elements, reflecting the fact that they are found in very small quantities.

Atomic Structure

Atoms consist of three major components:

- **Protons:** positively charged particles
- **Neutrons:** uncharged particles
- **Electrons:** negatively charged particles

Protons and neutrons together form the atomic nucleus (dense, central region of the atom), around which electrons move in a "cloud" (**figure 2.1**). Overall, the atom has no charge because the number of positive protons in the atomic nucleus is the same as the number of negative electrons in the cloud.

Each type of atom is distinguished by the number of protons in its nucleus, referred to as its **atomic number** (**table 2.1**). For example, a hydrogen atom has 1 proton, so its atomic number is 1; a carbon atom has 6 protons, so its atomic number is 6. Each atom also has a **mass number,** the sum of the number of protons and neutrons in the nucleus of that atom. A hydrogen atom with 1 proton and no neutrons has an atomic number of 1 as well as a mass number of 1. A carbon atom with 6 protons and 6 neutrons has an atomic number of 6 and a mass number of 12. Note that electrons are too light to contribute to the mass of an atom.

Atoms can be depicted in various ways to emphasize certain characteristics (**figure 2.2**). Some diagrams indicate the number of protons and neutrons in the nucleus as well as the number of electrons in the surrounding cloud. Frequently, however, the element symbol is used, with the atomic number in subscript to the left, and the mass number in superscript to the left. A depiction called a Lewis symbol uses the element symbol and indicates the number of valence electrons using dots (valence electrons are described next).

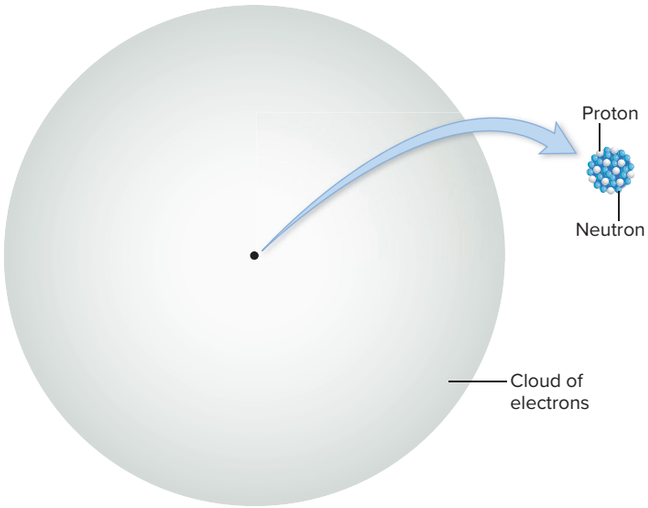


FIGURE 2.1 Atomic Structure A proton has a positive charge, a neutron has a neutral charge, and an electron has a negative charge. The electrons move around the nucleus in a cloud.

? How does the number of electrons in an atom compare with the number of protons?

TABLE 2.1 Characteristics of Atoms Common in Living Organisms

Atom	Symbol	Atomic Number	Mass Number
Hydrogen	H	1	1
Carbon	C	6	12
Nitrogen	N	7	14
Oxygen	O	8	16
Phosphorus	P	15	31
Sulfur	S	16	32

MicroByte

If the nucleus of an atom were a marble in the center of a football field, the electrons would occupy the entire space of the stadium.

The Role of Electrons

Electrons move in a cloud around an atom's nucleus, but at any point in time their precise location is impossible to determine (see figure 2.1). They are most likely to be in specific regions called shells, each of which is associated with a different energy level. The number of electrons a shell can hold is limited. Electrons are attracted to the protons in the nucleus, so shells closer to the nucleus are generally filled before

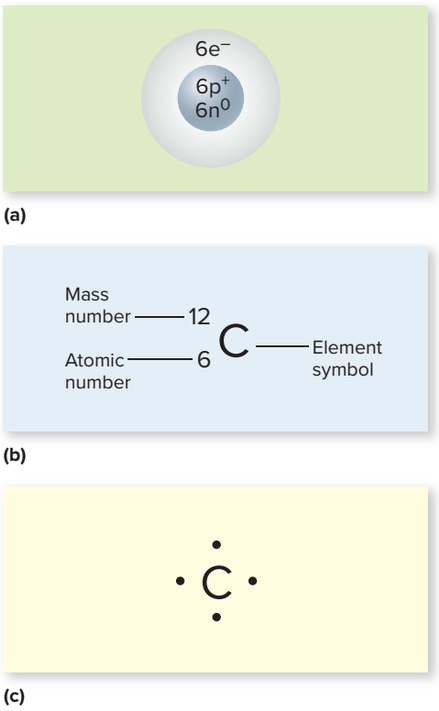


FIGURE 2.2 Depictions of a Carbon Atom **(a)** The number of protons (p^+) and neutrons (n^0) in the nucleus are indicated, along with the number of electrons (e^-) in the surrounding cloud. **(b)** The mass number and atomic number are indicated on the left of the element symbol. **(c)** The Lewis symbol uses dots to indicate the number of valence electrons.

Why are electrons not considered when determining the mass of an atom?

electrons occupy other shells. The shell closest to the nucleus holds no more than 2 electrons; these electrons are most highly attracted to the nucleus and have the lowest energy level in that particular atom. Once that shell is filled, additional electrons occupy the next shell, which holds 8 electrons. Larger atoms have additional shells that hold even more electrons, but most atoms of biological significance follow the "octet rule," meaning they are most stable when their outer shell contains 8 electrons. An important exception is hydrogen, with a single shell; recall that the first shell has a limit of 2 electrons.

Electrons largely determine the chemical reactivity of an atom. An atom's **valence electrons,** meaning the electrons in the outermost shell (called the valence shell), are the most important in that regard. Atoms with the maximum number of electrons in the outer shell are very stable. Atoms with an unfilled outer shell tend to react with other atoms that have an unfilled outer shell by sharing or transferring electrons. Lewis symbols provide a simple visual representation of an atom's outer shell, with each dot representing one valence electron (figure 2.2c); Lewis structures are similar, but they show the interaction of two or more atoms, with dots indicating shared and unshared valence electrons (see figure 2.6a).

Isotopes

All atoms of a given element have the same number of protons, but they can have different numbers of neutrons. That is, atoms of an element all have the same atomic number, but they can have different mass numbers. The various forms of atoms of an element are **isotopes** (*iso* means "same" and *tope* means "place"). For example, nearly 99% of naturally occurring carbon atoms have 6 neutrons, but some have 7 or 8. The fact that the atoms of an element can have different mass numbers is reflected in the term **atomic mass.** This is the average of the mass numbers of the atoms of the element, weighted according to the relative abundance of the naturally occurring isotopes. The atomic mass of carbon is 12.01.

Although the chemical behavior of different isotopes is similar to their more common counterparts, the structural differences can often be detected. Because of this, researchers and clinicians use isotopes to monitor the fate of specific atoms within a population of cells. Some isotopes are unstable and emit radiation that can be detected; others are tracked using special instruments that measure differences in mass. The energy emitted from radioactive isotopes (radioisotopes) is sometimes useful in medical diagnosis. For example, to evaluate proper functioning of the human thyroid gland (which produces the iodine-containing hormone thyroxine) doctors often administer radioactive iodine. The gland can then be scanned to determine if the amount and distribution of iodine is normal (**figure 2.3**).

MicroAssessment 2.1

The six most common elements in biology are carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur. The basic unit of all matter, the atom, is composed of protons, electrons, and neutrons. The reactivity of an atom is largely determined by the number of valence electrons. Isotopes are atoms of a given element that have a different number of neutrons.

1. What do the dots on a Lewis symbol represent?
2. What is the "octet rule" and its biologically important exception?
3. What do atoms of ^{14}C and ^{14}N have in common? How do they differ?

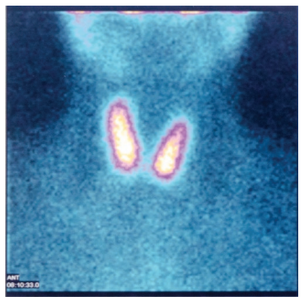


FIGURE 2.3 Radioisotopes A scan of the thyroid gland 24 hours after the patient received radioactive iodine. Stefania Arca/Shutterstock

Why is radioactive iodine concentrated in the thyroid gland?

2.2 ■ Chemical Bonds and Reactions

Learning Outcomes

3. Compare and contrast ionic bonds, covalent bonds, and hydrogen bonds.
4. Explain the role of an enzyme in a chemical reaction.

Atoms with an unfilled outer shell react with each other to lose, gain, or share their valence electrons. This allows them to achieve a more stable state and is the basis for chemical bond formation.

Ions and Ionic Bonds

An atom that gains or loses an electron is no longer neutral—it is an **ion** (**figure 2.4**). Atoms that gain an electron become negatively charged and are called **anions;** those that lose an electron become positively charged and are called **cations.** The type and amount of charge are indicated by a superscript to the right of the chemical symbol. For example, Na^+ indicates a sodium atom that has lost one electron and therefore carries a +1 (positive) charge; Mg^{2+} indicates a magnesium atom that has lost two electrons and therefore has a +2 charge. Note that a positive ion is formed by the loss of one or more valence electrons. The nucleus still contains the same number of protons, resulting in a positive charge. Na^+ cannot be formed by gaining a proton because gaining a proton would change the atomic number, and therefore the atom would no longer be sodium.

Ionic bonds form between cations and anions because of the attraction between positive and negative charges (see figure 2.4). The resulting product is called a salt. A common type of salt, sodium chloride (table salt), is composed of Na^+ (sodium cations) and Cl^- (chloride anions) and forms a solid crystal. The structure is highly ordered because the electrical attraction between positive and negative charges brings the ions together, but the like charges repel one another and are positioned as far apart as possible. Crystals continue to grow as new ions are added. Salts such as sodium chloride dissolve in water and are called **electrolytes,** meaning that they conduct electricity.

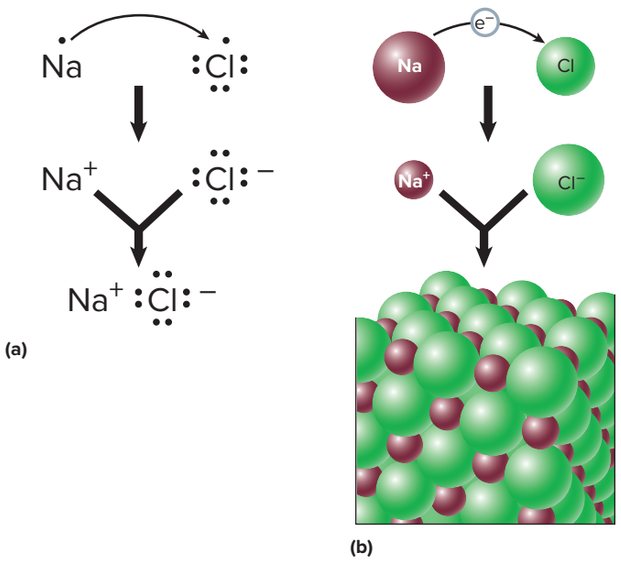


FIGURE 2.4 Ions and Ionic Bonds **(a)** Lewis symbols of sodium and chloride ions being formed, and an ionic bond between them. **(b)** Space-filling model of a salt crystal being formed by ionic bonding. Note that a cation is smaller than its neutral atom while an anion is larger.

? Which of the ions in this figure is an anion, and which is a cation?

MicroByte

Electrical charges from the heart are conducted by electrolytes and can be detected on the body surface as an electrocardiogram (ECG).

Covalent Bonds

Atoms do not always fill their valence shells by gaining or losing electrons. They may instead share pairs of valence electrons, forming **covalent bonds.** A hydrogen atom (H) has 1 electron, so 1 additional electron is required to fill its valence shell (outer shell); if two H atoms share their single electron so that they have two between them, both atoms gain stability. The covalent bond between the two atoms is indicated by a dash, as H—H. Some atoms share more than one pair of electrons with each other, forming a double or triple covalent bond, indicated by a corresponding number of lines between the atoms. An oxygen atom needs 2 electrons to fill its valence shell; if each of two oxygen atoms shares 2 of its electrons with the other, then a double covalent bond is formed, represented as O=O. Double bonds are stronger than single bonds, meaning that more energy is required to break them, and triple bonds are stronger still.

Two or more atoms joined together by covalent bonds form a **molecule.** A molecule is represented by a molecular formula that indicates how many atoms of each type are present. Thus, a hydrogen molecule is represented by the formula H_2. If atoms that make up a molecule are different elements, the term **compound** may be used. (Salts such as NaCl are called ionic compounds.) Water is a compound that contains two hydrogen atoms and one oxygen atom; it is represented by the formula H_2O. The molecular mass of a molecule is based on the mass numbers of the component atoms. The molecular mass of most water molecules is 1 + 1 + 16, or 18.

Carbon (C) is a particularly important element in biological systems because its bonding properties provide the basis for many diverse structures. A carbon atom has four valence electrons, so it needs four more to fill its valence shell. Because of this, the atom forms four covalent bonds, typically with the main elements that make up cells: CHONPS (carbon, hydrogen, oxygen, nitrogen, phosphate, and sulfur). One C atom sharing electrons with four H atoms is methane (CH_4) (**figure 2.5**). Molecules that contain at least carbon and hydrogen are **organic compounds;** those that do not are inorganic compounds.