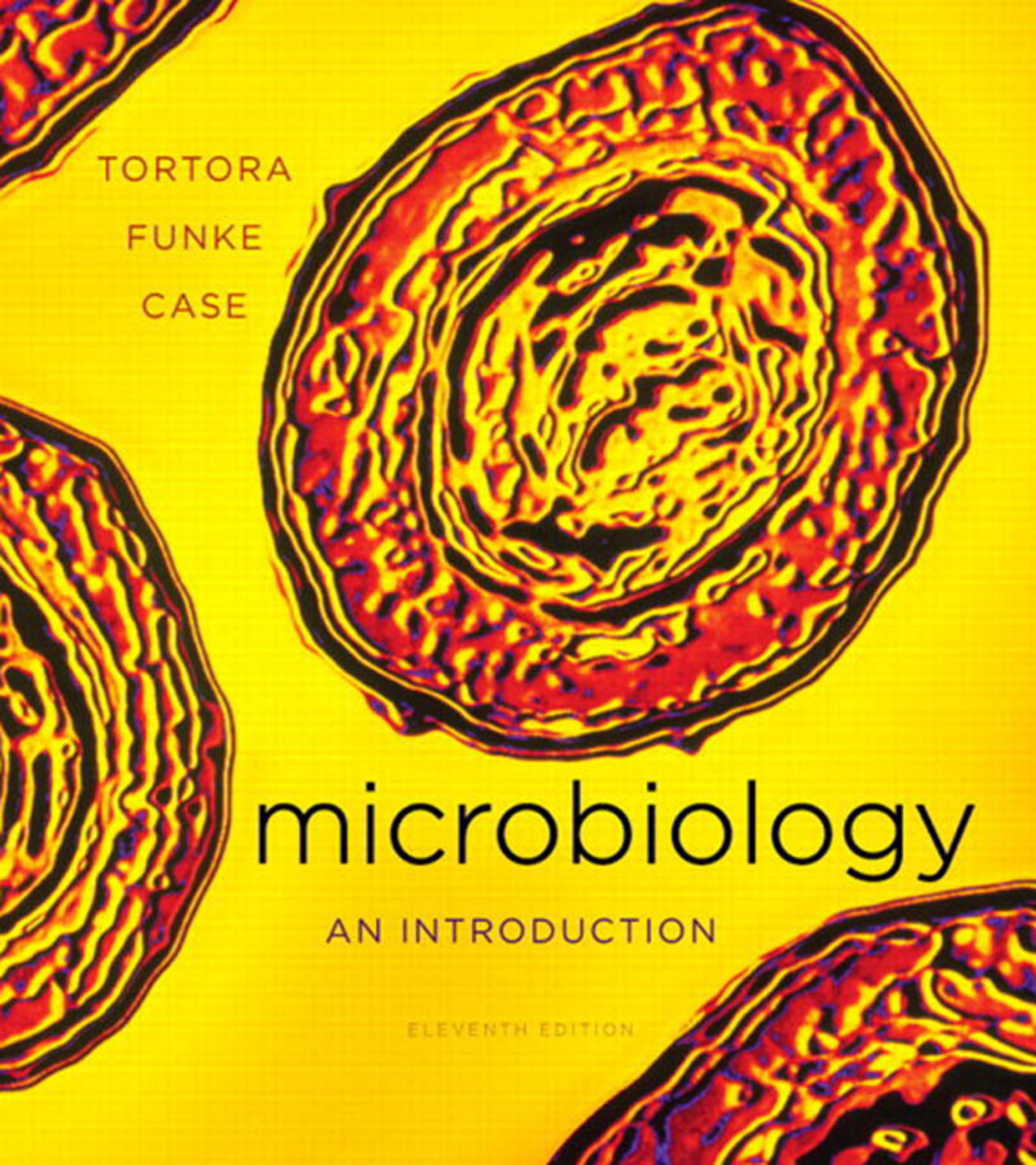
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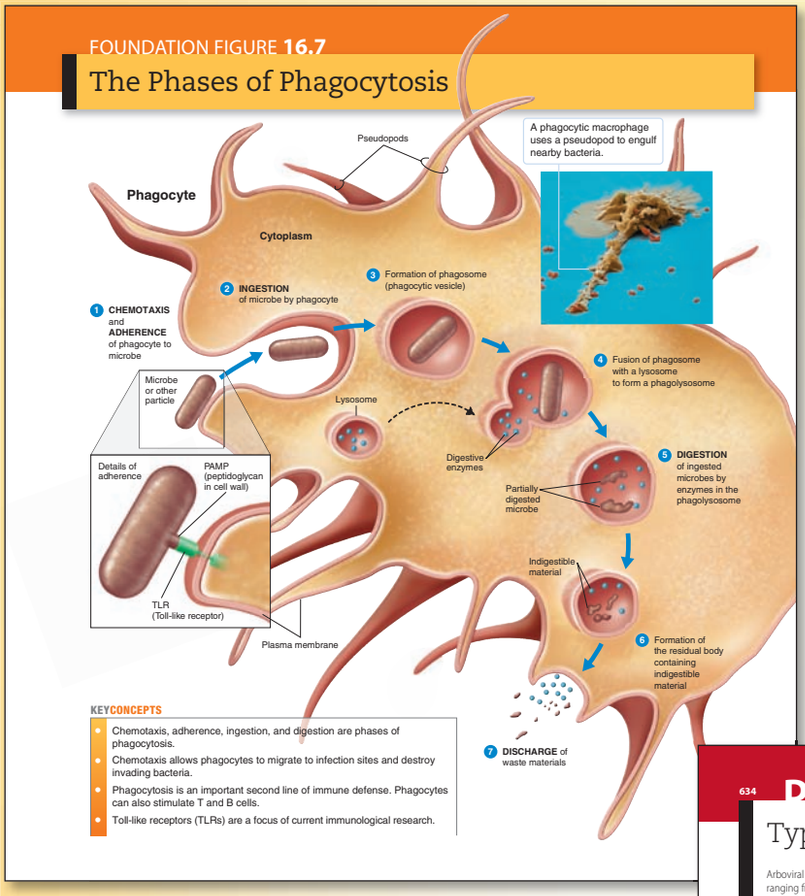
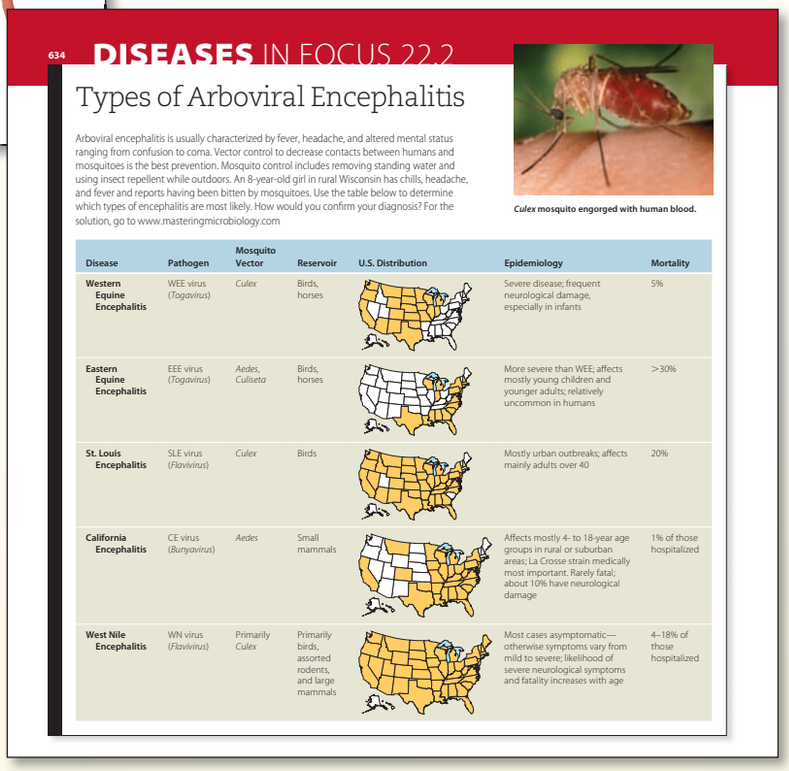
Make the Connection Between Lecture, Lab, and the Real World

In its Eleventh Edition, ***Microbiology: An Introduction*** helps you make the connection between microbiological theory presented in the text and real-world applications, encouraging you to see the connection between human health and microbiology.



Stunningly Revised Foundation Figures

Foundation Figures focus on especially important topics in microbiology. Clearly marked step-numbers make process-oriented figures easy to follow, while the "Key Concepts" highlight the take-away lessons for easy review. In MasteringMicrobiology,® Foundation Figures are highly interactive activities, designed to guide you through the essential concepts and processes of microbiology with in-depth, self-paced tutorials.



Disease in Focus

These boxes encourage you to think like a clinician by making a differential diagnosis based on a brief clinical overview. Diseases in Focus include disease tables, focusing on similar diseases or infections. These tables are organized around symptoms and pathogens in order to be as clinically relevant as possible. Disease in Focus activities in MasteringMicrobiology help you see the practical applications of microbiology to your future career.

Clinical Case: Microscopic Mayhem

Maryanne, a 42-year-old marketing executive and mother of three occasionally works from home, but she always feels that she isn't getting as much done at home as she does in the office. She has been experiencing recurrent stomach pain, which seems to be getting worse. She jokes with her husband that he should buy stock in Pepto-Bismol, because she buys so much of it. At her husband's urging, she finally makes an appointment to see her primary care physician. After hearing that Maryanne feels better immediately after taking Pepto-Bismol, the doctor suspects Maryanne may have a peptic ulcer associated with *Helicobacter pylori*.

What is *Helicobacter pylori*? Read on to find out.

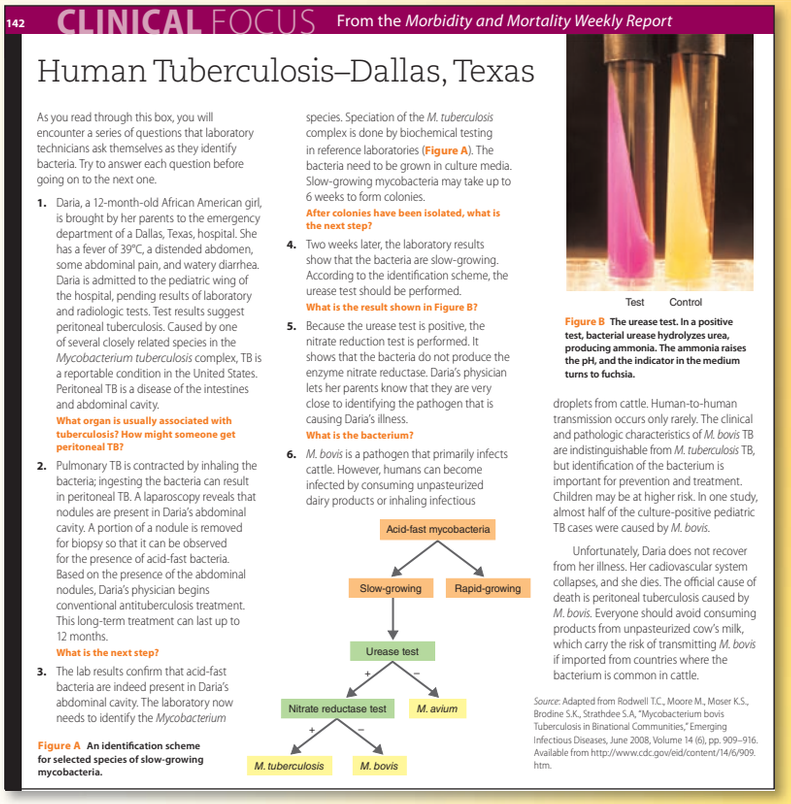
54 64 69 71

NEW! Clinical Cases

Clinical Cases in every chapter help motivate you to think critically about the chapter content and provide you with practical applications to your future allied health career. Each case segment includes a critical-thinking question related to the chapter material. In MasteringMicrobiology®, additional case studies come alive with images and questions, leading you through the process of disease diagnosis.

Clinical Focus

Clinical Focus boxes contain *Morbidity and Mortality Weekly Report* data from the Centers for Disease Control and Prevention (CDC) modified into clinical problem-solving scenarios with questions to help you develop your critical-thinking skills.



142 **CLINICAL FOCUS** From the *Morbidity and Mortality Weekly Report*

Human Tuberculosis–Dallas, Texas

As you read through this box, you will encounter a series of questions that laboratory technicians ask themselves as they identify bacteria. Try to answer each question before going on to the next one.

1. Daria, a 12-month-old African American girl, is brought by her parents to the emergency department of a Dallas, Texas, hospital. She has a fever of 39°C, a distended abdomen, some abdominal pain, and watery diarrhea. Daria is admitted to the pediatric wing of the hospital, pending results of laboratory and radiologic tests. Test results suggest peritoneal tuberculosis. Caused by one of several closely related species in the *Mycobacterium tuberculosis* complex, TB is a reportable condition in the United States. Peritoneal TB is a disease of the intestines and abdominal cavity.
   What organ is usually associated with tuberculosis? How might someone get peritoneal TB?
2. Pulmonary TB is contracted by inhaling the bacteria; ingesting the bacteria can result in peritoneal TB. A laparoscopy reveals that nodules are present in Daria's abdominal cavity. A portion of a nodule is removed for biopsy so that it can be observed for the presence of acid-fast bacteria. Based on the presence of the abdominal nodules, Daria's physician begins conventional antituberculosis treatment. This long-term treatment can last up to 12 months.
   What is the next step?
3. The lab results confirm that acid-fast bacteria are indeed present in Daria's abdominal cavity. The laboratory now needs to identify the *Mycobacterium* species. Speciation of the *M. tuberculosis* complex is done by biochemical testing in reference laboratories (**Figure A**). The bacteria need to be grown in culture media. Slow-growing mycobacteria may take up to 6 weeks to form colonies.
   After colonies have been isolated, what is the next step?
4. Two weeks later, the laboratory results show that the bacteria are slow-growing. According to the identification scheme, the urease test should be performed.
   What is the result shown in Figure B?
5. Because the urease test is positive, the nitrate reduction test is performed. It shows that the bacteria do not produce the enzyme nitrate reductase. Daria's physician lets her parents know that they are very close to identifying the pathogen that is causing Daria's illness.
   What is the bacterium?
6. *M. bovis* is a pathogen that primarily infects cattle. However, humans can become infected by consuming unpasteurized dairy products or inhaling infectious droplets from cattle. Human-to-human transmission occurs only rarely. The clinical and pathologic characteristics of *M. bovis* TB are indistinguishable from *M. tuberculosis* TB, but identification of the bacterium is important for prevention and treatment. Children may be at higher risk. In one study, almost half of the culture-positive pediatric TB cases were caused by *M. bovis*.

Unfortunately, Daria does not recover from her illness. Her cadiovascular system collapses, and she dies. The official cause of death is peritoneal tuberculosis caused by *M. bovis*. Everyone should avoid consuming products from unpasteurized cow's milk, which carry the risk of transmitting *M. bovis* if imported from countries where the bacterium is common in cattle.

Figure A **An identification scheme for selected species of slow-growing mycobacteria.**

Figure B **The urease test. In a positive test, bacterial urease hydrolyzes urea, producing ammonia. The ammonia raises the pH, and the indicator in the medium turns to fuchsia.**

Source: Adapted from Rodwell T.C., Moore M., Moser K.S., Brodine S.K., Strathdee S.A, "Mycobacterium bovis Tuberculosis in Binational Communities," Emerging Infectious Diseases, June 2008, Volume 14 (6), pp. 909–916. Available from http://www.cdc.gov/eid/content/14/6/909.htm.

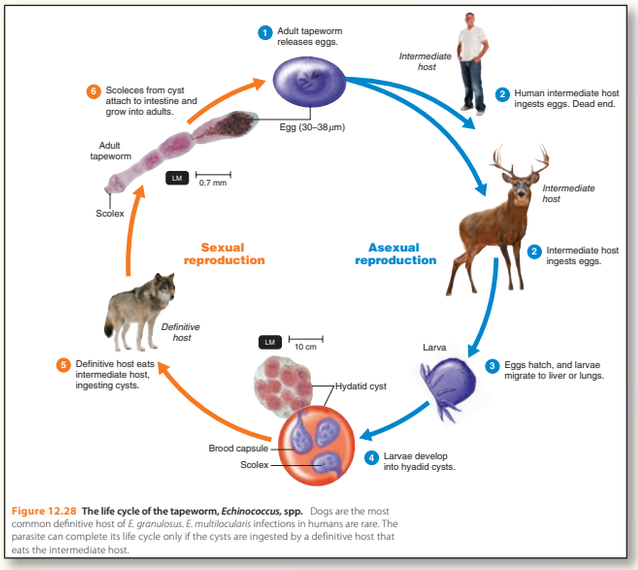


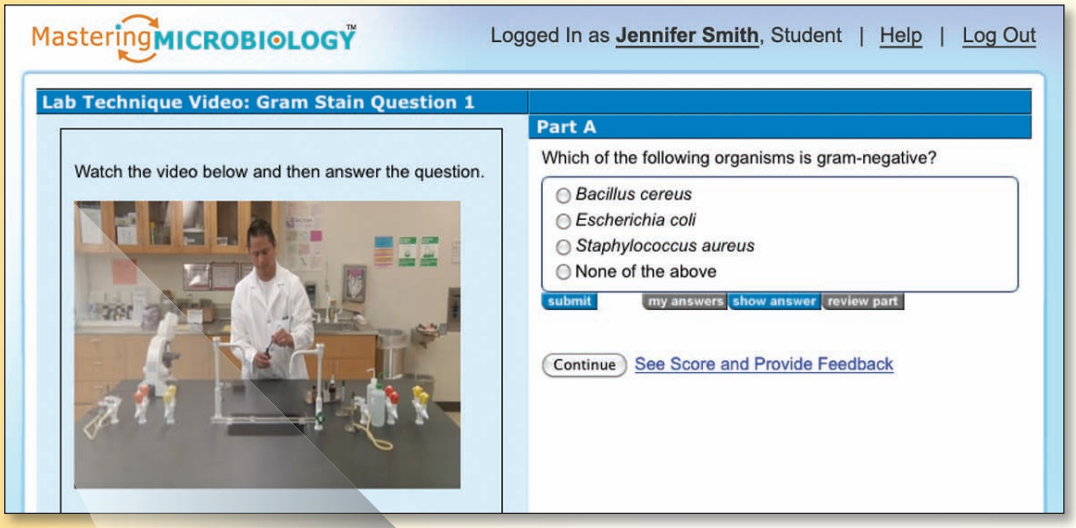
Figure 12.28 **The life cycle of the tapeworm, *Echinococcus*, spp.** Dogs are the most common definitive host of *E. granulosus*. *E. multilocularis* infections in humans are rare. The parasite can complete its life cycle only if the cysts are ingested by a definitive host that eats the intermediate host.

NEW! Life Cycle Figures

Life Cycle figures break down complex processes into more readily understandable steps. Each Life Cycle figure is color-coded to differentiate between steps that involve sexual or asexual reproduction.

Arrive prepared for lecture and lab

The Mastering online homework and tutoring system delivers self-paced tutorials that provide you with individualized coaching set to your professor's course objectives. MasteringMicrobiology helps you arrive better prepared for lecture and lab with reading questions, coaching activities, tutorials and more. Research shows that Mastering's immediate feedback and tutorial assistance help you understand and master microbiology concepts— meaning that you retain more knowledge and perform better in subsequent courses.



NEW! Lab Technique Videos

Lab Technique Videos are 3-5 minute videos, demonstrating specific lab techniques. These videos cover commonly performed procedures, such as aseptic technique, Gram staining, and preparation of smears. The videos help you get prepared for your wet lab and also allow you to review the techniques on your own time. Quizzes test your comprehension of the steps involved in each technique to make sure you get the most out of the videos.

▲ NEW! MicroLab Tutors

These tutors help you get the most out of lab time. Each MicroLab Tutor begins with clinical backround and a technique video. Select MicroLab Tutors, like the Gram Stain MicroLab Tutor, also contain an animation illustrating the procedure at the molecular level, helping you visualize each process. Each tutorial's questions contain hints and feedback that include photomicrographs, video clips or animation clips and are designed to make sure that you are prepared for lab by introducing and assessing your understanding of lab concepts and techniques outside of formal lecture and lab time. Select Tutors will contain an animation illustrating the procedure at the molecular level, as is the case in this sample for the Gram stain tutor.

What instructors are saying—

"The tutorial would cut down on lab time needed for explanation and allow more time for hands-on experience."

—Rita Moyes, Instructor
Texas A&M University

"This is the perfect thing to enhance student learning of the procedure along with providing feedback for both correct and incorrect procedures."

—Tanya Crider, Instructor
East Mississippi Community College

Unparalleled Online Resources for Additional Student Practice and Assessment

All of the resources previously found on the Microbiology Place™ website are now accessible and assignable in MasteringMicrobiology®. MasteringMicrobiology builds on these study tools and includes new content and assessments, enabling more frequent student practice and more meaningful course management.

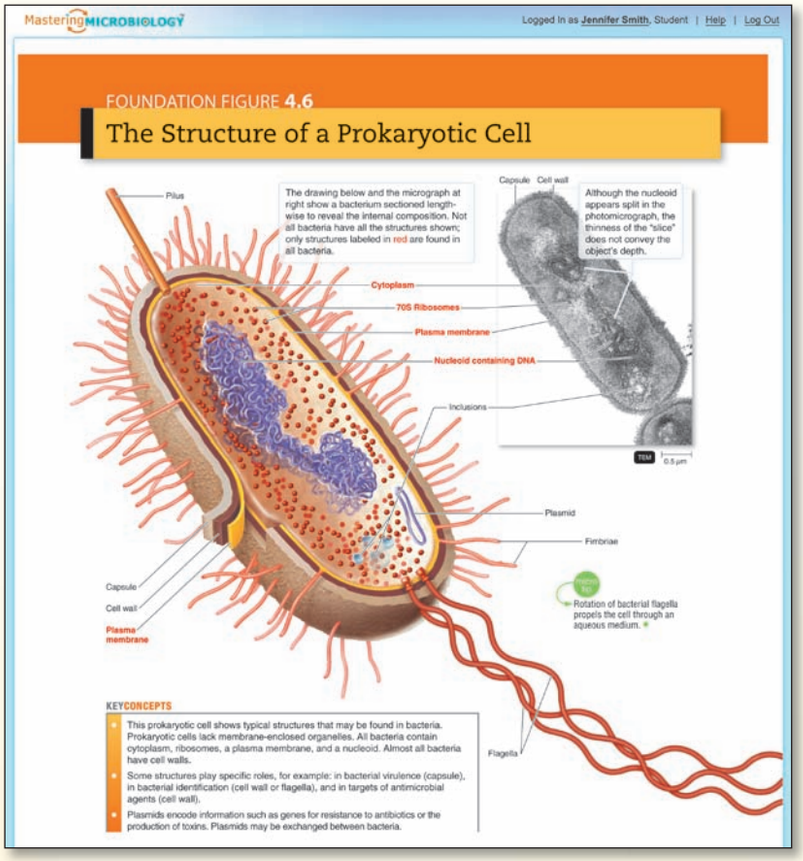
MicroFlix™

MicroFlix™ are 3D movie-quality animations with self-paced tutorials and gradable quizzes that help students master the three toughest topics in microbiology: metabolism, DNA replication, and immunology.

Students can review the fundamentals by viewing the animations, completing the tutorial, printing a personal review sheet, and taking the quiz. Students also have access to BioFlix® animations that help them review relevant concepts from general biology.

Foundation Figures Coaching Activities

Foundations Figures are reinforced in MasteringMicrobiology® with Coaching Activities that ensure students master the toughest topics before moving on in the chapter. The results of the Coaching Activities feed directly into the gradebook.



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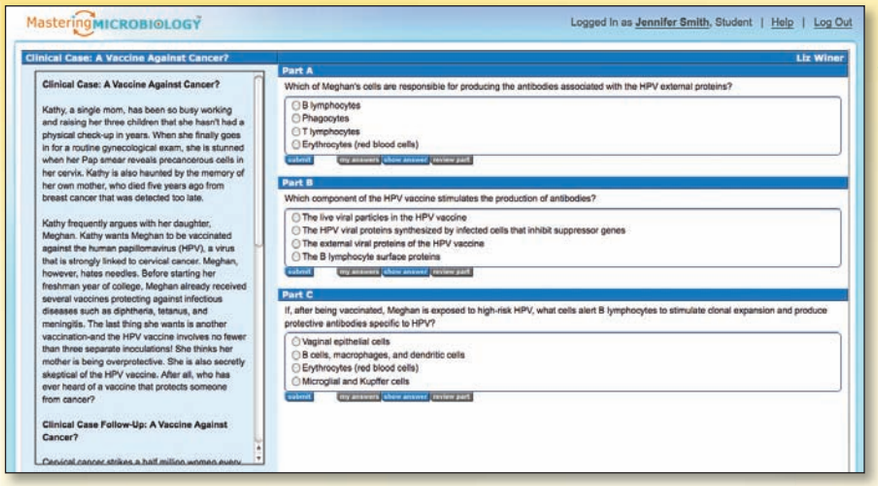
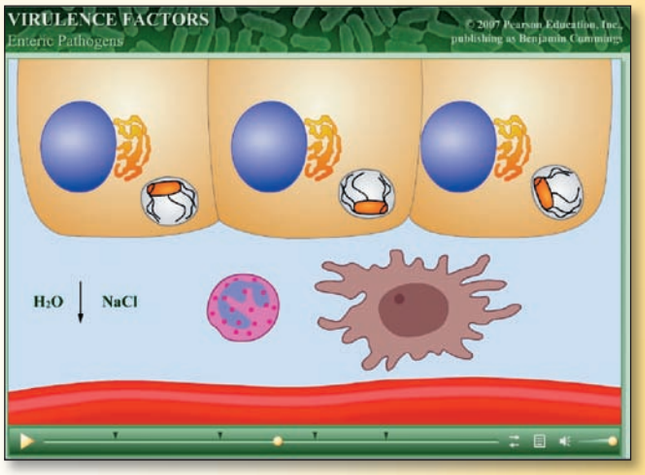


Case Study Coaching Activities

These activities in MasteringMicrobiology help students connect microbiological theory to real-world disease diagnosis and treatment, are assignable, and feed directly into the MasteringMicrobiology gradebook.

2-D Microbiology Animations

More than 120 multi-step Microbiology Animations explain and visually demonstrate core concepts, providing an additional opportunity for students to visualize and understand core microbiology concepts. They are accompanied by gradable quizzes. References to the Microbiology Animations appear throughout the chapters of the book.



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The Mastering platform was developed by scientists for science students and instructors, and has a proven history with more than 10 years of student use. Mastering currently has more than 1.5 million active registrations with active users in all 50 states and in 41 countries. The Mastering platform has 99.8% server reliability.

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Mastering questions are tied to the specific Learning Outcomes in Tortora, Funke, and Case as well as global science Learning Outcomes and those provided by the American Society of Microbiology Center for Undergraduate Educators. These provide a powerful tool for tracking individual student learning and assessing course objectives.

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MasteringMicrobiology can be successfully implemented in any environment—lab-based, hybrid, fully online, or traditional. Integrated usage of MasteringMicrobiology has demonstrated quantifiable differences in student retention, subsequent success and overall achievement.

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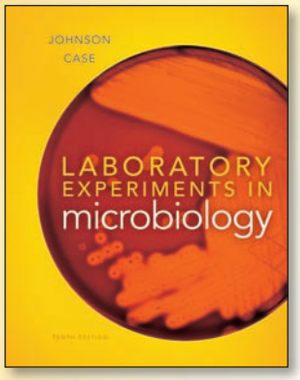

NEW! *Laboratory Experiments in Microbiology,* Tenth Edition

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and Christine L. Case
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Containing 57 thoroughly class-tested exercises, this manual provides engaging labs with instruction on performing basic microbiology techniques and applications in diverse areas, including the biological sciences, allied health sciences, agriculture, environmental science, nutrition, pharmacy, and various pre-professional programs. The **Tenth Edition** is easily customizable and features an updated art program and a full-color design, integrating valuable micrographs throughout each exercise. Additionally, many of the illustrations have been re-rendered in a modern, realistic, three-dimensional style to better visually engage students. Experiments have been refined throughout the manual and the Tenth Edition includes a new exercise using pGLO to demonstrate transformation in bacteria and introduce students to this important technique.

◂ Also available to help prepare your students for lab: *Preparation Guide for Laboratory Experiments in Microbiology,* Tenth Edition

by Ted R. Johnson and Christine L. Case
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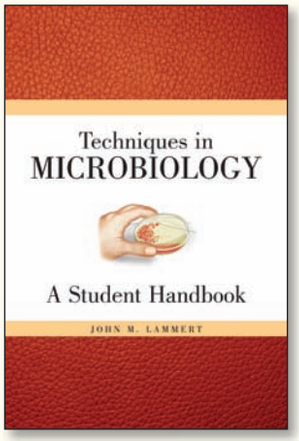

Techniques for Microbiology: A Student Handbook

by John M. Lammert
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Lammert's *Techniques in Microbiology* is highly visual and incorporates "voice balloons" that keep you focused on the relevant process. The techniques are those that will be used frequently for studying microbes in the laboratory, and include those identified by the American Society for Microbiology in its recommendations for the Microbiology Laboratory Core Curriculum (recommendations in which the author participated).

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Microbiology

An Introduction

ELEVENTH EDITION

Gerard J. Tortora
Bergen Community College

Berdell R. Funke
North Dakota State University

Christine L. Case
Skyline College

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Courtesy of Rev. Dr. James F. Tortora

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Berdell R. Funke Bert Funke received his Ph.D., M.S., and B.S. in microbiology from Kansas State University. He has spent his professional years as a professor of microbiology at North Dakota State University. He taught introductory microbiology, including laboratory sections, general microbiology, food microbiology, soil microbiology, clinical parasitology, and pathogenic microbiology. As a research scientist in the Experiment Station at North Dakota State, he has published numerous papers in soil microbiology and food microbiology.

Christine L. Case Chris Case is a registered microbiologist and a professor of microbiology at Skyline College in San Bruno, California, where she has taught for the past 40 years. She received her Ed.D. in curriculum and instruction from Nova Southeastern University and her M.A. in microbiology from San Francisco State University. She was Director for the Society for Industrial Microbiology (SIM) and is an active member of the ASM and Northern California SIM. She received the ASM and California Hayward outstanding educator awards. In 2008, Chris received the SACNAS Distinguished Community/Tribal College Mentor Award for her commitment to her students, several of whom have presented at undergraduate research conferences and won awards. In addition to teaching, Chris contributes regularly to the professional literature, develops innovative educational methodologies, and maintains a personal and professional commitment to conservation and the importance of science in society. Chris is also an avid photographer, and many of her photographs appear in this book.

PREFACE

Since the publication of the first edition nearly 30 years ago, well over one million students have used *Microbiology: An Introduction* at colleges and universities around the world, making it the leading textbook for non-majors microbiology. The eleventh edition continues to be a comprehensive beginning text, assuming no previous study of biology or chemistry. The text is appropriate for students in a wide variety of programs, including the allied health sciences, biological science, environmental science, animal science, forestry, agriculture, home economics, and the liberal arts.

The eleventh edition has retained the features that have made this book so popular:

- **An appropriate balance between microbiological fundamentals and applications, and between medical applications and other applied areas of microbiology.** Basic microbiological principles are given greater emphasis than are applications, and health-related applications are featured.
- **Straightforward presentation of complex topics.** Each section of the text is written with the student in mind.
- **Clear, accurate, and pedagogically effective illustrations and photos.** Step-by-step diagrams that closely coordinate with narrative descriptions aid student comprehension of concepts.
- **Flexible organization.** We have organized the book in what we think is a useful fashion while recognizing that the material might be effectively presented in other sequences. For instructors who wish to use a different order, we have made each chapter as independent as possible and have included numerous cross-references. The Instructor's Guide, written by Christine Case, provides detailed guidelines for organizing the material in several other ways.

NEW TO THE ELEVENTH EDITION

The visual introduction at the beginning of the book contains more details on the eleventh edition.

The eleventh edition meets all students at their respective levels of skill and understanding while addressing the biggest challenges that instructors face. Updates to the new eleventh edition enhance the book's consistent pedagogy and clear explanations. Some of the highlights of the eleventh edition follow:

- **Cutting-edge media integration.** MasteringMicrobiology (www.masteringmicrobiology.com) provides unprecedented, cutting-edge assessment resources for instructors as well as self-study tools for students. The 3-D MicroFlix and Microbiology Animations allow students to visualize key concepts; new Foundation Figure questions allow students to master the foundational material; new Case Studies stress real-world applications; and Lab Technique videos partner with the lab manual to prepare students so that they get the most out of lab time.
- **New Clinical Cases that relate the study of microbiology to real-world applications.** The Clinical Cases allow students to apply what they have learned to real-life scenarios. As the student reads the chapter they can follow along with the Clinical Case and answer critical thinking questions that directly relate to the material that they have just read.
- **Illustrations and photos that enhance student understanding**. The Foundation Figures and Life Cycle figures have been stunningly revised to foster student comprehension. The Foundation Figures, which integrate text and visuals to help students master the core concepts of microbiology, now include a bulleted list of Key Concepts. All stepwise figures (including Foundation Figures and Life Cycle figures) have been made to be entirely self-explanatory so that the student doesn't have to rely on lengthy captions to follow them. The new edition also includes over 100 new electron and light micrographs of quality unmatched in the market.
- **Addition of a Name It! activity to the Study Questions at the end of each chapter.** This question provides clues about the physical and biochemical nature of a microbe, signs and symptoms of the disease the microbe causes, information about treatment, etc., and then asks students to use their critical thinking skills to identify the microbe.

CHAPTER-BY-CHAPTER REVISIONS

Every chapter in this edition has been thoroughly revised, and data in the text, tables, Clinical Focus boxes, and figures have been updated through February 2011. The main changes to each chapter are summarized below.

Chapter 1

- A new section on H1N1 influenza (swine flu) has been added.
- A new section on multi-drug-resistant tuberculosis has been added.
- Figure 1.3 is now a Foundation Figure.

Chapter 2
- A new table on chemical bonds has been added.
- A new table compares DNA and RNA.

Chapter 5
- The discussion of photophosphorylation has been revised.

Chapter 6
- A new Applications of Microbiology box addresses life in extreme environments.

Chapter 8
- MicroRNA and epigenetic control are now included.

Chapter 9
- The discussions of gene silencing and forensic microbiology have been revised.
- Examples of veterinary uses of rDNA technology and nanotechnology are included.
- The Minimal Genome Project is introduced.

Chapter 10
- The tree of life has been revised to include new information on horizontal gene transfer between lineages.
- A molecular clock is introduced.
- Nucleic acid amplification tests are explained.

Chapter 11
- The section on the nonproteobacteria gram-negative bacteria has been reorganized.
- The material on purple and green photosynthetic bacteria has been extensively revised. A discussion of the deinococci has been added.

Chapter 12
- Newest changes to fungal and protozoan taxonomy are included.
- The chapter now includes discussion of microsporidia, emerging opportunistic pathogens.

Chapter 13
- Discussions on influenza epidemics and crossing the species barrier have been updated.

Chapter 14
- Data in the epidemiology graphs have been updated through 2010.
- A new section on the Human Microbiome Project has been added.
- A new section on health care–associated infections has been included.

Chapter 15
- A new Applications of Microbiology box addresses streptokinase.

Chapter 16
- The section on inflammation has been revised.
- The table on innate immunity responses has been revised.

Chapter 17
- A discussion of T_H17 T cells and the ineffectiveness of other T cells to deal with certain infections has been greatly expanded.

Chapter 18
- The discussion of the various types of vaccines has been extensively updated and revised.
- The discussion of adjuvants has been thoroughly updated and revised.
- A discussion of needle-free vaccines has been added.
- The significance of spelling of the names of monoclonal antibodies is now explained.

Chapter 19
- The discussion of HIV/AIDS has been extensively updated and revised.

Chapter 20
- Discussion of some of the newer antibiotics and antibiotic types, such as the pleuromutilins, has been added.
- A discussion of artemisinin-based malarial treatments is now included.
- The discussion of antibiotic-resistant superbugs has been expanded.
- An essay on the future of chemotherapeutic agents has been added.

Chapter 21
- An outbreak of *Pseudomonas* dermatitis is described in the Clinical Case.

Chapter 22
- The discussion of the meningococcal diseases and vaccines for polio has been extensively revised.
- Maps, graphs, and other art have been updated and revised.

Chapter 23
- The first case of dengue fever acquired in the United States is described in the Clinical Case.

Chapter 24
- The discussion of the etiology and symptoms of the common cold has been expanded.
- The diagnosis of tuberculosis has been updated and expanded.
- The discussion of influenza has been considerably expanded and updated.

Chapter 25
- The discussions of traveler's diarrhea (*E. coli* gastroenteritis) and hepatitis B infections have been revised extensively.
- Discussion of *Clostridium difficile*–associated diarrhea is now included.

Chapter 26

- The discussion of the gonococcus now describes Opa proteins.
- The discussion of neonatal herpes and genital warts has been updated and revised.

Chapter 27

- The figure depicting the sulfur cycle has been revised.

Chapter 28

- Quality assurance microbiology is demonstrated in the Clinical Case.

ACKNOWLEDGMENTS

In preparing this textbook, we have benefited from the guidance and advice of a large number of microbiology instructors across the country. These reviewers have provided constructive criticism and valuable suggestions at various stages of the revision. We gratefully acknowledge our debt to these individuals.

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Gerard J. Tortora ***Berdell R. Funke*** ***Christine L. Case***

BRIEF CONTENTS

CONTENTS

FEATURES

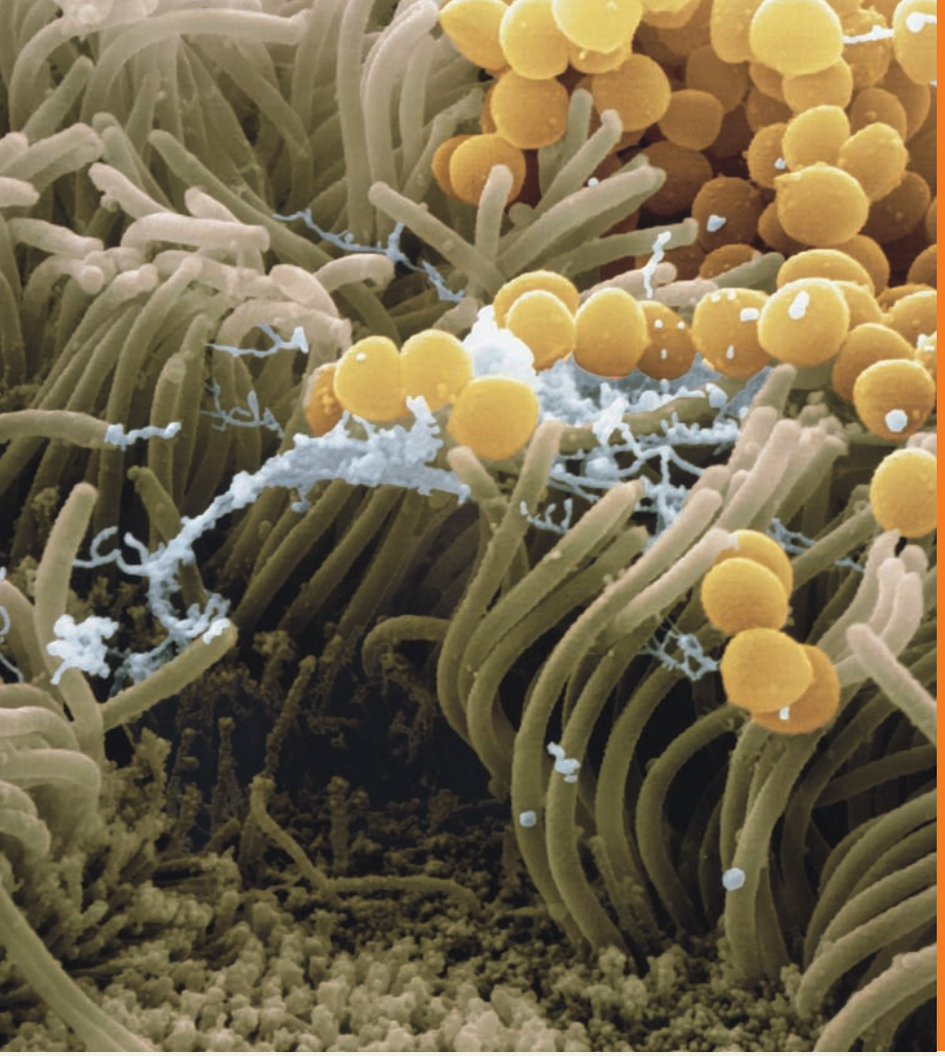
FOUNDATION FIGURES

LIFE CYCLE FIGURES

CLINICAL FOCUS

APPLICATIONS OF MICROBIOLOGY

DISEASES IN FOCUS



1

The Microbial World and You

MasteringMICROBIOLOGY™

Visualize microbiology and check your understanding with a pre-test at www.masteringmicrobiology.com.

The overall theme of this textbook is the relationship between microbes (very small organisms that usually require a microscope to be seen) and our lives. This relationship involves not only the familiar harmful effects of certain microorganisms, such as disease and food spoilage, but also their many beneficial effects. In this chapter we introduce you to some of the many ways microbes affect our lives. Microbes have been fruitful subjects of study for many years. We begin by introducing you to how organisms are named and classified, followed by a short history of microbiology that reveals how much we have learned in just a few hundred years. We then discuss the incredible diversity of microorganisms and their ecological importance, noting how they maintain balance in the environment by recycling chemical elements such as carbon and nitrogen among the soil, organisms, and the atmosphere. We also examine how microbes are used in commercial and industrial applications to produce foods, chemicals, and drugs (such as antibiotics); and to treat sewage, control pests, and clean up pollutants. We will discuss microbes as the cause of such diseases as avian (bird) flu, West Nile encephalitis, mad cow disease, diarrhea, hemorrhagic fever, and AIDS. We will also examine the growing public health problem of antibiotic-resistant bacteria. *Staphylococcus aureus* bacteria on human nasal epithelial cells are shown in the photograph. These bacteria live harmlessly on skin or inside the nose. Misuse of antibiotics allows the survival of bacteria with antibiotic-resistant genes such as methicillin-resistant *S. aureus* (MRSA). As illustrated in the Clinical Case, an infection caused by these bacteria is resistant to antibiotic treatment.

Microbes in Our Lives

LEARNING OBJECTIVE

1-1 List several ways in which microbes affect our lives.

For many people, the words *germ* and *microbe* bring to mind a group of tiny creatures that do not quite fit into any of the categories in that old question, "Is it animal, vegetable, or mineral?" **Microbes,** also called **microorganisms,** are minute living things that individually are usually too small to be seen with the unaided eye. The group includes bacteria (Chapter 11), fungi (yeasts and molds), protozoa, and microscopic algae (Chapter 12). It also includes viruses, those noncellular entities sometimes regarded as straddling the border between life and nonlife (Chapter 13). You will be introduced to each of these groups of microbes shortly.

We tend to associate these small organisms only with major diseases such as AIDS, uncomfortable infections, or such common inconveniences as spoiled food. However, the majority of microorganisms actually help maintain the balance of living organisms and chemicals in our environment. Marine and freshwater microorganisms form the basis of the food chain in oceans, lakes, and rivers. Soil microbes help break down wastes and incorporate nitrogen gas from the air into organic compounds, thereby recycling chemical elements between the soil, water, life, and air. Certain microbes play important roles in *photosynthesis,* a food- and oxygen-generating process that is critical to life on Earth. Humans and many other animals depend on the microbes in their intestines for digestion and the synthesis of some vitamins that their bodies require, including some B vitamins for metabolism and vitamin K for blood clotting.

Microorganisms also have many commercial applications. They are used in the synthesis of such chemical products as vitamins, organic acids, enzymes, alcohols, and many drugs. For example, microbes are used to produce acetone and butanol, and the vitamins B_2 (riboflavin) and B_{12} (cobalamin) are made biochemically. The process by which microbes produce acetone and butanol was discovered in 1914 by Chaim Weizmann, a Russian-born chemist working in England. With the outbreak of World War I in August of that year, the production of acetone became very important for making cordite (a smokeless form of gunpowder used in munitions). Weizmann's discovery played a significant role in determining the outcome of the war.

The food industry also uses microbes in producing, for example, vinegar, sauerkraut, pickles, soy sauce, cheese, yogurt, bread, and alcoholic beverages. In addition, enzymes from microbes can now be manipulated to cause the microbes to produce substances they normally do not synthesize, including cellulose, digestive aids, and drain cleaner, plus important therapeutic substances such as insulin. Microbial enzymes may even have helped produce your favorite pair of jeans (see the box on page 3).

Though only a minority of microorganisms are **pathogenic** (disease-producing), practical knowledge of microbes is necessary for medicine and the related health sciences. For example, hospital workers must be able to protect patients from common microbes that are normally harmless but pose a threat to the sick and injured.

Today we understand that microorganisms are found almost everywhere. Yet not long ago, before the invention of the microscope, microbes were unknown to scientists. Thousands of people died in devastating epidemics, the causes of which were not understood. Entire families died because vaccinations and antibiotics were not available to fight infections.

We can get an idea of how our current concepts of microbiology developed by looking at a few historic milestones in microbiology that have changed our lives. First, however, we will look at the major groups of microbes and how they are named and classified.

CHECK YOUR UNDERSTANDING

✓ Describe some of the destructive and beneficial actions of microbes. **1-1***

Clinical Case: A Simple Spider Bite?

Andrea is a normally healthy 22-year-old college student who lives at home with her mother and younger sister, a high school gymnast. She is trying to work on a paper for her psychology class but is having a hard time because a red, swollen sore on her right wrist is making typing difficult. "Why won't this spider bite heal?" she wonders. "It's been there for days!" She makes an appointment with her doctor so she can show him the painful lesion. Although Andrea does not have a fever, she does have an elevated white blood cell count that indicates a bacterial infection. Andrea's doctor suspects that this isn't a spider bite at all, but a staph infection. He prescribes a β-lactam antibiotic, cephalosporin. Learn more about the development of Andrea's illness on the following pages.

What is staph? Read on to find out.

2 17 19 20 21

Naming and Classifying Microorganisms

LEARNING OBJECTIVES

1-2 Recognize the system of scientific nomenclature that uses two names: a genus and a specific epithet.

1-3 Differentiate the major characteristics of each group of microorganisms.

1-4 List the three domains.

* The numbers following Check Your Understanding questions refer to the corresponding Learning Objectives.

Designer Jeans: Made by Microbes?

Denim blue jeans have become increasingly popular ever since Levi Strauss and Jacob Davis first made them for California gold miners in 1873. Now, companies that manufacture blue jeans are turning to microbiology to develop environmentally sound production methods that minimize toxic wastes and the associated costs.

Stone Washing?

A softer denim, called "stone-washed," was introduced in the 1980s. Enzymes, called cellulases, from *Trichoderma* fungus are used to digest some of the cellulose in the cotton, thereby softening it and giving the stone-washed appearance. Unlike many chemical reactions, enzymes usually operate at safe temperatures and pH. Moreover, enzymes are proteins, so they are readily degraded for removal from wastewater.

Fabric

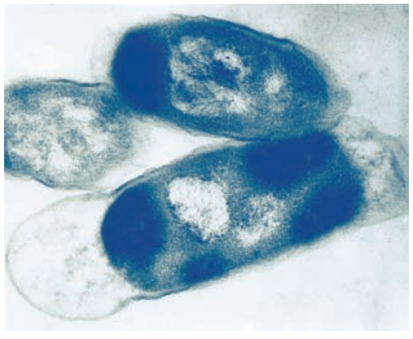
Cotton production requires large tracts of land, pesticides, and fertilizer, and the crop yield depends on the weather. However, bacteria can produce both cotton and polyester with less environmental impact. *Gluconacetobacter xylinus* bacteria make cellulose by attaching glucose units to simple chains in the outer membrane of the bacterial cell wall. The cellulose microfibrils are extruded through pores in the outer membrane, and bundles of microfibrils then twist into ribbons.

Bleaching

Peroxide is a safer bleaching agent than chlorine and can be easily removed from fabric and wastewater by enzymes. Researchers at Novo Nordisk Biotech cloned a mushroom peroxidase gene in yeast and grew the yeasts in washing machine conditions. The yeast that survived the washing machine were selected as the peroxidase producers.

Indigo

Chemical synthesis of indigo requires a high pH and produces waste that explodes in contact with air. However, a California biotechnology company, Genencor, has developed a method to produce indigo by using bacteria. Researchers identified a gene from a soil bacterium, *Pseudomonas putida*, for conversion of the bacterial by-product indole to indigo. This gene was put into *Escherichia coli* bacteria, which then turned blue.



Indigo-producing *E. coli* bacteria.

Bioplastic

Microbes can even make plastic zippers and packaging material for the jeans. Over 25 bacteria make polyhydroxyalkanoate (PHA) inclusion granules as a food reserve. PHAs are similar to common plastics, and because they are made by bacteria, they are also readily degraded by many bacteria. PHAs could provide a biodegradable alternative to conventional plastic, which is made from petroleum.

***E. coli* bacteria produce indigo from tryptophan.**

Nomenclature

The system of nomenclature (naming) for organisms in use today was established in 1735 by Carolus Linnaeus. Scientific names are latinized because Latin was the language traditionally used by scholars. Scientific nomenclature assigns each organism two names—the **genus** (plural: *genera*) is the first name and is always capitalized; the **specific epithet** (**species** name) follows and is not capitalized. The organism is referred to by both the genus and the specific epithet, and both names are underlined or italicized. By custom, after a scientific name has been mentioned once, it can be abbreviated with the initial of the genus followed by the specific epithet.

Scientific names can, among other things, describe an organism, honor a researcher, or identify the habitat of a species. For example, consider *Staphylococcus aureus* (staf-i-lō-kok′kus ô′rē-us), a bacterium commonly found on human skin. *Staphylo-* describes the clustered arrangement of the cells; *coccus* indicates that they are shaped like spheres. The specific epithet, *aureus,* is Latin for golden, the color of many colonies of this bacterium. The genus of the bacterium *Escherichia coli* (esh-ē-rik′-ē-ä kō′lī or kō′lē) is named for a scientist, Theodor Escherich, whereas its specific epithet, *coli,* reminds us that *E. coli* live in the colon, or large intestine. **Table 1.1** contains more examples.

CHECK YOUR UNDERSTANDING

Distinguish a genus from a specific epithet. **1-2**

Types of Microorganisms

The classification and identification of microorganisms is discussed in Chapter 10. Here is an overview of the major groups.

Bacteria

Bacteria (singular: **bacterium**) are relatively simple, single-celled (unicellular) organisms. Because their genetic material is not

TABLE 1.1 Making Scientific Names Familiar

Use the word roots guide in Appendix E to find out what the name means. The name will not seem so strange if you translate it. When you encounter a new name, practice saying it out loud. The exact pronunciation is not as important as the familiarity you will gain. Guidelines for pronunciation are given in Appendix D.

Following are some examples of microbial names you may encounter in the popular press as well as in the lab.

	Pronunciation	Source of Genus Name	Source of Specific Epithet
Salmonella enterica (bacterium)	sal-mōn-el′lä en-ter′i-kä	Honors public health microbiologist Daniel Salmon	Found in the intestines (*entero-*)
Streptococcus pyogenes (bacterium)	strep-tō-kok′kus pī-äj′en-ēz	Appearance of cells in chains (*strepto-*)	Forms pus (*pyo-*)
Saccharomyces cerevisiae (yeast)	sak-ä-rō-mī′ses se-ri-vis′ē-ī	Fungus (*-myces*) that uses sugar (*saccharo-*)	Makes beer (*cerevisia*)
Penicillium chrysogenum (fungus)	pen-i-sil′lē-um krī-so′jen-um	Tuftlike or paintbrush (*penicill-*) appearance microscopically	Produces a yellow (*chryso-*) pigment
Trypanosoma cruzi (protozoan)	tri-pa-nō-sō′mä krüz′ē	Corkscrew- (*trypano-*, borer; *soma-*, body)	Honors epidemiologist Oswaldo Cruz

enclosed in a special nuclear membrane, bacterial cells are called **prokaryotes** (prō-kar′e-ōts), from Greek words meaning prenucleus. Prokaryotes include both bacteria and archaea.

Bacterial cells generally appear in one of several shapes. *Bacillus* (bä-sil′lus) (rodlike), illustrated in **Figure 1.1a**, *coccus* (kok′kus) (spherical or ovoid), and *spiral* (corkscrew or curved) are among the most common shapes, but some bacteria are star-shaped or square (see Figures 4.1 through 4.5, pages 77–78). Individual bacteria may form pairs, chains, clusters, or other groupings; such formations are usually characteristic of a particular genus or species of bacteria.

Bacteria are enclosed in cell walls that are largely composed of a carbohydrate and protein complex called *peptidoglycan.* (By contrast, cellulose is the main substance of plant and algal cell walls.) Bacteria generally reproduce by dividing into two equal cells; this process is called *binary fission.* For nutrition, most bacteria use organic chemicals, which in nature can be derived from either dead or living organisms. Some bacteria can manufacture their own food by photosynthesis, and some can derive nutrition from inorganic substances. Many bacteria can "swim" by using moving appendages called *flagella.* (For a complete discussion of bacteria, see Chapter 11.)

Archaea

Like bacteria, **archaea** (är′kē-ä) consist of prokaryotic cells, but if they have cell walls, the walls lack peptidoglycan. Archaea, often found in extreme environments, are divided into three main groups. The *methanogens* produce methane as a waste product from respiration. The *extreme halophiles* (*halo* = salt; *philic* = loving) live in extremely salty environments such as the Great Salt Lake and the Dead Sea. The *extreme thermophiles* (*therm* = heat) live in hot sulfurous water, such as hot springs at Yellowstone National Park. Archaea are not known to cause disease in humans.

Fungi

Fungi (singular: **fungus**) are **eukaryotes** (yū-kar′ē-ōts), organisms whose cells have a distinct nucleus containing the cell's genetic material (DNA), surrounded by a special envelope called the nuclear membrane. Organisms in the Kingdom Fungi may be unicellular or multicellular (see Chapter 12, page 331). Large multicellular fungi, such as mushrooms, may look somewhat like plants, but unlike most plants, fungi cannot carry out photosynthesis. True fungi have cell walls composed primarily of a substance called *chitin.* The unicellular forms of fungi, *yeasts,* are oval microorganisms that are larger than bacteria. The most typical fungi are *molds* (**Figure 1.1b**). Molds form visible masses called *mycelia,* which are composed of long filaments (*hyphae*) that branch and intertwine. The cottony growths sometimes found on bread and fruit are mold mycelia. Fungi can reproduce sexually or asexually. They obtain nourishment by absorbing solutions of organic material from their environment—whether soil, seawater, freshwater, or an animal or plant host. Organisms called *slime molds* have characteristics of both fungi and amoebas. They are discussed in detail in Chapter 12.

Protozoa

Protozoa (singular: **protozoan**) are unicellular eukaryotic microbes (see Chapter 12, page 348). Protozoa move by pseudopods, flagella, or cilia. Amebae (**Figure 1.1c**) move by using extensions of their cytoplasm called *pseudopods* (false feet). Other protozoa have long *flagella* or numerous shorter appendages for locomotion

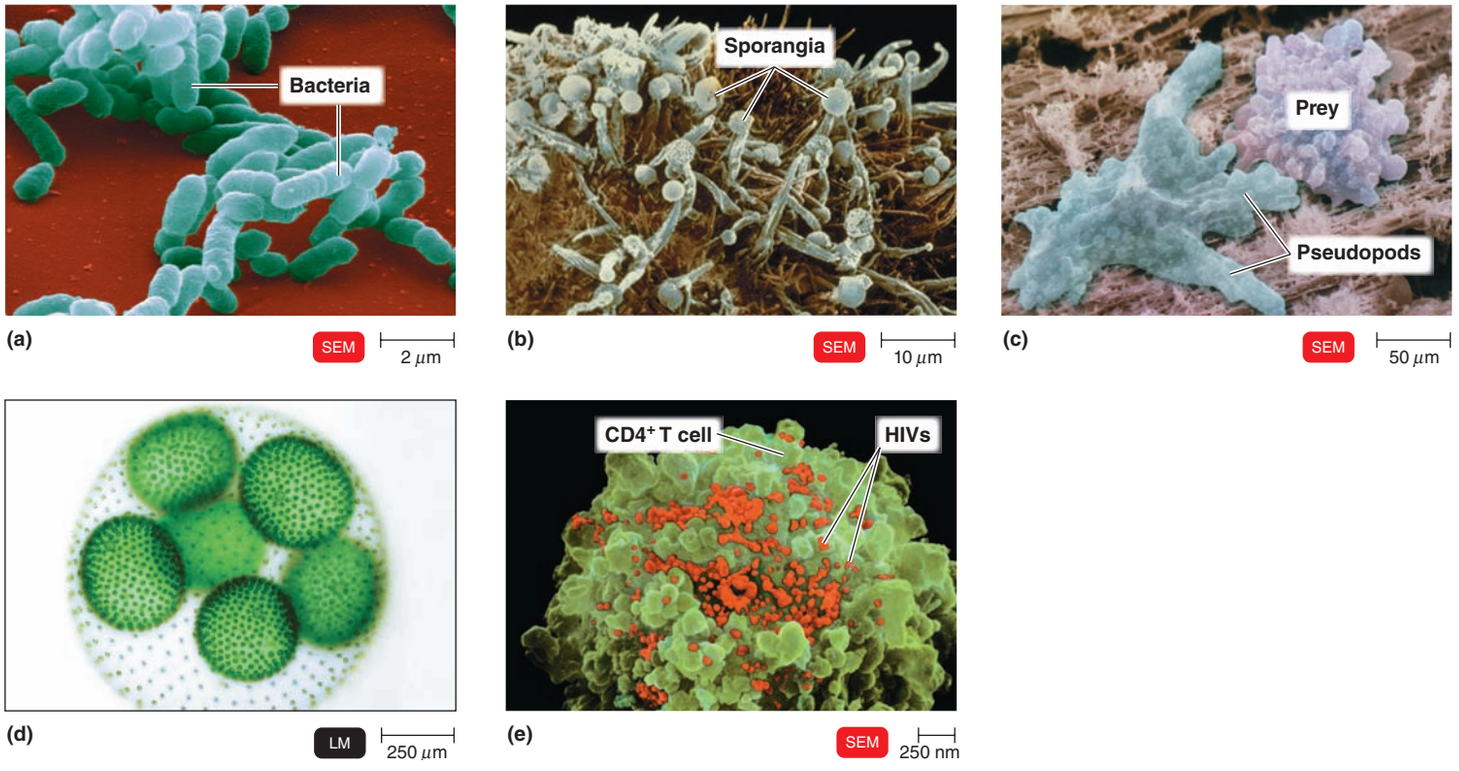


Figure 1.1 Types of microorganisms.
NOTE: Throughout the book, a red icon under a micrograph indicates that the micrograph has been artificially colored. (**a**) The rod-shaped bacterium *Haemophilus influenzae,* one of the bacterial causes of pneumonia. (**b**) *Mucor,* a common bread mold, is a type of fungus. When released from sporangia, spores that land on a favorable surface germinate into a network of hyphae (filaments) that absorb nutrients. (**c**) An ameba, a protozoan, approaching a food particle. (**d**) The pond alga *Volvox.* (**e**) Several human immunodeficiency viruses (HIVs), the causative agent of AIDS, budding from a $CD4^+$ T cell.

How are bacteria, archaea, fungi, protozoa, algae, and viruses distinguished on the basis of cellular structure?

called *cilia.* Protozoa have a variety of shapes and live either as free entities or as *parasites* (organisms that derive nutrients from living hosts) that absorb or ingest organic compounds from their environment. Some protozoa, such as *Euglena,* are photosynthetic. They use light as a source of energy and carbon dioxide as their chief source of carbon to produce sugars. Protozoa can reproduce sexually or asexually.

Algae

Algae (singular: **alga**) are photosynthetic eukaryotes with a wide variety of shapes and both sexual and asexual reproductive forms (Figure 1.1d). The algae of interest to microbiologists are usually unicellular (see Chapter 12, page 343). The cell walls of many algae, are composed of a carbohydrate called *cellulose.* Algae are abundant in freshwater and salt water, in soil, and in association with plants. As photosynthesizers, algae need light, water, and carbon dioxide for food production and growth, but they do not generally require organic compounds from the environment. As a result of photosynthesis, algae produce oxygen and carbohydrates that are then utilized by other organisms, including animals. Thus, they play an important role in the balance of nature.

Viruses

Viruses (Figure 1.1e) are very different from the other microbial groups mentioned here. They are so small that most can be seen only with an electron microscope, and they are acellular (not cellular). Structurally very simple, a virus particle contains a core made of only one type of nucleic acid, either DNA or RNA. This core is surrounded by a protein coat, which is sometimes encased by a lipid membrane called an envelope. All living cells have RNA *and* DNA, can carry out chemical reactions, and can reproduce as self-sufficient units. Viruses can reproduce only by using the cellular machinery of other organisms. Thus, on the one hand, viruses are considered to be living only when they multiply within host cells they infect. In this sense, viruses are parasites of other forms of life. On the other hand, viruses are not considered to be living because they are inert outside living hosts. (Viruses will be discussed in detail in Chapter 13.)

Multicellular Animal Parasites

Although multicellular animal parasites are not strictly microorganisms, they are of medical importance and therefore will be

discussed in this text. Animal parasites are eukaryotes. The two major groups of parasitic worms are the flatworms and the roundworms, collectively called **helminths** (see Chapter 12, page 354). During some stages of their life cycle, helminths are microscopic in size. Laboratory identification of these organisms includes many of the same techniques used for identifying microbes.

CHECK YOUR UNDERSTANDING

- Which groups of microbes are prokaryotes? Which are eukaryotes? **1-3**

Classification of Microorganisms

Before the existence of microbes was known, all organisms were grouped into either the animal kingdom or the plant kingdom. When microscopic organisms with characteristics of animals and plants were discovered late in the seventeenth century, a new system of classification was needed. Still, biologists could not agree on the criteria for classifying these new organisms until the late 1970s.

In 1978, Carl Woese devised a system of classification based on the cellular organization of organisms. It groups all organisms in three domains as follows:

1. Bacteria (cell walls contain a protein–carbohydrate complex called peptidoglycan)
2. Archaea (cell walls, if present, lack peptidoglycan)
3. Eukarya, which includes the following:
   - Protists (slime molds, protozoa, and algae)
   - Fungi (unicellular yeasts, multicellular molds, and mushrooms)
   - Plants (mosses, ferns, conifers, and flowering plants)
   - Animals (sponges, worms, insects, and vertebrates)

Classification will be discussed in more detail in Chapters 10 through 12.

CHECK YOUR UNDERSTANDING

- What are the three domains? **1-4**

A Brief History of Microbiology

LEARNING OBJECTIVES

1-5 Explain the importance of observations made by Hooke and van Leeuwenhoek.

1-6 Compare spontaneous generation and biogenesis.

1-7 Identify the contributions to microbiology made by Needham, Spallanzani, Virchow, and Pasteur.

1-8 Explain how Pasteur's work influenced Lister and Koch.

1-9 Identify the importance of Koch's postulates.

1-10 Identify the importance of Jenner's work.

1-11 Identify the contributions to microbiology made by Ehrlich and Fleming.

1-12 Define *bacteriology*, *mycology*, *parasitology*, *immunology*, and *virology*.

1-13 Explain the importance of microbial genetics and molecular biology.

The science of microbiology dates back only 200 years, yet the recent discovery of *Mycobacterium tuberculosis* (mī-kō-bak-ti′rē-um tü-bėr-ku-lō′sis) DNA in 3000-year-old Egyptian mummies reminds us that microorganisms have been around for much longer. In fact, bacterial ancestors were the first living cells to appear on Earth. Although we know relatively little about what earlier people thought about the causes, transmission, and treatment of disease, we know more about the history of the past few hundred years. Let's look now at some key developments in microbiology that have spurred the field to its current technological state.

The First Observations

One of the most important discoveries in biology occurred in 1665. After observing a thin slice of cork through a relatively crude microscope, an Englishman, Robert Hooke, reported to the world that life's smallest structural units were "little boxes," or "cells," as he called them. Using his improved version of a compound microscope (one that uses two sets of lenses), Hooke was able to see individual cells. Hooke's discovery marked the beginning of the **cell theory**—the theory that *all living things are composed of cells.* Subsequent investigations into the structure and function of cells were based on this theory.

Though Hooke's microscope was capable of showing large cells, it lacked the resolution that would have allowed him to see microbes clearly. The Dutch merchant and amateur scientist Anton van Leeuwenhoek was probably the first actually to observe live microorganisms through the magnifying lenses of more than 400 microscopes he constructed. Between 1673 and 1723, he wrote a series of letters to the Royal Society of London describing the "animalcules" he saw through his simple, single-lens microscope. Van Leeuwenhoek made detailed drawings of "animalcules" he found in rainwater, in his own feces, and in material scraped from his teeth. These drawings have since been identified as representations of bacteria and protozoa (**Figure 1.2**).

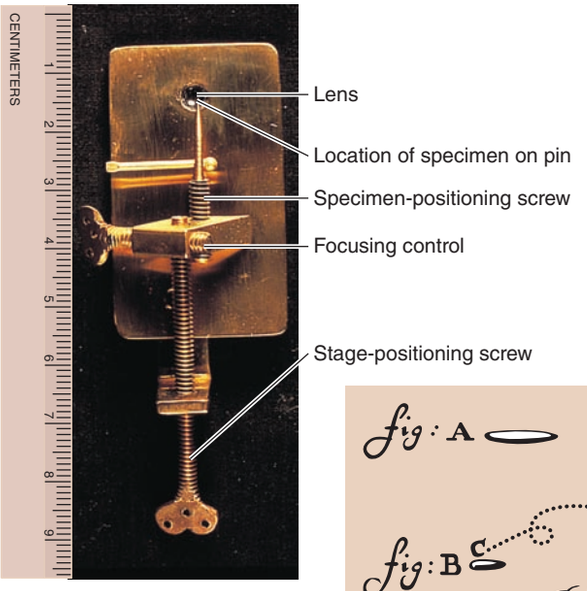
CHECK YOUR UNDERSTANDING

- What is the cell theory? **1-5**

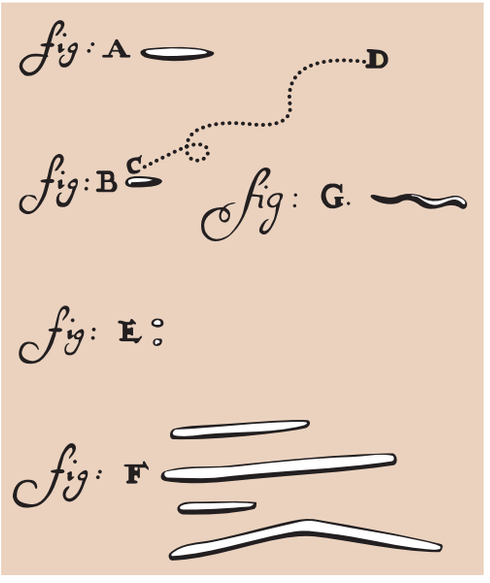
The Debate over Spontaneous Generation

After van Leeuwenhoek discovered the previously "invisible" world of microorganisms, the scientific community of the time became interested in the origins of these tiny living things. Until the second half of the nineteenth century, many scientists and philosophers believed that some forms of life could arise spontaneously from nonliving matter; they called this hypothetical process **spontaneous generation.** Not much more than 100 years ago, people commonly believed that toads, snakes, and mice could be born of moist soil; that flies could emerge from manure;

(a) Van Leeuwenhoek using his microscope



(b) Microscope replica



(c) Drawings of bacteria

Figure 1.2 Anton van Leeuwenhoek's microscopic observations. (**a**) By holding his brass microscope toward a source of light, van Leeuwenhoek was able to observe living organisms too small to be seen with the unaided eye. (**b**) The specimen was placed on the tip of the adjustable point and viewed from the other side through the tiny, nearly spherical lens. The highest magnification possible with his microscopes was about 300× (times). (**c**) Some of van Leeuwenhoek's drawings of bacteria, made in 1683. The letters represent various shapes of bacteria. C–D represents a path of motion he observed.

Q Why was van Leeuwenhoek's discovery so important?

and that maggots (which we now know are the larvae of flies) could arise from decaying corpses.

Evidence Pro and Con

A strong opponent of spontaneous generation, the Italian physician Francesco Redi set out in 1668 to demonstrate that maggots did not arise spontaneously from decaying meat. Redi filled two jars with decaying meat. The first was left unsealed; the flies laid their eggs on the meat, and the eggs developed into larvae. The second jar was sealed, and because the flies could not lay their eggs on the meat, no maggots appeared. Still, Redi's antagonists were not convinced; they claimed that fresh air was needed for spontaneous generation. So Redi set up a second experiment, in which he covered a jar with a fine net instead of sealing it. No larvae appeared in the gauze-covered jar, even though air was present. Maggots appeared only when flies were allowed to leave their eggs on the meat.

Redi's results were a serious blow to the long-held belief that large forms of life could arise from nonlife. However, many scientists still believed that small organisms, such as van Leeuwenhoek's "animalcules," were simple enough to be generated from nonliving materials.

The case for spontaneous generation of microorganisms seemed to be strengthened in 1745, when John Needham, an Englishman, found that even after he heated nutrient fluids (chicken broth and corn broth) before pouring them into covered flasks, the cooled solutions were soon teeming with microorganisms. Needham claimed that microbes developed spontaneously from the fluids. Twenty years later, Lazzaro Spallanzani, an Italian scientist, suggested that microorganisms from the air probably had entered Needham's solutions after they were boiled. Spallanzani showed that nutrient fluids heated *after* being sealed in a flask did not develop microbial growth. Needham responded by claiming the "vital force" necessary for spontaneous generation had been destroyed by the heat and was kept out of the flasks by the seals.

This intangible "vital force" was given all the more credence shortly after Spallanzani's experiment, when Anton Laurent Lavoisier showed the importance of oxygen to life. Spallanzani's observations were criticized on the grounds that there was not enough oxygen in the sealed flasks to support microbial life.

The Theory of Biogenesis

The issue was still unresolved in 1858, when the German scientist Rudolf Virchow challenged the case for spontaneous generation with the concept of **biogenesis,** the claim that living cells can arise only from preexisting living cells. Because he could offer no scientific proof, arguments about spontaneous generation continued until 1861, when the issue was finally resolved by the French scientist Louis Pasteur.

With a series of ingenious and persuasive experiments, Pasteur demonstrated that microorganisms are present in the air and can contaminate sterile solutions, but that air itself does not create microbes. He filled several short-necked flasks with beef broth and then boiled their contents. Some were then left open and allowed to cool. In a few days, these flasks were found to be contaminated with microbes. The other flasks, sealed after boiling, were free of microorganisms. From these results, Pasteur reasoned that microbes in the air were the agents responsible for contaminating nonliving matter.

Pasteur next placed broth in open-ended, long-necked flasks and bent the necks into S-shaped curves (Figure 1.3). The contents of these flasks were then boiled and cooled. The broth in the flasks did not decay and showed no signs of life, even after months. Pasteur's unique design allowed air to pass into the flask, but the curved neck trapped any airborne microorganisms that might contaminate the broth. (Some of these original vessels are still on display at the Pasteur Institute in Paris. They have been sealed but, like the flask shown in Figure 1.3, show no sign of contamination more than 100 years later.)

Pasteur showed that microorganisms can be present in nonliving matter—on solids, in liquids, and in the air. Furthermore, he demonstrated conclusively that microbial life can be destroyed by heat and that methods can be devised to block the access of airborne microorganisms to nutrient environments. These discoveries form the basis of **aseptic techniques,** techniques that prevent contamination by unwanted microorganisms, which are now the standard practice in laboratory and many medical procedures. Modern aseptic techniques are among the first and most important concepts that a beginning microbiologist learns.

Pasteur's work provided evidence that microorganisms cannot originate from mystical forces present in nonliving materials. Rather, any appearance of "spontaneous" life in nonliving solutions can be attributed to microorganisms that were already present in the air or in the fluids themselves. Scientists now believe that a form of spontaneous generation probably did occur on the primitive Earth when life first began, but they agree that this does not happen under today's environmental conditions.

CHECK YOUR UNDERSTANDING

- What evidence supported spontaneous generation? 1-6
- How was spontaneous generation disproved? 1-7

The Golden Age of Microbiology

The work that began with Pasteur started an explosion of discoveries in microbiology. The period from 1857 to 1914 has been appropriately named the Golden Age of Microbiology. During this period, rapid advances, spearheaded mainly by Pasteur and Robert Koch, led to the establishment of microbiology as a science. Discoveries during these years included both the agents of many diseases and the role of immunity in preventing and curing disease. During this productive period, microbiologists studied the chemical activities of microorganisms, improved the techniques for performing microscopy and culturing microorganisms, and developed vaccines and surgical techniques. Some of the major events that occurred during the Golden Age of Microbiology are listed in Figure 1.4.

Fermentation and Pasteurization

One of the key steps that established the relationship between microorganisms and disease occurred when a group of French merchants asked Pasteur to find out why wine and beer soured. They hoped to develop a method that would prevent spoilage when those beverages were shipped long distances. At the time, many scientists believed that air converted the sugars in these fluids into alcohol. Pasteur found instead that microorganisms called yeasts convert the sugars to alcohol in the absence of air. This process, called **fermentation** (see Chapter 5, page 130), is used to make wine and beer. Souring and spoilage are caused by different microorganisms called bacteria. In the presence of air, bacteria change the alcohol into vinegar (acetic acid).

Pasteur's solution to the spoilage problem was to heat the beer and wine just enough to kill most of the bacteria that caused the spoilage. The process, called **pasteurization,** is now commonly used to reduce spoilage and kill potentially harmful bacteria in milk as well as in some alcoholic drinks. Showing the connection between food spoilage and microorganisms was a major step toward establishing the relationship between disease and microbes.

The Germ Theory of Disease

As we have seen, the fact that many kinds of diseases are related to microorganisms was unknown until relatively recently. Before the time of Pasteur, effective treatments for many diseases were discovered by trial and error, but the causes of the diseases were unknown.

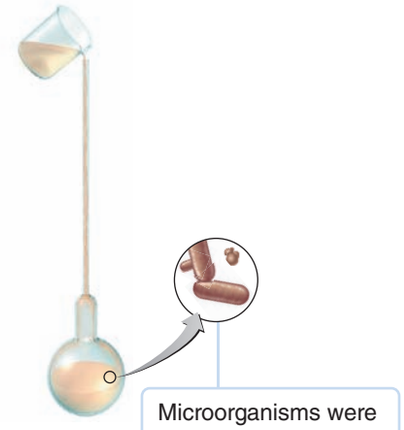
The realization that yeasts play a crucial role in fermentation was the first link between the activity of a microorganism and physical and chemical changes in organic materials. This discovery alerted scientists to the possibility that microorganisms might have similar relationships with plants and animals—specifically, that microorganisms might cause disease. This idea was known as the **germ theory of disease.**

FOUNDATION FIGURE **1.3**

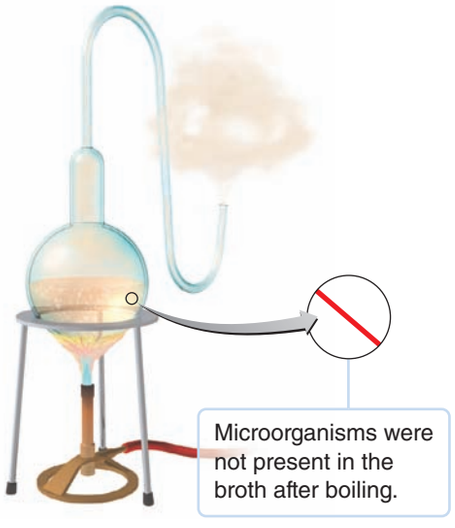
Disproving the Theory of Spontaneous Generation

According to the theory of spontaneous generation, life can arise spontaneously from nonliving matter, such as dead corpses and soil. Pasteur's experiment, described below, demonstrated that microbes are present in nonliving matter—air, liquids, and solids.

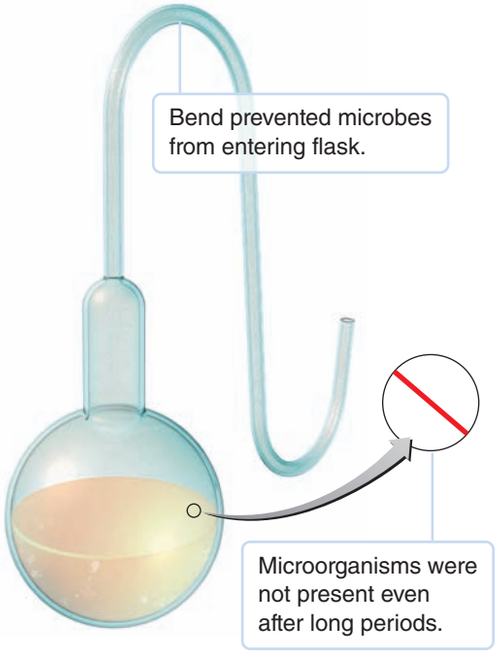
1 Pasteur first poured beef broth into a long-necked flask.



2 Next he heated the neck of the flask and bent it into an S-shape; then he boiled the broth for several minutes.



3 Microorganisms did not appear in the cooled solution, even after long periods.



KEY CONCEPTS

- Pasteur demonstrated that microbes are responsible for food spoilage, leading researchers to the connection between microbes and disease.
- His experiments and observations provided the basis of aseptic techniques, which are used to prevent microbial contamination, as shown in the photo at right.

The germ theory was a difficult concept for many people to accept at that time because for centuries disease was believed to be punishment for an individual's crimes or misdeeds. When the inhabitants of an entire village became ill, people often blamed the disease on demons appearing as foul odors from sewage or on poisonous vapors from swamps. Most people born in Pasteur's time found it inconceivable that "invisible" microbes could travel through the air to infect plants and animals or remain on clothing and bedding to be transmitted from one person to another. Despite these doubts scientists gradually accumulated the information needed to support the new germ theory.

In 1865, Pasteur was called upon to help fight silkworm disease, which was ruining the silk industry throughout Europe. Years earlier, in 1835, Agostino Bassi, an amateur microscopist, had proved that another silkworm disease was caused by a fungus. Using data provided by Bassi, Pasteur found that the more recent infection was caused by a protozoan, and he developed a method for recognizing afflicted silkworm moths.

In the 1860s, Joseph Lister, an English surgeon, applied the germ theory to medical procedures. Lister was aware that in the 1840s, the Hungarian physician Ignaz Semmelweis had demonstrated that physicians, who at the time did not disinfect their hands, routinely transmitted infections (puerperal, or childbirth, fever) from one obstetrical patient to another. Lister had also heard of Pasteur's work connecting microbes to animal diseases. Disinfectants were not used at the time, but Lister knew

1665 Hooke—First observation of cells
1673 van Leeuwenhoek—First observation of live microorganisms
1735 Linnaeus—Nomenclature for organisms
1798 Jenner—First vaccine
1835 Bassi—Silkworm fungus
1840 Semmelweis—Childbirth fever
1853 DeBary—Fungal plant disease

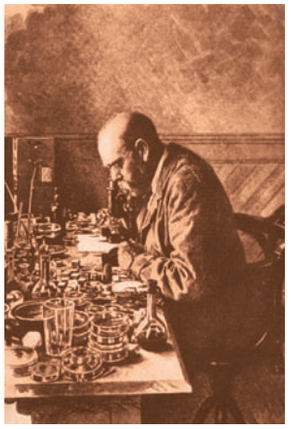
1857 Pasteur—Fermentation
1861 Pasteur—Disproved spontaneous generation
1864 Pasteur—Pasteurization
1867 Lister—Aseptic surgery
1876 *Koch—Germ theory of disease
1879 Neisser—*Neisseria gonorrhoeae*
1881 *Koch—Pure cultures
Finley—Yellow fever
1882 *Koch—*Mycobacterium tuberculosis*
Hess—Agar (solid) media
1883 *Koch—*Vibrio cholerae*
1884 *Metchnikoff—Phagocytosis
Gram—Gram-staining procedure
Escherich—*Escherichia coli*
1887 Petri—Petri dish
1889 Kitasato—*Clostridium tetani*
1890 *von Bering—Diphtheria antitoxin
*Ehrlich—Theory of immunity
1892 Winogradsky—Sulfur cycle
1898 Shiga—*Shigella dysenteriae*
1908 *Ehrlich—Syphilis
1910 Chagas—*Trypanosoma cruzi*
1911 * Rous—Tumor-causing virus (1966 Nobel Prize)

GOLDEN AGE OF MICROBIOLOGY

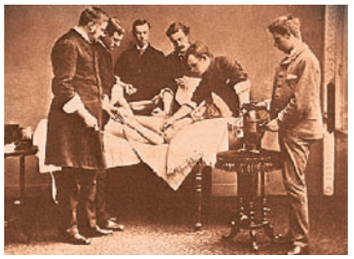
1928 *Fleming, Chain, Florey—Penicillin
Griffith—Transformation in bacteria
1934 Lancefield—Streptococcal antigens
1935 *Stanley, Northrup, Sumner—Crystallized virus
1941 Beadle and Tatum—Relationship between genes and enzymes
1943 *Delbrück and Luria—Viral infection of bacteria
1944 Avery, MacLeod, McCarty—Genetic material is DNA
1946 Lederberg and Tatum—Bacterial conjugation
1953 *Watson and Crick—DNA structure
1957 *Jacob and Monod—Protein synthesis regulation
1959 Stewart—Viral cause of human cancer
1962 *Edelman and Porter—Antibodies
1964 Epstein, Achong, Barr—Epstein-Barr virus as cause of human cancer
1971 *Nathans, Smith, Arber—Restriction enzymes (used for recombinant DNA technology)
1973 Berg—Genetic engineering
1975 Dulbecco, Temin, Baltimore—Reverse transcriptase
1978 Woese—Archaea
*Mitchell—Chemiosmotic mechanism
1981 Margulis—Origin of eukaryotic cells
1982 *Klug—Structure of tobacco mosaic virus
1983 *McClintock—Transposons

1988 *Deisenhofer, Huber, Michel—Bacterial photosynthesis pigments
1994 Cano—Reported to have cultured 40-million-year-old bacteria
1997 *Prusiner—Prions

Louis Pasteur (1822–1895)
Demonstrated that life did not arise spontaneously from nonliving matter.



Robert Koch (1843–1910)
Established experimental steps for directly linking a specific microbe to a specific disease.



Joseph Lister (1827–1912)
Performed surgery under antiseptic conditions using phenol. Proved that microbes caused surgical wound infections.

Rebecca C. Lancefield (1895–1981)
Classified streptococci according to serotypes (variants within a species)

Figure 1.4 Milestones in microbiology, highlighting those that occurred during the Golden Age of Microbiology. An asterisk (*) indicates a Nobel laureate.

Q Why do you think the Golden Age of Microbiology occurred when it did?

that phenol (carbolic acid) kills bacteria, so he began treating surgical wounds with a phenol solution. The practice so reduced the incidence of infections and deaths that other surgeons quickly adopted it. Lister's technique was one of the earliest medical attempts to control infections caused by microorganisms. In fact, his findings proved that microorganisms cause surgical wound infections.

The first proof that bacteria actually cause disease came from Robert Koch in 1876. Koch, a German physician, was Pasteur's young rival in the race to discover the cause of anthrax, a disease that was destroying cattle and sheep in Europe. Koch discovered rod-shaped bacteria now known as *Bacillus anthracis* (bä-sil′lus an-thrā′sis) in the blood of cattle that had died of anthrax. He cultured the bacteria on nutrients and then injected samples of the culture into healthy animals. When these animals became sick and died, Koch isolated the bacteria in their blood and compared them with the originally isolated bacteria. He found that the two sets of blood cultures contained the same bacteria.

Koch thus established **Koch's postulates,** a sequence of experimental steps for directly relating a specific microbe to a specific disease (see Figure 14.3, page 407). During the past 100 years, these same criteria have been invaluable in investigations proving that specific microorganisms cause many diseases. Koch's postulates, their limitations, and their application to disease will be discussed in greater detail in Chapter 14.

Vaccination

Often a treatment or preventive procedure is developed before scientists know why it works. The smallpox vaccine is an example. On May 4, 1796, almost 70 years before Koch established that a specific microorganism causes anthrax, Edward Jenner, a young British physician, embarked on an experiment to find a way to protect people from smallpox.

Smallpox epidemics were greatly feared. The disease periodically swept through Europe, killing thousands, and it wiped out 90% of the American Indians on the East Coast when European settlers first brought the infection to the New World.

When a young milkmaid informed Jenner that she couldn't get smallpox because she already had been sick from cowpox—a much milder disease—he decided to put the girl's story to the test. First Jenner collected scrapings from cowpox blisters. Then he inoculated a healthy 8-year-old volunteer with the cowpox material by scratching the person's arm with a pox-contaminated needle. The scratch turned into a raised bump. In a few days, the volunteer became mildly sick but recovered and never again contracted either cowpox or smallpox. The process was called *vaccination,* from the Latin word *vacca,* meaning cow. Pasteur gave it this name in honor of Jenner's work. The protection from disease provided by vaccination (or by recovery from the disease itself) is called **immunity.** We will discuss the mechanisms of immunity in Chapter 17.

Years after Jenner's experiment, in about 1880, Pasteur discovered why vaccinations work. He found that the bacterium that causes fowl cholera lost its ability to cause disease (lost its *virulence,* or became *avirulent*) after it was grown in the laboratory for long periods. However, it—and other microorganisms with decreased virulence—was able to induce immunity against subsequent infections by its virulent counterparts. The discovery of this phenomenon provided a clue to Jenner's successful experiment with cowpox. Both cowpox and smallpox are caused by viruses. Even though cowpox virus is not a laboratory-produced derivative of smallpox virus, it is so closely related to the smallpox virus that it can induce immunity to both viruses. Pasteur used the term *vaccine* for cultures of avirulent microorganisms used for preventive inoculation.

Jenner's experiment marked the first time in a Western culture that a living viral agent—the cowpox virus—was used to produce immunity. Physicians in China had immunized patients from smallpox by removing scales from drying pustules of a person suffering from a mild case of smallpox, grinding the scales to a fine powder, and inserting the powder into the nose of the person to be protected.

Some vaccines are still produced from avirulent microbial strains that stimulate immunity to the related virulent strain. Other vaccines are made from killed virulent microbes, from isolated components of virulent microorganisms, or by genetic engineering techniques.

CHECK YOUR UNDERSTANDING

- Summarize in your own words the germ theory of disease. **1-8**
- What is the importance of Koch's postulates? **1-9**
- What is the significance of Jenner's discovery? **1-10**

The Birth of Modern Chemotherapy: Dreams of a "Magic Bullet"

After the relationship between microorganisms and disease was established, medical microbiologists next focused on the search for substances that could destroy pathogenic microorganisms without damaging the infected animal or human. Treatment of disease by using chemical substances is called **chemotherapy.** (The term also commonly refers to chemical treatment of noninfectious diseases, such as cancer.) Chemicals produced naturally by bacteria and fungi to act against other microorganisms are called **antibiotics.** Chemotherapeutic agents prepared from chemicals in the laboratory are called **synthetic drugs.** The success of chemotherapy is based on the fact that some chemicals are more poisonous to microorganisms than to the hosts infected by the microbes. Antimicrobial therapy will be discussed in further detail in Chapter 20.

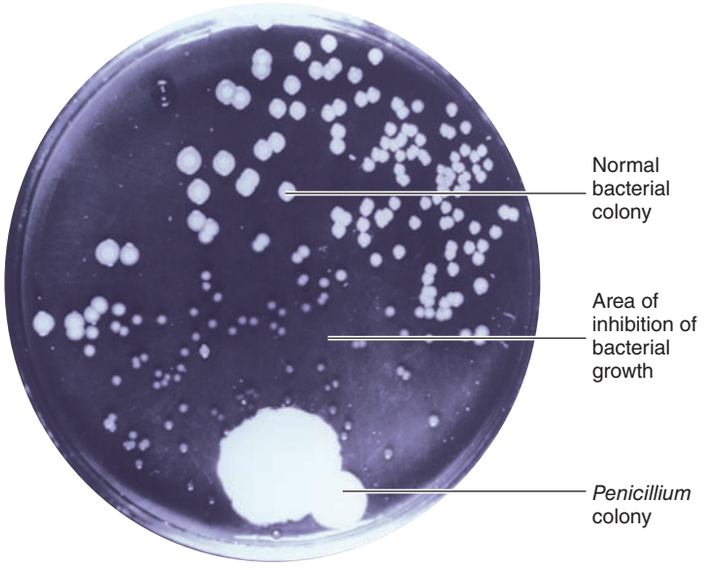


Figure 1.5 The discovery of penicillin. Alexander Fleming took this photograph in 1928. The colony of *Penicillium* mold accidentally contaminated the plate and inhibited nearby bacterial growth.

Q Why do you think penicillin is no longer as effective as it once was?

The First Synthetic Drugs

Paul Ehrlich, a German physician, was the imaginative thinker who fired the first shot in the chemotherapy revolution. As a medical student, Ehrlich speculated about a "magic bullet" that could hunt down and destroy a pathogen without harming the infected host. He then launched a search for such a bullet. In 1910, after testing hundreds of substances, he found a chemotherapeutic agent called *salvarsan,* an arsenic derivative effective against syphilis. The agent was named salvarsan because it was considered to offer salvation from syphilis and it contained arsenic. Before this discovery, the only known chemical in Europe's medical arsenal was an extract from the bark of a South American tree, *quinine,* which had been used by Spanish conquistadors to treat malaria.

By the late 1930s, researchers had developed several other synthetic drugs that could destroy microorganisms. Most of these drugs were derivatives of dyes. This came about because the dyes synthesized and manufactured for fabrics were routinely tested for antimicrobial qualities by microbiologists looking for a "magic bullet." In addition, *sulfonamides* (sulfa drugs) were synthesized at about the same time.

A Fortunate Accident—Antibiotics

In contrast to the sulfa drugs, which were deliberately developed from a series of industrial chemicals, the first antibiotic was discovered by accident. Alexander Fleming, a Scottish physician and bacteriologist, almost tossed out some culture plates that had been contaminated by mold. Fortunately, he took a second look at the curious pattern of growth on the contaminated plates. Around the mold was a clear area where bacterial growth had been inhibited (**Figure 1.5**). Fleming was looking at a mold that could inhibit the growth of a bacterium. The mold was later identified as *Penicillium notatum* (pen-i-sil′lē-um nō-tā′tum), later renamed *Penicillium chrysogenum* (krī-so′jen-um), and in 1928 Fleming named the mold's active inhibitor *penicillin.* Thus, penicillin is an antibiotic produced by a fungus. The enormous usefulness of penicillin was not apparent until the 1940s, when it was finally tested clinically and mass produced.

Since these early discoveries, thousands of other antibiotics have been discovered. Unfortunately, antibiotics and other chemotherapeutic drugs are not without problems. Many antimicrobial chemicals are too toxic to humans for practical use; they kill the pathogenic microbes, but they also damage the infected host. For reasons we will discuss later, toxicity to humans is a particular problem in the development of drugs for treating viral diseases. Viral growth depends on life processes of normal host cells. Thus, there are very few successful antiviral drugs, because a drug that would interfere with viral reproduction would also likely affect uninfected cells of the body.

Another major problem associated with antimicrobial drugs is the emergence and spread of new strains of microorganisms that are resistant to antibiotics. Over the years, more and more microbes have developed resistance to antibiotics that at one time were very effective against them. Drug resistance results from genetic changes in microbes that enables them to tolerate a certain amount of an antibiotic that would normally inhibit them (see the box in Chapter 26, page 757). For example a microbe might produce chemicals (enzymes) that inactivate antibiotics, or a microbe might undergo changes to its surface that prevent an antibiotic from attaching to it or entering it.

The recent appearance of vancomycin-resistant *Staphylococcus aureus* and *Enterococcus faecalis* (en-te-rō-kok′kus fe-kā′lis) has alarmed health care professionals because it indicates that some previously treatable bacterial infections may soon be impossible to treat with antibiotics.

CHECK YOUR UNDERSTANDING

✓ What was Ehrlich's "magic bullet"? **1-11**

Modern Developments in Microbiology

The quest to solve drug resistance, identify viruses, and develop vaccines requires sophisticated research techniques and correlated studies that were never dreamed of in the days of Koch and Pasteur.

The groundwork laid during the Golden Age of Microbiology provided the basis for several monumental achievements during the twentieth century (**Table 1.2**). New branches of microbiology were developed, including immunology and virology. Most recently, the development of a set of new methods called recombinant DNA technology has revolutionized research and practical applications in all areas of microbiology.

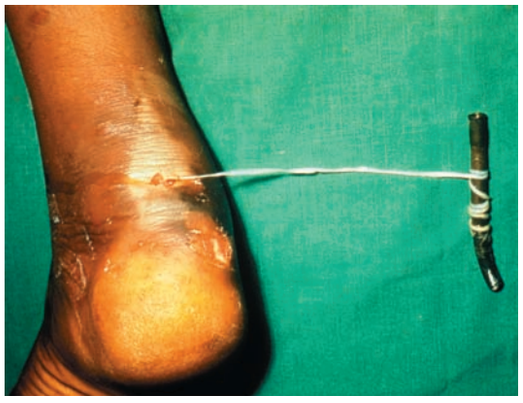
Bacteriology, Mycology, and Parasitology

Bacteriology, the study of bacteria, began with van Leeuwenhoek's first examination of tooth scrapings. New pathogenic

TABLE 1.2 Selected Nobel Prizes Awarded for Research in Microbiology

Nobel Laureates	Year of Presentation	Country of Birth	Contribution
Ronald Ross	1902	England	Discovered how malaria is transmitted
Selman A. Waksman	1952	Ukraine	Discovered streptomycin
Hans A. Krebs	1953	Germany	Discovered chemical steps of the Krebs cycle in carbohydrate metabolism
John F. Enders, Thomas H. Weller, and Frederick C. Robbins	1954	United States	Cultured poliovirus in cell cultures
Joshua Lederberg, George Beadle, and Edward Tatum	1958	United States	Described genetic control of biochemical reactions
Frank Macfarlane Burnet and Peter Brian Medawar	1960	Australia Great Britain	Discovered acquired immune tolerance
César Milstein, Georges J. F. Köhler, and Niels Kai Jerne	1984	Argentina Germany Denmark	Developed a technique for producing monoclonal antibodies (single pure antibodies)
Susumu Tonegawa	1987	Japan	Described the genetics of antibody production
J. Michael Bishop and Harold E. Varmus	1989	United States	Discovered cancer-causing genes called oncogenes
Joseph E. Murray and E. Donnall Thomas	1990	United States	Performed the first successful organ transplants by using immunosuppressive agents
Edmond H. Fisher and Edwin G. Krebs	1992	United States	Discovered protein kinases, enzymes that regulate cell growth
Richard J. Roberts and Phillip A. Sharp	1993	Great Britain United States	Discovered that a gene can be separated onto different segments of DNA
Kary B. Mullis	1993	United States	Discovered the polymerase chain reaction to amplify (make multiple copies of) DNA
Peter C. Doherty and Rolf M. Zinkernagel	1996	Australia Switzerland	Discovered how cytotoxic T cells recognize virus-infected cells prior to destroying them
Peter Agre and Roderick MacKirron	2003	United States	Discovered water and ion channels in plasma membranes
Aaron Ciechanover, Avram Hershko, and Irwin Rose	2004	Israel Israel United States	Discovered how cells dispose of unwanted proteins in proteasomes
Barry Marshall and J. Robin Warren	2005	Australia	Discovered that *Helicobacter pylori* causes peptic ulcers
Andrew Fire and Craig Mello	2006	United States	Discovered RNA interference (RNAi), or gene silencing, by double-stranded RNA
Harald zur Hausen	2008	Germany	Discovered that human papilloma viruses cause cervical cancer
Françoise Barré-Sinoussi and Luc Montagnier	2008	France	Discovered human immunodeficiency virus (HIV)
Venkatraman Ramakrishnan, Thomas A. Steitz, and Ada E. Yonath	2010	India United States Israel	Detailed study of the structure and function of ribosomes

(a) Rod of Asclepius, symbol of the medical profession.



(b) A parasitic guinea worm (*Dracunculus medinensis*) is removed from the subcutaneous tissue of a patient by winding it onto a stick. This procedure may have been used for the design of the symbol in part (a).

Figure 1.6 **Parasitology: the study of protozoa and parasitic worms.**

How do you think parasitic worms survive and live off a human host?

bacteria are still discovered regularly. Many bacteriologists, like Pasteur, look at the roles of bacteria in food and the environment. One intriguing discovery came in 1997, when Heide Schulz discovered a bacterium large enough to be seen with the unaided eye (0.2 mm wide). This bacterium, named *Thiomargarita namibiensis* (thī′o-mä-gär-e-tä na′mib-ē-ėn-sis), lives in the mud on the African coast. *Thiomargarita* is unusual because of its size and its ecological niche. The bacterium consumes hydrogen sulfide, which would be toxic to mud-dwelling animals (Figure 11.28, page 327).

Mycology, the study of fungi, includes medical, agricultural, and ecological branches. Recall that Bassi's work leading up to the germ theory of disease focused on a fungal pathogen. Fungal infection rates have been rising during the past decade, accounting for 10% of hospital-acquired infections. Climatic and environmental changes (severe drought) are thought to account for the tenfold increase in *Coccidioides immitis* (kok-sid-ē-oi′dēz im′mi-tis) infections in California. New techniques for diagnosing and treating fungal infections are currently being investigated.

Parasitology is the study of protozoa and parasitic worms. Because many parasitic worms are large enough to be seen with the unaided eye, they have been known for thousands of years. It has been speculated that the medical symbol, the rod of Asclepius, represents the removal of parasitic guinea worms (**Figure 1.6**). Asclepius was a Greek physician who practiced about 1200 B.C. and was deified as the god of medicine.

The clearing of rain forests has exposed laborers to previously undiscovered parasites. Previously unknown parasitic diseases are also being found in patients whose immune systems have been suppressed by organ transplants, cancer chemotherapy, or AIDS.

Bacteriology, mycology, and parasitology are currently going through a "golden age" of classification. Recent advances in **genomics,** the study of all of an organism's genes, have allowed scientists to classify bacteria and fungi according to their genetic relationships with other bacteria, fungi, and protozoa. These microorganisms were originally classified according to a limited number of visible characteristics.

Immunology

Immunology, the study of immunity, dates back in Western culture to Jenner's first vaccine in 1796. Since then, knowledge about the immune system has accumulated steadily and expanded rapidly. Vaccines are now available for numerous diseases, including measles, rubella (German measles), mumps, chickenpox, pneumococcal pneumonia, tetanus, tuberculosis, influenza, whooping cough, polio, and hepatitis B. The smallpox vaccine was so effective that the disease has been eliminated. Public health officials estimate that polio will be eradicated within a few years because of the polio vaccine.

A major advance in immunology occurred in 1933, when Rebecca Lancefield proposed that streptococci be classified according to serotypes (variants within a species) based on certain components in the cell walls of the bacteria. Streptococci are responsible for a variety of diseases, such as sore throat (strep throat), streptococcal toxic shock, and septicemia (blood poisoning). Her research permits the rapid identification of specific pathogenic streptococci based on immunological techniques.

In 1960, interferons, substances generated by the body's own immune system, were discovered. Interferons inhibit replication of viruses and have triggered considerable research related to the treatment of viral diseases and cancer. One of today's biggest challenges for immunologists is learning how the immune system might be stimulated to ward off the virus responsible for AIDS, a disease that destroys the immune system.

Virology

The study of viruses, **virology,** originated during the Golden Age of Microbiology. In 1892, Dmitri Iwanowski reported that the organism that caused mosaic disease of tobacco was so small that it passed through filters fine enough to stop all known bacteria. At the time, Iwanowski was not aware that the organism in question was a virus. In 1935, Wendell Stanley demonstrated that the organism, called tobacco mosaic virus (TMV), was fundamentally different from other microbes and so simple and homogeneous that it could be crystallized like a chemical compound. Stanley's work facilitated the study of viral structure and chemistry. Since the development of the electron microscope in the 1940s, microbiologists have been able to observe the structure of viruses in detail, and today much is known about their structure and activity.

Recombinant DNA Technology

Microorganisms can now be genetically modified to manufacture large amounts of human hormones and other urgently needed medical substances. In the late 1960s, Paul Berg showed that fragments of human or animal DNA (genes) that code for important proteins can be attached to bacterial DNA. The resulting hybrid was the

first example of **recombinant DNA.** When recombinant DNA is inserted into bacteria (or other microbes), it can be used to make large quantities of the desired protein. The technology that developed from this technique is called **recombinant DNA technology.** Its origins can be found in two related fields. The first, **microbial genetics,** studies the mechanisms by which microorganisms inherit traits. The second, **molecular biology,** specifically studies how genetic information is carried in molecules of DNA and how DNA directs the synthesis of proteins.

Although molecular biology encompasses all organisms, much of our knowledge of how genes determine specific traits has been revealed through experiments with bacteria. Through the 1930s, all genetic research was based on the study of plant and animal cells. But in the 1940s, scientists turned to unicellular organisms, primarily bacteria, which have several advantages for genetic and biochemical research. For one thing, bacteria are less complex than plants and animals. For another, the life cycles of many bacteria last less than an hour, so scientists can cultivate very large numbers of bacteria for study in a relatively short time.

Once science turned to the study of unicellular life, rapid progress was made in genetics. In 1941, George W. Beadle and Edward L. Tatum demonstrated the relationship between genes and enzymes. DNA was established as the hereditary material in 1944 by Oswald Avery, Colin MacLeod, and Maclyn McCarty. In 1946, Joshua Lederberg and Edward L. Tatum discovered that genetic material could be transferred from one bacterium to another by a process called conjugation. Then, in 1953, James Watson and Francis Crick proposed a model for the structure and replication of DNA. The early 1960s witnessed a further explosion of discoveries relating to the way DNA controls protein synthesis. In 1961, François Jacob and Jacques Monod discovered messenger RNA (ribonucleic acid), a chemical involved in protein synthesis, and later they made the first major discoveries about the regulation of gene function in bacteria. During the same period, scientists were able to break the genetic code and thus understand how the information for protein synthesis in messenger RNA is translated into the amino acid sequence for making proteins.

CHECK YOUR UNDERSTANDING

- Define *bacteriology*, *mycology*, *parasitology*, *immunology*, and *virology*. **1-12**
- Differentiate microbial genetics from molecular biology. **1-13**

Microbes and Human Welfare

LEARNING OBJECTIVES

1-14 List at least four beneficial activities of microorganisms.

1-15 Name two examples of biotechnology that use recombinant DNA technology and two examples that do not.

As mentioned earlier, only a minority of all microorganisms are pathogenic. Microbes that cause food spoilage, such as soft spots on fruits and vegetables, decomposition of meats, and rancidity of fats and oils, are also a minority. The vast majority of microbes benefit humans, other animals, and plants in many ways. For example, microbes produce methane and ethanol that can be used as alternative fuels to generate electricity and power vehicles. Biotechnology companies are using bacterial enzymes to break down plant cellulose so that yeast can metabolize the resulting simple sugars and produce ethanol. The following sections outline some of these beneficial activities. In later chapters, we will discuss these activities in greater detail.

Recycling Vital Elements

Discoveries made by two microbiologists in the 1880s have formed the basis for today's understanding of the biogeochemical cycles that support life on Earth. Martinus Beijerinck and Sergei Winogradsky were the first to show how bacteria help recycle vital elements between the soil and the atmosphere. **Microbial ecology,** the study of the relationship between microorganisms and their environment, originated with the work of these scientists. Today, microbial ecology has branched out and includes the study of how microbial populations interact with plants and animals in various environments. Among the concerns of microbial ecologists are water pollution and toxic chemicals in the environment.

The chemical elements carbon, nitrogen, oxygen, sulfur, and phosphorus are essential for life and abundant, but not necessarily in forms that organisms can use. Microorganisms are primarily responsible for converting these elements into forms that plants and animals can use. Microorganisms, primarily bacteria and fungi, return carbon dioxide to the atmosphere when they decompose organic wastes and dead plants and animals. Algae, cyanobacteria, and higher plants use the carbon dioxide during photosynthesis to produce carbohydrates for animals, fungi, and bacteria. Nitrogen is abundant in the atmosphere but in that form is not usable by plants and animals. Only bacteria can naturally convert atmospheric nitrogen to a form available to plants and animals.

Sewage Treatment: Using Microbes to Recycle Water

Our society's growing awareness of the need to preserve the environment has made people more conscious of the responsibility to recycle precious water and prevent the pollution of rivers and oceans. One major pollutant is sewage, which consists of human excrement, waste water, industrial wastes, and surface runoff. Sewage is about 99.9% water, with a few hundredths of 1% suspended solids. The remainder is a variety of dissolved materials.

Sewage treatment plants remove the undesirable materials and harmful microorganisms. Treatments combine various physical processes with the action of beneficial microbes. Large solids such as paper, wood, glass, gravel, and plastic are removed from sewage; left behind are liquid and organic materials that bacteria convert into such by-products as carbon dioxide, nitrates, phosphates, sulfates, ammonia, hydrogen sulfide, and methane. (We will discuss sewage treatment in detail in Chapter 27.)

Bioremediation: Using Microbes to Clean Up Pollutants

In 1988, scientists began using microbes to clean up pollutants and toxic wastes produced by various industrial processes. For example, some bacteria can actually use pollutants as energy sources; others produce enzymes that break down toxins into less harmful substances. By using bacteria in these ways—a process known as **bioremediation**—toxins can be removed from underground wells, chemical spills, toxic waste sites, and oil spills, such as the massive oil spill from an offshore drilling rig in the Gulf of Mexico on April 20, 2010 (see also the box in Chapter 2, page 32). In addition, bacterial enzymes are used in drain cleaners to remove clogs without adding harmful chemicals to the environment. In some cases, microorganisms indigenous to the environment are used; in others, genetically modified microbes are used. Among the most commonly used microbes are certain species of bacteria of the genera *Pseudomonas* (sū-dō-mō′nas) and *Bacillus* (bă-sil′lus). *Bacillus* enzymes are also used in household detergents to remove spots from clothing.

Insect Pest Control by Microorganisms

Besides spreading diseases, insects can cause devastating crop damage. Insect pest control is therefore important for both agriculture and the prevention of human disease.

The bacterium *Bacillus thuringiensis* (thůr-in-jē-en′sis) has been used extensively in the United States to control such pests as alfalfa caterpillars, bollworms, corn borers, cabbageworms, tobacco budworms, and fruit tree leaf rollers. It is incorporated into a dusting powder that is applied to the crops these insects eat. The bacteria produce protein crystals that are toxic to the digestive systems of the insects. The toxin gene also has been inserted into some plants to make them insect resistant.

By using microbial rather than chemical insect control, farmers can avoid harming the environment. Many chemical insecticides, such as DDT, remain in the soil as toxic pollutants and are eventually incorporated into the food chain.

Modern Biotechnology and Recombinant DNA Technology

Earlier, we touched on the commercial use of microorganisms to produce some common foods and chemicals. Such practical applications of microbiology are called **biotechnology.** Although biotechnology has been used in some form for centuries, techniques have become much more sophisticated in the past few decades. In the last several years, biotechnology has undergone a revolution through the advent of recombinant DNA technology to expand the potential of bacteria, viruses, and yeast cells and other fungi as miniature biochemical factories. Cultured plant and animal cells, as well as intact plants and animals, are also used as recombinant cells and organisms.

The applications of recombinant DNA technology are increasing with each passing year. Recombinant DNA techniques have been used thus far to produce a number of natural proteins, vaccines, and enzymes. Such substances have great potential for medical use; some of them are described in Table 9.1 on page 248.

A very exciting and important outcome of recombinant DNA techniques is **gene therapy**—inserting a missing gene or replacing a defective one in human cells. This technique uses a harmless virus to carry the missing or new gene into certain host cells, where the gene is picked up and inserted into the appropriate chromosome. Since 1990, gene therapy has been used to treat patients with adenosine deaminase (ADA) deficiency, a cause of severe combined immunodeficiency disease (SCID), in which cells of the immune system are inactive or missing; Duchenne's muscular dystrophy, a muscle-destroying disease; cystic fibrosis, a disease of the secreting portions of the respiratory passages, pancreas, salivary glands, and sweat glands; and LDL-receptor deficiency, a condition in which low-density lipoprotein (LDL) receptors are defective and LDL cannot enter cells. The LDL remains in the blood in high concentrations and increases the risk of atherosclerosis and coronary artery disease because it leads to fatty plaque formation in blood vessels. Results are still being evaluated. Other genetic diseases may also be treatable by gene therapy in the future, including hemophilia, an inability of the blood to clot normally; diabetes, elevated blood sugar levels; sickle cell disease, an abnormal kind of hemoglobin; and one type of hypercholesterolemia, high blood cholesterol.

Beyond medical applications, recombinant DNA techniques have also been applied to agriculture. For example, genetically altered strains of bacteria have been developed to protect fruit against frost damage, and bacteria are being modified to control insects that damage crops. Recombinant DNA has also been used to improve the appearance, flavor, and shelf life of fruits and vegetables. Potential agricultural uses of recombinant DNA include drought resistance, resistance to insects and microbial diseases, and increased temperature tolerance in crops.

CHECK YOUR UNDERSTANDING

- Name two beneficial uses of bacteria. **1-14**
- Differentiate biotechnology from recombinant DNA technology. **1-15**

Microbes and Human Disease

LEARNING OBJECTIVES

1-16 Define *normal microbiota* and *resistance.*

1-17 Define *biofilm.*

1-18 Define *emerging infectious disease.*

Normal Microbiota

We all live from birth until death in a world filled with microbes, and we all have a variety of microorganisms on and inside our

bodies. These microorganisms make up our **normal microbiota,** or *flora** (Figure 1.7). The normal microbiota not only do us no harm, but also in some cases can actually benefit us. For example, some normal microbiota protect us against disease by preventing the overgrowth of harmful microbes, and others produce useful substances such as vitamin K and some B vitamins. Unfortunately, under some circumstances normal microbiota can make us sick or infect people we contact. For instance, when some normal microbiota leave their habitat, they can cause disease.

When is a microbe a welcome part of a healthy human, and when is it a harbinger of disease? The distinction between health and disease is in large part a balance between the natural defenses of the body and the disease-producing properties of microorganisms. Whether our bodies overcome the offensive tactics of a particular microbe depends on our **resistance**—the ability to ward off diseases. Important resistance is provided by the barrier of the skin, mucous membranes, cilia, stomach acid, and antimicrobial chemicals such as interferons. Microbes can be destroyed by white blood cells, by the inflammatory response, by fever, and by specific responses of our immune system. Sometimes, when our natural defenses are not strong enough to overcome an invader, they have to be supplemented by antibiotics or other drugs.

Clinical Case

Staph is the common name for *Staphylococcus aureus* bacteria, which are carried on the skin of about 30% of the human population. Although Andrea is diligent about taking her antibiotic as prescribed, she doesn't seem to be improving. After 3 days, the lesion on her wrist is even larger than before and is now draining yellow pus. Andrea also develops a fever. Her mother insists that she call her doctor to tell him about the latest developments.

Why does Andrea's infection persist after treatment?

2 **17** 19 20 21

Biofilms

In nature, microorganisms may exist as single cells that float or swim independently in a liquid, or they may attach to each other and/or some usually solid surface. This latter mode of behavior is called a **biofilm,** a complex aggregation of microbes. The slime covering a rock in a lake is a biofilm. Use your tongue to feel the biofilm on your teeth. Biofilms can be beneficial. They protect your mucous membranes from harmful microbes, and biofilms in lakes are an important food for aquatic animals. Biofilms can also be harmful. They can clog water pipes, and on medical implants such as joint prostheses and catheters (Figure 1.8), they can cause such infections as endocarditis (inflammation of the heart). Bacteria in biofilms are often resistant to antibiotics because the biofilm offers a protective barrier. See the box in Chapter 3 on page 56. Biofilms will be discussed in Chapter 6.

* At one time, bacteria and fungi were thought to be plants, and thus the term *flora* was used.

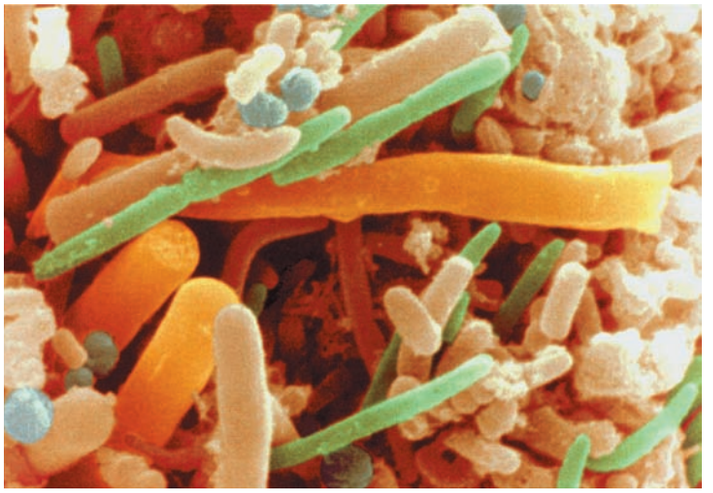


Figure 1.7 Several types of bacteria found as part of the normal microbiota on the surface of the human tongue.

Q How do we benefit from the production of vitamin K by microbes?

Infectious Diseases

An **infectious disease** is a disease in which pathogens invade a susceptible host, such as a human or an animal. In the process, the pathogen carries out at least part of its life cycle inside the host, and disease frequently results. By the end of World War II, many people believed that infectious diseases were under control. They thought malaria would be eradicated through the use of the insecticide DDT to kill mosquitoes, that a vaccine would prevent diphtheria, and that improved sanitation measures would help prevent cholera transmission. Malaria is far from eliminated. Since 1986, local outbreaks have been identified in New Jersey, California, Florida, New York, and Texas, and the disease infects 300 million people worldwide. In 1994, diphtheria appeared in the United States, brought by travelers from the newly independent states of the former Soviet Union, which were experiencing a massive diphtheria epidemic. The epidemic was brought under control in 1998. Cholera outbreaks still occur in less-developed parts of the world.

Emerging Infectious Diseases

These recent outbreaks point to the fact that infectious diseases are not disappearing, but rather seem to be reemerging and increasing. In addition, a number of new diseases—**emerging infectious diseases (EIDs)**—have cropped up in recent years. These are diseases that are new or changing and are increasing

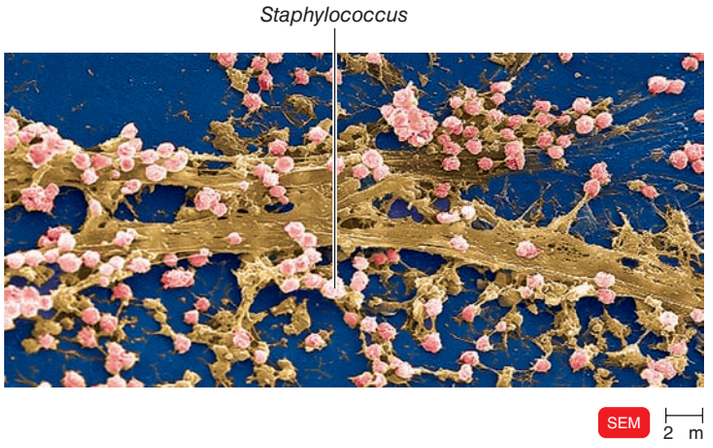


Figure 1.8 Biofilm on a catheter. *Staphylococcus* bacteria stick to solid surfaces, forming a slimy layer. Bacteria that break away from this biofilm can cause infections.

Q How does a biofilm's protective barrier make it resistant to antibiotics?

or have the potential to increase in incidence in the near future. Some of the factors that have contributed to the development of EIDs are evolutionary changes in existing organisms (e.g., *Vibrio cholerae;* vib′rē-ō kol′-er-ī); the spread of known diseases to new geographic regions or populations by modern transportation (e.g., West Nile virus); and increased human exposure to new, unusual infectious agents in areas that are undergoing ecologic changes such as deforestation and construction (e.g., Venezuelan hemorrhagic virus). EIDs also develop as a result of antimicrobial resistance (e.g., vancomycin-resistant *S. aureus*). An increasing number of incidents in recent years highlights the extent of the problem.

H1N1 influenza (flu), also known as *swine flu,* is a type of influenza caused by a new virus called *influenza H1N1*. H1N1 was first detected in the United States in april 2009. In June 2009, the World Health Organization declared H1N1 flu to be a *global pandemic disease* (a disease that affects large numbers of individuals in a short period of time and occurs worldwide).

Avian influenza A (H5N1), or **bird flu,** caught the attention of the public in 2003, when it killed millions of poultry and 24 people in eight countries in southeast Asia. Avian influenza viruses occur in birds worldwide. Certain wild birds, particularly waterfowl, do not get sick but carry the virus in their intestines and shed it in saliva, nasal secretions, and feces. Most often, the wild birds spread influenza to domesticated birds, in which the virus causes death.

Influenza A viruses are found in many different animals, including ducks, chickens, pigs, whales, horses, and seals. Normally, each subtype of influenza A virus is specific to certain species. However, influenza A viruses normally seen in one species sometimes can cross over and cause illness in another species, and all subtypes of influenza A virus can infect pigs. Although it is unusual for people to get influenza infections directly from animals, sporadic human infections and outbreaks caused by certain avian influenza A viruses and pig influenza viruses have been reported. As of 2008, avian influenza had sickened 242 people, and about half of them died. Fortunately, the virus has not yet evolved to be transmitted successfully among humans.

Human infections with avian influenza viruses detected since 1997 have not resulted in sustained human-to-human transmission. However, because influenza viruses have the potential to change and gain the ability to spread easily between people, monitoring for human infection and person-to-person transmission is important (see the box in Chapter 13 on page 374). The U.S. Food and Drug Administration (FDA) approved a human vaccine against the avian influenza virus in April 2007.

Antibiotics are critical in treating bacterial infections. However, years of overuse and misuse of these drugs have created environments in which antibiotic-resistant bacteria thrive. Random mutations in bacterial genes can make a bacterium resistant to an antibiotic. In the presence of that antibiotic, this bacterium has an advantage over other, susceptible bacteria and is able to proliferate. Antibiotic-resistant bacteria have become a global health crisis.

Staphylococcus aureus causes a wide range of human infections from pimples and boils to pneumonia, food poisoning, and surgical wound infections, and it is a significant cause of hospital-associated infections. After penicillin's initial success in treating *S. aureus* infection, penicillin-resistant *S. aureus* became a major threat in hospitals in the 1950s, requiring the use of methicillin. In the 1980s, **methicillin-resistant *S. aureus,*** called **MRSA,** emerged and became endemic in many hospitals, leading to increasing use of vancomycin. In the late 1990s, *S. aureus* infections that were less sensitive to vancomycin (**vancomycin-intermediate *S. aureus,*** or **VISA**) were reported. In 2002, an infection caused by **vancomycin-resistant *S. aureus* (VRSA)** in a patient in the United States was reported.

In March 2010, the World Health Organization (WHO) reported that in some parts of the world (such as northwestern Russia) about 28% of all individuals with tuberculosis (TB) had the multidrug-resistant form of the disease (MDR-TB). Multidrug-resistant TB is caused by bacteria that are resistant to at least the antibiotics isoniazid and rifampicin, the most effective drugs against tuberculosis.

The antibacterial substances added to various household cleaning products are similar to antibiotics in many ways. When used correctly, they inhibit bacterial growth. However, wiping every household surface with these antibacterial agents creates an environment in which the resistant bacteria survive. Unfortunately, when you really need to disinfect your homes and hands—for example, when a family member comes home from a hospital and is still vulnerable to infection—you may encounter mainly resistant bacteria.

Routine housecleaning and handwashing are necessary, but standard soaps and detergents (without added antibacterials) are fine for these tasks. In addition, quickly evaporating chemicals, such as chlorine bleach, alcohol, ammonia, and hydrogen peroxide, remove potentially pathogenic bacteria but do not leave residues that encourage the growth of resistant bacteria.

Clinical Case

The S. *aureus* bacterium responsible for Andrea's infection is resistant to the β-lactam antibiotic prescribed by Andrea's doctor. Concerned about what his patient is telling him, Andrea's doctor calls the local hospital to let them know he is sending a patient over. In the emergency department, a nurse swabs Andrea's wound and sends it to the hospital lab for culturing. The culture shows that Andrea's infection is caused by methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA produces β-lactamase, an enzyme that destroys β-lactam antibiotics. The attending physician surgically drains the pus from the sore on Andrea's wrist.

How does antibiotic resistance develop?

2 17 **19** 20 21

West Nile encephalitis (WNE) is inflammation of the brain caused by West Nile virus (see Chapter 8). WNE was first diagnosed in the West Nile region of Uganda in 1937. In 1999 the virus made its first North American appearance in humans in New York City. In 2007, West Nile virus infected over 3600 people in 43 states. West Nile virus is now established in nonmigratory birds in 48 states. The virus, which is carried by birds, is transmitted between birds—and to horses and humans—by mosquitoes. West Nile virus may have arrived in the United States in an infected traveler or in migratory birds.

In 1996, countries worldwide were refusing to import beef from the United Kingdom, where hundreds of thousands of cattle born after 1988 had to be killed because of an epidemic of **bovine spongiform encephalopathy** (en-sef-a-lop′a-thē), also called **BSE** or **mad cow disease.** BSE first came to the attention of microbiologists in 1986 as one of a handful of diseases caused by an infectious protein called a *prion.* Studies suggest that the source of disease was cattle feed prepared from sheep infected with their own version of the disease. Cattle are herbivores (planteaters), but adding protein to their feed improves their growth and health. **Creutzfeldt-Jakob disease** (kroits′felt yä′kôb), or **CJD,** is a human disease also caused by a prion. The incidence of CJD in the United Kingdom is similar to the incidence in other countries. However, by 2005 the United Kingdom reported 154 human cases of CJD caused by a new variant related to the bovine disease (see Chapter 22).

Escherichia coli is a normal inhabitant of the large intestine of vertebrates, including humans, and its presence is beneficial because it helps produce certain vitamins and breaks down otherwise undigestible foodstuffs (see Chapter 25). However, a strain called ***E. coli* O157:H7** causes bloody diarrhea when it grows in the intestines. This strain was first recognized in 1982 and since then has emerged as a public health problem. It is now one of the leading causes of diarrhea worldwide. In 1996, some 9000 people in Japan became ill, and 7 died, as a result of infection by *E. coli* O157:H7. The recent outbreaks of *E. coli* O157:H7 in the United States, associated with contamination of undercooked meat and unpasteurized beverages, have led public health officials to call for the development of new methods of testing for bacteria in food.

In 1995, infections of so-called **flesh-eating bacteria** were reported on the front pages of major newspapers. The bacteria are more correctly named invasive group A *Streptococcus* (strep-tō-kok′kus), or IGAS. Rates of IGAS in the United States, Scandinavia, England, and Wales have been increasing.

In 1995, a hospital laboratory technician in Democratic Republic of Congo (DROC) who had fever and bloody diarrhea underwent surgery for a suspected perforated bowel. Afterward he started hemorrhaging, and his blood began clotting in his blood vessels. A few days later, health care workers in the hospital where he was staying developed similar symptoms. One of them was transferred to a hospital in a different city; personnel in the second hospital who cared for this patient also developed symptoms. By the time the epidemic was over, 315 people had contracted **Ebola hemorrhagic fever** (hem-ôr-raj′ik), or **EHF,** and over 75% of them died. The epidemic was controlled when microbiologists instituted training on the use of protective equipment and educational measures in the community. Close personal contact with infectious blood or other body fluids or tissue (see Chapter 23) leads to human-to-human transmission.

Microbiologists first isolated Ebola viruses from humans during earlier outbreaks in DROC in 1976. (The virus is named after Congo's Ebola River.) In 2008, an Ebola virus outbreak occurred in Uganda with 149 cases. In 1989 and 1996, outbreaks among monkeys imported into the United States from the Philippines were caused by another Ebola virus but were not associated with human disease.

Recorded cases of **Marburg virus,** another hemorrhagic fever virus, are rare. The first cases were laboratory workers in Europe who handled African green monkeys from Uganda. Four outbreaks were identified in Africa between 1975 and 1998, involving 2 to 154 people with 56% mortality. In 2004, an outbreak killed 227 people. Microbiologists have been studying many animals but have not yet discovered the natural reservoir (source) of EHF and Marburg viruses.

In 1993, an outbreak of **cryptosporidiosis** (krip-tō-spô-rid-ē-ō′sis) transmitted through the public water supply in Milwaukee, Wisconsin, resulted in diarrheal illness in an estimated 403,000 persons. The microorganism responsible for this outbreak was the protozoan *Cryptosporidium* (krip-tō-spô-ri′dē-um). First

reported as a cause of human disease in 1976, it is responsible for up to 30% of the diarrheal illness in developing countries. In the United States, transmission has occurred via drinking water, swimming pools, and contaminated hospital supplies.

AIDS (acquired immunodeficiency syndrome) first came to public attention in 1981 with reports from Los Angeles that a few young homosexual men had died of a previously rare type of pneumonia known as *Pneumocystis* (nü-mō-sis′tis) pneumonia. These men had experienced a severe weakening of the immune system, which normally fights infectious diseases. Soon these cases were correlated with an unusual number of occurrences of a rare form of cancer, Kaposi's sarcoma, among young homosexual men. Similar increases in such rare diseases were found among hemophiliacs and intravenous drug users.

Researchers quickly discovered that the cause of AIDS was a previously unknown virus (see Figure 1.1e). The virus, now called **human immunodeficiency virus (HIV),** destroys $CD4^+$ T cells, one type of white blood cell important to immune system defenses. Sickness and death result from microorganisms or cancerous cells that might otherwise have been defeated by the body's natural defenses. So far, the disease has been inevitably fatal once symptoms develop.

By studying disease patterns, medical researchers found that HIV could be spread through sexual intercourse, by contaminated needles, from infected mothers to their newborns via breast milk, and by blood transfusions—in short, by the transmission of body fluids from one person to another. Since 1985, blood used for transfusions has been carefully checked for the presence of HIV, and it is now quite unlikely that the virus can be spread by this means.

By the end of 2010, over 1 million people in the United States are living with AIDS. Over 50,000 Americans become infected and 18,000 die each year. As of 2010, health officials estimated that 1.3 million Americans have HIV infection. In 2009, the World Health Organization (WHO) estimated that over 33 million people worldwide are living with HIV/AIDS and that 7500 new infections occur every day.

Since 1994, new treatments have extended the life span of people with AIDS; however, approximately 40,000 new cases occur annually in the United States. The majority of individuals with AIDS are in the sexually active age group. Because heterosexual partners of AIDS sufferers are at high risk of infection, public health officials are concerned that even more women and minorities will contract AIDS. In 1997, HIV diagnoses began increasing among women and minorities. Among the AIDS cases reported in 2009, 26% were women, and 49% were African American.

In the months and years to come, scientists will continue to apply microbiological techniques to help them learn more about the structure of the deadly HIV, how it is transmitted, how it grows in cells and causes disease, how drugs can be directed against it, and whether an effective vaccine can be developed. Public health officials have also focused on prevention through education.

AIDS poses one of this century's most formidable health threats, but it is not the first serious epidemic of a sexually transmitted disease. Syphilis was also once a fatal epidemic disease. As recently as 1941, syphilis caused an estimated 14,000 deaths per year in the United States. With few drugs available for treatment and no vaccines to prevent it, efforts to control the disease focused mainly on altering sexual behavior and on the use of condoms. The eventual development of drugs to treat syphilis contributed significantly to preventing the spread of the disease. According to the Centers for Disease Control and Prevention (CDC), reported cases of syphilis dropped from a record high of 575,000 in 1943 to an all-time low of 5979 cases in 2004. Since then, however, the number of cases has been increasing.

Just as microbiological techniques helped researchers in the fight against syphilis and smallpox, they will help scientists discover the causes of new emerging infectious diseases in the twenty-first century. Undoubtedly there will be new diseases. Ebola virus and *Influenzavirus* are examples of viruses that may be changing their abilities to infect different host species. Emerging infectious diseases will be discussed further in Chapter 14 on page 417.

Infectious diseases may reemerge because of antibiotic resistance (see the box in Chapter 26 on page 757) and through the use of microorganisms as weapons. (See the box in Chapter 23 on page 651.) The breakdown of public health measures for previously controlled infections has resulted in unexpected cases of tuberculosis, whooping cough, and diphtheria (see Chapter 24).

Clinical Case

Mutations develop randomly in bacteria: some mutations are lethal, some have no effect, and some may be beneficial. Once these mutations develop, the offspring of the mutated parent cells also carry the same mutation. Because they have an advantage in the presence of the antibiotic, bacteria that are resistant to antibiotics soon outnumber those that are susceptible to antibiotic therapy. The widespread use of antibiotics selectively allows the resistant bacteria to grow, whereas the susceptible bacteria are killed. Eventually, almost the entire population of bacteria is resistant to the antibiotic.

The emergency department physician prescribes a different antibiotic, vancomycin, which will kill the MRSA in Andrea's wrist. She also explains to Andrea what MRSA is and why it's important they find out where Andrea acquired the potentially lethal bacteria.

What can the emergency department physician tell Andrea about MRSA?

2 17 19 **20** 21

CHECK YOUR UNDERSTANDING

- Differentiate normal microbiota and infectious disease. **1-16**
- Why are biofilms important? **1-17**
- What factors contribute to the emergence of an infectious disease? **1-18**

* * *

The diseases we have mentioned are caused by viruses, bacteria, protozoa, and prions—types of microorganisms. This book introduces you to the enormous variety of microscopic organisms. It shows you how microbiologists use specific techniques and procedures to study the microbes that cause such diseases as AIDS and diarrhea—and diseases that have yet to be discovered. You will also learn how the body responds to microbial infection and how certain drugs combat microbial diseases. Finally, you will learn about the many beneficial roles that microbes play in the world around us.

Clinical Case Resolved

The first MRSA was health care–associated MRSA (HA-MRSA), transmitted between staff and patients in health care settings. In the 1990s, infections by a genetically different strain, community-associated MRSA (CA-MRSA), emerged as a major cause of skin disease in the United States. CA-MRSA enters skin abrasions from environmental surfaces or other people. Andrea has never been hospitalized before now, so they are able to rule out the hospital as the source of infection. Her college courses are all online, so she didn't contract MRSA at the university, either. The local health department sends someone to her family home to swab for the bacteria there.

MRSA is isolated from Andrea's living room sofa, but how did it get there? After speaking with the family, the representative from the health department, knowing that clusters of CA-MRSA infections have been seen among athletes suggests swabbing the mats used by the gymnasts at the school Andrea's sister attends. The cultures come back positive for MRSA. Andrea's sister, although not infected, transferred the bacteria from her skin to the sofa, where Andrea laid her arm. (A person can carry MRSA on the skin without becoming infected.) The bacteria entered through a scratch on Andrea's wrist.

2 17 19 20 **21**

Study Outline

MasteringMICROBIOLOGY™

Test your understanding with quizzes, microbe review, and a chapter post-test at www.masteringmicrobiology.com.

Microbes in Our Lives (p. 2)

1. Living things too small to be seen with the unaided eye are called microorganisms.
2. Microorganisms are important in maintaining Earth's ecological balance.
3. Some microorganisms live in humans and other animals and are needed to maintain good health.
4. Some microorganisms are used to produce foods and chemicals.
5. Some microorganisms cause disease.

Naming and Classifying Microorganisms (pp. 2–6)

Nomenclature (p. 3)

1. In a nomenclature system designed by Carolus Linnaeus (1735), each living organism is assigned two names.
2. The two names consist of a genus and a specific epithet, both of which are underlined or italicized.

Types of Microorganisms (pp. 3–6)

3. Bacteria are unicellular organisms. Because they have no nucleus, the cells are described as prokaryotic.
4. The three major basic shapes of bacteria are bacillus, coccus, and spiral.
5. Most bacteria have a peptidoglycan cell wall; they divide by binary fission, and they may possess flagella.
6. Bacteria can use a wide range of chemical substances for their nutrition.
7. Archaea consist of prokaryotic cells; they lack peptidoglycan in their cell walls.
8. Archaea include methanogens, extreme halophiles, and extreme thermophiles.
9. Fungi (mushrooms, molds, and yeasts) have eukaryotic cells (cells with a true nucleus). Most fungi are multicellular.
10. Fungi obtain nutrients by absorbing organic material from their environment.
11. Protozoa are unicellular eukaryotes.
12. Protozoa obtain nourishment by absorption or ingestion through specialized structures.
13. Algae are unicellular or multicellular eukaryotes that obtain nourishment by photosynthesis.
14. Algae produce oxygen and carbohydrates that are used by other organisms.
15. Viruses are noncellular entities that are parasites of cells.
16. Viruses consist of a nucleic acid core (DNA or RNA) surrounded by a protein coat. An envelope may surround the coat.
17. The principal groups of multicellular animal parasites are flatworms and roundworms, collectively called helminths.
18. The microscopic stages in the life cycle of helminths are identified by traditional microbiological procedures.

Classification of Microorganisms (p. 6)

19. All organisms are classified into Bacteria, Archaea, and Eukarya. Eukarya include protists, fungi, plants, and animals.

A Brief History of Microbiology (pp. 6–15)

The First Observations (p. 6)

1. Robert Hooke observed that cork was composed of "little boxes"; he introduced the term *cell* (1665).
2. Hooke's observations laid the groundwork for development of the cell theory, the concept that all living things are composed of cells.
3. Anton van Leeuwenhoek, using a simple microscope, was the first to observe microorganisms (1673).

The Debate over Spontaneous Generation (pp. 6–8)

4. Until the mid-1880s, many people believed in spontaneous generation, the idea that living organisms could arise from nonliving matter.
5. Francesco Redi demonstrated that maggots appear on decaying meat only when flies are able to lay eggs on the meat (1668).
6. John Needham claimed that microorganisms could arise spontaneously from heated nutrient broth (1745).
7. Lazzaro Spallanzani repeated Needham's experiments and suggested that Needham's results were due to microorganisms in the air entering his broth (1765).
8. Rudolf Virchow introduced the concept of biogenesis: living cells can arise only from preexisting cells (1858).
9. Louis Pasteur demonstrated that microorganisms are in the air everywhere and offered proof of biogenesis (1861).
10. Pasteur's discoveries led to the development of aseptic techniques used in laboratory and medical procedures to prevent contamination by microorganisms.

The Golden Age of Microbiology (pp. 8–11)

11. The science of microbiology advanced rapidly between 1857 and 1914.
12. Pasteur found that yeasts ferment sugars to alcohol and that bacteria can oxidize the alcohol to acetic acid.
13. A heating process called pasteurization is used to kill bacteria in some alcoholic beverages and milk.
14. Agostino Bassi (1835) and Pasteur (1865) showed a causal relationship between microorganisms and disease.
15. Joseph Lister introduced the use of a disinfectant to clean surgical wounds in order to control infections in humans (1860s).
16. Robert Koch proved that microorganisms cause disease. He used a sequence of procedures, now called Koch's postulates (1876), that are used today to prove that a particular microorganism causes a particular disease.
17. In a vaccination, immunity (resistance to a particular disease) is conferred by inoculation with a vaccine.
18. In 1798, Edward Jenner demonstrated that inoculation with cowpox material provides humans with immunity to smallpox.
19. About 1880, Pasteur discovered that avirulent bacteria could be used as a vaccine for fowl cholera; he coined the word *vaccine*.
20. Modern vaccines are prepared from living avirulent microorganisms or killed pathogens, from isolated components of pathogens, and by recombinant DNA techniques.

The Birth of Modern Chemotherapy: Dreams of a "Magic Bullet" (pp. 11–12)

21. Chemotherapy is the chemical treatment of a disease.
22. Two types of chemotherapeutic agents are synthetic drugs (chemically prepared in the laboratory) and antibiotics (substances produced naturally by bacteria and fungi to inhibit the growth of other microorganisms).
23. Paul Ehrlich introduced an arsenic-containing chemical called salvarsan to treat syphilis (1910).
24. Alexander Fleming observed that the *Penicillium* fungus inhibited the growth of a bacterial culture. He named the active ingredient penicillin (1928).
25. Penicillin has been used clinically as an antibiotic since the 1940s.
26. Researchers are tackling the problem of drug-resistant microbes.

Modern Developments in Microbiology (pp. 12–15)

27. Bacteriology is the study of bacteria, mycology is the study of fungi, and parasitology is the study of parasitic protozoa and worms.
28. Microbiologists are using genomics, the study of all of an organism's genes, to classify bacteria, fungi, and protozoa.
29. The study of AIDS, analysis of the action of interferons, and the development of new vaccines are among the current research interests in immunology.
30. New techniques in molecular biology and electron microscopy have provided tools for advancing our knowledge of virology.
31. The development of recombinant DNA technology has helped advance all areas of microbiology.

Microbes and Human Welfare (pp. 15–16)

1. Microorganisms degrade dead plants and animals and recycle chemical elements to be used by living plants and animals.
2. Bacteria are used to decompose organic matter in sewage.
3. Bioremediation processes use bacteria to clean up toxic wastes.
4. Bacteria that cause diseases in insects are being used as biological controls of insect pests. Biological controls are specific for the pest and do not harm the environment.
5. Using microbes to make products such as foods and chemicals is called biotechnology.
6. Using recombinant DNA, bacteria can produce important substances such as proteins, vaccines, and enzymes.
7. In gene therapy, viruses are used to carry replacements for defective or missing genes into human cells.
8. Genetically modified bacteria are used in agriculture to protect plants from frost and insects and to improve the shelf life of produce.

Microbes and Human Disease (pp. 16–21)

1. Everyone has microorganisms in and on the body; these make up the normal microbiota, or flora.
2. The disease-producing properties of a species of microbe and the host's resistance are important factors in determining whether a person will contract a disease.
3. Bacterial communities that form slimy layers on surfaces are called biofilms.
4. An infectious disease is one in which pathogens invade a susceptible host.
5. An emerging infectious disease (EID) is a new or changing disease showing an increase in incidence in the recent past or a potential to increase in the near future.

Study Questions

Answers to the Review and Multiple Choice questions can be found by turning to the Answers tab at the back of the textbook.

Review

1. How did the idea of spontaneous generation come about?
2. Briefly state the role microorganisms play in each of the following:
   - **a.** biological control of pests
   - **b.** recycling of elements
   - **c.** normal microbiota
   - **d.** sewage treatment
   - **e.** human insulin production
   - **f.** vaccine production
   - **g.** biofilms
3. Into which field of microbiology would the following scientists best fit?

Researcher Who	Field
_____ **a.** Studies biodegradation of toxic wastes	**1.** Biotechnology
_____ **b.** Studies the causative agent of Ebola hemorrhagic fever	**2.** Immunology
_____ **c.** Studies the production of human proteins by bacteria	**3.** Microbial ecology
_____ **d.** Studies the symptoms of AIDS	**4.** Microbial genetics
_____ **e.** Studies the production of toxin by *E. coli*	**5.** Microbial physiology
_____ **f.** Studies the life cycle of *Cryptosporidium*	**6.** Molecular biology
_____ **g.** Develops gene therapy for a disease	**7.** Mycology
_____ **h.** Studies the fungus *Candida albicans*	**8.** Virology

4. Match the microorganisms in column A to their descriptions in column B.

Column A	Column B
_____ **a.** Archaea	**1.** Not composed of cells
_____ **b.** Algae	**2.** Cell wall made of chitin
_____ **c.** Bacteria	**3.** Cell wall made of peptidoglycan
_____ **d.** Fungi	**4.** Cell wall made of cellulose; photosynthetic
_____ **e.** Helminths	**5.** Unicellular, complex cell structure lacking a cell wall
_____ **f.** Protozoa	**6.** Multicellular animals
_____ **g.** Viruses	**7.** Prokaryote without peptidoglycan cell wall

5. Match the people in column A to their contribution toward the advancement of microbiology, in column B.

Column A	Column B
_____ **a.** Avery, MacLeod, and McCarty	**1.** Developed vaccine against smallpox
_____ **b.** Beadle and Tatum	**2.** Discovered how DNA controls protein synthesis in a cell
_____ **c.** Berg	**3.** Discovered penicillin
_____ **d.** Ehrlich	**4.** Discovered that DNA can be transferred from one bacterium to another
_____ **e.** Fleming	**5.** Disproved spontaneous generation
_____ **f.** Hooke	**6.** First to characterize a virus
_____ **g.** Iwanowski	**7.** First to use disinfectants in surgical procedures
_____ **h.** Jacob and Monod	**8.** First to observe bacteria
_____ **i.** Jenner	**9.** First to observe cells in plant material and name them
_____ **j.** Koch	**10.** Observed that viruses are filterable
_____ **k.** Lancefield	**11.** Proved that DNA is the hereditary material
_____ **l.** Lederberg and Tatum	**12.** Proved that microorganisms can cause disease
_____ **m.** Lister	**13.** Said living cells arise from preexisting living cells
_____ **n.** Pasteur	**14.** Showed that genes code for enzymes
_____ **o.** Stanley	**15.** Spliced animal DNA to bacterial DNA
_____ **p.** van Leeuwenhoek	**16.** Used bacteria to produce acetone
_____ **q.** Virchow	**17.** Used the first synthetic chemotherapeutic agent
_____ **r.** Weizmann	**18.** Proposed a classification system for streptococci based on antigens in their cell walls

6. The genus name of a bacterium is "erwinia," and the specific epithet is "amylovora." Write the scientific name of this organism correctly. Using this name as an example, explain how scientific names are chosen.
7. It is possible to purchase the following microorganisms in a retail store. Provide a reason for buying each.
   a. *Bacillus thuringiensis*
   b. *Saccharomyces*
8. DRAW IT Show where airborne microbes ended up in Pasteur's experiment.

9. NAME IT What type of microorganism has a peptidoglycan cell wall, has DNA that is not contained in a nucleus, and has flagella?

Multiple Choice

1. Which of the following is a scientific name?
   a. *Mycobacterium tuberculosis*
   b. Tubercle bacillus
2. Which of the following is *not* a characteristic of bacteria?
   a. are prokaryotic
   b. have peptidoglycan cell walls
   c. have the same shape
   d. grow by binary fission
   e. have the ability to move
3. Which of the following is the most important element of Koch's germ theory of disease? The animal shows disease symptoms when
   a. the animal has been in contact with a sick animal.
   b. the animal has a lowered resistance.
   c. a microorganism is observed in the animal.
   d. a microorganism is inoculated into the animal.
   e. microorganisms can be cultured from the animal.
4. Recombinant DNA is
   a. DNA in bacteria.
   b. the study of how genes work.
   c. the DNA resulting when genes of two different organisms are mixed.
   d. the use of bacteria in the production of foods.
   e. the production of proteins by genes.
5. Which of the following statements is the best definition of *biogenesis*?
   a. Nonliving matter gives rise to living organisms.
   b. Living cells can only arise from preexisting cells.
   c. A vital force is necessary for life.
   d. Air is necessary for living organisms.
   e. Microorganisms can be generated from nonliving matter.
6. Which of the following is a beneficial activity of microorganisms?
   a. Some microorganisms are used as food for humans.
   b. Some microorganisms use carbon dioxide.
   c. Some microorganisms provide nitrogen for plant growth.
   d. Some microorganisms are used in sewage treatment processes.
   e. all of the above
7. It has been said that bacteria are essential for the existence of life on Earth. Which of the following is the essential function performed by bacteria?
   a. control insect populations
   b. directly provide food for humans
   c. decompose organic material and recycle elements
   d. cause disease
   e. produce human hormones such as insulin
8. Which of the following is an example of bioremediation?
   a. application of oil-degrading bacteria to an oil spill
   b. application of bacteria to a crop to prevent frost damage
   c. fixation of gaseous nitrogen into usable nitrogen
   d. production by bacteria of a human protein such as interferon
   e. all of the above
9. Spallanzani's conclusion about spontaneous generation was challenged because Lavoisier had just shown that oxygen was the vital component of air. Which of the following statements is true?
   a. All life requires air.
   b. Only disease-causing organisms require air.
   c. Some microbes do not require air.
   d. Pasteur kept air out of his biogenesis experiments.
   e. Lavoisier was mistaken.
10. Which of the following statements about *E. coli* is *false*?
    a. *E. coli* was the first disease-causing bacterium identified by Koch.
    b. *E. coli* is part of the normal microbiota of humans.
    c. *E. coli* is beneficial in human intestines.
    d. A disease-causing strain of *E. coli* causes bloody diarrhea.
    e. none of the above

Critical Thinking

1. How did the theory of biogenesis lead the way for the germ theory of disease?
2. Even though the germ theory of disease was not demonstrated until 1876, why did Semmelweis (1840) and Lister (1867) argue for the use of aseptic techniques?
3. Find at least three supermarket products made by microorganisms. (*Hint:* The label will state the scientific name of the organism or include the word *culture, fermented,* or *brewed.*)
4. People once believed all microbial diseases would be controlled by the twenty-first century. Name one emerging infectious disease. List three reasons why we are identifying new diseases now.

Clinical Applications

1. The prevalence of arthritis in the United States is 1 in 100,000 children. However, 1 in 10 children in Lyme, Connecticut, developed arthritis between June and September 1973. Allen Steere, a rheumatologist at Yale University, investigated the cases in Lyme and found that 25% of the patients remembered having a skin rash during their arthritic episode and that the disease was treatable with penicillin. Steere concluded that this was a new infectious disease and did not have an environmental, genetic, or immunologic cause.
   a. What was the factor that caused Steere to reach his conclusion?
   b. What is the disease?
   c. Why was the disease more prevalent between June and September?
2. In 1864, Lister observed that patients recovered completely from simple fractures, but that compound fractures had "disastrous consequences." He knew that the application of phenol (carbolic acid) to fields in the town of Carlisle prevented cattle disease. Lister treated compound fractures with phenol, and his patients recovered without complications. How was Lister influenced by Pasteur's work? Why was Koch's work still needed?

2

Chemical Principles

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We can see a tree rot and smell milk going sour, but we might not realize what is happening on a microscopic level. In both cases, microbes are conducting chemical operations. The tree rots when microorganisms decompose the wood. Milk turns sour from the production of lactic acid by bacteria. Most of the activities of microorganisms are the result of a series of chemical reactions.

Like all organisms, microorganisms use nutrients to make chemical building blocks for growth and other functions essential to life. For most microorganisms, synthesizing these building blocks requires them to break down nutrient substances and use the energy released to assemble the resulting molecular fragments into new substances.

The chemistry of microbes is one of the most important concerns of microbiologists. Knowledge of chemistry is essential to understanding what roles microorganisms play in nature, how they cause disease, how methods for diagnosing disease are developed, how the body's defenses combat infection, and how antibiotics and vaccines are produced to combat the harmful effects of microbes. The *Bacillus anthracis* bacteria in the photograph make a capsule that is not readily digested by animal cells. As discussed in the Clinical Case, these bacteria can grow in mammals by avoiding host defenses. Researchers are investigating ways to identify unique chemicals made by *B. anthracis* and other potential biological weapons in order to detect bioterrorism. To understand the changes that occur in microorganisms and the changes microbes make in the world around us, we need to know how molecules are formed and how they interact.

The Structure of Atoms

LEARNING OBJECTIVE

2-1 Describe the structure of an atom and its relation to the physical properties of elements.

All matter—whether air, rock, or a living organism—is made up of small units called atoms. An **atom** is the smallest component of a pure substance that exhibits physical and chemical properties of that substance; an atom cannot be subdivided into smaller substances without losing its properties. Atoms interact with each other in certain combinations to form **molecules.** Living cells are made up of molecules, some of which are very complex. The science of the interaction between atoms and molecules is called **chemistry.**

Atoms are the smallest units of matter that enter into chemical reactions. Every atom has a centrally located **nucleus** and particles called **electrons** that move around the nucleus in regions called electron shells (**Figure 2.1**). The nuclei of most atoms are stable—that is, they do not change spontaneously—and nuclei do not participate in chemical reactions. The nucleus is made up of positively (+) charged particles called **protons** and uncharged (neutral) particles called **neutrons.** The nucleus, therefore, bears a net positive charge. A **charge** is a property of some subatomic particles that produces an attractive or repulsive force between them; particles of opposite charge attract each other, and particles of the same charge repel each other. Neutrons and protons have approximately the same weight, which is about 1840 times that of an electron. The charge on electrons is negative (−), and in all atoms the number of electrons is equal to the number of protons. Because the total positive charge of the nucleus equals the total negative charge of the electrons, each atom is electrically neutral.

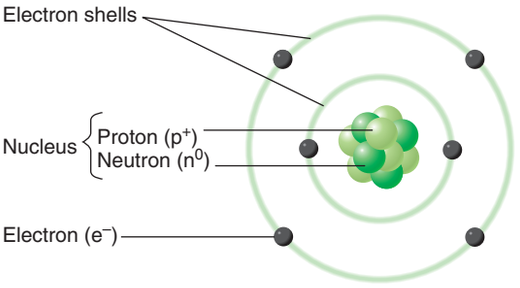


Figure 2.1 The structure of an atom. In this simplified diagram of a carbon atom, note the central location of the nucleus. The nucleus contains six neutrons and six protons, although not all the protons are visible in this view. The six electrons move about the nucleus in regions called electron shells, shown here as circles.

Q What is the atomic number of this atom?

The number of protons in an atomic nucleus ranges from one (in a hydrogen atom) to more than 100 (in the largest atoms known). Atoms are often listed by their **atomic number,** the number of protons in the nucleus. The total number of protons and neutrons in an atom is its approximate **atomic weight.**

Clinical Case: Drumming Up Dust

Jonathan, a 52-year-old drummer, is doing his best to ignore the cold sweat that is breaking out all over his body. He and his bandmates are performing in a local Philadelphia nightclub, and they are just about finished with the second set of the evening. Jonathan hasn't been feeling well for a while, actually; he has been feeling weak and short of breath for the last 3 days or so. Jonathan makes it to the end of the song, but the noise from the clapping and cheering audience seems to come from far away. He stands up to bow and collapses. Jonathan is admitted to a local emergency department with a mild fever and severe shaking. He is able to tell the admitting nurse that he also has had a dry cough for the last few days. The attending physician orders a chest X-ray exam and sputum culture. Jonathan is diagnosed with bilateral pneumonia caused by *Bacillus anthracis*. The attending physician is astonished by this diagnosis.

How did Jonathan become infected by *B. anthracis*? Read on to find out.

26 43 44 48

Chemical Elements

All atoms with the same number of protons behave the same way chemically and are classified as the same **chemical element.** Each element has its own name and a one- or two-letter symbol, usually derived from the English or Latin name for the element. For example, the symbol for the element hydrogen is H, and the symbol for carbon is C. The symbol for sodium is Na—the first two letters of its Latin name, *natrium*—to distinguish it from nitrogen, N, and from sulfur, S. There are 92 naturally occurring elements. However, only about 26 elements are commonly found in living things. **Table 2.1** lists some of the chemical elements found in living organisms.

Most elements have several **isotopes**—atoms with different numbers of neutrons in their nuclei. All isotopes of an element have the same number of protons in their nuclei, but their atomic weights differ because of the difference in the number of neutrons. For example, in a natural sample of oxygen, all the atoms contain eight protons. However, 99.76% of the atoms have eight neutrons, 0.04% contain nine neutrons, and the remaining 0.2% contain ten neutrons. Therefore, the three isotopes composing a natural sample of oxygen have atomic weights of 16, 17, and 18, although all will have the atomic number 8. Atomic numbers are written as a subscript to the left of an element's chemical

TABLE 2.1 The Elements of Life*

Element	Symbol	Atomic Number	Approximate Atomic Weight
Hydrogen	H	1	1
Carbon	C	6	12
Nitrogen	N	7	14
Oxygen	O	8	16
Sodium	Na	11	23
Magnesium	Mg	12	24
Phosphorus	P	15	31
Sulfur	S	16	32
Chlorine	Cl	17	35
Potassium	K	19	39
Calcium	Ca	20	40
Iron	Fe	26	56
Iodine	I	53	127

*Hydrogen, carbon, nitrogen, and oxygen are the most abundant chemical elements in living organisms.

symbol. Atomic weights are written as a superscript above the atomic number. Thus, natural oxygen isotopes are represented as ${}^{16}_{8}O$, ${}^{17}_{8}O$, and ${}^{18}_{8}O$. Isotopes of certain elements are extremely useful in biological research, medical diagnosis, the treatment of some disorders, and some forms of sterilization.

Electronic Configurations

In an atom, electrons are arranged in **electron shells,** which are regions corresponding to different **energy levels.** The arrangement is called an **electronic configuration.** Shells are layered outward from the nucleus, and each shell can hold a characteristic maximum number of electrons—two electrons in the innermost shell (lowest energy level), eight electrons in the second shell, and eight electrons in the third shell, if it is the atom's outermost (valence) shell. The fourth, fifth, and sixth electron shells can each accommodate 18 electrons, although there are some exceptions to this generalization. **Table 2.2** shows the electronic configurations for atoms of some elements found in living organisms.

The outermost shell tends to be filled with the maximum number of electrons. An atom can give up, accept, or share electrons with other atoms to fill this shell. The chemical properties of atoms are largely a function of the number of electrons in the outermost electron shell. When its outer shell is filled, the atom is chemically stable, or inert: it does not tend to react with other atoms. Helium (atomic number 2) and neon (atomic number 10) are examples of atoms of inert gases whose outer shells are filled.

When an atom's outer electron shell is only partially filled, the atom is chemically unstable. Such an atom reacts with other atoms, and this reaction depends, in part, on the degree to which the outer energy levels are filled. Notice the number of electrons in the outer energy levels of the atoms in Table 2.2. We will see later how the number correlates with the chemical reactivity of the elements.

CHECK YOUR UNDERSTANDING

How does ${}^{14}_{6}C$ differ from ${}^{12}_{6}C$? What is the atomic number of each carbon atom? The atomic weight? **2-1**

How Atoms Form Molecules: Chemical Bonds

LEARNING OBJECTIVES

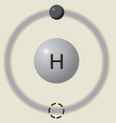
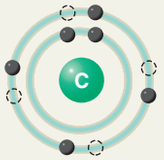
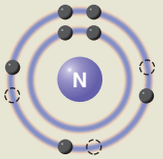
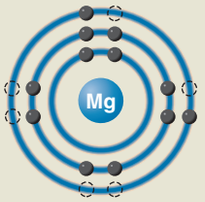
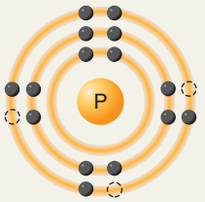
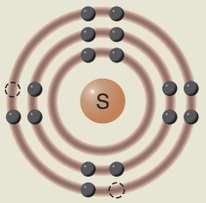
2-2 Define *ionic bond, covalent bond, hydrogen bond, molecular weight,* and *mole.*

When the outermost energy level of an atom is not completely filled by electrons, you can think of it as having either unfilled spaces or extra electrons in that energy level, depending on whether it is easier for the atom to gain or lose electrons. For example, an atom of oxygen, with two electrons in the first energy level and six in the second, has two unfilled spaces in the second electron shell; an atom of magnesium has two extra electrons in its outermost shell. The most chemically stable configuration for any atom is to have its outermost shell filled. Therefore, for these two atoms to attain that state, oxygen must gain two electrons, and magnesium must lose two electrons. Because all atoms tend to combine so that the extra electrons in the outermost shell of one atom fill the spaces of the outermost shell of the other atom, oxygen and magnesium combine so that the outermost shell of each atom has the full complement of eight electrons.

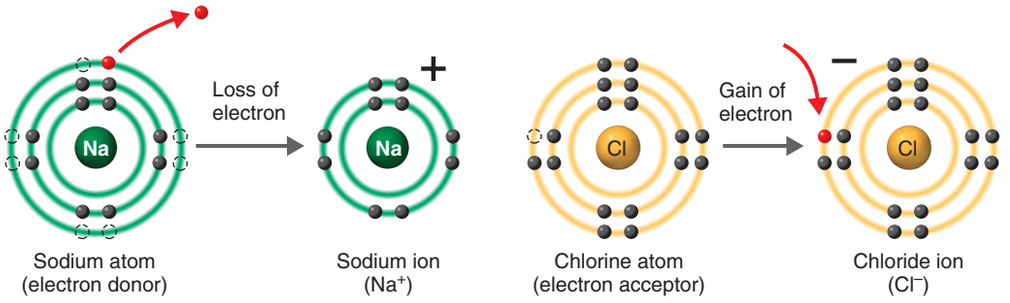
The **valence,** or combining capacity, of an atom is the number of extra or missing electrons in its outermost electron shell. For example, hydrogen has a valence of 1 (one unfilled space, or one extra electron), oxygen has a valence of 2 (two unfilled spaces), carbon has a valence of 4 (four unfilled spaces, or four extra electrons), and magnesium has a valence of 2 (two extra electrons).

Basically, atoms achieve the full complement of electrons in their outermost energy shells by combining to form molecules, which are made up of atoms of one or more elements. A molecule that contains at least two different kinds of atoms, such as H_2O (the water molecule), is called a **compound.** In H_2O, the subscript 2 indicates that there are two atoms of hydrogen; the absence of a subscript indicates that there is only one atom of oxygen. Molecules hold together because the valence electrons of the combining atoms form attractive forces, called **chemical bonds,** between the atomic nuclei. Therefore, valence may also be viewed as the bonding capacity of an element. Because energy is required for chemical bond formation, each chemical bond possesses a certain amount of potential chemical energy.

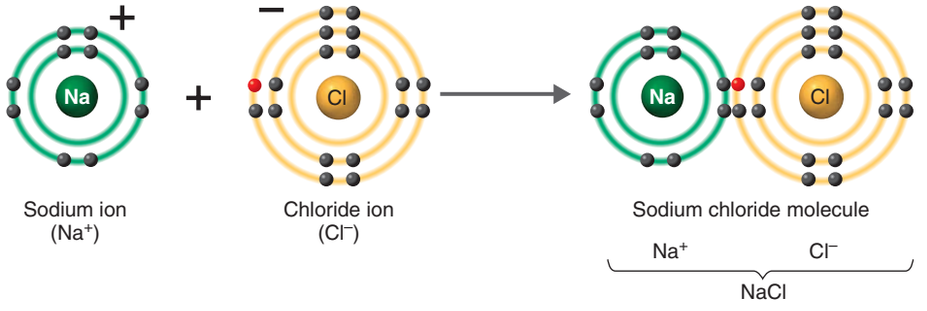
TABLE 2.2 Electronic Configurations for the Atoms of Some Elements Found in Living Organisms

Element	First Electron Shell (2)*	Second Electron Shell (8)*	Third Electron Shell (8)*	Diagram	Number of Valence (Outermost) Shell Electrons	Number of Unfilled Spaces	Maximum Number of Bonds Formed
Hydrogen	1	—	—		1	1	1
Carbon	2	4	—		4	4	4
Nitrogen	2	5	—		5	3	5
Oxygen	2	6	—		6	2	2
Magnesium	2	8	2		2	6	2
Phosphorus	2	8	5		5	3	5
Sulfur	2	8	6		6	2	6

*Numbers in parentheses indicate the maximum number of electrons in their respective shells.



(a) A sodium atom (Na) loses one electron to an electron acceptor and forms a sodium ion (Na^+). A chlorine atom (Cl) accepts one electron from an electron donor to become a chloride ion (Cl^-).



(b) The sodium and chloride ions are attracted because of their opposite charges and are held together by an ionic bond to form a molecule of sodium chloride.

Figure 2.2 **Ionic bond formation.**

Q What is an ionic bond?

In general, atoms form bonds in one of two ways: by either gaining or losing electrons from their outer electron shell, or by sharing outer electrons. When atoms have gained or lost outer electrons, the chemical bond is called an *ionic bond*. When outer electrons are shared, the bond is called a *covalent bond*. Although we will discuss ionic and covalent bonds separately, the kinds of bonds actually found in molecules do not belong entirely to either category. Instead, bonds range from the highly ionic to the highly covalent.

Ionic Bonds

Atoms are electrically neutral when the number of positive charges (protons) equals the number of negative charges (electrons). But when an isolated atom gains or loses electrons, this balance is upset. If the atom gains electrons, it acquires an overall negative charge; if the atom loses electrons, it acquires an overall positive charge. Such a negatively or positively charged atom (or group of atoms) is called an **ion.**

Consider the following examples. Sodium (Na) has 11 protons and 11 electrons, with one electron in its outer electron shell. Sodium tends to lose the single outer electron; it is an *electron donor* (Figure 2.2a). When sodium donates an electron to another atom, it is left with 11 protons and only 10 electrons and so has an overall charge of +1. This positively charged sodium atom is called a sodium ion and is written as Na^+. Chlorine (Cl) has a total of 17 electrons, seven of them in the outer electron shell. Because this outer shell can hold eight electrons, chlorine tends to pick up an electron that has been lost by another atom; it is an *electron acceptor* (see Figure 2.2a). By accepting an electron, chlorine totals 18 electrons. However, it still has only 17 protons in its nucleus. The chloride ion therefore has a charge of −1 and is written as Cl^-.

The opposite charges of the sodium ion (Na^+) and chloride ion (Cl^-) attract each other. The attraction, an ionic bond, holds the two atoms together, and a molecule is formed (Figure 2.2b). The formation of this molecule, called sodium chloride (NaCl) or table salt, is a common example of ionic bonding. Thus, an **ionic bond** is an attraction between ions of opposite charge that holds them together to form a stable molecule. Put another way, an ionic bond is an attraction between atoms in which one atom loses electrons and another atom gains electrons. Strong ionic bonds, such as those that hold Na^+ and Cl^- together in salt crystals, have limited importance in living cells. But the weaker ionic bonds formed in aqueous (water) solutions are important in biochemical reactions in microbes and other organisms. For example, weaker ionic bonds assume a role in certain antigen–antibody reactions—that is, reactions in which molecules produced by the immune system (antibodies) combine with foreign substances (antigens) to combat infection.

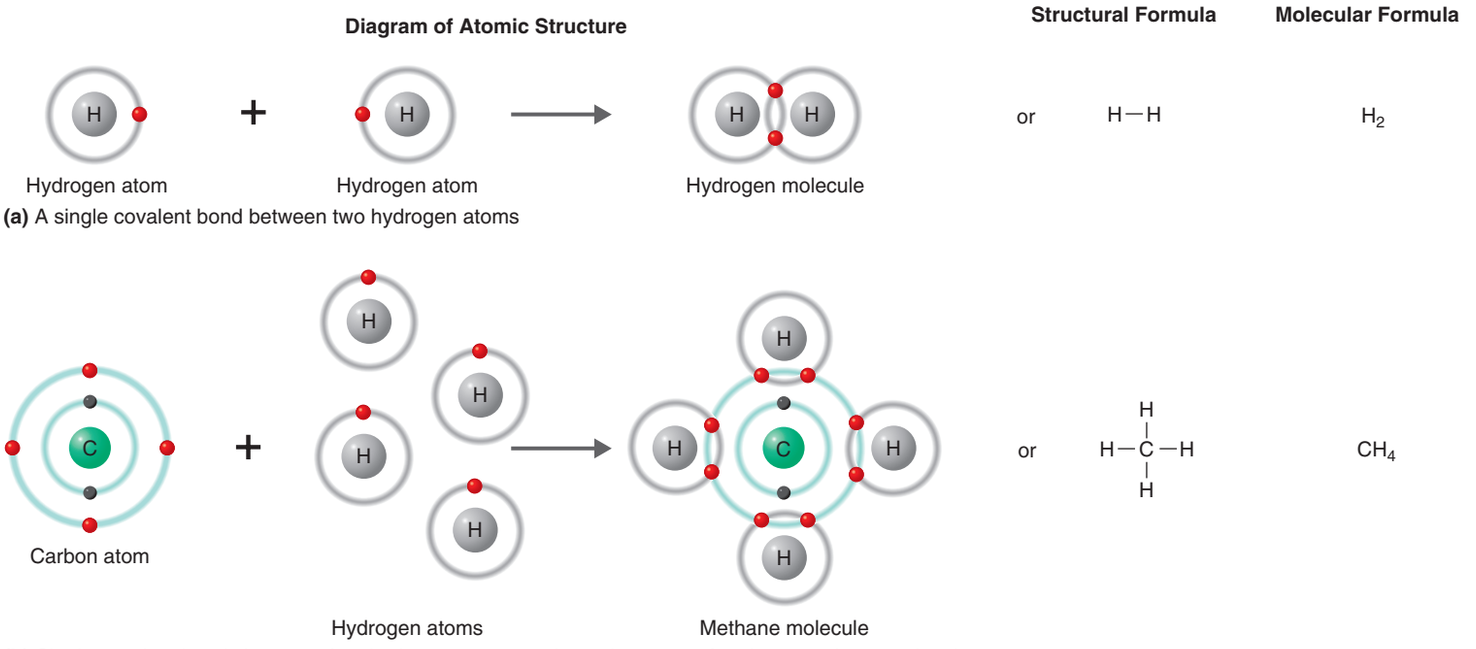


Figure 2.3 Covalent bond formation. On the right are simpler ways to represent molecules. In structural formulas, each covalent bond is written as a straight line between the symbols for two atoms. In molecular formulas, the number of atoms in each molecule is noted by subscripts.

Q What is a covalent bond?

In general, an atom whose outer electron shell is less than half-filled will lose electrons and form positively charged ions, called **cations.** Examples of cations are the potassium ion (K^+), calcium ion (Ca^{2+}), and sodium ion (Na^+). When an atom's outer electron shell is more than half-filled, the atom will gain electrons and form negatively charged ions, called **anions.** Examples are the iodide ion (I^-), chloride ion (Cl^-), and sulfide ion (S^{2-}).

Covalent Bonds

A **covalent bond** is a chemical bond formed by two atoms sharing one or more pairs of electrons. Covalent bonds are stronger and far more common in organisms than are true ionic bonds. In the hydrogen molecule, H_2, two hydrogen atoms share a pair of electrons. Each hydrogen atom has its own electron plus one electron from the other atom (**Figure 2.3a**). The shared pair of electrons actually orbits the nuclei of both atoms. Therefore, the outer electron shells of both atoms are filled. Atoms that share only one pair of electrons form a *single covalent bond.* For simplicity, a single covalent bond is expressed as a single line between the atoms (H—H). Atoms that share two pairs of electrons form a *double covalent bond,* expressed as two single lines (=). A *triple covalent bond,* expressed as three single lines (≡), occurs when atoms share three pairs of electrons.

The principles of covalent bonding that apply to atoms of the same element also apply to atoms of different elements. Methane (CH_4) is an example of covalent bonding between atoms of different elements (**Figure 2.3b**). The outer electron shell of the carbon atom can hold eight electrons but has only four; each hydrogen atom can hold two electrons but has only one. Consequently, in the methane molecule the carbon atom gains four hydrogen electrons to complete its outer shell, and each hydrogen atom completes its pair by sharing one electron from the carbon atom. Each outer electron of the carbon atom orbits both the carbon nucleus and a hydrogen nucleus. Each hydrogen electron orbits both its own nucleus and the carbon nucleus.

Elements such as hydrogen and carbon, whose outer electron shells are half-filled, form covalent bonds quite easily. In fact, in living organisms, carbon almost always forms covalent bonds; it almost never becomes an ion. *Remember:* Covalent bonds are formed by the *sharing* of electrons between atoms. Ionic bonds are formed by *attraction* between atoms that have lost or gained electrons and are therefore positively or negatively charged.

Hydrogen Bonds

Another chemical bond of special importance to all organisms is the **hydrogen bond,** in which a hydrogen atom that is covalently bonded to one oxygen or nitrogen atom is attracted to another oxygen or nitrogen atom. Such bonds are weak and do not bind atoms into molecules. However, they do serve as bridges between different molecules or between various portions of the same molecule.

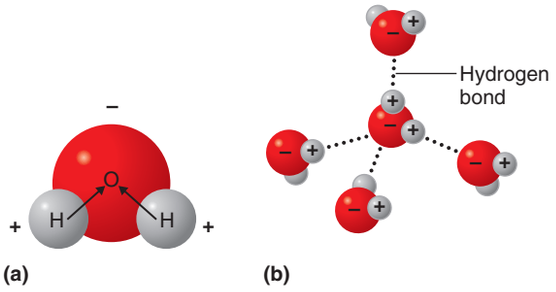


Figure 2.4 Hydrogen bond formation in water. (**a**) In a water molecule, the electrons of the hydrogen atoms are strongly attracted to the oxygen atom. Therefore, the part of the water molecule containing the oxygen atom has a slightly negative charge, and the part containing hydrogen atoms has a slightly positive charge. (**b**) In a hydrogen bond between water molecules, the hydrogen of one water molecule is attracted to the oxygen of another water molecule. Many water molecules may be attracted to each other by hydrogen bonds (black dots).

Q Which chemical elements are usually involved in hydrogen bonding?

When hydrogen combines with atoms of oxygen or nitrogen, the relatively large nucleus of these larger oxygen or nitrogen atoms has more protons and attracts the hydrogen electron more strongly than does the small hydrogen nucleus. Thus, in a molecule of water (H_2O), all the electrons tend to be closer to the oxygen nucleus than to the hydrogen nuclei. As a result, the oxygen portion of the molecule has a slightly negative charge, and the hydrogen portion of the molecule has a slightly positive charge (**Figure 2.4a**). When the positively charged end of one molecule is attracted to the negatively charged end of another molecule, a hydrogen bond is formed (**Figure 2.4b**). This attraction can also occur between hydrogen and other atoms of the same molecule, especially in large molecules. Oxygen and nitrogen are the elements most frequently involved in hydrogen bonding.

Hydrogen bonds are considerably weaker than either ionic or covalent bonds; they have only about 5% of the strength of covalent bonds. Consequently, hydrogen bonds are formed and broken relatively easily. This property accounts for the temporary bonding that occurs between certain atoms of large and complex molecules, such as proteins and nucleic acids. Even though hydrogen bonds are relatively weak, large molecules containing several hundred of these bonds have considerable strength and stability. A summary of ionic, covalent and hydrogen bonds is shown in **Table 2.3**.

Molecular Weight and Moles

You have seen that bond formation results in the creation of molecules. Molecules are often discussed in terms of units of measure called molecular weight and moles. The **molecular weight** of a molecule is the sum of the atomic weights of all its atoms. To relate the molecular level to the laboratory level, we use a unit called the mole. One **mole** of a substance is its molecular weight expressed in grams. For example, 1 mole of water weighs 18 grams because the molecular weight of H_2O is 18, or $[(2 \times 1) + 16]$.

TABLE 2.3 Comparison among Ionic, Covalent, and Hydrogen Bonds

Type of Bond	Definition and Importance
Ionic	An attraction between ions of opposite charge that holds them together to form a stable molecule. Weaker ionic bonds are important in biochemical reactions such as antigen–antibody reactions.
Covalent	A bond formed by two atoms that share one or more pairs of electrons. Covalent bonds are the most common type of chemical bond in organisms and are responsible for holding together the atoms of most molecules in organisms.
Hydrogen	A relatively weak bond in which a hydrogen atom that is covalently bonded to one oxygen or nitrogen atom is attracted to another oxygen or nitrogen atom. Hydrogen bonds do not bind atoms into molecules, but rather serve as bridges between different molecules or different portions of the same molecule, for example, within proteins and nucleic acids.

CHECK YOUR UNDERSTANDING

Differentiate an ionic bond from a covalent bond. **2.2**

Chemical Reactions

LEARNING OBJECTIVE

2-3 Diagram three basic types of chemical reactions.

As we said earlier, **chemical reactions** involve the making or breaking of bonds between atoms. After a chemical reaction, the total number of atoms remains the same, but there are new molecules with new properties because the atoms have been rearranged.

Energy in Chemical Reactions

Chemical energy occurs whenever bonds between atoms are formed or broken during chemical reactions. All chemical bonds require energy when they are broken and release chemical energy when they are formed. A chemical reaction that absorbs more energy than it releases is called an **endergonic reaction** (*endo* = within), meaning that energy is directed inward. A chemical reaction that releases more energy than it absorbs is called an **exergonic reaction** (*exo* = out), meaning that energy is directed outward.

In this section we will look at three basic types of chemical reactions common to all living cells. By becoming familiar with these reactions, you will be able to understand the specific chemical reactions we will discuss later, particularly in Chapter 5.

Bioremediation—Bacteria Clean Up Pollution

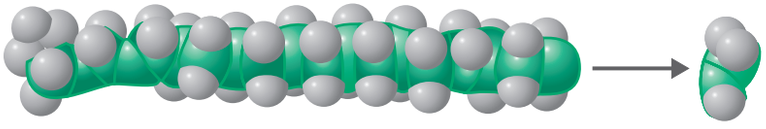
Although many bacteria have dietary requirements similar to ours—that's why they cause food spoilage—others metabolize (or chemically process) substances that are toxic to most plants and animals: heavy metals, sulfur, petroleum, and mercury.

Oil in the environment can come from natural oil that seeps from petroleum deposits, and it can also come from oil spills. Although there are oil-degrading bacteria in soil and sediments, these bacteria are in such small numbers that they cannot deal with large-scale contamination efficiently. Scientists are now working to improve the efficiency of natural pollution fighters. Using bacteria to degrade pollutants is called *bioremediation*.

One of the most promising successes for bioremediation occurred on an Alaskan beach following the *Exxon Valdez* oil spill in 1989. Several naturally occurring *Pseudomonas* bacteria are able to degrade oil for their carbon and energy requirements. In the presence of air, they remove two carbon atoms at a time from a large petroleum molecule (see the figure).

The bacteria degrade the oil too slowly to clean up an oil spill. However, scientists hit on a very simple way to speed up the process: they simply dumped ordinary nitrogen and phosphorus plant fertilizers (bioenhancers) onto a test beach. The number of oil-degrading bacteria increased compared with that on unfertilized control beaches, and oil was quickly cleared from the test beach.

This technique works on land but has not been studied in open water. A number of questions need to be addressed: Will the fertilizer stay near the oil? Will the fertilizers stimulate toxic algae?



Typical saturated hydrocarbon found in petroleum

Two-carbon unit can be metabolized in cell

Synthesis Reactions

When two or more atoms, ions, or molecules combine to form new and larger molecules, the reaction is called a **synthesis reaction.** To synthesize means to put together, and a synthesis reaction *forms new bonds.* Synthesis reactions can be expressed in the following way:

$$\underset{\substack{\text{Atom, ion,}\\\text{or molecule A}}}{A} + \underset{\substack{\text{Atom, ion,}\\\text{or molecule B}}}{B} \xrightarrow{\text{Combine to form}} \underset{\substack{\text{New molecule}\\AB}}{AB}$$

The combining substances, A and B, are called the *reactants;* the substance formed by the combination, AB, is the *product.* The arrow indicates the direction in which the reaction proceeds.

Pathways of synthesis reactions in living organisms are collectively called anabolic reactions, or simply **anabolism** (an-ab′ō-lizm). The combining of sugar molecules to form starch and of amino acids to form proteins are two examples of anabolism.

Decomposition Reactions

The reverse of a synthesis reaction is a **decomposition reaction.** To decompose means to break down into smaller parts, and in a decomposition reaction *bonds are broken.* Typically, decomposition reactions split large molecules into smaller molecules, ions, or atoms. A decomposition reaction occurs in the following way:

$$\underset{\text{Molecule AB}}{AB} \xrightarrow{\text{Breaks down into}} \underset{\substack{\text{Atom, ion,}\\\text{or molecule A}}}{A} + \underset{\substack{\text{Atom, ion,}\\\text{or molecule B}}}{B}$$

Decomposition reactions that occur in living organisms are collectively called catabolic reactions, or simply **catabolism** (ka-tab′ō-lizm). An example of catabolism is the breakdown of sucrose (table sugar) into simpler sugars, glucose and fructose, during digestion. Bacterial decomposition of petroleum is discussed in the box above.

Exchange Reactions

All chemical reactions are based on synthesis and decomposition. Many reactions, such as **exchange reactions,** are actually part synthesis and part decomposition. An exchange reaction works in the following way:

$$AB + CD \xrightarrow{\text{Recombine to form}} AD + BC$$

First, the bonds between A and B and between C and D are broken in a decomposition process. New bonds are then formed between A and D and between B and C in a synthesis process. For example, an exchange reaction occurs when sodium hydroxide (NaOH) and hydrochloric acid (HCl) react to form table salt (NaCl) and water (H_2O), as follows:

$$NaOH + HCl \longrightarrow NaCl + H_2O$$

The Reversibility of Chemical Reactions

All chemical reactions are, in theory, reversible; that is, they can occur in either direction. In practice, however, some reactions do this more easily than others. A chemical reaction that is readily reversible (when the end product can revert to the original molecules) is termed a **reversible reaction** and is indicated by two arrows, as shown here:

$$A + B \underset{\text{Breaks down into}}{\overset{\text{Combines to form}}{\rightleftharpoons}} AB$$

Some reversible reactions occur because neither the reactants nor the end products are very stable. Other reactions reverse only under special conditions:

$$A + B \underset{\text{Water}}{\overset{\text{Heat}}{\rightleftharpoons}} AB$$

Whatever is written above or below the arrows indicates the special condition under which the reaction in that direction occurs. In this case, A and B react to produce AB only when heat is applied, and AB breaks down into A and B only in the presence of water. See Figure 2.8 on page 38 for another example.

In Chapter 5 we will examine the various factors that affect chemical reactions.

CHECK YOUR UNDERSTANDING

This chemical reaction below is used to remove chlorine from water. What type of reaction is it? **2-3**

$$HClO + Na_2SO_3 \longrightarrow Na_2SO_4 + HCl$$

Important Biological Molecules

Biologists and chemists divide compounds into two principal classes: inorganic and organic. **Inorganic compounds** are defined as molecules, usually small and structurally simple, which typically lack carbon and in which ionic bonds may play an important role. Inorganic compounds include water, molecular oxygen (O_2), carbon dioxide, and many salts, acids, and bases.

Organic compounds always contain carbon and hydrogen and typically are structurally complex. Carbon is a unique element because it has four electrons in its outer shell and four unfilled spaces. It can combine with a variety of atoms, including other carbon atoms, to form straight or branched chains and rings. Carbon chains form the basis of many organic compounds in living cells, including sugars, amino acids, and vitamins. Organic compounds are held together mostly or entirely by covalent bonds. Some organic molecules, such as polysaccharides, proteins, and nucleic acids, are very large and usually contain thousands of atoms. Such giant molecules are called *macromolecules*. In the following section we will discuss inorganic and organic compounds that are essential for cells.

Inorganic Compounds

LEARNING OBJECTIVES

2-4 List several properties of water that are important to living systems.

2-5 Define *acid*, *base*, *salt*, and *pH*.

Water

All living organisms require a wide variety of inorganic compounds for growth, repair, maintenance, and reproduction. Water is one of the most important, as well as one of the most abundant, of these compounds, and it is particularly vital to microorganisms. Outside the cell, nutrients are dissolved in water, which facilitates their passage through cell membranes. And inside the cell, water is the medium for most chemical reactions. In fact, water is by far the most abundant component of almost all living cells. Water makes up at least 5–95% of every cell, on average between 65% and 75%. Simply stated, no organism can survive without water.

Water has structural and chemical properties that make it particularly suitable for its role in living cells. As we discussed, the total charge on the water molecule is neutral, but the oxygen region of the molecule has a slightly negative charge, and the hydrogen region has a slightly positive charge (see Figure 2.4a). Any molecule having such an unequal distribution of charges is called a **polar molecule.** The polar nature of water gives it four characteristics that make it a useful medium for living cells.

First, every water molecule is capable of forming four hydrogen bonds with nearby water molecules (see Figure 2.4b). This property results in a strong attraction between water molecules. Because of this strong attraction, a great deal of heat is required to separate water molecules from each other to form water vapor; thus, water has a relatively high boiling point (100°C). Because water has such a high boiling point, it exists in the liquid state on most of the Earth's surface. Furthermore, the hydrogen bonding between water molecules affects the density of water, depending on whether it occurs as ice or a liquid. For example, the hydrogen bonds in the crystalline structure of water (ice) make ice take up more space. As a result, ice has fewer molecules than an equal volume of liquid water. This makes its crystalline

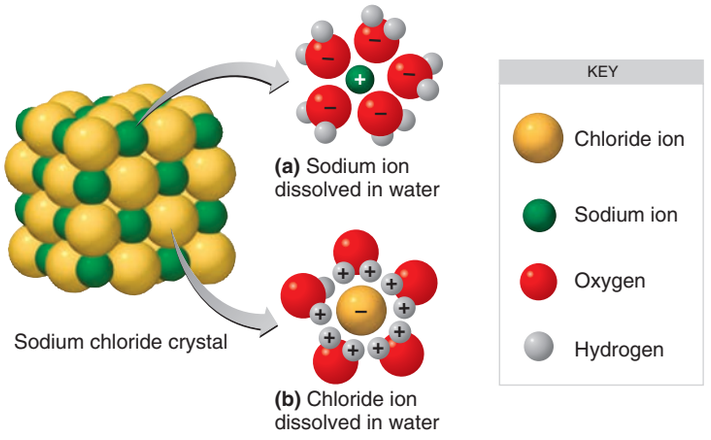


Figure 2.5 How water acts as a solvent for sodium chloride (NaCl). (**a**) The positively charged sodium ion (Na^+) is attracted to the negative part of the water molecule. (**b**) The negatively charged chloride ion (Cl^-) is attracted to the positive part of the water molecule. In the presence of water molecules, the bonds between the Na^+ and Cl^- are disrupted, and the NaCl dissolves in the water.

Q What happens during ionization?

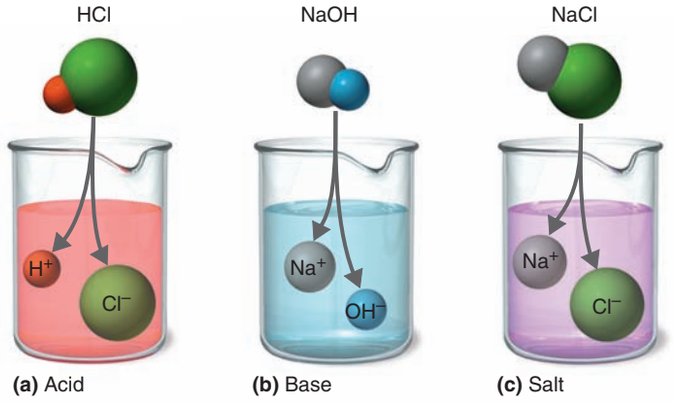


Figure 2.6 Acids, bases, and salts. (**a**) In water, hydrochloric acid (HCl) dissociates into H^+ and Cl^-. (**b**) Sodium hydroxide (NaOH), a base, dissociates into OH^- and Na^+ in water. (**c**) In water, table salt (NaCl) dissociates into positive ions (Na^+) and negative ions (Cl^-), neither of which are H^+ or OH^-.

Q How do acids and bases differ?

structure less dense than liquid water. For this reason, ice floats and can serve as an insulating layer on the surfaces of lakes and streams that harbor living organisms.

Second, the polarity of water makes it an excellent dissolving medium, or **solvent.** Many polar substances undergo **dissociation,** or separation, into individual molecules in water—that is, they dissolve. The negative part of the water molecules is attracted to the positive part of the molecules in the **solute,** or dissolving substance, and the positive part of the water molecules is attracted to the negative part of the solute molecules. Substances (such as salts) that are composed of atoms (or groups of atoms) held together by ionic bonds tend to dissociate into separate cations and anions in water. Thus, the polarity of water allows molecules of many different substances to separate and become surrounded by water molecules (Figure 2.5).

Third, polarity accounts for water's characteristic role as a reactant or product in many chemical reactions. Its polarity facilitates the splitting and rejoining of hydrogen ions (H^+) and hydroxide ions (OH^-). Water is a key reactant in the digestive processes of organisms, whereby larger molecules are broken down into smaller ones. Water molecules are also involved in synthetic reactions; water is an important source of the hydrogen and oxygen that are incorporated into numerous organic compounds in living cells.

Finally, the relatively strong hydrogen bonding between water molecules (see Figure 2.4b) makes water an excellent temperature buffer. Compared with many other substances, a given quantity of water requires a great gain of heat to increase its temperature and a great loss of heat to decrease its temperature. Normally, heat absorption by molecules increases their kinetic energy and thus increases their rate of motion and their reactivity. In water, however, heat absorption first breaks hydrogen bonds rather than increasing the rate of motion. Therefore, much more heat must be applied to raise the temperature of water than to raise the temperature of a non–hydrogen-bonded liquid. The reverse is true as water cools. Thus, water more easily maintains a constant temperature than other solvents and tends to protect a cell from fluctuations in environmental temperatures.

Acids, Bases, and Salts

As we saw in Figure 2.5, when inorganic salts such as sodium chloride (NaCl) are dissolved in water, they undergo **ionization** or *dissociation;* that is, they break apart into ions. Substances called acids and bases show similar behavior.

An **acid** can be defined as a substance that dissociates into one or more hydrogen ions (H^+) and one or more negative ions (anions). Thus, an acid can also be defined as a proton (H^+) donor. A **base** dissociates into one or more positive ions (cations) plus one or more negatively charged hydroxide ions (OH^-) that can accept, or combine with, protons. Thus, sodium hydroxide (NaOH) is a base because it dissociates to release OH^-, which has a strong attraction for protons and is among the most important proton acceptors. A **salt** is a substance that dissociates in water into cations and anions, neither of which is H^+ or OH^-. Figure 2.6 shows common examples of each type of compound and how they dissociate in water.

Acid–Base Balance: The Concept of pH

An organism must maintain a fairly constant balance of acids and bases to remain healthy. For example, if a particular acid or base concentration is too high or too low, enzymes change in shape and no longer effectively promote chemical reactions in a cell. In the aqueous environment within organisms, acids dissociate into hydrogen

ions (H^+) and anions. Bases, in contrast, dissociate into hydroxide ions (OH^-) and cations. The more hydrogen ions that are free in a solution, the more acidic the solution is. Conversely, the more hydroxide ions that are free in a solution, the more basic, or alkaline, it is.

Biochemical reactions—that is, chemical reactions in living systems—are extremely sensitive to even small changes in the acidity or alkalinity of the environments in which they occur. In fact, H^+ and OH^- are involved in almost all biochemical processes, and any deviation from a cell's narrow band of normal H^+ and OH^- concentrations can dramatically modify the cell's functions. For this reason, the acids and bases that are continually formed in an organism must be kept in balance.

It is convenient to express the amount of H^+ in a solution by a logarithmic **pH** scale, which ranges from 0 to 14 (Figure 2.7). The term *pH* means potential of hydrogen. On a logarithmic scale, a change of one whole number represents a *tenfold* change from the previous concentration. Thus, a solution of pH 1 has ten times more hydrogen ions than a solution of pH 2 and has 100 times more hydrogen ions than a solution of pH 3.

A solution's pH is calculated as $-\log_{10}[H^+]$, the negative logarithm to the base 10 of the hydrogen ion concentration (denoted by brackets), determined in moles per liter $[H^+]$. For example, if the H^+ concentration of a solution is 1.0×10^{-4} moles/liter, or 10^{-4}, its pH equals $-\log_{10}10^{-4} = -(-4) = 4$; this is about the pH value of wine (see Appendix B). The pH values of some human body fluids and other common substances are also shown in Figure 2.7. In the laboratory, you will usually measure the pH of a solution with a pH meter or with chemical test papers.

Acidic solutions contain more H^+ than OH^- and have a pH lower than 7. If a solution has more OH^- than H^+, it is a basic, or alkaline, solution. In pure water, a small percentage of the molecules are dissociated into H^+ and OH^-, so it has a pH of 7. Because the concentrations of H^+ and OH^- are equal, this pH is said to be the pH of a neutral solution.

Keep in mind that the pH of a solution can be changed. We can increase its acidity by adding substances that will increase the concentration of hydrogen ions. As a living organism takes up nutrients, carries out chemical reactions, and excretes wastes, its balance of acids and bases tends to change, and the pH fluctuates. Fortunately, organisms possess natural pH **buffers,** compounds that help keep the pH from changing drastically. But the pH in our environment's water and soil can be altered by waste products from organisms, pollutants from industry, or fertilizers used in agricultural fields or gardens. When bacteria are grown in a laboratory medium, they excrete waste products such as acids that can alter the pH of the medium. If this effect were to continue, the medium would become acidic enough to inhibit bacterial enzymes and kill the bacteria. To prevent this problem, pH buffers are added to the culture medium. One very effective pH buffer for some culture media uses a mixture of K_2HPO_4 and KH_2PO_4 (see Table 6.3, page 163).

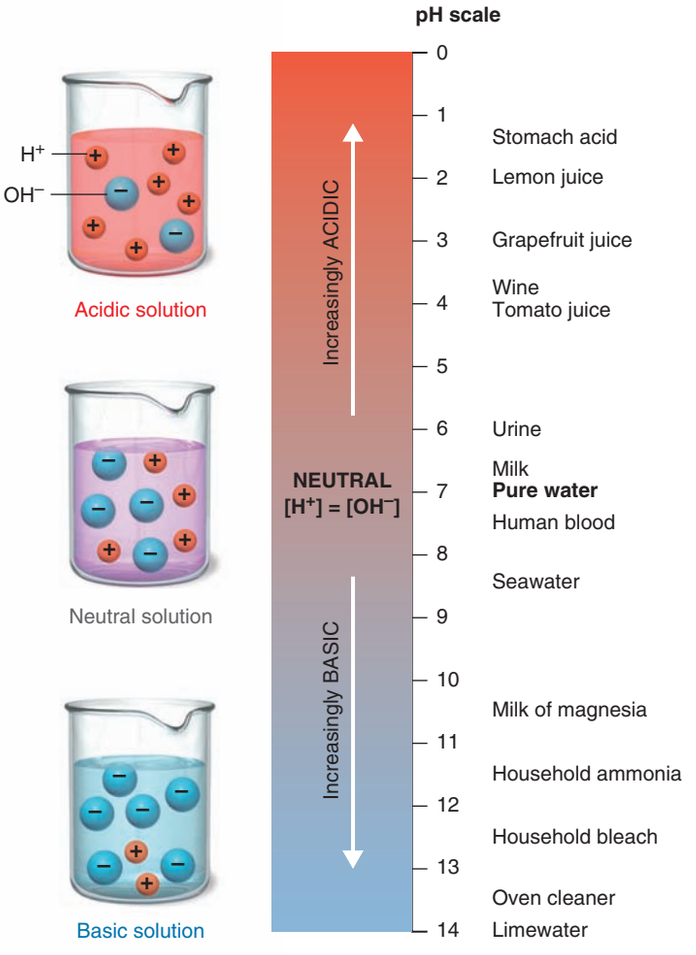


Figure 2.7 The pH scale.
As pH values decrease from 14 to 0, the H^+ concentration increases. Thus, the lower the pH, the more acidic the solution; the higher the pH, the more basic the solution. If the pH value of a solution is below 7, the solution is acidic; if the pH is above 7, the solution is basic (alkaline). The approximate pH values of some human body fluids and common substances are shown next to the pH scale.

Q At what pH are the concentrations of H^+ and OH^- equal?

Different microbes function best within different pH ranges, but most organisms grow best in environments with a pH value between 6.5 and 8.5. Among microbes, fungi are best able to tolerate acidic conditions, whereas the prokaryotes called cyanobacteria tend to do well in alkaline habitats. *Propionibacterium acnes* (prō-pē-on-ē-bak-ti′rē-um ak′nēz), a bacterium that causes acne, has as its natural environment human skin, which tends to be slightly acidic, with a pH of about 4. *Thiobacillus ferrooxidans* (thī-ō-bä-sil′lus fer-rō-oks′i-danz) is a bacterium that metabolizes elemental sulfur and produces sulfuric acid (H_2SO_4). Its pH range for optimum growth is from 1 to 3.5. The sulfuric acid produced by this bacterium in mine water is important in dissolving uranium and copper from low-grade ore (see Chapter 28).

CHECK YOUR UNDERSTANDING

- Why is the polarity of a water molecule important? **2-4**
- Antacids neutralize acid by the following reaction.
  $Mg(OH)_2 + 2HCl \rightarrow MgCl_2 + H_2O$
  Identify the acid, base, and salt. **2-5**

Organic Compounds

LEARNING OBJECTIVES

2-6 Distinguish organic and inorganic compounds.
2-7 Define *functional group.*
2-8 Identify the building blocks of carbohydrates.
2-9 Differentiate simple lipids, complex lipids, and steroids.
2-10 Identify the building blocks and structure of proteins.
2-11 Identify the building blocks of nucleic acids.
2-12 Describe the role of ATP in cellular activities.

Inorganic compounds, excluding water, constitute about 1–1.5% of living cells. These relatively simple components, whose molecules have only a few atoms, cannot be used by cells to perform complex biological functions. Organic molecules, whose carbon atoms can combine in an enormous variety of ways with other carbon atoms and with atoms of other elements, are relatively complex and thus are capable of more complex biological functions.

Structure and Chemistry

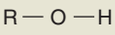
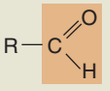
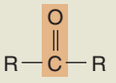
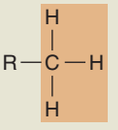
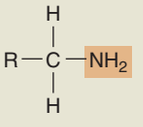
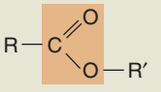
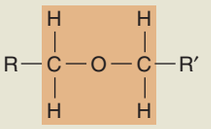
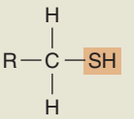
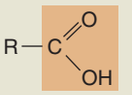
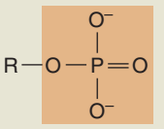
In the formation of organic molecules, carbon's four outer electrons can participate in up to four covalent bonds, and carbon atoms can bond to each other to form straight-chain, branched-chain, or ring structures.

In addition to carbon, the most common elements in organic compounds are hydrogen (which can form one bond), oxygen (two bonds), and nitrogen (three bonds). Sulfur (two bonds) and phosphorus (five bonds) appear less often. Other elements are found, but only in relatively few organic compounds. The elements that are most abundant in living organisms are the same as those that are most abundant in organic compounds (see Table 2.1).

The chain of carbon atoms in an organic molecule is called the **carbon skeleton;** a huge number of combinations is possible for carbon skeletons. Most of these carbons are bonded to hydrogen atoms. The bonding of other elements with carbon and hydrogen forms characteristic **functional groups,** specific groups of atoms that are most commonly involved in chemical reactions and are responsible for most of the characteristic chemical properties and many of the physical properties of a particular organic compound (**Table 2.4**).

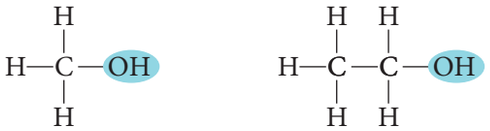
Different functional groups confer different properties on organic molecules. For example, the hydroxyl group of alcohols is hydrophilic (water-loving) and thus attracts water molecules to it. This attraction helps dissolve organic molecules containing hydroxyl groups. Because the carboxyl group is a source of hydrogen ions, molecules containing it have acidic properties. Amino groups, by contrast, function as bases because they readily accept hydrogen ions. The sulfhydryl group helps stabilize the intricate structure of many proteins.

TABLE 2.4 Representative Functional Groups and the Compounds in Which They Are Found

Structure	Name of Group	Biological Importance
	Alcohol	Lipids, carbohydrates
	Aldehyde*	Reducing sugars such as glucose; polysaccharides
	Ketone*	Metabolic intermediates
	Methyl	DNA; energy metabolism
	Amino	Proteins
	Ester	Bacterial and eukaryotic plasma membranes
	Ether	Archaeal plasma membranes
	Sulfhydryl	Energy metabolism; protein structure
	Carboxyl	Organic acids, lipids, proteins
	Phosphate	ATP, DNA

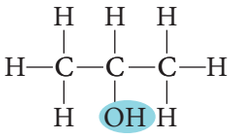
*In an aldehyde, a C═O is at the end of a molecule, in contrast to the internal C═O in a ketone.

Functional groups help us classify organic compounds. For example, the —OH group is present in each of the following molecules:



Methanol

Ethanol

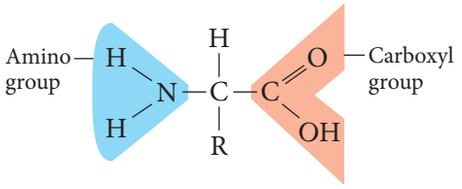


Isopropanol

Because the characteristic reactivity of the molecules is based on the —OH group, they are grouped together in a class called alcohols. The —OH group is called the *hydroxyl group* and is not to be confused with the *hydroxide ion* (OH^-) of bases. The hydroxyl group of alcohols does not ionize at neutral pH; it is covalently bonded to a carbon atom.

When a class of compounds is characterized by a certain functional group, the letter *R* can be used to stand for the remainder of the molecule. For example, alcohols in general may be written R—OH.

Frequently, more than one functional group is found in a single molecule. For example, an amino acid molecule contains both amino and carboxyl groups. The amino acid glycine has the following structure:



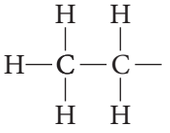
Most of the organic compounds found in living organisms are quite complex; a large number of carbon atoms form the skeleton, and many functional groups are attached. In organic molecules, it is important that each of the four bonds of carbon be satisfied (attached to another atom) and that each of the attaching atoms have its characteristic number of bonds satisfied. Because of this, such molecules are chemically stable.

Small organic molecules can be combined into very large molecules called **macromolecules** (*macro* = large). Macromolecules are usually **polymers** (*poly* = many; *mers* = parts): polymers are formed by covalent bonding of many repeating small molecules called **monomers** (*mono* = one). When two monomers join together, the reaction usually involves the elimination of a hydrogen atom from one monomer and a hydroxyl group from the other; the hydrogen atom and the hydroxyl group combine to produce water:

This type of exchange reaction is called **dehydration synthesis** (*de* = from; *hydro* = water), or a **condensation reaction,** because a molecule of water is released (**Figure 2.8a**). Such macromolecules as carbohydrates, lipids, proteins, and nucleic acids are assembled in the cell, essentially by dehydration synthesis. However, other molecules must also participate to provide energy for bond formation. ATP, the cell's chief energy provider, is discussed at the end of this chapter.

CHECK YOUR UNDERSTANDING

- Define *organic*. **2-6**
- Add the appropriate functional group(s) to the ethyl group below to produce each of the following compounds: ethanol, acetic acid, acetaldehyde, ethanolamine, diethyl ether. **2-7**



Carbohydrates

The **carbohydrates** are a large and diverse group of organic compounds that includes sugars and starches. Carbohydrates perform a number of major functions in living systems. For instance, one type of sugar (deoxyribose) is a building block of deoxyribonucleic acid (DNA), the molecule that carries hereditary information. Other sugars are needed for the cell walls. Simple carbohydrates are used in the synthesis of amino acids and fats or fatlike substances, which are used to build cell membranes and other structures. Macromolecular carbohydrates function as food reserves. The principal function of carbohydrates, however, is to fuel cell activities with a ready source of energy.

Carbohydrates are made up of carbon, hydrogen, and oxygen atoms. The ratio of hydrogen to oxygen atoms is always 2:1 in simple carbohydrates. This ratio can be seen in the formulas for the carbohydrates ribose ($C_5H_{10}O_5$), glucose ($C_6H_{12}O_6$), and sucrose ($C_{12}H_{22}O_{11}$). Although there are exceptions, the general formula for carbohydrates is $(CH_2O)_n$, where *n* indicates that there are three or more CH_2O units. Carbohydrates can be classified into three major groups on the basis of size: monosaccharides, disaccharides, and polysaccharides.

Monosaccharides

Simple sugars are called **monosaccharides** (*sacchar* = sugar); each molecule contains from three to seven carbon atoms. The number of carbon atoms in the molecule of a simple sugar is indicated by the prefix in its name. For example, simple sugars with three carbons are called trioses. There are also tetroses (four-carbon sugars), pentoses (five-carbon sugars), hexoses (six-carbon sugars), and heptoses (seven-carbon sugars). Pentoses and hexoses are extremely important to living organisms. Deoxyribose is a pentose found in DNA. Glucose, a very common hexose, is the main energy-supplying molecule of living cells.

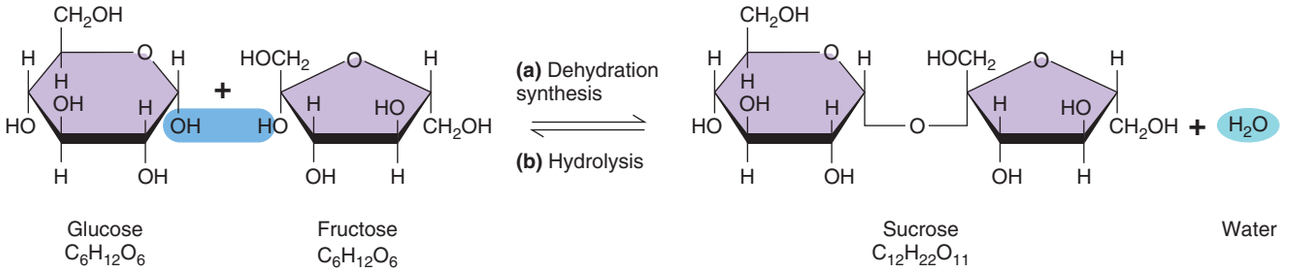


Figure 2.8 Dehydration synthesis and hydrolysis. (**a**) In dehydration synthesis (left to right), the monosaccharides glucose and fructose combine to form a molecule of the disaccharide sucrose. A molecule of water is released in the reaction. (**b**) In hydrolysis (right to left), the sucrose molecule breaks down into the smaller molecules glucose and fructose. For the hydrolysis reaction to proceed, water must be added to the sucrose.

Q What is the difference between a polymer and a monomer?

Disaccharides

Disaccharides (*di* = two) are formed when two monosaccharides bond in a dehydration synthesis reaction.* For example, molecules of two monosaccharides, glucose and fructose, combine to form a molecule of the disaccharide sucrose (table sugar) and a molecule of water (see Figure 2.8a). Similarly, the dehydration synthesis of the monosaccharides glucose and galactose forms the disaccharide lactose (milk sugar).

It may seem odd that glucose and fructose have the same chemical formula (see Figure 2.8), even though they are different monosaccharides. The positions of the oxygens and carbons differ in the two different molecules, and consequently the molecules have different physical and chemical properties. Two molecules with the same chemical formula but different structures and properties are called **isomers** (*iso* = same).

Disaccharides can be broken down into smaller, simpler molecules when water is added. This chemical reaction, the reverse of dehydration synthesis, is called **hydrolysis** (*hydro* =water; *lysis* = to loosen) (Figure 2.8b). A molecule of sucrose, for example, may be hydrolyzed (digested) into its components of glucose and fructose by reacting with the H^+ and OH^- of water.

As you will see in Chapter 4, the cell walls of bacterial cells are composed of disaccharides and proteins (together called peptidoglycan).

Polysaccharides

Carbohydrates in the third major group, the **polysaccharides,** consist of tens or hundreds of monosaccharides joined through dehydration synthesis. Polysaccharides often have side chains branching off the main structure and are classified as macromolecules. Like disaccharides, polysaccharides can be split apart into their constituent sugars through hydrolysis. Unlike monosaccharides and disaccharides, however, they usually lack the characteristic sweetness of sugars such as fructose and sucrose and usually are not soluble in water.

*Carbohydrates composed of 2 to about 20 monosaccharides are called **oligosaccharides** (*oligo* = few). Disaccharides are the most common oligosaccharides.

One important polysaccharide is *glycogen,* which is composed of glucose subunits and is synthesized as a storage material by animals and some bacteria. *Cellulose,* another important glucose polymer, is the main component of the cell walls of plants and most algae. Although cellulose is the most abundant carbohydrate on Earth, it can be digested by only a few organisms that have the appropriate enzyme. The polysaccharide *dextran,* which is produced as a sugary slime by certain bacteria, is used in a blood plasma substitute. *Chitin* is a polysaccharide that makes up part of the cell wall of most fungi and the exoskeletons of lobsters, crabs, and insects. *Starch* is a polymer of glucose produced by plants and used as food by humans.

Many animals, including humans, produce enzymes called *amylases* that can break the bonds between the glucose molecules in glycogen. However, this enzyme cannot break the bonds in cellulose. Bacteria and fungi that produce enzymes called *cellulases* can digest cellulose. Cellulases from the fungus *Trichoderma* (trik′ō-dėr-mä) are used for a variety of industrial purposes. One of the more unusual uses is producing stonewashed denim. Because washing the fabric with rocks would damage washing machines, cellulase is used to digest, and therefore soften, the cotton. (See the box in Chapter 1, page 3.)

CHECK YOUR UNDERSTANDING

✓ Give an example of a monosaccharide, a disaccharide, and a polysaccharide. 2-8

Lipids

If lipids were suddenly to disappear from the Earth, all living cells would collapse in a pool of fluid, because lipids are essential to the structure and function of membranes that separate living cells from their environment. **Lipids** (*lip* = fat) are a second major group of organic compounds found in living matter. Like carbohydrates, they are composed of atoms of carbon, hydrogen, and oxygen, but lipids lack the 2:1 ratio between hydrogen and oxygen atoms. Even though lipids are a very diverse group of compounds, they share one common characteristic: they are *nonpolar* molecules so, unlike water, do not have a positive and a negative end (pole). Therefore,

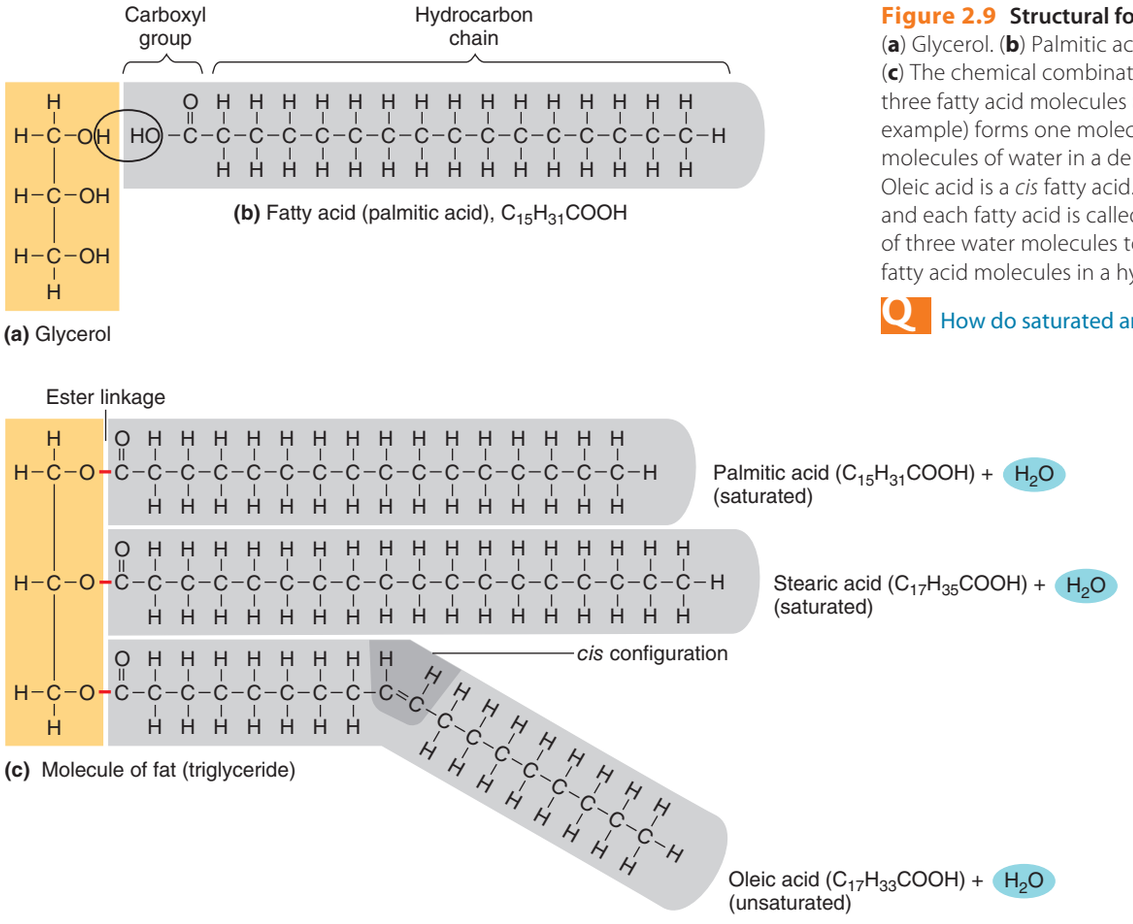


Figure 2.9 Structural formulas of simple lipids. (**a**) Glycerol. (**b**) Palmitic acid, a saturated fatty acid. (**c**) The chemical combination of a molecule of glycerol and three fatty acid molecules (palmitic, stearic, and oleic in this example) forms one molecule of fat (triglyceride) and three molecules of water in a dehydration synthesis reaction. Oleic acid is a *cis* fatty acid. The bond between glycerol and each fatty acid is called an ester linkage. The addition of three water molecules to a fat forms glycerol and three fatty acid molecules in a hydrolysis reaction.

Q How do saturated and unsaturated fatty acids differ?

most lipids are insoluble in water but dissolve readily in nonpolar solvents, such as ether and chloroform. Lipids provide the structure of membranes and some cell walls and function in energy storage.

Simple Lipids

Simple lipids, called *fats* or *triglycerides,* contain an alcohol called *glycerol* and a group of compounds known as *fatty acids*. Glycerol molecules have three carbon atoms to which are attached three hydroxyl (—OH) groups (**Figure 2.9a**). Fatty acids consist of long hydrocarbon chains (composed only of carbon and hydrogen atoms) ending in a carboxyl (—COOH, organic acid) group (**Figure 2.9b**). Most common fatty acids contain an even number of carbon atoms.

A molecule of fat is formed when a molecule of glycerol combines with one to three fatty acid molecules. The number of fatty acid molecules determines whether the fat molecule is a monoglyceride, diglyceride, or triglyceride (**Figure 2.9c**). In the reaction, one to three molecules of water are formed (dehydration), depending on the number of fatty acid molecules reacting. The chemical bond formed where the water molecule is removed is called an *ester linkage.* In the reverse reaction, hydrolysis, a fat molecule is broken down into its component fatty acid and glycerol molecules.

Because the fatty acids that form lipids have different structures, there is a wide variety of lipids. For example, three molecules of fatty acid A might combine with a glycerol molecule. Or one molecule each of fatty acids A, B, and C might unite with a glycerol molecule (see Figure 2.9c).

The primary function of lipids is to form plasma membranes that enclose cells. A plasma membrane supports the cell and allows nutrients and wastes to pass in and out; therefore, the lipids must maintain the same viscosity, regardless of the surrounding temperature. The membrane must be about as viscous as olive oil, without getting too fluid when warmed or too thick when cooled. As everyone who has ever cooked a meal knows, animal fats (such as butter) are usually solid at room temperature, whereas vegetable oils are usually liquid at room temperature. The difference in their respective melting points is due to the degrees of saturation of the fatty acid chains. A fatty acid is said to be *saturated* when it has no double bonds, in which case the carbon skeleton contains the maximum number of hydrogen atoms (see Figure 2.9c and **Figure 2.10a**). Saturated chains become solid more easily because they are relatively straight and are thus able to pack together more closely than unsaturated chains. The double bonds of *unsaturated* chains create kinks in the chain, which keep the chains apart from

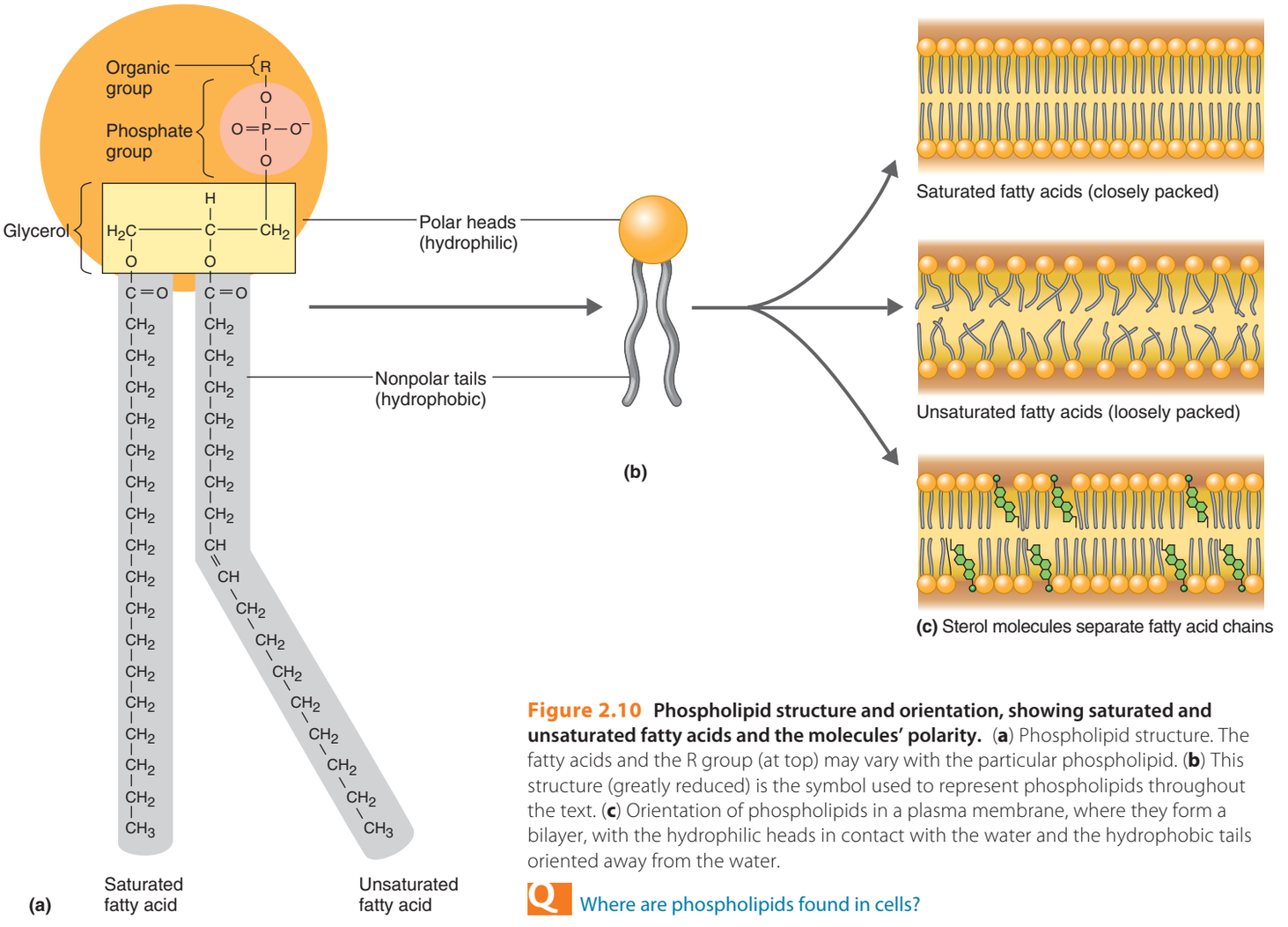


Figure 2.10 Phospholipid structure and orientation, showing saturated and unsaturated fatty acids and the molecules' polarity. (**a**) Phospholipid structure. The fatty acids and the R group (at top) may vary with the particular phospholipid. (**b**) This structure (greatly reduced) is the symbol used to represent phospholipids throughout the text. (**c**) Orientation of phospholipids in a plasma membrane, where they form a bilayer, with the hydrophilic heads in contact with the water and the hydrophobic tails oriented away from the water.

Q Where are phospholipids found in cells?

one another (**Figure 2.10b**). Note in Figure 2.9c that the H atoms on either side of the double bond in oleic acid are on the same side of the unsaturated fatty acid. Such an unsaturated fatty acid is called a *cis* fatty acid. If, instead, the H atoms are on opposite sides of the double bond, the unsaturated acid is called a *trans* fatty acid.

Complex Lipids

Complex lipids contain such elements as phosphorus, nitrogen, and sulfur, in addition to the carbon, hydrogen, and oxygen found in simple lipids. The complex lipids called *phospholipids* are made up of glycerol, two fatty acids, and, in place of a third fatty acid, a phosphate group bonded to one of several organic groups (see Figure 2.10a). Phospholipids are the lipids that build membranes; they are essential to a cell's survival. Phospholipids have polar as well as nonpolar regions (Figure 2.10a and b; see also Figure 4.14, page 89). When placed in water, phospholipid molecules twist themselves in such a way that all polar (hydrophilic) portions orient themselves toward the polar water molecules, with which they then form hydrogen bonds. (Recall that *hydrophilic* means water-loving.) This forms the basic structure of a plasma membrane (**Figure 2.10c**). Polar portions consist of a phosphate group and glycerol. In contrast to the polar regions, all nonpolar (hydrophobic) parts of the phospholipid make contact only with the nonpolar portions of neighboring molecules. (*Hydrophobic* means water-fearing.) Nonpolar portions consist of fatty acids. This characteristic behavior makes phospholipids particularly suitable for their role as a major component of the membranes that enclose cells. Phospholipids enable the membrane to act as a barrier that separates the contents of the cell from the water-based environment in which it lives.

Some complex lipids are useful in identifying certain bacteria. For example, the cell wall of *Mycobacterium tuberculosis* (mī-kō-bak-ti′rē-um tü-bėr-kū-lō′sis), the bacterium that causes tuberculosis, is distinguished by its lipid-rich content. The cell wall contains complex lipids such as waxes and glycolipids (lipids with carbohydrates attached) that give the bacterium distinctive staining characteristics. Cell walls rich in such complex lipids are characteristic of all members of the genus *Mycobacterium*.

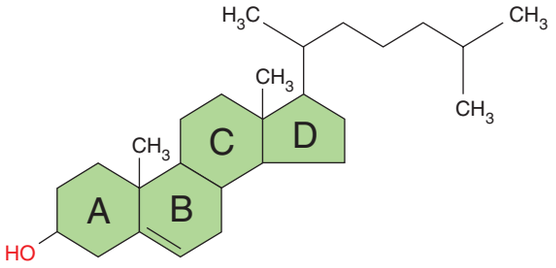


Figure 2.11 Cholesterol, a steroid. Note the four "fused" carbon rings (labeled A–D), which are characteristic of steroid molecules. The hydrogen atoms attached to the carbons at the corners of the rings have been omitted. The —OH group (colored red) makes this molecule a sterol.

Q Where are sterols found in cells?

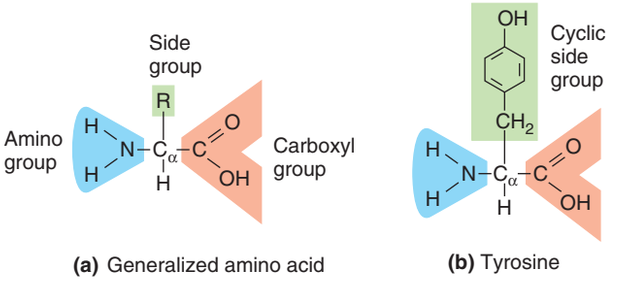


Figure 2.12 Amino acid structure. (**a**) The general structural formula for an amino acid. The alpha-carbon (C_α) is shown in the center. Different amino acids have different R groups, also called side groups. (**b**) Structural formula for the amino acid tyrosine, which has a cyclic side group.

Q What distinguishes one amino acid from another?

Steroids

Steroids are structurally very different from lipids. **Figure 2.11** shows the structure of the steroid cholesterol, with the four interconnected carbon rings that are characteristic of steroids. When an —OH group is attached to one of the rings, the steroid is called a *sterol* (an alcohol). Sterols are important constituents of the plasma membranes of animal cells and of one group of bacteria (mycoplasmas), and they are also found in fungi and plants. The sterols separate the fatty acid chains and thus prevent the packing that would harden the plasma membrane at low temperatures (see Figure 2.10c).

CHECK YOUR UNDERSTANDING

How do simple lipids differ from complex lipids? **2-9**

Proteins

Proteins are organic molecules that contain carbon, hydrogen, oxygen, and nitrogen. Some also contain sulfur. If you were to separate and weigh all the groups of organic compounds in a living cell, the proteins would tip the scale. Hundreds of different proteins can be found in any single cell, and together they make up 50% or more of a cell's dry weight.

Proteins are essential ingredients in all aspects of cell structure and function. *Enzymes* are the proteins that speed up biochemical reactions. But proteins have other functions as well. *Transporter proteins* help transport certain chemicals into and out of cells. Other proteins, such as the *bacteriocins* produced by many bacteria, kill other bacteria. Certain *toxins,* called exotoxins, produced by some disease-causing microorganisms are also proteins. Some proteins play a role in the *contraction* of animal muscle cells and the *movement* of microbial and other types of cells. Other proteins are integral parts of *cell structures* such as walls, membranes, and cytoplasmic components. Still others, such as the *hormones* of certain organisms, have regulatory functions. As we will see in Chapter 17, proteins called *antibodies* play a role in vertebrate immune systems.

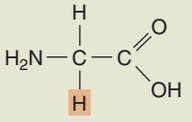
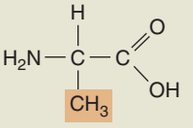
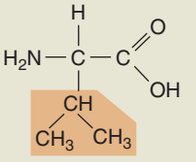
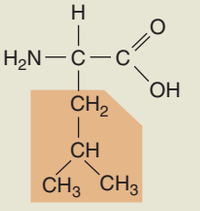
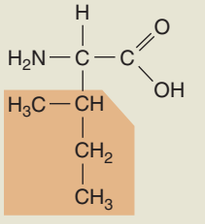
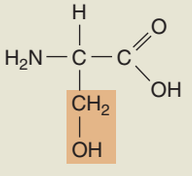
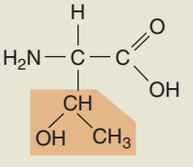
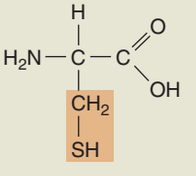
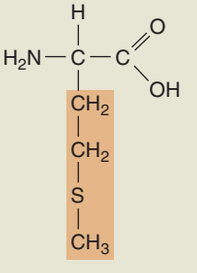
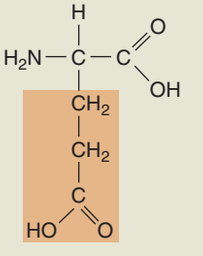
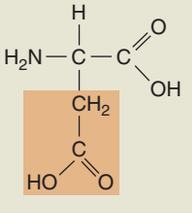
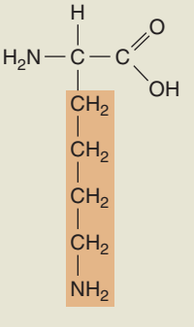
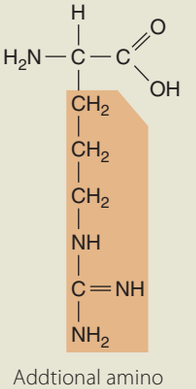
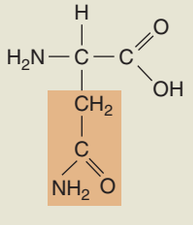
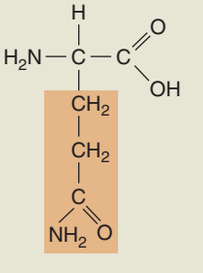
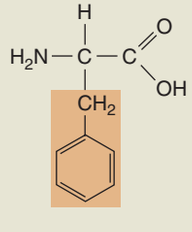
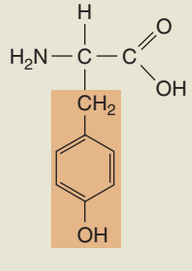
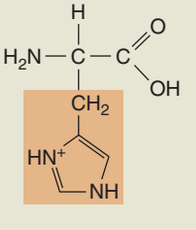
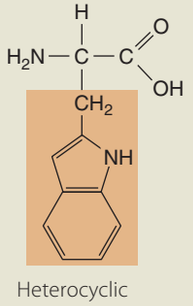
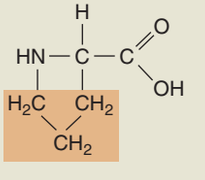
Amino Acids

Just as monosaccharides are the building blocks of larger carbohydrate molecules, and just as fatty acids and glycerol are the building blocks of fats, **amino acids** are the building blocks of proteins. Amino acids contain at least one carboxyl (—COOH) group and one amino ($-NH_2$) group attached to the same carbon atom, called an alpha-carbon (written C_α) (**Figure 2.12a**). Such amino acids are called *alpha-amino acids.* Also attached to the alpha-carbon is a side group (R group), which is the amino acid's distinguishing feature. The side group can be a hydrogen atom, an unbranched or branched chain of atoms, or a ring structure that is cyclic (all carbon) or heterocyclic (when an atom other than carbon is included in the ring). **Figure 2.12b** shows the structural formula of tyrosine, an amino acid that has a cyclic side group. The side group can contain functional groups, such as the sulfhydryl group (—SH), the hydroxyl group (—OH), or additional carboxyl or amino groups. These side groups and the carboxyl and alpha-amino groups affect the total structure of a protein, described later. The structures and standard abbreviations of the 20 amino acids found in proteins are shown in **Table 2.5**.

Most amino acids exist in either of two configurations called **stereoisomers,** designated by D and L. These configurations are mirror images, corresponding to "right-handed" (D) and "left-handed" (L) three-dimensional shapes (**Figure 2.13**). The amino acids found in proteins are always the L-isomers (except for glycine, the simplest amino acid, which does not have stereoisomers). However, D-amino acids occasionally occur in nature—for example, in certain bacterial cell walls and antibiotics. (Many other kinds of organic molecules also can exist in D and L forms. One example is the sugar glucose, which occurs in nature as D-glucose.)

Although only 20 different amino acids occur naturally in proteins, a single protein molecule can contain from 50 to hundreds of amino acid molecules, which can be arranged in an almost infinite number of ways to make proteins of different lengths, compositions, and structures. The number of proteins is practically endless, and every living cell produces many different proteins.

TABLE 2.5 The 20 Amino Acids Found in Proteins*

Glycine (Gly)	**Alanine (Ala)**	**Valine (Val)**	**Leucine (Leu)**	**Isoleucine (Ile)**
				
Hydrogen atom	Unbranched chain	Branched chain	Branched chain	Branched chain
Serine (Ser)	**Threonine (Thr)**	**Cysteine (Cys)**	**Methionine (Met)**	**Glutamic acid (Glu)**
				
Hydroxyl (—OH) group	Hydroxyl (—OH) group	Sulphur-containing (—SH) group	Thioether (SC) group	Additional carboxyl (—COOH) group, acidic
Aspartic acid (Asp)	**Lysine (Lys)**	**Arginine (Arg)**	**Asparagine (Asn)**	**Glutamine (Gln)**
				
Addtional Carboxyl (—COOH) group, acidic	Addtional amino ($—NH_2$) group, basic	Addtional amino ($—NH_2$) group, basic	Addtional amino ($—NH_2$) group, basic	Addtional amino ($—NH_2$) group, basic
Phenylalanine (Phe)	**Tyrosine (Tyr)**	**Histidine (His)**	**Tryptophan (Trp)**	**Proline (Pro)**
				
Cyclic	Cyclic	Heterocyclic	Heterocyclic	Heterocyclic

*Shown are the amino acid names, including the three-letter abbreviation in parentheses (above), their structural formulas (center), and characteristic R group (below). Note that cysteine and methionine are the only amino acids that contain sulfur.

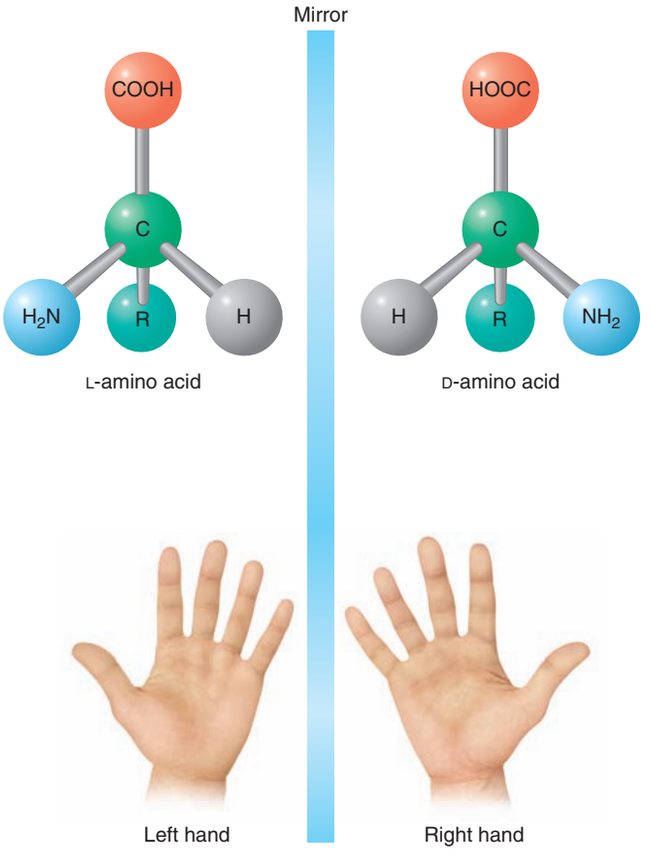


Figure 2.13 The L- and D-isomers of an amino acid, shown with ball-and-stick models. The two isomers, like left and right hands, are mirror images of each other and cannot be superimposed on one another. (Try it!)

Q Which isomer is always found in proteins?

Peptide Bonds

Amino acids bond between the carbon atom of the carboxyl (—COOH) group of one amino acid and the nitrogen atom of the amino ($—NH_2$) group of another (**Figure 2.14**). The bonds between amino acids are called **peptide bonds.** For every peptide bond formed between two amino acids, one water molecule is released; thus, peptide bonds are formed by dehydration synthesis. The resulting compound in Figure 2.14 is called a *dipeptide* because it consists of two amino acids joined by a peptide bond. Adding another amino acid to a dipeptide would form a *tripeptide*. Further additions of amino acids would produce a long, chainlike molecule called a *peptide* (4–9 amino acids) or *polypeptide* (10–2000 or more amino acids).

Levels of Protein Structure

Proteins vary tremendously in structure. Different proteins have different architectures and different three-dimensional shapes. This variation in structure is directly related to their diverse functions.

Clinical Case

While Jonathan is in intensive care, his wife, DeeAnn, and adult daughter talk with his physician and an investigator from the Centers for Disease Control and Prevention (CDC) to find the source of Jonathan's *B. anthracis* infection. Environmental investigations uncover *B. anthracis* at Jonathan's home, in his van, and in his workplace, but neither his wife nor children show signs of infection. His bandmates are also tested; they are all negative for *B. anthracis*. The CDC investigator explains to Jonathan's family that *B. anthracis* forms endospores that can survive in soil for up to 60 years. It is rare in humans; however, grazing animals and people who handle their hides or other by-products can become infected. *B. anthracis* cells have capsules that are composed of poly-D-glutamic acid.

Why are the capsules resistant to digestion by phagocytes? (Phagocytes are white blood cells that engulf and destroy bacteria.)

26 **43** 44 48

When a cell makes a protein, the polypeptide chain folds spontaneously to assume a certain shape. One reason for folding of the polypeptide is that some parts of a protein are attracted to water and other parts are repelled by it. In practically every case, the function of a protein depends on its ability to recognize and bind to some other molecule. For example, an enzyme binds specifically with its substrate. A hormonal protein binds to a receptor on a cell whose function it will alter. An antibody binds to an antigen (foreign substance) that has invaded the body. The unique shape of each protein permits it to interact with specific other molecules in order to carry out specific functions.

Proteins are described in terms of four levels of organization: primary, secondary, tertiary, and quaternary. The *primary structure* is the unique sequence in which the amino acids are linked together to form a polypeptide chain (**Figure 2.15a**). This sequence is genetically determined. Alterations in sequence can have profound metabolic effects. For example, a single incorrect amino acid in a blood protein can produce the deformed hemoglobin molecule characteristic of sickle cell disease. But proteins do not exist as long, straight chains. Each polypeptide chain folds and coils in specific ways into a relatively compact structure with a characteristic three-dimensional shape.

A protein's *secondary structure* is the localized, repetitious twisting or folding of the polypeptide chain. This aspect of a protein's shape results from hydrogen bonds joining the atoms of peptide bonds at different locations along the polypeptide chain.

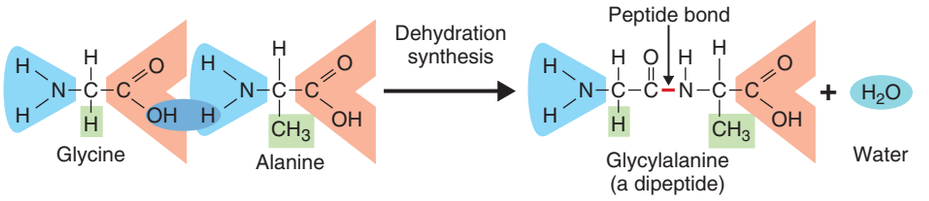


Figure 2.14 Peptide bond formation by dehydration synthesis. The amino acids glycine and alanine combine to form a dipeptide. The newly formed bond between the carbon atom of glycine and the nitrogen atom of alanine is called a peptide bond.

Q How are amino acids related to proteins?

The two types of secondary protein structures are clockwise spirals called *helices* (singular: *helix*) and pleated sheets, which form from roughly parallel portions of the chain (Figure 2.15b). Both structures are held together by hydrogen bonds between oxygen or nitrogen atoms that are part of the polypeptide's backbone.

Tertiary structure refers to the overall three-dimensional structure of a polypeptide chain (Figure 2.15c). The folding is not repetitive or predictable, as in secondary structure. Whereas secondary structure involves hydrogen bonding between atoms of the amino and carboxyl groups involved in the peptide bonds, tertiary structure involves several interactions between various amino acid side groups in the polypeptide chain. For example, amino acids with nonpolar (hydrophobic) side groups usually interact at the core of the protein, out of contact with water. This *hydrophobic interaction* helps contribute to tertiary structure. Hydrogen bonds between side groups, and ionic bonds between oppositely charged side groups, also contribute to tertiary structure. Proteins that contain the amino acid cysteine form strong covalent bonds called *disulfide bridges*. These bridges form when two cysteine molecules are brought close together by the folding of the protein. Cysteine molecules contain sulfhydryl groups (—SH), and the sulfur of one cysteine molecule bonds to the sulfur on another, forming (by the removal of hydrogen atoms) a disulfide bridge (S—S) that holds parts of the protein together.

Some proteins have a *quaternary structure,* which consists of an aggregation of two or more individual polypeptide chains (subunits) that operate as a single functional unit. Figure 2.15d shows a hypothetical protein consisting of two polypeptide chains. More commonly, proteins have two or more kinds of polypeptide subunits. The bonds that hold a quaternary structure together are basically the same as those that maintain tertiary structure. The overall shape of a protein may be globular (compact and roughly spherical) or fibrous (threadlike).

If a protein encounters a hostile environment in terms of temperature, pH, or salt concentrations, it may unravel and lose its characteristic shape. This process is called **denaturation** (see Figure 5.6, page 117). As a result of denaturation, the protein is no longer functional. This process will be discussed in more detail in Chapter 5 with regard to denaturation of enzymes.

The proteins we have been discussing are *simple proteins,* which contain only amino acids. *Conjugated proteins* are combinations of amino acids with other organic or inorganic components. Conjugated proteins are named by their non–amino acid component. Thus, glycoproteins contain sugars, nucleoproteins contain nucleic acids, metalloproteins contain metal atoms, lipoproteins contain lipids, and phosphoproteins contain phosphate groups. Phosphoproteins are important regulators of activity in eukaryotic cells. Bacterial synthesis of phosphoproteins may be important for the survival of bacteria such as *Legionella pneumophila* that grow inside host cells.

CHECK YOUR UNDERSTANDING

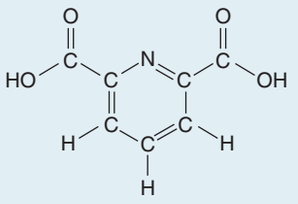
✓ What two functional groups are in all amino acids? 2-10

Nucleic Acids

In 1944, three American microbiologists—Oswald Avery, Colin MacLeod, and Maclyn McCarty—discovered that a substance called **deoxyribonucleic acid (DNA)** is the substance of which genes are made. Nine years later, James Watson and Francis Crick,

Clinical Case

The host's phagocytes cannot easily digest D-forms of amino acids, such as D-glutamic acid found in the capsules of *B. anthracis*. Therefore, infection can develop. The CDC investigator's mention of animal hides gives DeeAnn an idea. Jonathan plays West African drums called *djembe;* the drum skins are made from dried imported goat hides from West Africa. Although most of these hides are legally imported, some slip through the cracks. It's possible that the hides on Jonathan's drums have been illegally imported and therefore have not been inspected by the U.S. Department of Agriculture. To create *djembe* drums, the hides are soaked in water, stretched over the drum body, and then scraped and sanded. The scraping and sanding generates a large amount of aerosolized dust as the hides dry. Sometimes this dust contains *B. anthracis* endospores, which contain dipicolinic acid.



What is the functional group in dipicolinic acid? See the figure above.

26 43 **44** 48

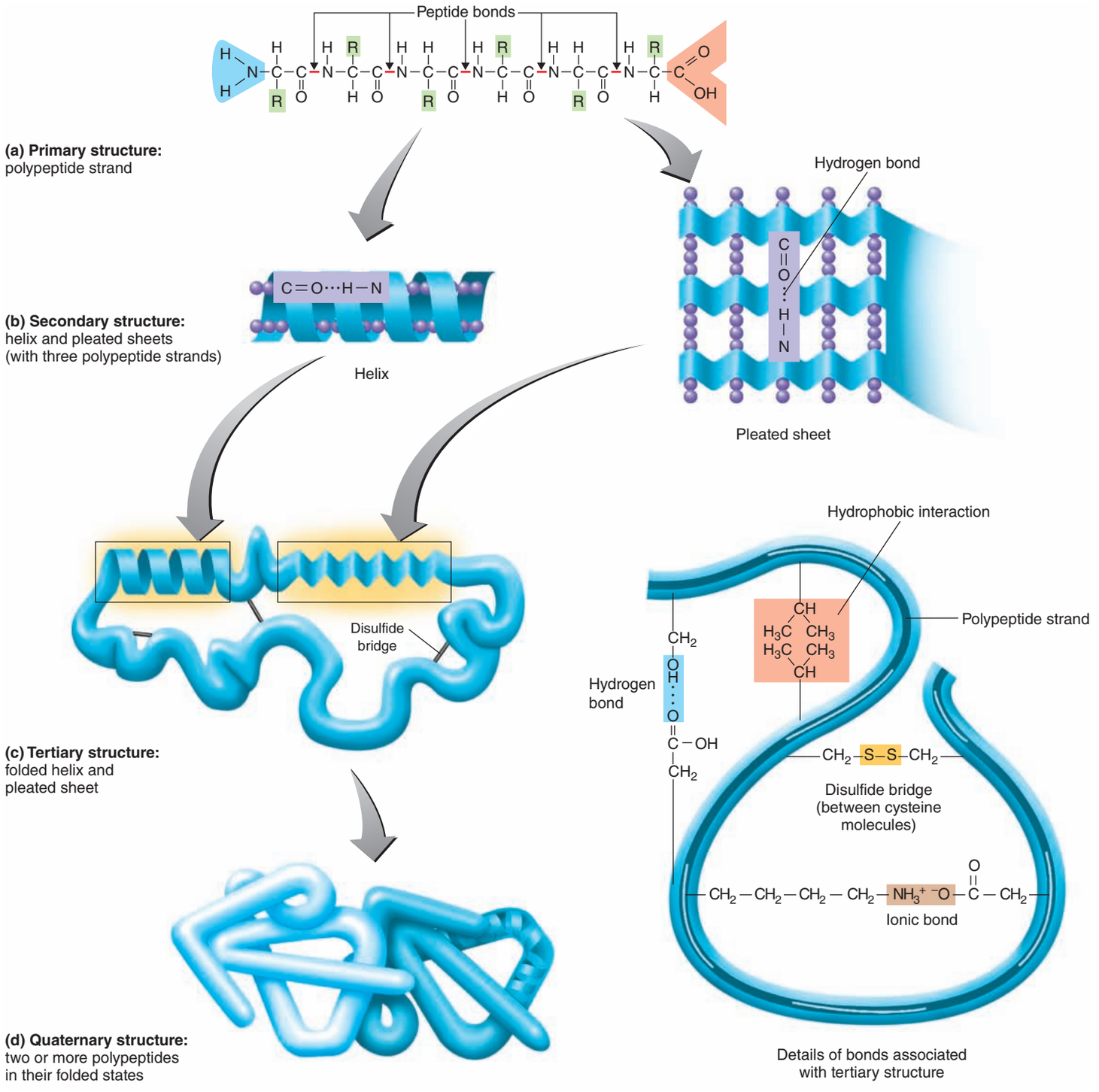


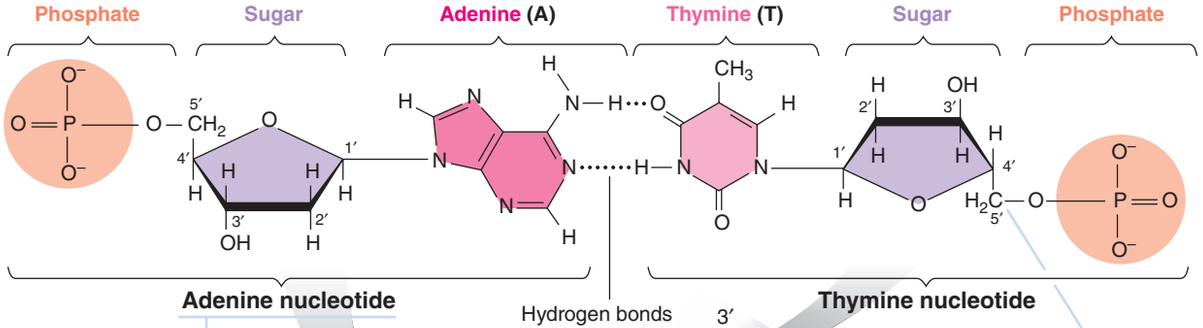
Figure 2.15 **Protein structure.** (**a**) Primary structure, the amino acid sequence. (**b**) Secondary structures: helix and pleated sheet. (**c**) Tertiary structure, the overall three-dimensional folding of a polypeptide chain. (**d**) Quaternary structure, the relationship between several polypeptide chains that make up a protein. Shown here is the quaternary structure of a hypothetical protein composed of two polypeptide chains.

Q What property of a protein enables it to carry out specific functions?

FOUNDATION FIGURE **2.16**

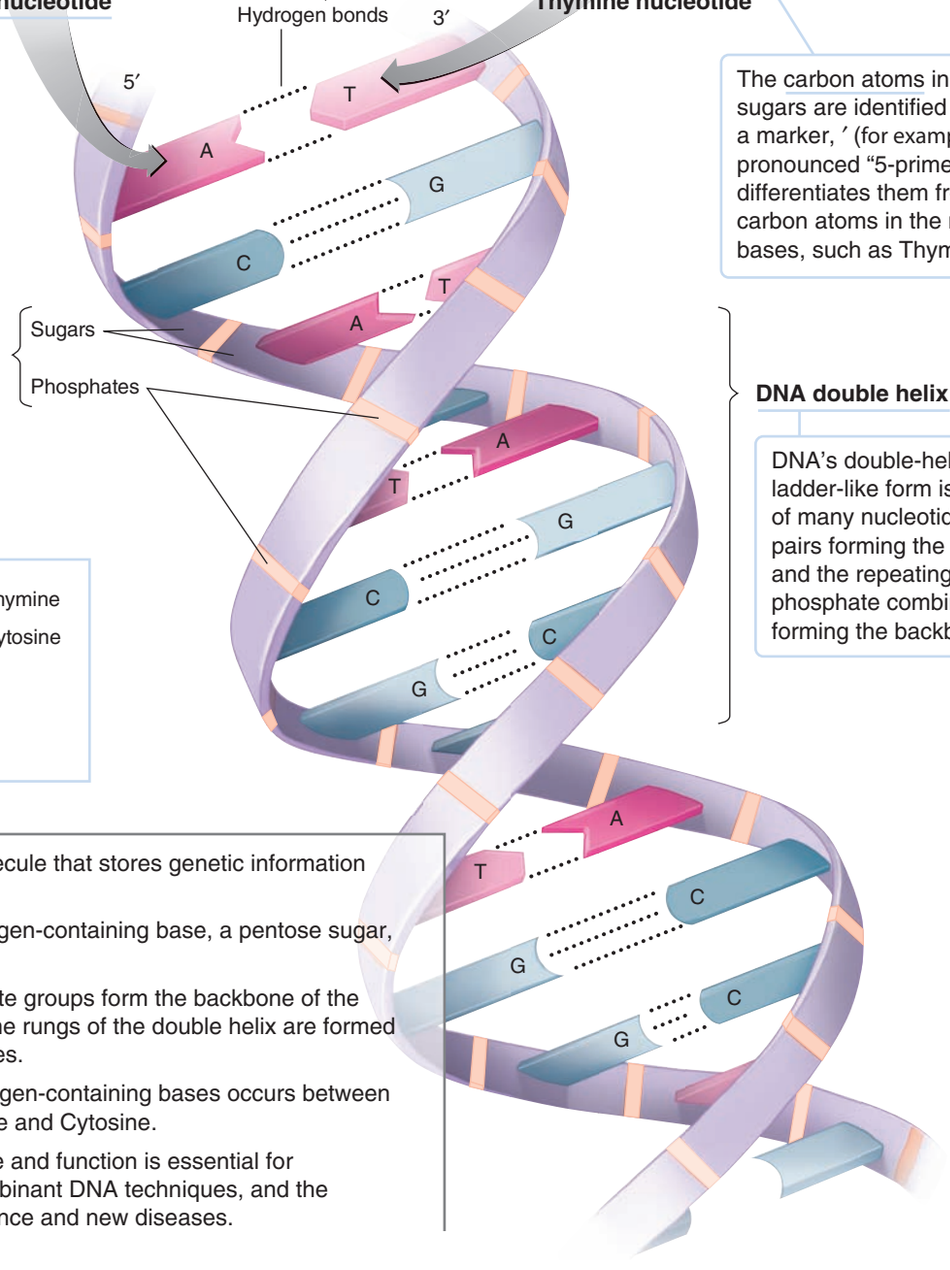
The Structure of DNA

Adenine and Thymine (as well as Cytosine and Guanine, not shown here) are nitrogenous bases or nucleobases.



Individual DNA nucleotides are composed of a deoxyribose sugar molecule covalently bonded to a phosphate group at the 5′ carbon, and to a nitrogen-containing base at the 3′ carbon. The two nucleotides shown here are held together by hydrogen bonds.

The carbon atoms in the sugars are identified by adding a marker, ′ (for example, 5′, pronounced "5-prime"). This differentiates them from the carbon atoms in the nucleobases, such as Thymine.



Sugar-phosphate backbone

The sugar-phosphate backbone of one strand is upside down, or antiparallel, relative to the backbone of the other strand.

DNA double helix

DNA's double-helical, ladder-like form is made up of many nucleotides base pairs forming the rungs, and the repeating sugar-phosphate combination, forming the backbone.

Key

Adenine	A	T	Thymine
Guanine	G	C	Cytosine
Deoxyribose sugar			
Phosphate			
Hydrogen bond			

KEYCONCEPTS

- DNA is a double-stranded molecule that stores genetic information in all cells.
- A nucleotide consists of a nitrogen-containing base, a pentose sugar, and a phosphate group.
- Alternating sugar and phosphate groups form the backbone of the double helix (twisted ladder); the rungs of the double helix are formed by the nitrogen-containing bases.
- Complementary pairing of nitrogen-containing bases occurs between Adenine and Thymine; Guanine and Cytosine.
- Familiarity with DNA's structure and function is essential for understanding genetics, recombinant DNA techniques, and the emergence of antibiotic resistance and new diseases.

working with molecular models and X-ray information supplied by Maurice Wilkins and Rosalind Franklin, identified the physical structure of DNA. In addition, Crick suggested a mechanism for DNA replication and how it works as the hereditary material. DNA and another substance called **ribonucleic acid (RNA)** are together referred to as **nucleic acids** because they were first discovered in the nuclei of cells. Just as amino acids are the structural units of proteins, nucleotides are the structural units of nucleic acids.

Each **nucleotide** has three parts: a nitrogen-containing base, a pentose (five-carbon) sugar (either **deoxyribose** or **ribose**), and a phosphate group (phosphoric acid). The nitrogen-containing bases are cyclic compounds made up of carbon, hydrogen, oxygen, and nitrogen atoms. The bases are named adenine (A), thymine (T), cytosine (C), guanine (G), and uracil (U). A and G are double-ring structures called **purines,** whereas T, C, and U are single-ring structures referred to as **pyrimidines.**

Nucleotides are named according to their nitrogen-containing base. Thus, a nucleotide containing thymine is a *thymine nucleotide,* one containing adenine is an *adenine nucleotide,* and so on. The term **nucleoside** refers to the combination of a purine or pyrimidine plus a pentose sugar; it does not contain a phosphate group.

DNA

According to the model proposed by Watson and Crick, a DNA molecule consists of two long strands wrapped around each other to form a **double helix** (Figure 2.16). The double helix looks like a twisted ladder, and each strand is composed of many nucleotides.

Every strand of DNA composing the double helix has a "backbone" consisting of alternating deoxyribose sugar and phosphate groups. The deoxyribose of one nucleotide is joined to the phosphate group of the next. (Refer to Figure 8.3, page 211, to see how nucleotides are bonded.) The nitrogen-containing bases make up the rungs of the ladder. Note that the purine A is always paired with the pyrimidine T and that the purine G is always paired with the pyrimidine C. The bases are held together by hydrogen bonds; A and T are held by two hydrogen bonds, and G and C by three. DNA does not contain uracil (U).

The order in which the nitrogen base pairs occur along the backbone is extremely specific and in fact contains the genetic instructions for the organism. Nucleotides form genes, and a single DNA molecule may contain thousands of genes. Genes determine all hereditary traits, and they control all the activities that take place within cells.

One very important consequence of nitrogen-containing base pairing is that if the sequence of bases of one strand is known, then the sequence of the other strand is also known. For example, if one strand has the sequence . . . ATGC . . . , then the other strand has the sequence . . . TACG . . . . Because the sequence of bases of one strand is determined by the sequence of bases of the other, the bases are said to be *complementary.* The actual transfer of information becomes possible because of DNA's unique structure and will be discussed further in Chapter 8.

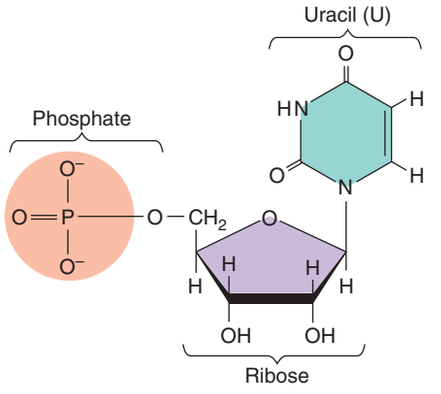


Figure 2.17 **A uracil nucleotide of RNA.**

Q How are DNA and RNA similar in structure?

RNA

RNA, the second principal kind of nucleic acid, differs from DNA in several respects. Whereas DNA is double-stranded, RNA is usually single-stranded. The five-carbon sugar in the RNA nucleotide is ribose, which has one more oxygen atom than deoxyribose. Also, one of RNA's bases is uracil (U) instead of thymine (Figure 2.17). The other three bases (A, G, C) are the same as DNA. Three major kinds of RNA have been identified in cells. They are **messenger RNA (mRNA), ribosomal RNA (rRNA),** and **transfer RNA (tRNA).** As we will see in Chapter 8, each type of RNA has a specific role in protein synthesis.

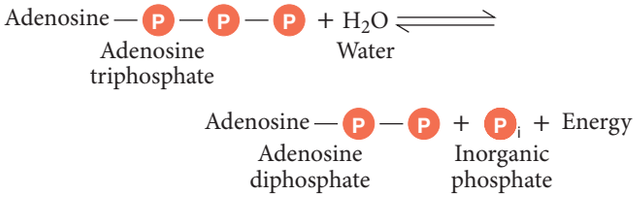
A comparison between DNA and RNA is presented in Table 2.6.

CHECK YOUR UNDERSTANDING

How do DNA and RNA differ? 2-11

Adenosine Triphosphate (ATP)

Adenosine triphosphate (ATP) is the principal energy-carrying molecule of all cells and is indispensable to the life of the cell. It stores the chemical energy released by some chemical reactions, and it provides the energy for reactions that require energy. ATP consists of an adenosine unit, composed of adenine and ribose, with three phosphate groups (Ⓟ) attached (Figure 2.18). In other words, it is an adenine nucleotide (also called adenosine monophosphate, or AMP) with two extra phosphate groups. ATP is called a high-energy molecule because it releases a large amount of usable energy when the third phosphate group is hydrolyzed to become **adenosine diphosphate (ADP).** This reaction can be represented as follows:



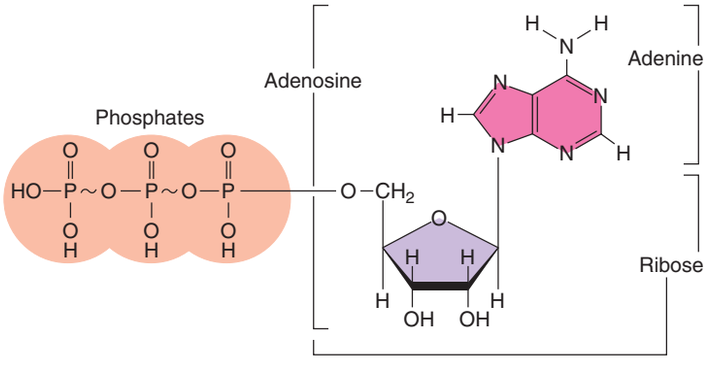


Figure 2.18 The structure of ATP. High-energy phosphate bonds are indicated by wavy lines. When ATP breaks down to ADP and inorganic phosphate, a large amount of chemical energy is released for use in other chemical reactions.

How is ATP similar to a nucleotide in RNA? In DNA?

A cell's supply of ATP at any particular time is limited. Whenever the supply needs replenishing, the reaction goes in the reverse direction; the addition of a phosphate group to ADP and the input of energy produces more ATP. The energy required to attach the terminal phosphate group to ADP is supplied by the cell's various oxidation reactions, particularly the oxidation of glucose. ATP can be stored in every cell, where its potential energy is not released until needed.

Clinical Case Resolved

The functional group in dipicolinic acid is carboxyl. *B. anthracis* infection is contracted by contact, ingestion, or inhalation of the endospores. In Jonathan's case, the process of stretching, scraping, and sanding the goat hides had created dust that settled on the drum skin and any surrounding crevices. *B. anthracis* endospores became airborne, or aerosolized, whenever Jonathan beat on the drum. He makes a full recovery, and from now on he makes certain that all parts of any drum he purchases have been legally imported .

26 43 44 **48**

CHECK YOUR UNDERSTANDING

Which can provide more energy for a cell and why: ATP or ADP? **2-12**

TABLE 2.6 Comparison between DNA and RNA

Backbone	DNA	RNA
Strands	Double-stranded in cells and most DNA viruses to form a double helix; single-stranded in some viruses (parvoviruses).	Single-stranded in celis and most RNA viruses; double-stranded in some viruses (reoviruses).
Composition	The sugar is deoxyribose.	The sugar is ribose.
	The nitrogen-containing bases are adenine (A), thymine (T), cytosine (C), and guanine (G).	The nitrogen-containing bases are adenine (A), thymine (T), cytosine (C), and uracil (U).
Function	Determines all hereditary traits.	Protein synthesis.

Study Outline

MasteringMICROBIOLOGY™

Test your understanding with quizzes, microbe review, and a chapter post-test at www.masteringmicrobiology.com.

Introduction (p.25)

1. The science of the interaction between atoms and molecules is called chemistry.
2. The metabolic activities of microorganisms involve complex chemical reactions.
3. Microbes break down nutrients to obtain energy and to make new cells.

The Structure of Atoms (pp. 26–27)

1. An atom is the smallest unit of a chemical element that exhibits the properties of that element.
2. Atoms consist of a nucleus, which contains protons and neutrons, and electrons, which move around the nucleus.
3. The atomic number is the number of protons in the nucleus; the total number of protons and neutrons is the atomic weight.

Chemical Elements (pp. 26–27)

4. Atoms with the same number of protons and the same chemical behavior are classified as the same chemical element.
5. Chemical elements are designated by abbreviations called chemical symbols.
6. About 26 elements are commonly found in living cells.
7. Atoms that have the same atomic number (are of the same element) but different atomic weights are called isotopes.

Electronic Configurations (p. 27)

8. In an atom, electrons are arranged around the nucleus in electron shells.
9. Each shell can hold a characteristic maximum number of electrons.
10. The chemical properties of an atom are due largely to the number of electrons in its outermost shell.

How Atoms Form Molecules: Chemical Bonds (pp. 27–31)

1. Molecules are made up of two or more atoms; molecules consisting of at least two different kinds of atoms are called compounds.
2. Atoms form molecules in order to fill their outermost electron shells.
3. Attractive forces that bind two atoms together are called chemical bonds.
4. The combining capacity of an atom—the number of chemical bonds the atom can form with other atoms—is its valence.

Ionic Bonds (pp. 29–30)

5. A positively or negatively charged atom or group of atoms is called an ion.
6. A chemical attraction between ions of opposite charge is called an ionic bond.
7. To form an ionic bond, one ion is an electron donor, and the other ion is an electron acceptor.

Covalent Bonds (p. 30)

8. In a covalent bond, atoms share pairs of electrons.
9. Covalent bonds are stronger than ionic bonds and are far more common in organic molecules.

Hydrogen Bonds (pp. 30–31)

10. A hydrogen bond exists when a hydrogen atom covalently bonded to one oxygen or nitrogen atom is attracted to another oxygen or nitrogen atom.
11. Hydrogen bonds form weak links between different molecules or between parts of the same large molecule.

Molecular Weight and Moles (p. 31)

12. The molecular weight is the sum of the atomic weights of all the atoms in a molecule.
13. A mole of an atom, ion, or molecule is equal to its atomic or molecular weight expressed in grams.

Chemical Reactions (pp. 31–33)

1. Chemical reactions are the making or breaking of chemical bonds between atoms.
2. A change of energy occurs during chemical reactions.
3. Endergonic reactions require more energy than they release; exergonic reactions release more energy.
4. In a synthesis reaction, atoms, ions, or molecules are combined to form a larger molecule.
5. In a decomposition reaction, a larger molecule is broken down into its component molecules, ions, or atoms.
6. In an exchange reaction, two molecules are decomposed, and their subunits are used to synthesize two new molecules.
7. The products of reversible reactions can readily revert to form the original reactants.

Important Biological Molecules (pp. 33–48)

Inorganic Compounds (pp. 33–36)

1. Inorganic compounds are usually small, ionically bonded molecules.
2. Water and many common acids, bases, and salts are examples of inorganic compounds.

Water (pp. 33–34)

3. Water is the most abundant substance in cells.
4. Because water is a polar molecule, it is an excellent solvent.
5. Water is a reactant in many of the decomposition reactions of digestion.
6. Water is an excellent temperature buffer.

Acids, Bases, and Salts (p. 34)

7. An acid dissociates into H^+ and anions.
8. A base dissociates into OH^- and cations.
9. A salt dissociates into negative and positive ions, neither of which is H^+ or OH^-.

Acid–Base Balance: The Concept of pH (pp. 34–36)

10. The term *pH* refers to the concentration of H^+ in a solution.
11. A solution of pH 7 is neutral; a pH value below 7 indicates acidity; pH above 7 indicates alkalinity.
12. The pH inside a cell and in culture media is stabilized with pH buffers.

Organic Compounds (pp. 36–48)

1. Organic compounds always contain carbon and hydrogen.
2. Carbon atoms form up to four bonds with other atoms.
3. Organic compounds are mostly or entirely covalently bonded, and many of them are large molecules.

Structure and Chemistry (pp. 36–37)

4. A chain of carbon atoms forms a carbon skeleton.
5. Functional groups of atoms are responsible for most of the properties of organic molecules.
6. The letter *R* may be used to denote the remainder of an organic molecule.
7. Frequently encountered classes of molecules are R—OH (alcohols) and R—COOH (organic acids).
8. Small organic molecules may combine into very large molecules called macromolecules.
9. Monomers usually bond together by dehydration synthesis, or condensation reactions, that form water and a polymer.
10. Organic molecules may be broken down by hydrolysis, a reaction involving the splitting of water molecules.

Carbohydrates (pp. 37–38)

11. Carbohydrates are compounds consisting of atoms of carbon, hydrogen, and oxygen, with hydrogen and oxygen in a 2:1 ratio.

12. Carbohydrates include sugars and starches.

13. Carbohydrates can be classified as monosaccharides, disaccharides, and polysaccharides.

14. Monosaccharides contain from three to seven carbon atoms.

15. Isomers are two molecules with the same chemical formula but different structures and properties—for example, glucose ($C_6H_{12}O_6$) and fructose ($C_6H_{12}O_6$).

16. Monosaccharides may form disaccharides and polysaccharides by dehydration synthesis.

Lipids (pp. 38–41)

17. Lipids are a diverse group of compounds distinguished by their insolubility in water.

18. Simple lipids (fats) consist of a molecule of glycerol and three molecules of fatty acids.

19. A saturated lipid has no double bonds between carbon atoms in the fatty acids; an unsaturated lipid has one or more double bonds. Saturated lipids have higher melting points than unsaturated lipids.

20. Phospholipids are complex lipids consisting of glycerol, two fatty acids, and a phosphate group.

21. Steroids have carbon ring structures; sterols have a functional hydroxyl group.

Proteins (pp. 41–44)

22. Amino acids are the building blocks of proteins.

23. Amino acids consist of carbon, hydrogen, oxygen, nitrogen, and sometimes sulfur.

24. Twenty amino acids occur naturally in proteins.

25. By linking amino acids, peptide bonds (formed by dehydration synthesis) allow the formation of polypeptide chains.

26. Proteins have four levels of structure: primary (sequence of amino acids), secondary (helices or pleats), tertiary (overall three-dimensional structure of a polypeptide), and quaternary (two or more polypeptide chains).

27. Conjugated proteins consist of amino acids combined with inorganic or other organic compounds.

Nucleic Acids (pp. 44–47)

28. Nucleic acids—DNA and RNA—are macromolecules consisting of repeating nucleotides.

29. A nucleotide is composed of a pentose, a phosphate group, and a nitrogen-containing base. A nucleoside is composed of a pentose and a nitrogen-containing base.

30. A DNA nucleotide consists of deoxyribose (a pentose) and one of the following nitrogen-containing bases: thymine or cytosine (pyrimidines) or adenine or guanine (purines).

31. DNA consists of two strands of nucleotides wound in a double helix. The strands are held together by hydrogen bonds between purine and pyrimidine nucleotides: AT and GC.

32. Genes consist of sequences of nucleotides.

33. An RNA nucleotide consists of ribose (a pentose) and one of the following nitrogen-containing bases: cytosine, guanine, adenine, or uracil.

Adenosine Triphosphate (ATP) (pp. 47–48)

34. ATP stores chemical energy for various cellular activities.

35. When the bond to ATP's terminal phosphate group is hydrolyzed, energy is released.

36. The energy from oxidation reactions is used to regenerate ATP from ADP and inorganic phosphate.

Study Questions

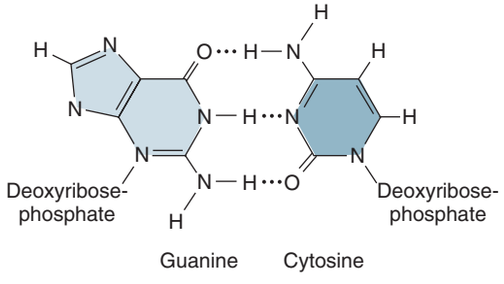
Answers to the Review and Multiple Choice questions can be found by turning to the Answers tab at the back of the textbook.

Review

1. What is a chemical element?

2. DRAW IT Diagram the electronic configuration of a carbon atom.

3. What type of bond holds the following atoms together?
- **a.** Li^+ and Cl^- in LiCl
- **b.** carbon and oxygen atoms in methanol
- **c.** oxygen atoms in O_2
- **d.** a hydrogen atom of one nucleotide to a nitrogen or oxygen atom of another nucleotide in:



4. Classify the following types of chemical reactions.
- **a.** glucose + fructose → sucrose + H_2O
- **b.** lactose → glucose + galactose
- **c.** $NH_4Cl + H_2O \rightarrow NH_4OH + HCl$
- **d.** ATP $\rightleftharpoons$ ADP + P_i

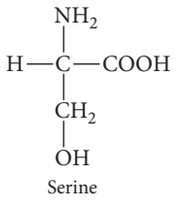
5. Bacteria use the enzyme urease to obtain nitrogen in a form they can use from urea in the following reaction:

$$\underset{\text{Urea}}{CO(NH_2)_2} + H_2O \rightarrow \underset{\text{Ammonia}}{2NH_3} + \underset{\text{Carbon dioxide}}{CO_2}$$

What purpose does the enzyme serve in this reaction? What type of reaction is this?

6. Classify the following as subunits of either a carbohydrate, lipid, protein, or nucleic acid.
- **a.** $CH_3-(CH_2)_7-CH=CH-(CH_2)_7-COOH$ (Oleic acid)

b.

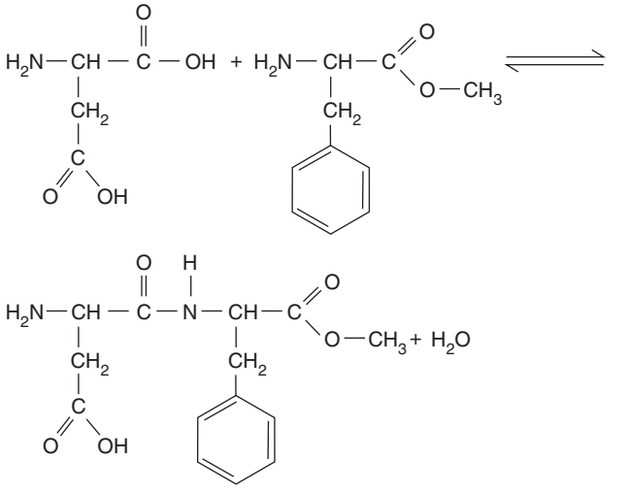


Serine

c. $C_6H_{12}O_6$

d. Thymine nucleotide

7. DRAW IT The artificial sweetener aspartame, or NutraSweet, is made by joining aspartic acid to methylated phenylalanine, as shown below.



a. What types of molecules are aspartic acid and phenylalanine?

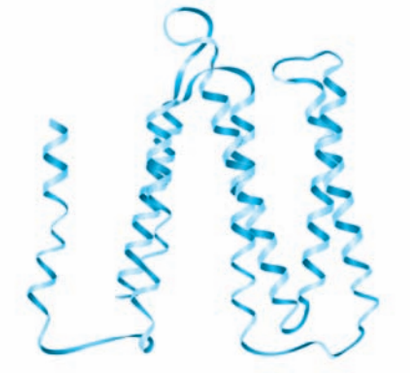
b. What direction is the hydrolysis reaction (left to right or right to left)?

c. What direction is the dehydration synthesis reaction?

d. Circle the atoms involved in the formation of water.

e. Identify the peptide bond.

8. DRAW IT The following diagram shows the bacteriorhodopsin protein. Indicate the regions of primary, secondary, and tertiary structure. Does this protein have quaternary structure?



9. DRAW IT Draw a simple lipid, and show how it could be modified to a phospholipid.

10. NAME IT What type of microorganism has a chitin cell wall, has DNA that is contained in a nucleus, and has ergosterol in its plasma membrane?

Multiple Choice

Radioisotopes are frequently used to label molecules in a cell. The fate of atoms and molecules in a cell can then be followed. This process is the basis for questions 1–3.

1. Assume *E. coli* bacteria are grown in a nutrient medium containing the radioisotope ^{16}N. After a 48-hour incubation period, the ^{16}N would most likely be found in the *E. coli*'s
a. carbohydrates.
b. lipids.
c. proteins.
d. water.
e. none of the above

2. If *Pseudomonas* bacteria are supplied with radioactively labeled cytosine, after a 24-hour incubation period this cytosine would most likely be found in the cells'
a. carbohydrates.
b. DNA.
c. lipids.
d. water.
e. proteins.

3. If *E. coli* were grown in a medium containing the radioactive isotope ^{32}P, the ^{32}P would be found in all of the following molecules of the cell *except*
a. ATP.
b. carbohydrates.
c. DNA.
d. plasma membrane.
e. none of the above

4. The optimum pH of *Thiobacillus* bacteria (pH 3,) is _____ times more acid than blood (pH 7).
a. 4
b. 10
c. 100
d. 1000
e. 10,000

5. The best definition of ATP is that it is
a. a molecule stored for food use.
b. a molecule that supplies energy to do work.
c. a molecule stored for an energy reserve.
d. a molecule used as a source of phosphate.

6. Which of the following is an organic molecule?
a. H_2O (water)
b. O_2 (oxygen)
c. $C_{18}H_{29}SO_3$ (Styrofoam)
d. FeO (iron oxide)
e. $F_2C{=}CF_2$ (Teflon)

Classify each of the molecules on the left as an acid, base, or salt. The dissociation products of the molecules are shown to help you.

7. $HNO_3 \rightarrow H^+ + NO_3^-$
8. $H_2SO_4 \rightarrow 2H^+ + SO_4^{2-}$
9. $NaOH \rightarrow Na^+ + OH^-$
10. $MgSO_4 \rightarrow Mg^{2+} + SO_4^{2-}$

a. acid
b. base
c. salt

Critical Thinking

1. When you blow bubbles into a glass of water, the following reactions take place:

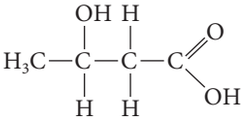
$$H_2O + CO_2 \xrightarrow{A} H_2CO_3 \xrightarrow{B} H^+ + HCO_3^-$$

   a. What type of reaction is *A*?
   b. What does reaction *B* tell you about the type of molecule H_2CO_3 is?

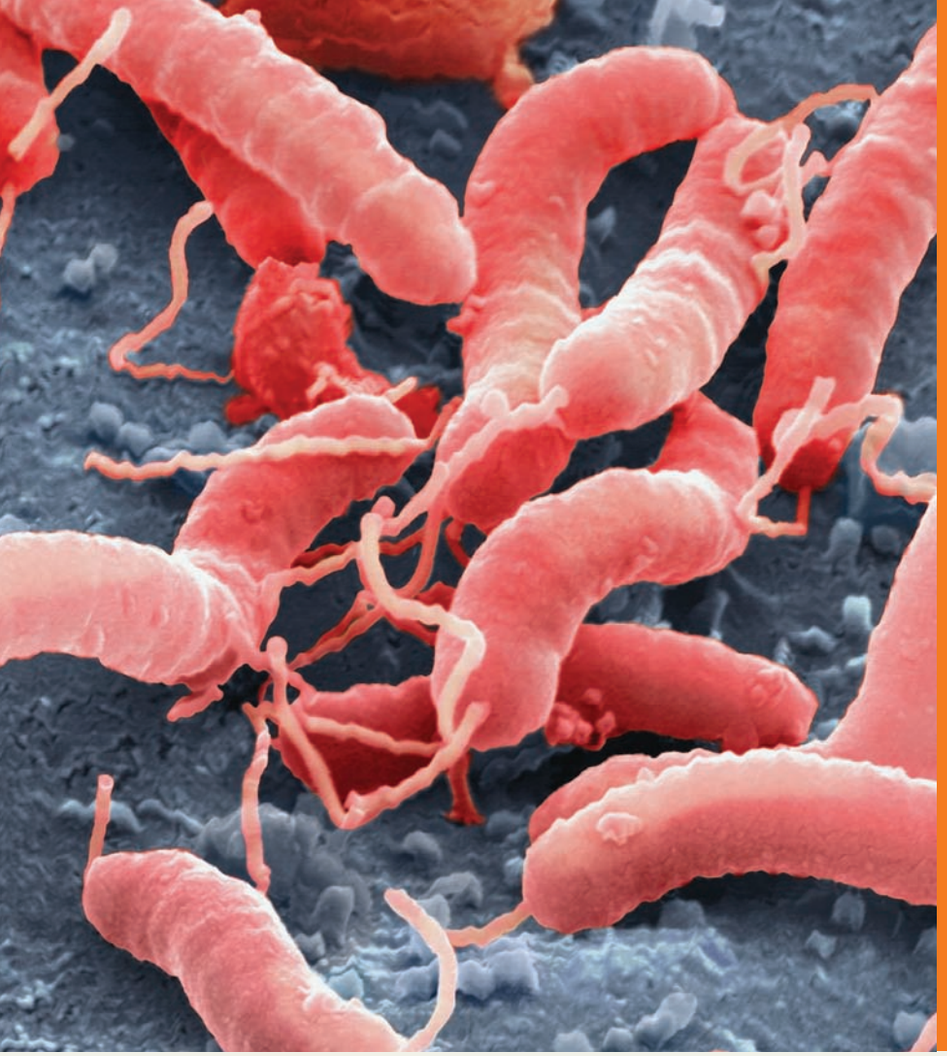
2. What are the common structural characteristics of ATP and DNA molecules?
3. What happens to the relative amount of unsaturated lipids in the plasma membrane when *E. coli* bacteria grown at 25°C are then grown at 37°C?
4. Giraffes, termites, and koalas eat only plant matter. Because animals cannot digest cellulose, how do you suppose these animals get nutrition from the leaves and wood they eat?

Clinical Applications

1. *Ralstonia* bacteria make poly-β-hydroxybutyrate (PHB), which is used to make a biodegradable plastic. PHB consists of many of the monomers shown below. What type of molecule is PHB? What is the most likely reason a cell would store this molecule?



2. *Thiobacillus ferrooxidans* was responsible for destroying buildings in the Midwest by causing changes in the earth. The original rock, which contained lime ($CaCO_3$) and pyrite (FeS_2), expanded as bacterial metabolism caused gypsum ($CaSO_4$) crystals to form. How did *T. ferrooxidans* bring about the change from lime to gypsum?
3. Newborn babies are tested for phenylketonuria (PKU), an inherited disease. Individuals with this disease are missing an enzyme to convert phenylalanine (phe) to tyrosine; the resulting accumulation of phe can cause mental retardation, brain damage, and seizures. The Guthrie test for PKU involves culturing *Bacillus subtilis,* which requires phe to grow. The bacteria are grown on media with a drop of the baby's blood.
   a. What type of chemical is phenylalanine?
   b. What does "no growth" in the Guthrie test mean?
   c. Why must individuals with PKU avoid the sweetener aspartame?
4. The antibiotic amphotericin B causes leaks in cells by combining with sterols in the plasma membrane. Would you expect to use amphotericin B against a bacterial infection? A fungal infection? Offer a reason why amphotericin B has severe side effects in humans.
5. You can smell sulfur when boiling eggs. What amino acids do you expect in the egg?



3

Observing Microorganisms Through a Microscope

MasteringMICROBIOLOGY™

Visualize microbiology and check your understanding with a pre-test at www.masteringmicrobiology.com.

Microorganisms are much too small to be seen with the unaided eye; they must be observed with a microscope. The word *microscope* is derived from the Latin word *micro* (small) and the Greek word *skopos* (to look at). Modern microbiologists use microscopes that produce, with great clarity, magnifications that range from ten to thousands of times greater than those of van Leeuwenhoek's single lens (see Figure 1.2b on page 7). This chapter describes how different types of microscopes function and why one type might be used in preference to another. *Helicobacter pylori*, shown in the photograph, is a spiral-shaped bacterium that was first seen in cadaver stomachs in 1886. The bacterium was largely ignored until the resolving ability of microscopes was improved. Microscopic examination of these bacteria is described in the Clinical Case.

Some microbes are more readily visible than others because of their larger size or more easily observable features. Many microbes, however, must undergo several staining procedures before their cell walls, capsules, and other structures lose their colorless natural state. The last part of this chapter explains some of the more commonly used methods of preparing specimens for examination through a light microscope.

You may wonder how we are going to sort, count, and measure the specimens we will study. To answer these questions, this chapter opens with a discussion of how to use the metric system for measuring microbes.

Units of Measurement

LEARNING OBJECTIVES

3-1 List the metric units of measurement that are used for microorganisms.

Because microorganisms and their component parts are so very small, they are measured in units that are unfamiliar to many of us in everyday life. When measuring microorganisms, we use the metric system. The standard unit of length in the metric system is the meter (m). A major advantage of the metric system is that the units are related to each other by factors of 10. Thus, 1 m equals 10 decimeters (dm) or 100 centimeters (cm) or 1000 millimeters (mm). Units in the U.S. system of measure do not have the advantage of easy conversion by a single factor of 10. For example, we use 3 feet or 36 inches to equal 1 yard.

Microorganisms and their structural components are measured in even smaller units, such as micrometers and nanometers. A **micrometer (μm)** is equal to 0.000001 m (10^{-6} m). The prefix *micro* indicates that the unit following it should be divided by 1 million, or 10^6 (see the "Exponential Notation" section in Appendix B). A **nanometer (nm)** is equal to 0.000000001 m (10^{-9} m). Angstrom (Å) was previously used for 10^{-10} m, or 0.1 nm.

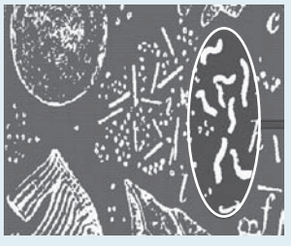
Table 3.1 presents the basic metric units of length and some of their U.S. equivalents. In Table 3.1, you can compare the microscopic units of measurement with the commonly known macroscopic units of measurement, such as centimeters, meters, and kilometers. If you look ahead to Figure 3.2, you will see the relative sizes of various organisms on the metric scale.

CHECK YOUR UNDERSTANDING

If a microbe measures 10 μm in length, how long is it in nanometers? **3-1**

Clinical Case: Microscopic Mayhem

Maryanne, a 42-year-old marketing executive and mother of three occasionally works from home, but she always feels that she isn't getting as much done at home as she does in the office. She has been experiencing recurrent stomach pain, which seems to be getting worse. She jokes with her husband that he should buy stock in Pepto-Bismol, because she buys so much of it. At her husband's urging, she finally makes an appointment to see her primary care physician. After hearing that Maryanne feels better immediately after taking Pepto-Bismol, the doctor suspects Maryanne may have a peptic ulcer associated with *Helicobacter pylori*.



What is *Helicobacter pylori*? Read on to find out.

54 64 69 71

Microscopy: The Instruments

LEARNING OBJECTIVES

3-2 Diagram the path of light through a compound microscope.

3-3 Define *total magnification* and *resolution*.

3-4 Identify a use for darkfield, phase-contrast, differential interference contrast, fluorescence, confocal, two-photon, and scanning acoustic microscopy, and compare each with brightfield illumination.

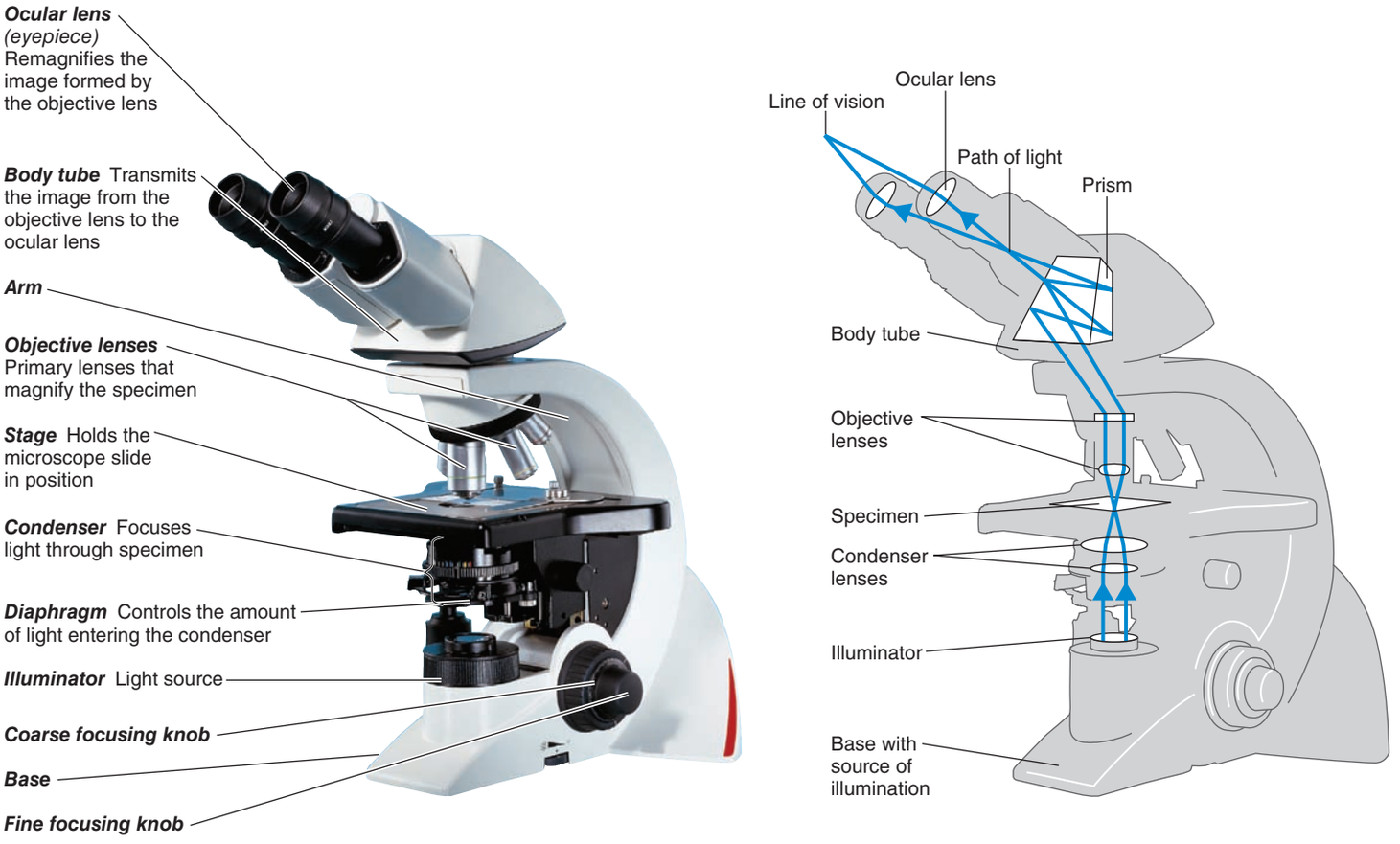
3-5 Explain how electron microscopy differs from light microscopy.

3-6 Identify one use for the TEM, SEM, and scanned-probe microscopes.

The simple microscope used by van Leeuwenhoek in the seventeenth century had only one lens and was similar to a magnifying

TABLE 3.1 Metric Units of Length and U.S. Equivalents

Metric Unit	Meaning of Prefix	Metric Equivalent	U.S. Equivalent
1 kilometer (km)	*kilo* = 1000	1000 m = 10^3 m	3280.84 ft or 0.62 mi; 1 mi = 1.61 km
1 meter (m)		Standard unit of length	39.37 in or 3.28 ft or 1.09 yd
1 decimeter (dm)	*deci* = 1/10	0.1 m = 10^{-1} m	3.94 in
1 centimeter (cm)	*centi* = 1/100	0.01 m = 10^{-2} m	0.394 in; 1 in = 2.54 cm
1 millimeter (mm)	*milli* = 1/1000	0.001 m = 10^{-3} m	
1 micrometer (μm)	*micro* = 1/1,000,000	0.000001 m = 10^{-6} m	
1 nanometer (nm)	*nano* = 1/1,000,000,000	0.000000001 m = 10^{-9} m	
1 picometer (pm)	*pico* = 1/1,000,000,000,000	0.000000000001 m = 10^{-12} m	



(a) Principal parts and functions

(b) The path of light (bottom to top)

Figure 3.1 The compound light microscope.

Q What is the total magnification of a compound light microscope with objective lens magnification of 40× and ocular lens of 10×?

glass. However, van Leeuwenhoek was the best lens grinder in the world in his day. His lenses were ground with such precision that a single lens could magnify a microbe 300×. His simple microscopes enabled him to be the first person to see bacteria (see Figure 1.2, page 7).

Contemporaries of van Leeuwenhoek, such as Robert Hooke, built compound microscopes, which have multiple lenses. In fact, a Dutch spectacle maker, Zaccharias Janssen, is credited with making the first compound microscope around 1600. However, these early compound microscopes were of poor quality and could not be used to see bacteria. It was not until about 1830 that a significantly better microscope was developed by Joseph Jackson Lister (the father of Joseph Lister). Various improvements to Lister's microscope resulted in the development of the modern compound microscope, the kind used in microbiology laboratories today. Microscopic studies of live specimens have revealed dramatic interactions between microbes (see the Applications of Microbiology box on page 56.)

MM **Animation** Microscopy and Staining: Overview

Light Microscopy

Light microscopy refers to the use of any kind of microscope that uses visible light to observe specimens. Here we examine several types of light microscopy.

Compound Light Microscopy

A modern **compound light microscope** has a series of lenses and uses visible light as its source of illumination (**Figure 3.1a**). With a compound light microscope, we can examine very small specimens as well as some of their fine detail. A series of finely ground lenses (**Figure 3.1b**) forms a clearly focused image that is many times larger than the specimen itself. This magnification is achieved when light rays from an **illuminator,** the light source, pass through a **condenser,** which has lenses that direct the light rays through the specimen. From here, light rays pass into the **objective lenses,** the lenses closest to the specimen. The image of the specimen is magnified again by the **ocular lens,** or *eyepiece.*

We can calculate the **total magnification** of a specimen by multiplying the objective lens magnification (power) by the ocular

What Is That Slime?

When bacteria grow, they often stay together in packs called biofilms. This can result in a slimy film on rocks, on food, inside pipes, and on implanted medical devices. Bacterial cells interact and exhibit multicellular organization (**Figure A**).

Pseudomonas aeruginosa can grow within a human without causing disease until the bacteria form a biofilm that overcomes the host's immune system. Biofilm-forming *P. aeruginosa* bacteria colonize the lungs of cystic fibrosis patients and are a leading cause of death in these patients (**Figure B**). Perhaps biofilms that lead to disease can be prevented by new drugs that destroy the inducer (discussed shortly).

Figure A ***Paenibacillus*. As one small colony moves away from the parent colony, other groups of cells follow the first colony. Soon, all of the other bacteria join the relocation to form this spiraling colony.**

Myxobacteria

Myxobacteria are found in decaying organic material and freshwater throughout the world. Although they are bacteria, many myxobacteria never exist as individual cells. *Myxococcus xanthus* cells appear to hunt in packs. In their natural aqueous habitat, *M. xanthus* cells form spherical colonies that surround prey bacteria, where they can secrete digestive enzymes and absorb the nutrients. On solid substrates, other myxobacterial cells glide over a solid surface, leaving slime trails that are followed by other cells. When food is scarce, the cells aggregate to form a mass. Cells within the mass differentiate into a fruiting body that consists of a slime stalk and clusters of spores, as shown in **Figure C**.

Vibrio

Aliivibrio fischeri is a bioluminescent bacterium that lives as a symbiont in the light-producing organ of squid and certain fish. When free-living, the bacteria are at a low concentration and do not give off light. However, when they grow in their host, they are highly concentrated, and each cell is induced to produce the enzyme luciferase, which is used in the chemical pathway of bioluminescence.

How Bacterial Group Behavior Works

Cell density alters gene expression in bacterial cells in a process called quorum sensing. In law, a quorum is the minimum number of members necessary to conduct business. *Quorum sensing* is the ability of bacteria to communicate and coordinate behavior. Bacteria that use quorum sensing produce and secrete a signaling chemical called an *inducer*. As the inducer diffuses into the surrounding medium, other bacterial cells move toward the source and begin producing inducer. The concentration of inducer increases with increasing cell numbers. This, in turn, attracts more cells and initiates synthesis of more inducer.

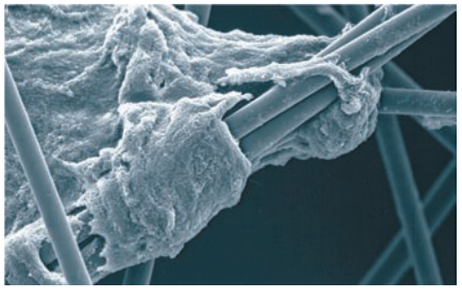
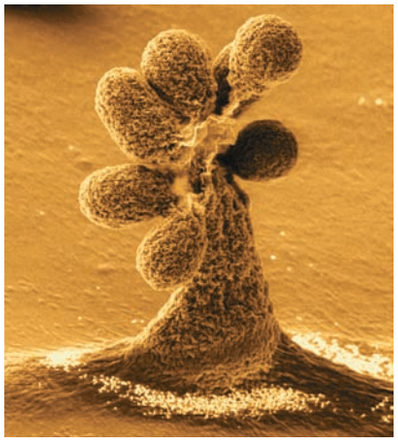


Figure B ***Pseudomonas aeruginosa* biofilm.**

5 μm

SEM

Figure C **A fruiting body of a myxobacterium.**



10 μm

SEM

lens magnification (power). Most microscopes used in microbiology have several objective lenses, including 10× (low power), 40× (high power), and 100× (oil immersion, which is described on page 58). Most ocular lenses magnify specimens by a factor of 10. Multiplying the magnification of a specific objective lens with that of the ocular, we see that the total magnifications would be 100× for low power, 400× for high power, and 1000× for oil immersion. Some compound light microscopes can achieve a total magnification of 2000× with the oil immersion lens.

Resolution (also called *resolving power*) is the ability of the lenses to distinguish fine detail and structure. Specifically, it refers to the ability of the lenses to distinguish two points a

specified distance apart. For example, if a microscope has a resolving power of 0.4 nm, it can distinguish two points if they are at least 0.4 nm apart. A general principle of microscopy is that the shorter the wavelength of light used in the instrument, the greater the resolution. The white light used in a compound light microscope has a relatively long wavelength and cannot resolve structures smaller than about 0.2 μm. This fact and other practical considerations limit the magnification achieved by even the best compound light microscopes to about 2000×. By comparison, van Leeuwenhoek's microscopes had a resolution of 1 μm.

Figure 3.2 shows various specimens that can be resolved by the human eye, light microscope, and electron microscope.

To obtain a clear, finely detailed image under a compound light microscope, specimens must be made to contrast sharply with their *medium* (substance in which they are suspended). To attain such contrast, we must change the refractive index of specimens from that of their medium. The **refractive index** is a measure of the light-bending ability of a medium. We change the refractive index of specimens by staining them, a procedure we will discuss shortly. Light rays move in a straight line through a single medium. After the specimen is stained, when light rays pass through the two materials (the specimen and its medium) with different refractive indexes, the rays change direction (refract) from a straight path by bending or changing angle at the boundary between the materials and increase the image's contrast between the specimen and the medium. As the light rays travel away from the specimen, they spread out and enter the objective lens, and the image is thereby magnified.

To achieve high magnification (1000×) with good resolution, the objective lens must be small. Although we want light traveling through the specimen and medium to refract differently, we do not want to lose light rays after they have passed through the stained specimen. To preserve the direction of light rays at the highest magnification, immersion oil is placed between the glass slide and the oil immersion objective lens (**Figure 3.3**).The immersion oil has the same refractive index as glass, so the oil becomes part of the optics of the glass of the microscope. Unless immersion oil is used, light rays are refracted as they enter the air from the slide, and the objective lens would have to be increased in diameter to capture most of them. The oil has the same effect as increasing the objective lens diameter; therefore, it improves the resolving power of the lenses. If oil is not used with an oil immersion objective lens, the image becomes fuzzy, with poor resolution.

Under usual operating conditions, the field of vision in a compound light microscope is brightly illuminated. By focusing the light, the condenser produces a **brightfield illumination** (**Figure 3.4a**).

It is not always desirable to stain a specimen. However, an unstained cell has little contrast with its surroundings and is therefore difficult to see. Unstained cells are more easily observed with the modified compound microscopes described in the next section.

CHECK YOUR UNDERSTANDING

- Through what lenses does light pass in a compound microscope? **3-2**
- What does it mean when a microscope has a resolution of 0.2 nm? **3-3**

Darkfield Microscopy

A **darkfield microscope** is used to examine live microorganisms that either are invisible in the ordinary light microscope, cannot be stained by standard methods, or are so distorted by staining that their characteristics then cannot be identified. Instead of the normal condenser, a darkfield microscope uses a darkfield condenser that contains an opaque disk. The disk blocks light that would enter the objective lens directly. Only light that is reflected off (turned away from) the specimen enters the objective lens. Because there is no direct background light, the specimen appears light against a black background—the dark field (**Figure 3.4b**). This technique is frequently used to examine unstained microorganisms suspended in liquid. One use for darkfield microscopy is the examination of very thin spirochetes, such as *Treponema pallidum* (tre-pō-nē′mä pal′li-dum), the causative agent of syphilis.

Phase-Contrast Microscopy

Another way to observe microorganisms is with a **phase-contrast microscope.** Phase-contrast microscopy is especially useful because it permits detailed examination of internal structures in *living* microorganisms. In addition, it is not necessary to fix (attach the microbes to the microscope slide) or stain the specimen—procedures that could distort or kill the microorganisms.

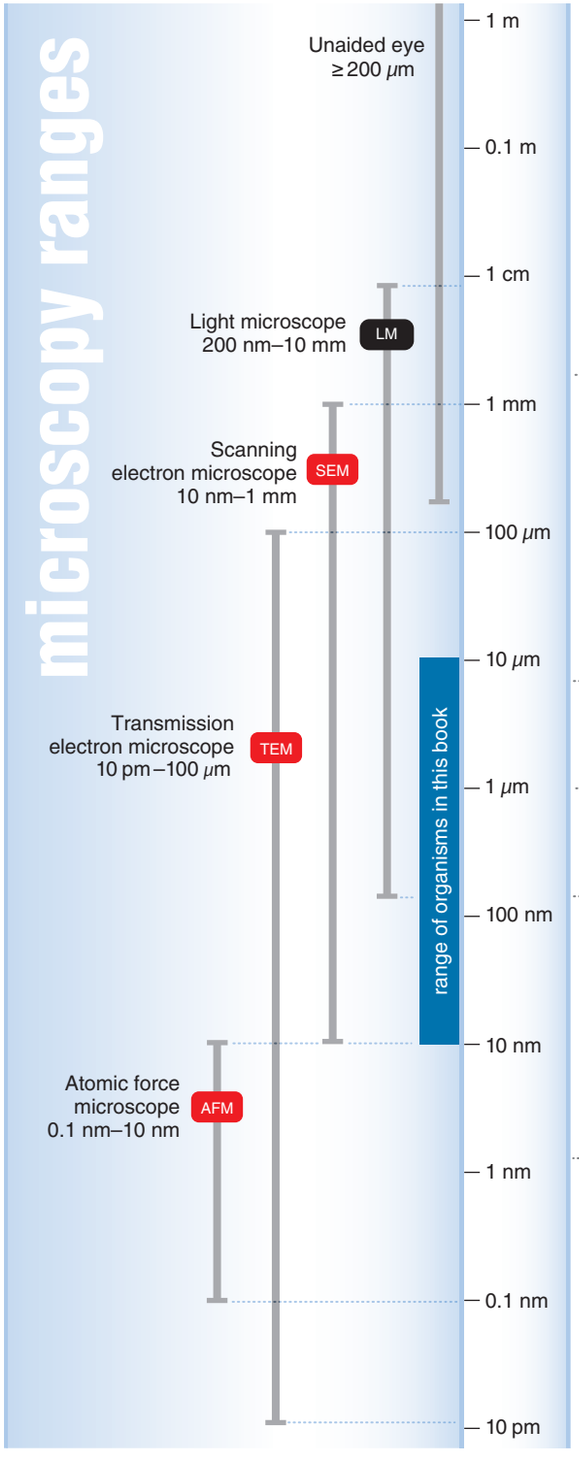
The principle of phase-contrast microscopy is based on the wave nature of light rays and the fact that light rays can be *in phase* (their peaks and valleys match) or *out of phase*. If the wave peak of light rays from one source coincides with the wave peak of light rays from another source, the rays interact to produce *reinforcement* (relative brightness). However, if the wave peak from one light source coincides with the wave trough from another light source, the rays interact to produce *interference* (relative darkness). In a phase-contrast microscope, one set of light rays comes directly from the light source. The other set comes from light that is reflected or diffracted from a particular structure in the specimen. (*Diffraction* is the scattering of light rays as they "touch" a specimen's edge. The diffracted rays are bent away from the parallel light rays that pass farther from the specimen.) When the two sets of light rays—direct rays and reflected or diffracted rays—are brought together, they form an image of the specimen on the ocular lens, containing areas that are relatively light (in phase), through shades of gray, to black (out of phase; **Figure 3.4c**). In phase-contrast microscopy, the internal structures of a cell become more sharply defined.

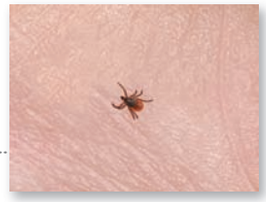
FOUNDATION FIGURE **3.2**

Microscopes and Magnification

KEYCONCEPTS

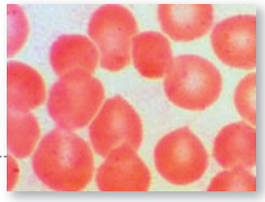
- Microscopes are used to magnify small objects.
- Because different microscopes have different resolution ranges, the size of a specimen determines which microscopes can be used to view the specimen effectively.
- Most micrographs shown in this textbook (like the ones below) have size bars and symbols to help you identify the actual size of the specimen and the type of microscope used for that image.
- A red icon indicates that a micrograph has been artificially colorized.





Tick

Actual size

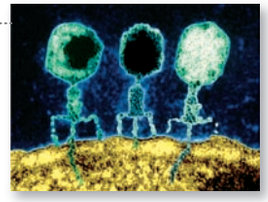


Red blood cells

LM 5 μm

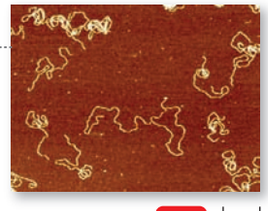
E. coli bacteria

SEM 2 μm



T-even bacteriophages (viruses)

TEM 50 nm



DNA double helix

AFM 10 nm

micro tip

If a bacterium is one micrometer in length and your index finger is 6.5 cm long, how many of the bacteria can you place end-to-end on your finger? Answer: 32,500.

Differential Interference Contrast (DIC) Microscopy

Differential interference contrast (DIC) microscopy is similar to phase-contrast microscopy in that it uses differences in refractive indexes. However, a DIC microscope uses two beams of light instead of one. In addition, prisms split each light beam, adding contrasting colors to the specimen. Therefore, the resolution of a DIC microscope is higher than that of a standard phase-contrast microscope. Also, the image is brightly colored and appears nearly three-dimensional (Figure 3.5).

Fluorescence Microscopy

Fluorescence microscopy takes advantage of **fluorescence,** the ability of substances to absorb short wavelengths of light (ultraviolet) and give off light at a longer wavelength (visible). Some organisms fluoresce naturally under ultraviolet light; if the specimen to be viewed does not naturally fluoresce, it is stained with one of a group of fluorescent dyes called *fluorochromes.* When microorganisms stained with a fluorochrome are examined under a fluorescence microscope with an ultraviolet or near-ultraviolet light source, they appear as luminescent, bright objects against a dark background.

Fluorochromes have special attractions for different microorganisms. For example, the fluorochrome auramine O, which glows yellow when exposed to ultraviolet light, is strongly absorbed by *Mycobacterium tuberculosis,* the bacterium that causes tuberculosis. When the dye is applied to a sample of material suspected of containing the bacterium, the bacterium can be detected by the appearance of bright yellow organisms against a dark background. *Bacillus anthracis,* the causative agent of anthrax, appears apple green when stained with another fluorochrome, fluorescein isothiocyanate (FITC).

The principal use of fluorescence microscopy is a diagnostic technique called the **fluorescent-antibody (FA) technique,** or **immunofluorescence. Antibodies** are natural defense molecules that are produced by humans and many animals in reaction to a foreign substance, or **antigen.** Fluorescent antibodies for a particular antigen are obtained as follows: an animal is injected with a specific antigen, such as a bacterium, and the animal then begins to produce antibodies against that antigen. After a sufficient time, the antibodies are removed from the serum of the animal. Next, as shown in Figure 3.6a, a fluorochrome is chemically combined with the antibodies. These fluorescent antibodies are then added to a microscope slide containing an unknown bacterium. If this unknown bacterium is the same bacterium that was injected into the animal, the fluorescent antibodies bind to antigens on the surface of the bacterium, causing it to fluoresce.

This technique can detect bacteria or other pathogenic microorganisms, even within cells, tissues, or other clinical specimens (Figure 3.6b). Of paramount importance, it can be used to identify a microbe in minutes. Immunofluorescence is especially useful in diagnosing syphilis and rabies. We will say more about antigen–antibody reactions and immunofluorescence in Chapter 18.

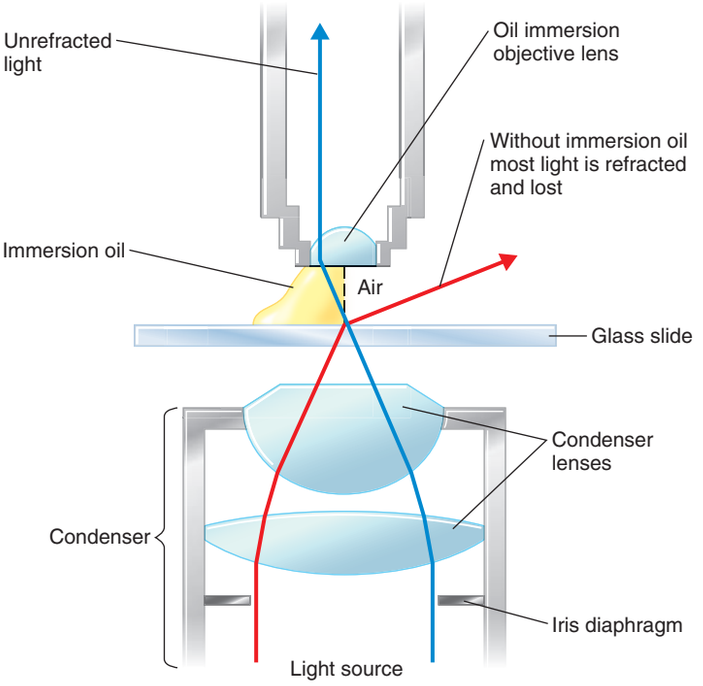


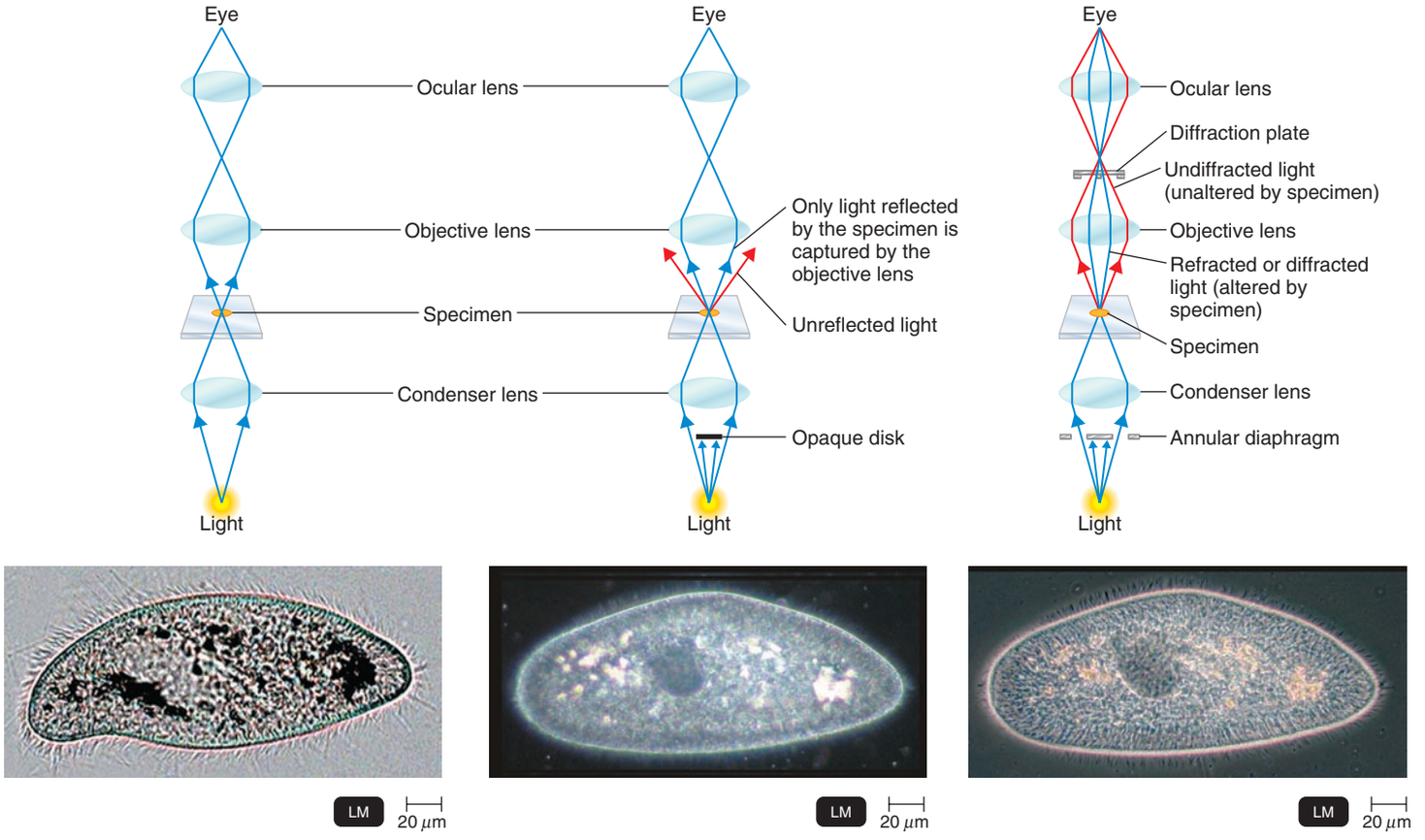
Figure 3.3 Refraction in the compound microscope using an oil immersion objective lens. Because the refractive indexes of the glass microscope slide and immersion oil are the same, the light rays do not refract when passing from one to the other when an oil immersion objective lens is used. Use of immersion oil is necessary at magnifications greater than 900×.

Q Why is immersion oil necessary at 1000× but not with the lower power objective?

Confocal Microscopy

Confocal microscopy is a technique in light microscopy used to reconstruct three-dimensional images. Like fluorescent microscopy, specimens are stained with fluorochromes so they will emit, or return, light. But instead of illuminating the entire field, in confocal microscopy, one plane of a small region of a specimen is illuminated with a short-wavelength (blue) light which passes the returned light through an aperture aligned with the illuminated region. Each plane corresponds to an image of a fine slice that has been physically cut from a specimen. Successive planes and regions are illuminated until the entire specimen has been scanned. Because confocal microscopy uses a pinhole aperture, it eliminates the blurring that occurs with other microscopes. As a result, exceptionally clear two-dimensional images can be obtained, with improved resolution of up to 40% over that of other microscopes.

Most confocal microscopes are used in conjunction with computers to construct three-dimensional images. The scanned planes of a specimen, which resemble a stack of images, are converted to a digital form that can be used by a computer to construct a three-dimensional representation. The reconstructed images



(a) Brightfield. (Top) The path of light in brightfield microscopy, the type of illumination produced by regular compound light microscopes. (Bottom) Brightfield illumination shows internal structures and the outline of the transparent pellicle (external covering).

(b) Darkfield. (Top) The darkfield microscope uses a special condenser with an opaque disk that eliminates all light in the center of the beam. The only light that reaches the specimen comes in at an angle; thus, only light reflected by the specimen (blue rays) reaches the objective lens. (Bottom) Against the black background seen with darkfield microscopy, edges of the cell are bright, some internal structures seem to sparkle, and the pellicle is almost visible.

(c) Phase-contrast. (Top) In phase-contrast microscopy, the specimen is illuminated by light passing through an annular (ring-shaped) diaphragm. Direct light rays (unaltered by the specimen) travel a different path from light rays that are reflected or diffracted as they pass through the specimen. These two sets of rays are combined at the eye. Reflected or diffracted light rays are indicated in blue; direct rays are red. (Bottom) Phase-contrast microscopy shows greater differentiation of internal structures and clearly shows the pellicle.

Figure 3.4 Brightfield, darkfield, and phase-contrast microscopy. The illustrations show the contrasting light pathways of each of these types of microscopy. The photographs compare the protozoan *Paramecium* using these three different microscopy techniques.

Q What are the advantages of brightfield, darkfield, and phase-contrast microscopy?

can be rotated and viewed in any orientation. This technique has been used to obtain three-dimensional images of entire cells and cellular components (Figure 3.7). In addition, confocal microscopy can be used to evaluate cellular physiology by monitoring the distributions and concentrations of substances such as ATP and calcium ions. MM **Animation** Light Microscopy

Two-Photon Microscopy

As in confocal microscopy, specimens are stained with a fluorochrome for **two-photon microscopy (TPM).** Two-photon microscopy uses long-wavelength (red) light, and therefore two photons, instead of one, are needed to excite the fluorochrome to emit light. The longer wavelength allows imaging of living cells in tissues up to 1 mm (1000 μm) deep (Figure 3.8). Confocal microscopy can image cells in detail only to a depth of less than 100 μm. Additionally, the longer wavelength is less likely to generate singlet oxygen, which damages cells (see page 159). Another advantage of TPM is that it can track the activity of cells in real time. For example, cells of the immune system have been observed responding to an antigen.

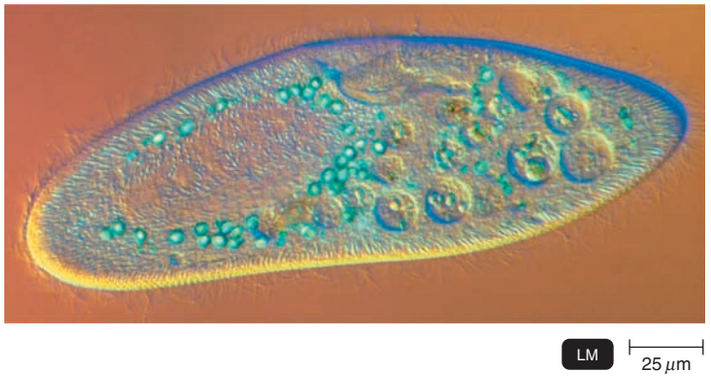


Figure 3.5 Differential interference contrast (DIC) microscopy. Like phase-contrast, DIC uses differences in refractive indexes to produce an image, in this case a *Paramecium*. The colors in the image are produced by prisms that split the two light beams used in this process.

Q Why does the image from a DIC microscope appear brightly colored?

Scanning Acoustic Microscopy

Scanning acoustic microscopy (SAM) basically consists of interpreting the action of a sound wave sent through a specimen. A sound wave of a specific frequency travels through the specimen, and a portion of it is reflected back every time it hits an interface within the material. The resolution is about 1 μm. SAM is used to study living cells attached to another surface, such as cancer cells, artery plaque, and bacterial biofilms that foul equipment (Figure 3.9).

CHECK YOUR UNDERSTANDING

How are brightfield, darkfield, phase-contrast, and fluorescence microscopy similar? 3-4

Electron Microscopy

Objects smaller than about 0.2 μm, such as viruses or the internal structures of cells, must be examined with an **electron microscope.** In electron microscopy, a beam of electrons is used instead of light. Like light, free electrons travel in waves. The resolving power of the electron microscope is far greater than that of the other microscopes described here so far. The better resolution of electron microscopes is due to the shorter wavelengths of electrons; the wavelengths of electrons are about 100,000 times smaller than the wavelengths of visible light. Thus, electron microscopes are used to examine structures too small to be resolved with light microscopes. Images produced by electron microscopes are always black and white, but they may be colored artificially to accentuate certain details.

Instead of using glass lenses, an electron microscope uses electromagnetic lenses to focus a beam of electrons onto a specimen. There are two types of electron microscopes: the transmission electron microscope and the scanning electron microscope.

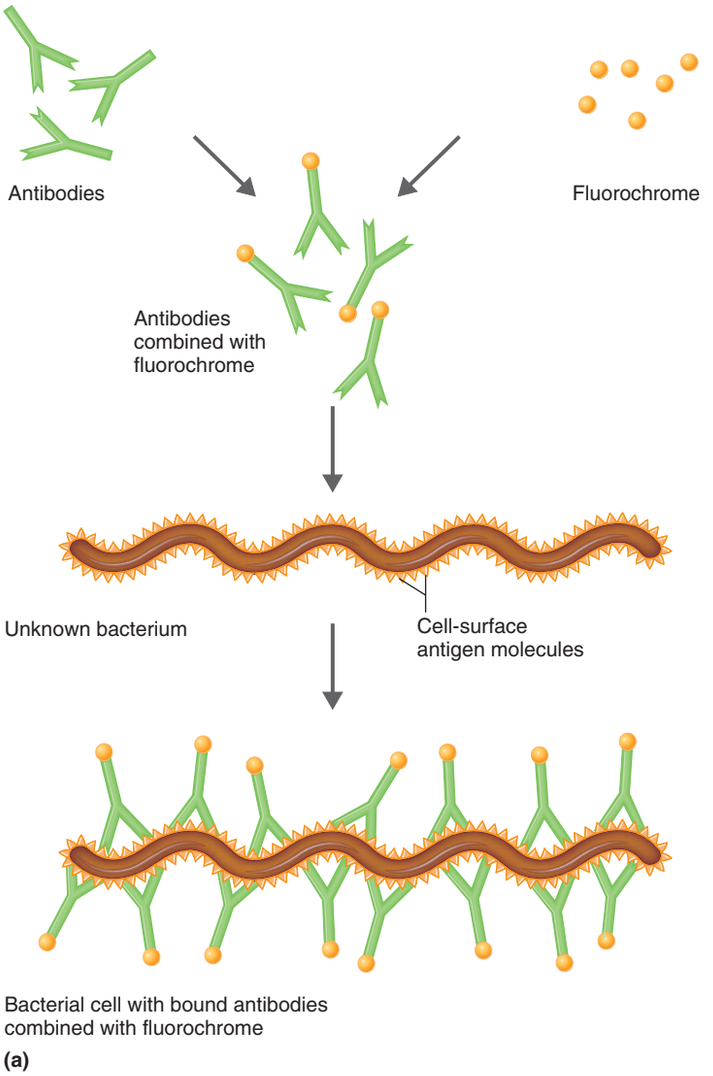


Figure 3.6 The principle of immunofluorescence. (**a**) A type of fluorochrome is combined with antibodies against a specific type of bacterium. When the preparation is added to bacterial cells on a microscope slide, the antibodies attach to the bacterial cells, and the cells fluoresce when illuminated with ultraviolet light. (**b**) In the fluorescent treponemal antibody absorption (FTA-ABS) test for syphilis shown here, *Treponema pallidum* shows up as green cells against a darker background.

Q Why won't other bacteria fluoresce in the FTA-ABS test?

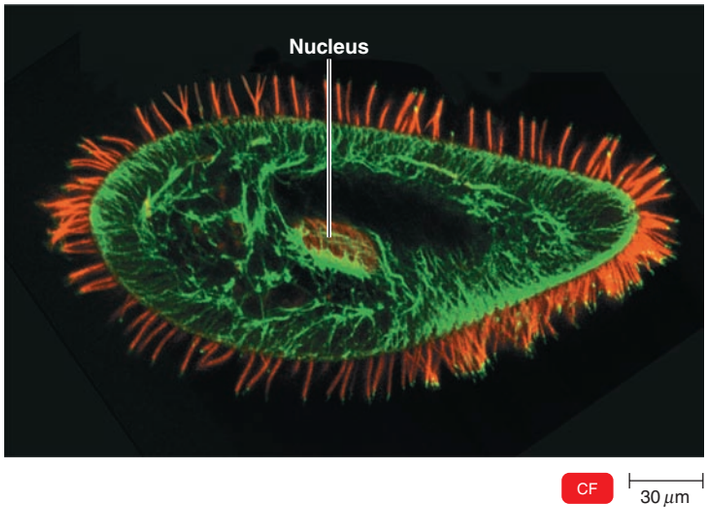


Figure 3.7 Confocal microscopy. Confocal microscopy produces three-dimensional images and can be used to look inside cells. Shown here is the nucleus in *Paramecium multimicronucleatum*.

Q What are the advantages of confocal microscopy?

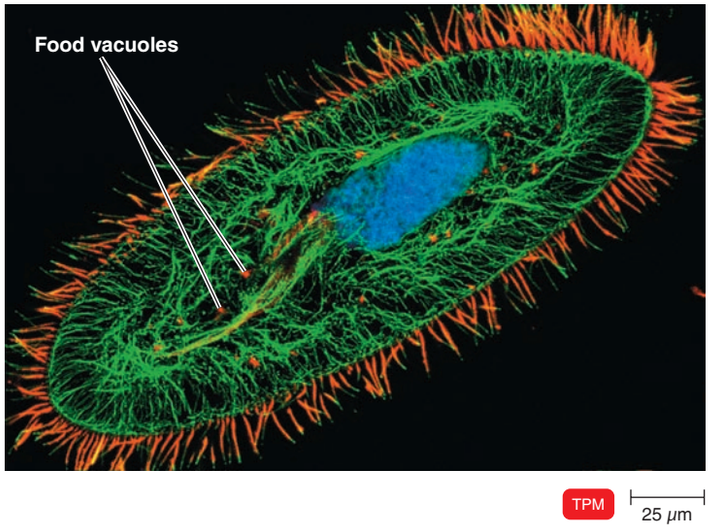


Figure 3.8 Two-photon microscopy (TPM). This procedure makes it possible to image living cells up to 1 mm deep in detail. This image shows food vacuoles in a living *Paramecium*.

Q What are the differences between TPM and confocal microscopy?

Transmission Electron Microscopy

In the **transmission electron microscope (TEM),** a finely focused beam of electrons from an electron gun passes through a specially prepared, ultrathin section of the specimen (**Figure 3.10a**). The beam is focused on a small area of the specimen by an electromagnetic condenser lens that performs roughly the same function as the condenser of a light microscope—directing the beam of electrons in a straight line to illuminate the specimen.

Electron microscopes use electromagnetic lenses to control illumination, focus, and magnification. Instead of being placed on a glass slide, as in light microscopes, the specimen is usually placed on a copper mesh grid. The beam of electrons passes through the specimen and then through an electromagnetic objective lens, which magnifies the image. Finally, the electrons are focused by an electromagnetic projector lens (rather than by an ocular lens as in a light microscope) onto a fluorescent screen or photographic plate. The final image, called a *transmission electron micrograph,* appears as many light and dark areas, depending on the number of electrons absorbed by different areas of the specimen.

The transmission electron microscope can resolve objects as close together as 10 pm, and objects are generally magnified 10,000 to 100,000×. Because most microscopic specimens are so thin, the contrast between their ultrastructures and the background is weak. Contrast can be greatly enhanced by using a "stain" that absorbs electrons and produces a darker image in the stained region. Salts of various heavy metals, such as lead, osmium, tungsten, and uranium, are commonly used as stains. These metals can be fixed onto the specimen (*positive staining*) or used to increase the electron opacity of the surrounding field (*negative staining*). Negative staining is useful for the study of the very smallest specimens, such as virus particles, bacterial flagella, and protein molecules.

In addition to positive and negative staining, a microbe can be viewed by a technique called *shadow casting.* In this procedure, a heavy metal such as platinum or gold is sprayed at an angle of about 45° so that it strikes the microbe from only one side. The metal piles up on one side of the specimen, and the uncoated area on the opposite side of the specimen leaves a clear area behind it as a shadow. This gives a three-dimensional effect to the specimen

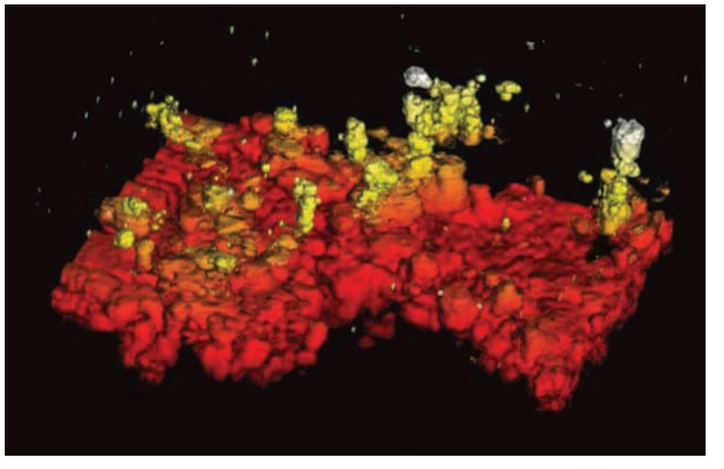
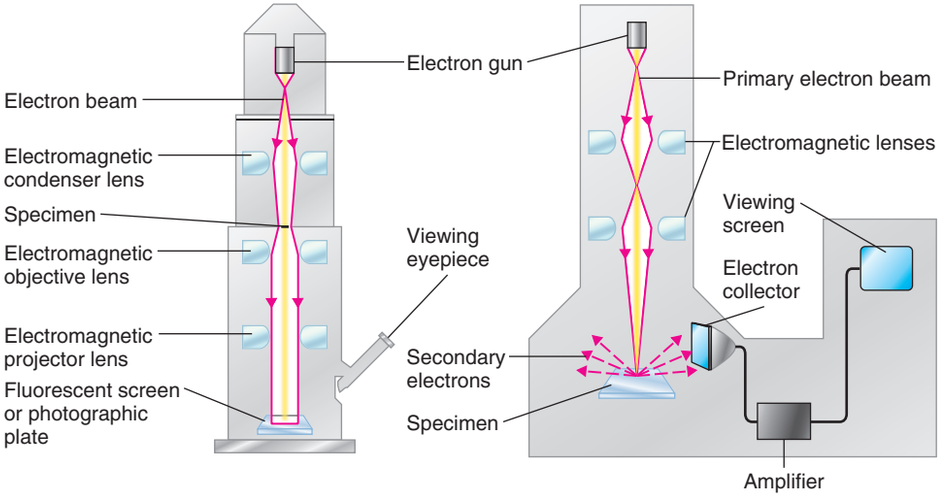
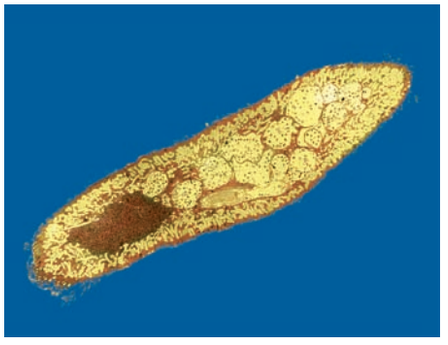


Figure 3.9 Scanning acoustic microscopy (SAM) of a bacterial biofilm on glass. Scanning acoustic microscopy essentially consists of interpreting the action of sound waves through a specimen. © 2006 IEEE.

Q What is the principal use of SAM?





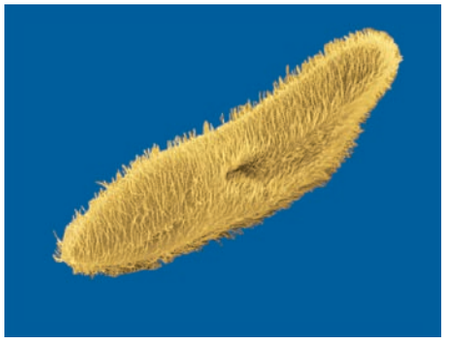


Figure 3.10 Transmission and scanning electron microscopy. The illustrations show the pathways of electron beams used to create images of the specimens. The photographs show a *Paramecium* viewed with both of these types of electron microscopes. Although electron micrographs are normally black and white, these and other electron micrographs in this book have been artificially colorized for emphasis.

Q How do TEM and SEM images of the same organism differ?

(a) Transmission. (Top) In a transmission electron microscope, electrons pass through the specimen and are scattered. Magnetic lenses focus the image onto a fluorescent screen or photographic plate. (Bottom) This colorized transmission electron micrograph (TEM) shows a thin slice of *Paramecium*. In this type of microscopy, the internal structures present in the slice can be seen.

(b) Scanning. (Top) In a scanning electron microscope, primary electrons sweep across the specimen and knock electrons from its surface. These secondary electrons are picked up by a collector, amplified, and transmitted onto a viewing screen or photographic plate. (Bottom) In this colorized scanning electron micrograph (SEM), the surface structures of *Paramecium* can be seen. Note the three-dimensional appearance of this cell, in contrast to the two-dimensional appearance of the transmission electron micrograph in part (a).

and provides a general idea of the size and shape of the specimen (see the TEM in Figure 4.6, page 79).

Transmission electron microscopy has high resolution and is extremely valuable for examining different layers of specimens. However, it does have certain disadvantages. Because electrons have limited penetrating power, only a very thin section of a specimen (about 100 nm) can be studied effectively. Thus, the specimen has no three-dimensional aspect. In addition, specimens must be fixed, dehydrated, and viewed under a high vacuum to prevent electron scattering. These treatments not only kill the specimen, but also cause some shrinkage and distortion, sometimes to the extent that there may appear to be additional structures in a prepared cell. Structures that appear as a result of the method of preparation are called *artifacts*.

Scanning Electron Microscopy

The **scanning electron microscope (SEM)** overcomes the problem of sectioning associated with a transmission electron microscope. A scanning electron microscope provides striking three-dimensional views of specimens (**Figure 3.10b**). In scanning electron microscopy, an electron gun produces a finely focused beam of electrons called the primary electron beam. These electrons pass through electromagnetic lenses and are directed over the surface of the specimen. The primary electron beam knocks electrons out of the surface of the specimen, and the secondary electrons thus produced are transmitted to an electron collector, amplified, and used to produce an image on a viewing screen or photographic plate. The image is called a *scanning electron micrograph*. This microscope is especially

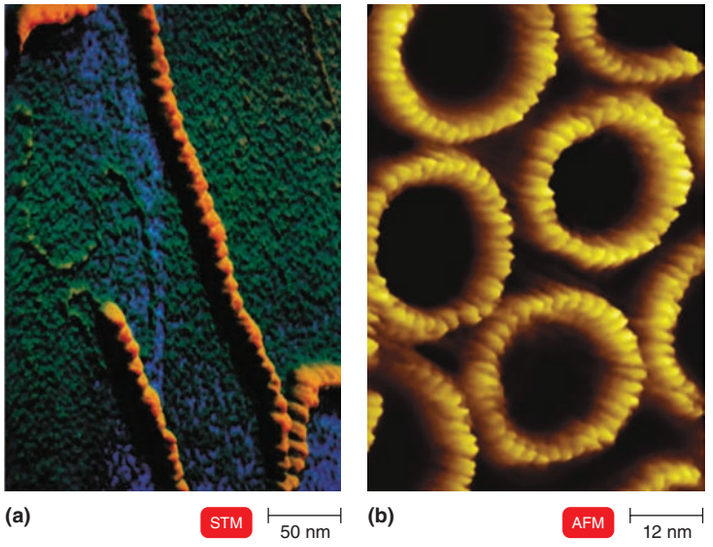


Figure 3.11 Scanned-probe microscopy. (**a**) Scanning tunneling microscopy (STM) image of RecA protein from *E. coli*. This protein is involved in repair of DNA. (**b**) Atomic force microscopy (AFM) image of perfringolysin O toxin from *Clostridium perfringens*. This protein makes holes in human plasma membranes.

What is the principle employed in scanned-probe microscopy?

useful in studying the surface structures of intact cells and viruses. In practice, it can resolve objects as close together as 10 nm, and objects are generally magnified 1000 to 10,000×.

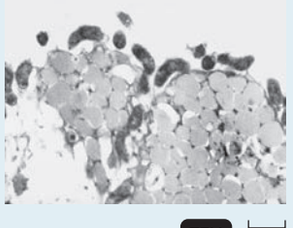
Animation Electron Microscopy

CHECK YOUR UNDERSTANDING

Why do electron microscopes have greater resolution than light microscopes? **3-5**

Clinical Case

Helicobacter pylori is a spiral-shaped, gram-negative bacterium with multiple flagella. It is the most common cause of peptic ulcers in humans and can also cause stomach cancer. The first electron micrograph of *H. pylori* was viewed in the 1980s, when Australian physician Robin Warren used an electron microscope to see *H. pylori* in stomach tissue.



Why was an electron microscope necessary to see the *H. pylori* bacteria?

54 **64** 69 71

Scanned-Probe Microscopy

Since the early 1980s, several new types of microscopes, called **scanned-probe microscopes,** have been developed. They use various kinds of probes to examine the surface of a specimen using electric current, which does not modify the specimen or expose it to damaging, high-energy radiation. Such microscopes can be used to map atomic and molecular shapes, to characterize magnetic and chemical properties, and to determine temperature variations inside cells. Among the new scanned-probe microscopes are the scanning tunneling microscope and the atomic force microscope, discussed next.

Scanning Tunneling Microscopy

Scanning tunneling microscopy (STM) uses a thin metal (tungsten) probe that scans a specimen and produces an image revealing the bumps and depressions of the atoms on the surface of the specimen (**Figure 3.11a**). The resolving power of an STM is much greater than that of an electron microscope; it can resolve features that are only about 1/100 the size of an atom. Moreover, special preparation of the specimen for observation is not needed. STMs are used to provide incredibly detailed views of molecules such as DNA.

Atomic Force Microscopy

In **atomic force microscopy (AFM),** a metal-and-diamond probe is gently forced down onto a specimen. As the probe moves along the surface of the specimen, its movements are recorded, and a three-dimensional image is produced (**Figure 3.11b**). As with STM, AFM does not require special specimen preparation. AFM is used to image both biological substances (in nearly atomic detail) (See also Figure 17.3b on page 482.) and molecular processes (such as the assembly of fibrin, a component of a blood clot).

The various types of microscopy just described are summarized in **Table 3.2** (pp. 65–67).

CHECK YOUR UNDERSTANDING

For what is TEM used? SEM? Scanned-probe microscopy? **3-6**

Preparation of Specimens for Light Microscopy

LEARNING OBJECTIVES

3-7 Differentiate an acidic dye from a basic dye.

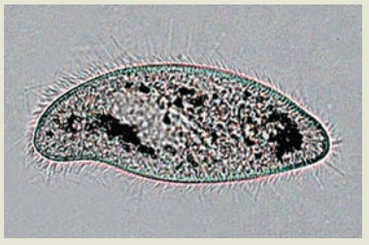
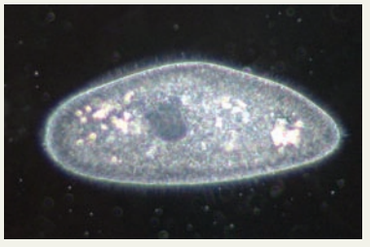
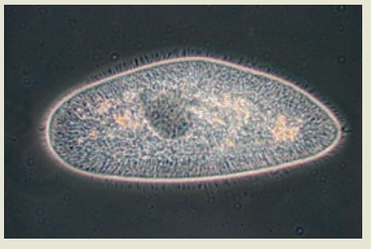
3-8 Explain the purpose of simple staining.

3-9 List the steps in preparing a Gram stain, and describe the appearance of gram-positive and gram-negative cells after each step.

3-10 Compare and contrast the Gram stain and the acid-fast stain.

3-11 Explain why each of the following is used: capsule stain, endospore stain, flagella stain.

TABLE 3.2 A Summary of Various Types of Microscopes

Microscope Type	Distinguishing Features	Typical Image	Principal Uses
Light			
Brightfield	Uses visible light as a source of illumination; cannot resolve structures smaller than about 0.2 µm; specimen appears against a bright background. Inexpensive and easy to use.	 *Paramecium* LM 25 µm	To observe various stained specimens and to count microbes; does not resolve very small specimens, such as viruses.
Darkfield	Uses a special condenser with an opaque disk that blocks light from entering the objective lens directly; light reflected by specimen enters the objective lens, and the specimen appears light against a black background.	 *Paramecium* LM 25 µm	To examine living microorganisms that are invisible in brightfield microscopy, do not stain easily, or are distorted by staining; frequently used to detect *Treponema pallidum* in the diagnosis of syphilis.
Phase-contrast	Uses a special condenser containing an annular (ring-shaped) diaphragm. The diaphragm allows direct light to pass through the condenser, focusing light on the specimen and a diffraction plate in the objective lens. Direct and reflected or diffracted light rays are brought together to produce the image. No staining required.	 *Paramecium* LM 25 µm	To facilitate detailed examination of the internal structures of living specimens.
Differential interference contrast (DIC)	Like phase-contrast, uses differences in refractive indexes to produce images. Uses two beams of light separated by prisms; the specimen appears colored as a result of the prism effect. No staining required.	 *Paramecium* LM 23 µm	To provide three-dimensional images.
Fluorescence	Uses an ultraviolet or near-ultraviolet source of illumination that causes fluorescent compounds (green-colored) in a specimen to emit light.	 *Treponema pallidum* LM 2 µm	For fluorescent-antibody techniques (immunofluorescence) to rapidly detect and identify microbes in tissues or clinical specimens.

(continued)

TABLE 3.2 A Summary of Various Types of Microscopes *(continued)*

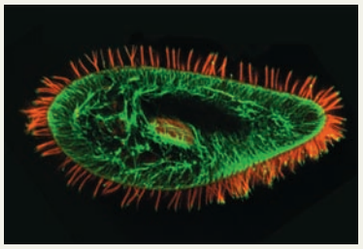
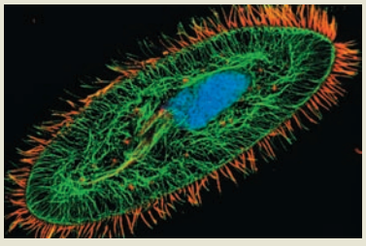
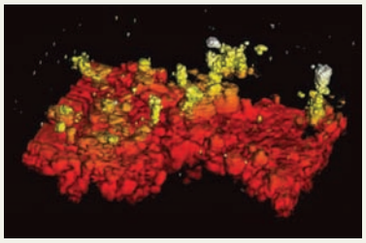
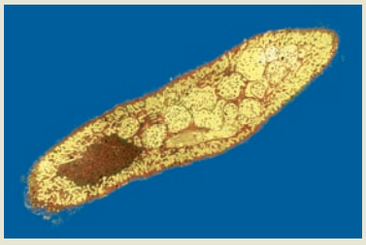
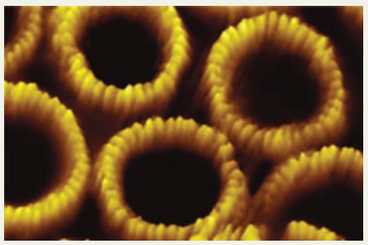
Microscope Type	Distinguishing Features	Typical Image	Principal Uses
Confocal	Uses a single photon to illuminate one plane of a specimen at a time.	 *Paramecium* CF 25 μm	To obtain two- and three-dimensional images of cells for biomedical applications.
Two-Photon	Uses two photons to illuminate a specimen.	 *Paramecium* TPM 22 μm	To image living cells, up to depth of 1 mm, reduce phototoxicity, and observe cell activity in real time.
Scanning Acoustic	Uses a sound wave of specific frequency that travels through the specimen with a portion being reflected when it hits an interface within the material.	 Biofilm SAM 180 μm	To examine living cells attached to another surface, such as cancer cells, artery plaque, and biofilms.
Electron			
Transmission	Uses a beam of electrons instead of light; electrons pass through the specimen; because of the shorter wavelength of electrons, structures smaller than 0.2 μm can be resolved. The image produced is two-dimensional.	 *Paramecium* TEM 25 μm	To examine viruses or the internal ultrastructure in thin sections of cells (usually magnified10,000–100,000×).
Scanning	Uses a beam of electrons instead of light; electrons are reflected from the specimen; because of the shorter wavelength of electrons, structures smaller than 0.2 μm can be resolved. The image produced appears three-dimensional.	 *Paramecium* SEM 25 μm	To study the surface features of cells and viruses (usually magnified 1000–10,000×).

TABLE 3.2 *(continued)*

Microscope Type	Distinguishing Features	Typical Image	Principal Uses
Scanned-Probe			
Scanning tunneling	Uses a thin metal probe that scans a specimen and produces an image revealing the bumps and depressions of the atoms on the surface of the specimen. Resolving power is much greater than that of an electron microscope. No special preparation required.	 RecA protein from *E. coli* STM 45 nm	Provides very detailed views of molecules inside cells.
Atomic force	Uses a metal-and-diamond probe gently forced down along the surface of the specimen. Produces a three-dimensional image. No special preparation required.	 Perfringolysin O toxin from *Clostridium perfringens* AFM 9 nm	Provides three-dimensional images of biological specimens at high resolution in nearly atomic detail and can measure physical properties of biological specimens and molecular processes.

Because most microorganisms appear almost colorless when viewed through a standard light microscope, we often must prepare them for observation. One way to do this is to stain (color) the specimen. Next we will discuss several different staining procedures.

Preparing Smears for Staining

Most initial observations of microorganisms are made with stained preparations. **Staining** simply means coloring the microorganisms with a dye that emphasizes certain structures. Before the microorganisms can be stained, however, they must be **fixed** (attached) to the microscope slide. Fixing simultaneously kills the microorganisms and fixes them to the slide. It also preserves various parts of microbes in their natural state with only minimal distortion.

When a specimen is to be fixed, a thin film of material containing the microorganisms is spread over the surface of the slide. This film, called a **smear,** is allowed to air dry. In most staining procedures the slide is then fixed by passing it through the flame of a Bunsen burner several times, smear side up, or by covering the slide with methyl alcohol for 1 minute. Stain is applied and then washed off with water; then the slide is blotted with absorbent paper. Without fixing, the stain might wash the microbes off the slide. The stained microorganisms are now ready for microscopic examination.

Stains are salts composed of a positive and a negative ion, one of which is colored and is known as the *chromophore*. The color of so-called **basic dyes** is in the positive ion; in **acidic dyes,** it is in the negative ion. Bacteria are slightly negatively charged at pH 7. Thus, the colored positive ion in a basic dye is attracted to the negatively charged bacterial cell. Basic dyes, which include crystal violet, methylene blue, malachite green, and safranin, are more commonly used than acidic dyes. Acidic dyes are not attracted to most types of bacteria because the dye's negative ions are repelled by the negatively charged bacterial surface, so the stain colors the background instead. Preparing colorless bacteria against a colored background is called **negative staining.** It is valuable for observing overall cell shapes, sizes, and capsules because the cells are made highly visible against a contrasting dark background (see Figure 3.14a on page 70). Distortions of cell size and shape are minimized because fixing is not necessary and the cells do not pick up the stain. Examples of acidic dyes are eosin, acid fuchsin, and nigrosin.

To apply acidic or basic dyes, microbiologists use three kinds of staining techniques: simple, differential, and special.

Simple Stains

A **simple stain** is an aqueous or alcohol solution of a single basic dye. Although different dyes bind specifically to different parts of cells, the primary purpose of a simple stain is to highlight the entire microorganism so that cellular shapes and basic structures are visible. The stain is applied to the fixed smear for a certain length of time and then washed off, and the slide is dried and

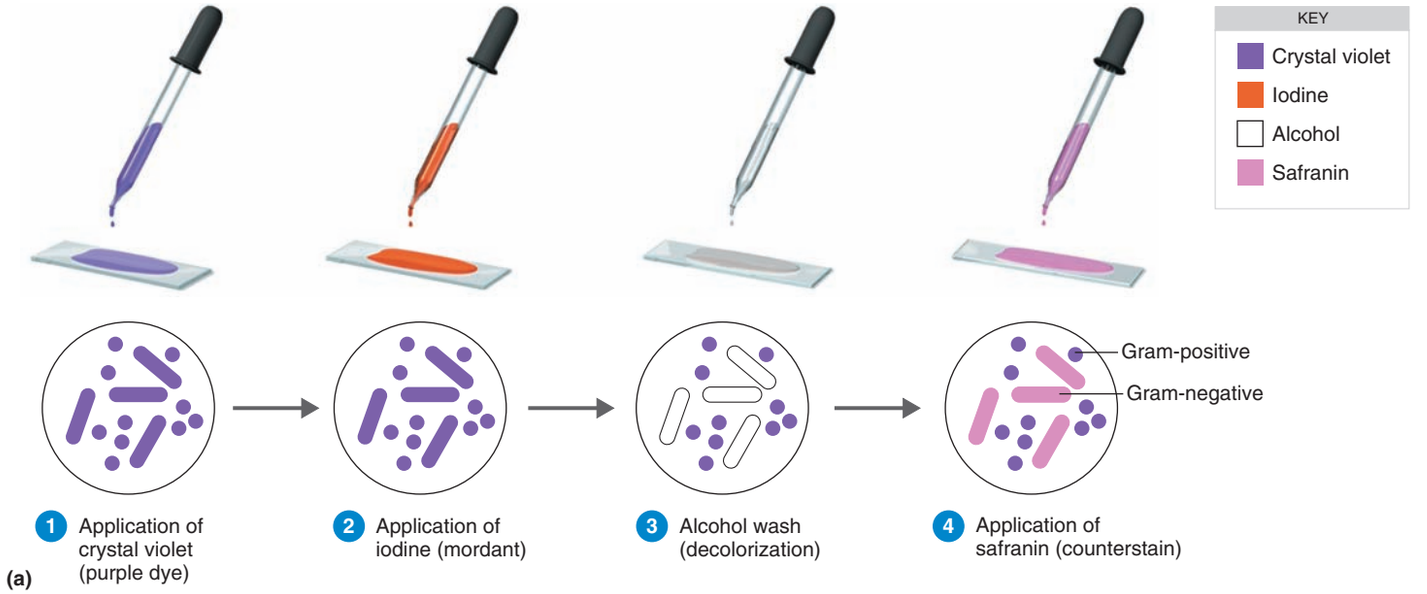


Figure 3.12 Gram staining. (**a**) Procedure. (**b**) Micrograph of gram-stained bacteria. The cocci (purple) are gram-positive, and the rods (pink) are gram-negative.

Q How can the Gram reaction be useful in prescribing antibiotic treatment?

examined. Occasionally, a chemical is added to the solution to intensify the stain; such an additive is called a **mordant.** One function of a mordant is to increase the affinity of a stain for a biological specimen; another is to coat a structure (such as a flagellum) to make it thicker and easier to see after it is stained with a dye. Some of the simple stains commonly used in the laboratory are methylene blue, carbolfuchsin, crystal violet, and safranin.

CHECK YOUR UNDERSTANDING

- Why doesn't a negative stain color a cell? **3-7**
- Why is fixing necessary for most staining procedures? **3-8**

Differential Stains

Unlike simple stains, **differential stains** react differently with different kinds of bacteria and thus can be used to distinguish them. The differential stains most frequently used for bacteria are the Gram stain and the acid-fast stain.

Gram Stain

The **Gram stain** was developed in 1884 by the Danish bacteriologist Hans Christian Gram. It is one of the most useful staining procedures because it classifies bacteria into two large groups: gram-positive and gram-negative.

In this procedure (**Figure 3.12a**),

1. A heat-fixed smear is covered with a basic purple dye, usually crystal violet. Because the purple stain imparts its color to all cells, it is referred to as a **primary stain.**
2. After a short time, the purple dye is washed off, and the smear is covered with iodine, a mordant. When the iodine is washed off, both gram-positive and gram-negative bacteria appear dark violet or purple.
3. Next, the slide is washed with alcohol or an alcohol-acetone solution. This solution is a **decolorizing agent,** which removes the purple from the cells of some species but not from others.
4. The alcohol is rinsed off, and the slide is then stained with safranin, a basic red dye. The smear is washed again, blotted dry, and examined microscopically.

The purple dye and the iodine combine in the cytoplasm of each bacterium and color it dark violet or purple. Bacteria that retain this color after the alcohol has attempted to decolorize

them are classified as **gram-positive;** bacteria that lose the dark violet or purple color after decolorization are classified as **gram-negative** (Figure 3.12b). Because gram-negative bacteria are colorless after the alcohol wash, they are no longer visible. This is why the basic dye safranin is applied; it turns the gram-negative bacteria pink. Stains such as safranin that have a contrasting color to the primary stain are called **counterstains.** Because gram-positive bacteria retain the original purple stain, they are not affected by the safranin counterstain.

As you will see in Chapter 4, different kinds of bacteria react differently to the Gram stain because structural differences in their cell walls affect the retention or escape of a combination of crystal violet and iodine, called the crystal violet–iodine (CV–I) complex. Among other differences, gram-positive bacteria have a thicker peptidoglycan (disaccharides and amino acids) cell wall than gram-negative bacteria. In addition, gram-negative bacteria contain a layer of lipopolysaccharide (lipids and polysaccharides) as part of their cell wall (see Figure 4.13, page 85). When applied to both gram-positive and gram-negative cells, crystal violet and then iodine readily enter the cells. Inside the cells, the crystal violet and iodine combine to form CV–I. This complex is larger than the crystal violet molecule that entered the cells, and, because of its size, it cannot be washed out of the intact peptidoglycan layer of gram-positive cells by alcohol. Consequently, gram-positive cells retain the color of the crystal violet dye. In gram-negative cells, however, the alcohol wash disrupts the outer lipopolysaccharide layer, and the CV–I complex is washed out through the thin layer of peptidoglycan. As a result, gram-negative cells are colorless until counterstained with safranin, after which they are pink.

In summary, gram-positive cells retain the dye and remain purple. Gram-negative cells do not retain the dye; they are colorless until counterstained with a red dye.

The Gram method is one of the most important staining techniques in medical microbiology. But Gram staining results are not universally applicable, because some bacterial cells stain poorly or not at all. The Gram reaction is most consistent when it is used on young, growing bacteria.

The Gram reaction of a bacterium can provide valuable information for the treatment of disease. Gram-positive bacteria tend to be killed easily by penicillins and cephalosporins. Gram-negative bacteria are generally more resistant because the antibiotics cannot penetrate the lipopolysaccharide layer. Some resistance to these antibiotics among both gram-positive and gram-negative bacteria is due to bacterial inactivation of the antibiotics.

Acid-Fast Stain

Another important differential stain (one that differentiates bacteria into distinctive groups) is the **acid-fast stain,** which binds strongly only to bacteria that have a waxy material in their cell walls. Microbiologists use this stain to identify all bacteria in the genus *Mycobacterium,* including the two important pathogens *Mycobacterium tuberculosis,* the causative agent of tuberculosis, and *Mycobacterium leprae* (lep′rī), the causative agent of leprosy. This stain is also used to identify the pathogenic strains of the genus *Nocardia* (nō-kär′dē-ä). Bacteria in the genera *Mycobacterium* and *Nocardia* are acid-fast.

In the acid-fast staining procedure, the red dye carbolfuchsin is applied to a fixed smear, and the slide is gently heated for several minutes. (Heating enhances penetration and retention of the dye.) Then the slide is cooled and washed with water. The smear is next treated with acid-alcohol, a decolorizer, which removes the red stain from bacteria that are not acid-fast. The acid-fast microorganisms retain the pink or red color because the carbolfuchsin is more soluble in the cell wall lipids than in the acid-alcohol (Figure 3.13). In non–acid-fast bacteria, whose cell walls lack the lipid components, the carbolfuchsin is rapidly removed during decolorization, leaving the cells colorless. The smear is then stained with a methylene blue counterstain. Non–acid-fast cells appear blue after the counterstain is applied.

CHECK YOUR UNDERSTANDING

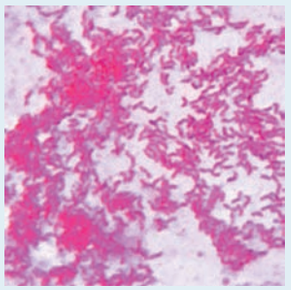
- Why is the Gram stain so useful? **3-9**
- Which stain would be used to identify microbes in the genera *Mycobacterium* and *Nocardia*? **3-10**

Special Stains

Special stains are used to color and isolate specific parts of microorganisms, such as endospores and flagella, and to reveal the presence of capsules.

Clinical Case

The resolving power of the electron microscope is much greater than that of a light microscope. The higher resolution provided unequivocal proof of the presence of spiral bacteria.



Although bismuth (the key ingredient in Pepto-Bismol) kills *H. pylori,* it is not a cure. Maryanne's physician prescribes the antibiotic clarithromycin. However, one week after finishing treatment, Maryanne's symptoms continue. To see whether *H. pylori* is still present, her physician orders a stomach biopsy to obtain a sample of the mucous lining of Maryanne's stomach. The lab uses light microscoy and a Gram stain to view the sample.

What does the gram stain above show?

54 64 **69** 71

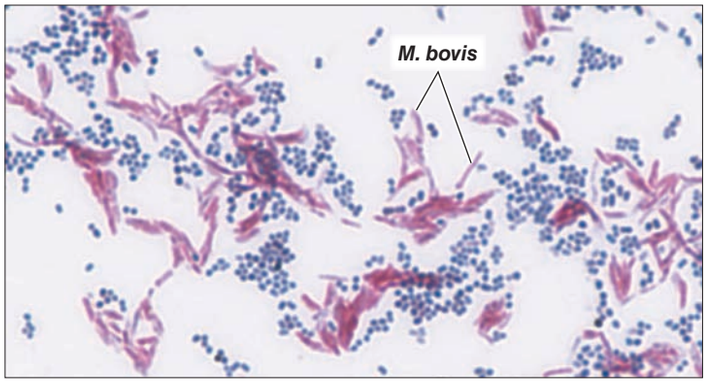


Figure 3.13 **Acid-fast bacteria.** The *Mycobacterium bovis* bacteria that have infected this tissue have been stained pink or red with an acid-fast stain. Non–acid-fast cells (*Staphylococcus*) are stained with the methylene blue counterstain.

Q Why is *Mycobacterium tuberculosis* easily identified by the acid-fast stain ?

Negative Staining for Capsules

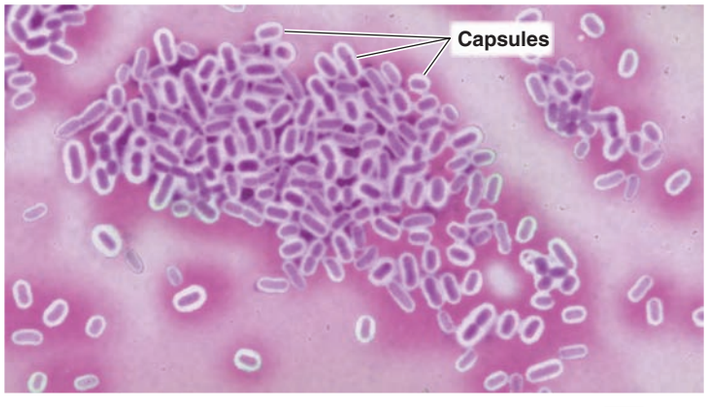
Many microorganisms contain a gelatinous covering called a **capsule,** which we will discuss in our examination of the prokaryotic cell in Chapter 4. In medical microbiology, demonstrating the presence of a capsule is a means of determining the organism's **virulence,** the degree to which a pathogen can cause disease.

Capsule staining is more difficult than other types of staining procedures because capsular materials are soluble in water and may be dislodged or removed during rigorous washing. To demonstrate the presence of capsules, a microbiologist can mix the bacteria in a solution containing a fine colloidal suspension of colored particles (usually India ink or nigrosin) to provide a contrasting background and then stain the bacteria with a simple stain, such as safranin (Figure 3.14a). Because of their chemical composition, capsules do not accept most biological dyes, such as safranin, and thus appear as halos surrounding each stained bacterial cell.

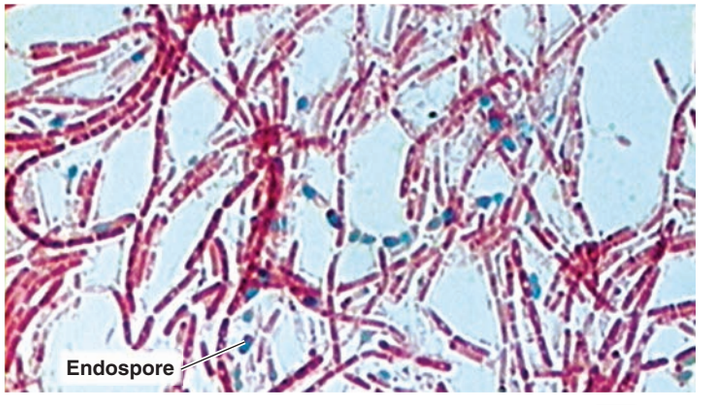
Endospore (Spore) Staining

An **endospore** is a special resistant, dormant structure formed within a cell that protects a bacterium from adverse environmental conditions. Although endospores are relatively uncommon in bacterial cells, they can be formed by a few genera of bacteria. Endospores cannot be stained by ordinary methods, such as simple staining and Gram staining, because the dyes do not penetrate the wall of the endospore.

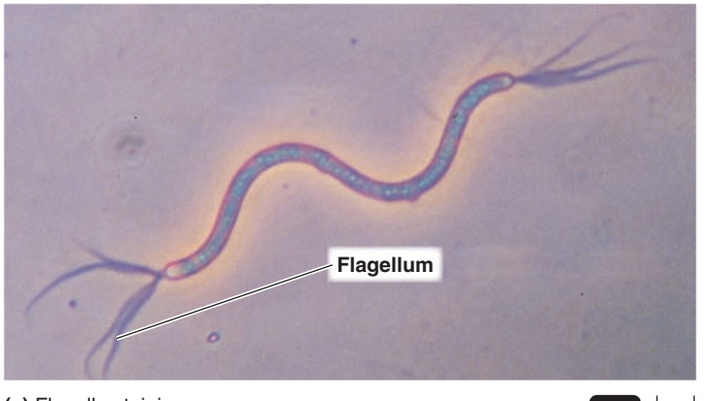
The most commonly used endospore stain is the *Schaeffer-Fulton endospore stain* (Figure 3.14b). Malachite green, the



(a) Negative staining



(b) Endospore staining



(c) Flagella staining

Figure 3.14 **Special staining.** (**a**) Capsule staining provides a contrasting background, so the capsules of these bacteria, *Klebsiella pneumoniae,* show up as light areas surrounding the stained cells. (**b**) Endospores are seen as green ovals in these rod-shaped cells of the bacterium *Bacillus cereus,* using the Schaeffer-Fulton endospore stain. (**c**) Flagella appear as wavy extensions from the ends of these cells of the bacterium *Spirillum volutans.* In relation to the body of the cell, the flagella are much thicker than normal because layers of the stain have accumulated from treatment of the specimen with a mordant.

Q Of what value are capsules, endospores, and flagella to bacteria?

TABLE 3.3 A Summary of Various Stains and Their Uses

Stain	Principal Uses
Simple (methylene blue, carbolfuchsin, crystal violet, safranin)	Used to highlight microorganisms to determine cellular shapes and arrangements. Aqueous or alcohol solution of a single basic dye stains cells. (Sometimes a mordant is added to intensify the stain.)
Differential	Used to distinguish different kinds of bacteria.
Gram	Classifies bacteria into two large groups: gram-positive and gram-negative. Gram-positive bacteria retain the crystal violet stain and appear purple. Gram-negative bacteria do not retain the crystal violet stain; they remain colorless until counterstained with safranin and then appear pink.
Acid-fast	Used to distinguish *Mycobacterium* species and some species of *Nocardia*. Acid-fast bacteria, once stained with carbolfuchsin and treated with acid-alcohol, remain pink or red because they retain the carbolfuchsin stain. Non–acid-fast bacteria, when stained and treated the same way and then stained with methylene blue, appear blue because they lose the carbolfuchsin stain and are then able to accept the methylene blue stain.
Special	Used to color and isolate various structures, such as capsules, endospores, and flagella; sometimes used as a diagnostic aid.
Negative	Used to demonstrate the presence of capsules. Because capsules do not accept most stains, the capsules appear as unstained halos around bacterial cells and stand out against a contrasting background.
Endospore	Used to detect the presence of endospores in bacteria. When malachite green is applied to a heat-fixed smear of bacterial cells, the stain penetrates the endospores and stains them green. When safranin (red) is then applied, it stains the remainder of the cells red or pink.
Flagella	Used to demonstrate the presence of flagella. A mordant is used to build up the diameters of flagella until they become visible microscopically when stained with carbolfuchsin.

primary stain, is applied to a heat-fixed smear and heated to steaming for about 5 minutes. The heat helps the stain penetrate the endospore wall. Then the preparation is washed for about 30 seconds with water to remove the malachite green from all of the cells' parts except the endospores. Next, safranin, a counterstain, is applied to the smear to stain portions of the cell other than endospores. In a properly prepared smear, the endospores appear green within red or pink cells. Because endospores are highly refractive, they can be detected under the light microscope when unstained, but without a special stain they cannot be differentiated from inclusions of stored material.

Flagella Staining

Bacterial **flagella** (singular: **flagellum**) are structures of locomotion too small to be seen with a light microscope without staining. A tedious and delicate staining procedure uses a mordant and the stain carbolfuchsin to build up the diameters of the flagella until they become visible under the light microscope (**Figure 3.14c**). Microbiologists use the number and arrangement of flagella as diagnostic aids. **Animation** Staining

CHECK YOUR UNDERSTANDING

How do unstained endospores appear? Stained endospores? **3-11**

A summary of stains is presented in **Table 3.3**. In the next chapters we will take a closer look at the structure of microbes and how they protect, nourish, and reproduce themselves.

Clinical Case Resolved

Because it is gram-negative, *H. pylori* stains pink after the counterstain is applied. The results from the lab indicate that the *H. pylori* is still present in Maryanne's stomach lining. Suspecting that the bacteria are resistant to clarithromycin, Maryanne's physician now prescribes two other antibiotics: tetracycline and metronidazole. This time, Maryanne's symptoms do not return. Soon, she is feeling like her old self again and is back in the office full time.

54 64 69 **71**

Study Outline

MasteringMICROBIOLOGY™

Test your understanding with quizzes, microbe review, and a chapter post-test at www.masteringmicrobiology.com.

Units of Measurement (p. 54)

1. The standard unit of length is the meter (m).
2. Microorganisms are measured in micrometers, μm (10^{-6} m), and in nanometers, nm (10^{-9} m).

Microscopy: The Instruments (pp. 54–64)

1. A simple microscope consists of one lens; a compound microscope has multiple lenses.

Light Microscopy (pp. 55–61)

2. The most common microscope used in microbiology is the compound light microscope (LM).
3. The total magnification of an object is calculated by multiplying the magnification of the objective lens by the magnification of the ocular lens.
4. The compound light microscope uses visible light.
5. The maximum resolution, or resolving power (the ability to distinguish two points) of a compound light microscope is 0.2 µm; maximum magnification is 2000×.
6. Specimens are stained to increase the difference between the refractive indexes of the specimen and the medium.
7. Immersion oil is used with the oil immersion lens to reduce light loss between the slide and the lens.
8. Brightfield illumination is used for stained smears.
9. Unstained cells are more productively observed using darkfield, phase-contrast, or DIC microscopy.
10. The darkfield microscope shows a light silhouette of an organism against a dark background.
11. It is most useful for detecting the presence of extremely small organisms.
12. A phase-contrast microscope brings direct and reflected or diffracted light rays together (in phase) to form an image of the specimen on the ocular lens.
13. It allows the detailed observation of living organisms.
14. The DIC microscope provides a colored, three-dimensional image of the object being observed.
15. It allows detailed observations of living cells.
16. In fluorescence microscopy, specimens are first stained with fluorochromes and then viewed through a compound microscope by using an ultraviolet light source.
17. The microorganisms appear as bright objects against a dark background.
18. Fluorescence microscopy is used primarily in a diagnostic procedure called fluorescent-antibody (FA) technique, or immunofluorescence.
19. In confocal microscopy, a specimen is stained with a fluorescent dye and illuminated with short-wavelength light.
20. Using a computer to process the images, two-dimensional and three-dimensional images of cells can be produced.

Two-Photon Microscopy (p. 61)

21. In TPM, a live specimen is stained with a fluorescent dye and illuminated with long-wavelength light.

Scanning Acoustic Microscopy (pp. 61–62)

22. Scanning acoustic microscopy (SAM) is based on the interpretation of sound waves through a specimen.
23. It is used to study living cells attached to surfaces such as cancer cells, artery plaque, and biofilms.

Electron Microscopy (pp. 62–63)

24. Instead of light, a beam of electrons is used with an electron microscope.
25. Instead of glass lenses, electromagnets control focus, illumination, and magnification.
26. Thin sections of organisms can be seen in an electron micrograph produced using a transmission electron microscope (TEM). Magnification: 10,000–100,000×. Resolving power: 10 pm.
27. Three-dimensional views of the surfaces of whole microorganisms can be obtained with a scanning electron microscope (SEM). Magnification: 1000–10,000×. Resolution: 10 nm.

Scanned-Probe Microscopy (pp. 63–64)

28. Scanning tunneling microscopy (STM) and atomic force microscopy (AFM) produce three-dimensional images of the surface of a molecule.

Preparation of Specimens for Light Microscopy (pp. 64–71)

Preparing Smears for Staining (pp. 64–67)

1. Staining means coloring a microorganism with a dye to make some structures more visible.
2. Fixing uses heat or alcohol to kill and attach microorganisms to a slide.
3. A smear is a thin film of material used for microscopic examination.
4. Bacteria are negatively charged, and the colored positive ion of a basic dye will stain bacterial cells.
5. The colored negative ion of an acidic dye will stain the background of a bacterial smear; a negative stain is produced.

Simple Stains (pp. 67–68)

6. A simple stain is an aqueous or alcohol solution of a single basic dye.
7. It is used to make cellular shapes and arrangements visible.
8. A mordant may be used to improve bonding between the stain and the specimen.

Differential Stains (pp. 68–69)

9. Differential stains, such as the Gram stain and acid-fast stain, differentiate bacteria according to their reactions to the stains.
10. The Gram stain procedure uses a purple stain (crystal violet), iodine as a mordant, an alcohol decolorizer, and a red counterstain.
11. Gram-positive bacteria retain the purple stain after the decolorization step; gram-negative bacteria do not and thus appear pink from the counterstain.
12. Acid-fast microbes, such as members of the genera *Mycobacterium* and *Nocardia,* retain carbolfuchsin after acid-alcohol decolorization and appear red; non–acid-fast microbes take up the methylene blue counterstain and appear blue.

Special Stains (pp. 69–71)

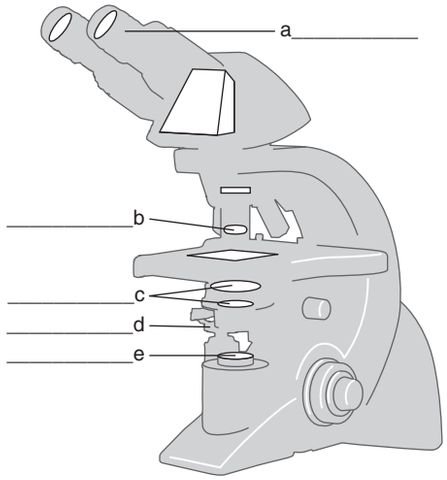
13. Negative staining is used to make microbial capsules visible.
14. The endospore stain and flagella stain are special stains that are used to visualize specific structures in bacterial cells.

Study Questions

Answers to the Review and Multiple Choice questions can be found by turning to the Answers tab at the back of the textbook.

Review

1. Fill in the following blanks.
   - **a.** 1 µm = _____ m
   - **b.** 1 _____ = 10^{-9} m
   - **c.** 1 µm = _____ nm
2. Which type of microscope would be best to use to observe each of the following?
   - **a.** a stained bacterial smear
   - **b.** unstained bacterial cells: the cells are small, and no detail is needed
   - **c.** unstained live tissue when it is desirable to see some intracellular detail
   - **d.** a sample that emits light when illuminated with ultraviolet light
   - **e.** intracellular detail of a cell that is 1 µm long
   - **f.** unstained live cells in which intracellular structures are shown in color
3. DRAW IT Label the parts of the compound light microscope in the figure below, and then draw the path of light from the illuminator to your eye.



4. Calculate the total magnification of the nucleus of a cell being observed through a compound light microscope with a 10× ocular lens and an oil immersion lens.
5. The maximum magnification of a compound microscope is (a) _____; that of an electron microscope, (b) _____. The maximum resolution of a compound microscope is (c) _____; that of an electron microscope, (d) _____. One advantage of a scanning electron microscope over a transmission electron microscope is (e) _____.
6. Why is a mordant used in the Gram stain? In the flagella stain?
7. What is the purpose of a counterstain in the acid-fast stain?
8. What is the purpose of a decolorizer in the Gram stain? In the acid-fast stain?
9. Fill in the following table regarding the Gram stain:

	Appearance After This Step of	
Steps	**Gram-Positive Cells**	**Gram-Negative Cells**
Crystal violet	a. _____	e. _____
Iodine	b. _____	f. _____
Alcohol-acetone	c. _____	g. _____
Safranin	d. _____	h. _____

10. NAME IT A sputum sample from Calle, a 30-year-old Asian elephant, was smeared onto a slide and air dried. The smear was fixed, covered with carbolfuchsin, and heated for 5 minutes. After washing with water, acid-alcohol was placed on the smear for 30 seconds. Finally, the smear was stained with methylene blue for 30 seconds, washed with water, and dried. On examination at 1000×, the zoo veterinarian saw red rods on the slide. (Calle was treated and recovered.) What microbe do these results suggest?

Multiple Choice

1. Assume you stain *Bacillus* by applying malachite green with heat and then counterstain with safranin. Through the microscope, the green structures are
   - **a.** cell walls.
   - **b.** capsules.
   - **c.** endospores.
   - **d.** flagella.
   - **e.** impossible to identify.
2. Three-dimensional images of live cells can be produced with
   - **a.** darkfield microscopy.
   - **b.** fluorescence microscopy.
   - **c.** transmission electron microscopy.
   - **d.** confocal microscopy.
   - **e.** phase-contrast microscopy.
3. Carbolfuchsin can be used as a simple stain and a negative stain. As a simple stain, the pH is
   - **a.** 2.
   - **b.** higher than the negative stain.
   - **c.** lower than the negative stain.
   - **d.** the same as the negative stain.
4. Looking at the cell of a photosynthetic microorganism, you observe that the chloroplasts are green in brightfield microscopy and red in fluorescence microscopy. You conclude that
   - **a.** chlorophyll is fluorescent.
   - **b.** the magnification has distorted the image.
   - **c.** you're not looking at the same structure in both microscopes.
   - **d.** the stain masked the green color.
   - **e.** none of the above
5. Which of the following is *not* a functionally analogous pair of stains?
   - **a.** nigrosin and malachite green
   - **b.** crystal violet and carbolfuchsin
   - **c.** safranin and methylene blue
   - **d.** ethanol-acetone and acid-alcohol
   - **e.** none of the above

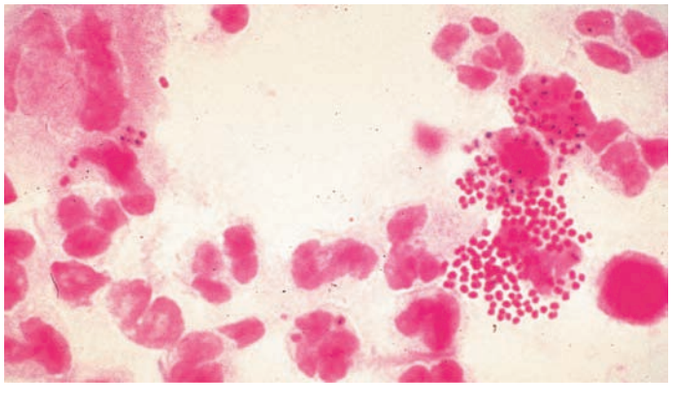
6. Which of the following pairs is *mismatched*?
   a. capsule—negative stain
   b. cell arrangement—simple stain
   c. cell size—negative stain
   d. Gram stain—bacterial identification
   e. none of the above
7. Assume you stain *Clostridium* by applying a basic stain, carbolfuchsin, with heat, decolorizing with acid-alcohol, and counterstaining with an acidic stain, nigrosin. Through the microscope, the endospores are ____1____, and the cells are stained ____2____.
   a. 1—red; 2—black
   b. 1—black; 2—colorless
   c. 1—colorless; 2—black
   d. 1—red; 2—colorless
   e. 1—black; 2—red
8. Assume that you are viewing a Gram-stained field of red cocci and blue bacilli through the microscope. You can safely conclude that you have
   a. made a mistake in staining.
   b. two different species.
   c. old bacterial cells.
   d. young bacterial cells.
   e. none of the above
9. In 1996, scientists described a new tapeworm parasite that had killed at least one person. The initial examination of the patient's abdominal mass was most likely made using
   a. brightfield microscopy.
   b. darkfield microscopy.
   c. electron microscopy.
   d. phase-contrast microscopy.
   e. fluorescence microscopy.
10. Which of the following is *not* a modification of a compound light microscope?
    a. brightfield microscopy
    b. darkfield microscopy
    c. electron microscopy
    d. phase-contrast microscopy
    e. fluorescence microscopy

Critical Thinking

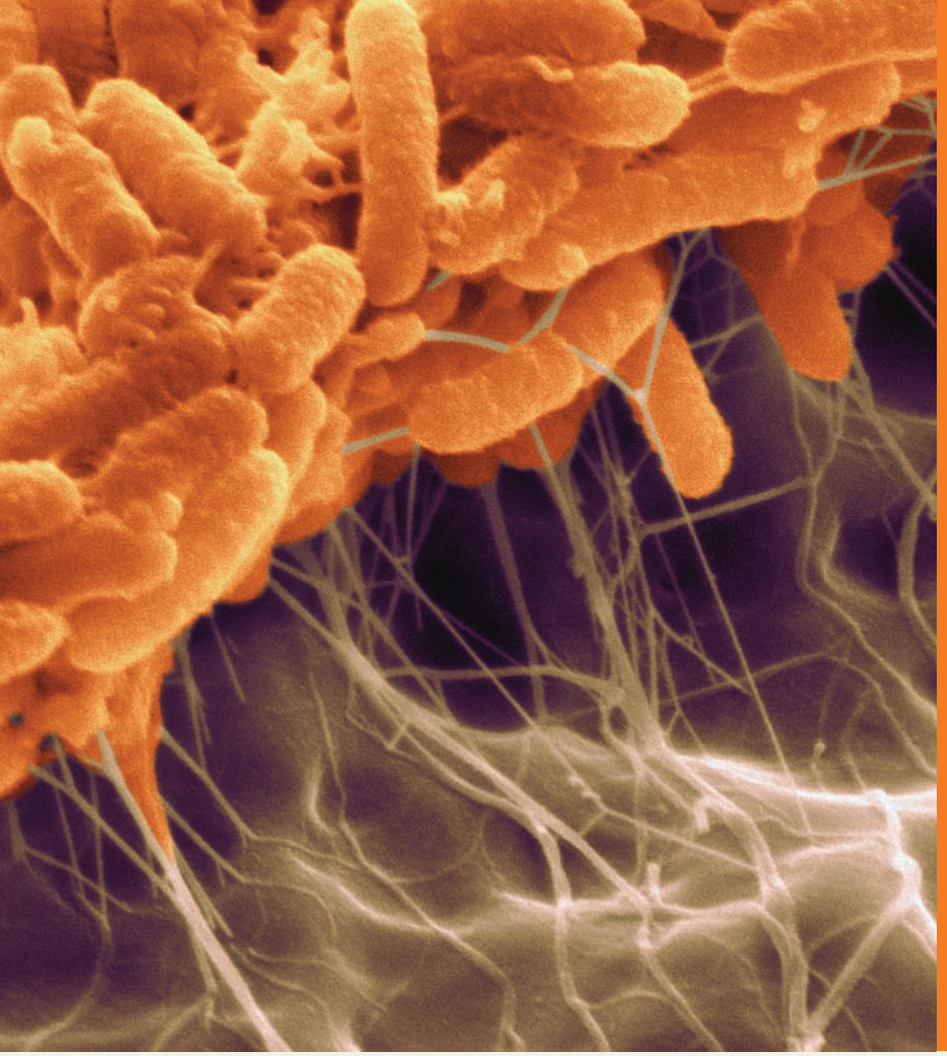
1. In a Gram stain, one step could be omitted and still allow differentiation between gram-positive and gram-negative cells. What is that one step?
2. Using a good compound light microscope with a resolving power of 0.3 µm, a 10× ocular lens, and a 100× oil immersion lens, would you be able to discern two objects separated by 3 µm? 0.3 µm? 300 nm?
3. Why isn't the Gram stain used on acid-fast bacteria? If you did Gram stain acid-fast bacteria, what would their Gram reaction be? What is the Gram reaction of non–acid-fast bacteria?
4. Endospores can be seen as refractile structures in unstained cells and as colorless areas in Gram-stained cells. Why is it necessary to do an endospore stain to verify the presence of endospores?

Clinical Applications

1. In 1882, German bacteriologist Paul Erhlich described a method for staining *Mycobacterium* and noted, "It may be that all disinfecting agents which are acidic will be without effect on this [tubercle] bacillus, and one will have to be limited to alkaline agents." How did he reach this conclusion without testing disinfectants?
2. Laboratory diagnosis of *Neisseria gonorrhoeae* infection is based on microscopic examination of Gram-stained pus. Locate the bacteria in this light micrograph. What is the disease?



3. Assume that you are viewing a Gram-stained sample of vaginal discharge. Large (10 µm) nucleated red cells are coated with small (0.5 µm wide by 1.5 µm long) blue cells on their surfaces. What is the most likely explanation for the red and blue cells?



4

Functional Anatomy of Prokaryotic and Eukaryotic Cells

Despite their complexity and variety, all living cells can be classified into two groups, prokaryotes and eukaryotes, based on certain structural and functional characteristics. In general, prokaryotes are structurally simpler and smaller than eukaryotes. The DNA (genetic material) of prokaryotes is usually a single, circularly arranged chromosome and is not surrounded by a membrane; the DNA of eukaryotes is found in multiple chromosomes in a membrane-enclosed nucleus. Prokaryotes lack membrane-enclosed organelles, specialized structures that carry on various activities.

Plants and animals are entirely composed of eukaryotic cells. In the microbial world, bacteria and archaea are prokaryotes. Other cellular microbes—fungi (yeasts and molds), protozoa, and algae—are eukaryotes. Both eukaryotic and prokaryotic cells can have a sticky glycocalyx surrounding them. In nature, most bacteria are found sticking to solid surfaces including other cells rather than free-floating. The glycocalyx is the glue that holds the cells in place. The *Serratia* bacteria in the photograph are attached to plastic; the sticky glycocalyx dried into filaments during microscopic examination. An example of the problem posed by biofilms in hospital water supplies is described in the Clinical Case.

Comparing Prokaryotic and Eukaryotic Cells: An Overview

LEARNING OBJECTIVE

4-1 Compare and contrast the overall cell structure of prokaryotes and eukaryotes.

Prokaryotes and eukaryotes are chemically similar, in the sense that they both contain nucleic acids, proteins, lipids, and carbohydrates. They use the same kinds of chemical reactions to metabolize food, build proteins, and store energy. It is primarily the structure of cell walls and membranes, and the absence of *organelles* (specialized cellular structures that have specific functions), that distinguish prokaryotes from eukaryotes.

The chief distinguishing characteristics of **prokaryotes** (from the Greek words meaning prenucleus) are as follows:

Clinical Case: Infection Detection

Irene Matthews, an infection control nurse in a hospital in Atlanta, Georgia, is in a quandary. Three patients in her hospital have all contracted postprocedure bacterial septicemia. All three have a fever and dangerously low blood pressure. These three patients are in separate areas of the hospital, in different units, and they have all undergone different procedures. The first patient, Joe, a 32-year-old construction worker, is recovering from rotator cuff surgery. He is in relatively good health, otherwise. The second patient, Jessie, a 16-year-old student in intensive care, is in critical condition following an automobile accident. She is on a ventilator and cannot breathe on her own. The third patient, Maureen, a 57-year-old grandmother, is recovering from coronary artery bypass surgery. As far as Irene can tell, the only thing these patients have in common is the infectious agent—*Klebsiella pneumoniae*.

How can three patients in different parts of a hospital contract *Klebsiella pneumoniae*? Read on to find out.

76 86 88 95 97

1. Their DNA is not enclosed within a membrane and is usually a singular circularly arranged chromosome. (Some bacteria, such as *Vibrio cholerae,* have two chromosomes, and some bacteria have a linearly arranged chromosome.)
2. Their DNA is not associated with histones (special chromosomal proteins found in eukaryotes); other proteins are associated with the DNA.
3. They lack membrane-enclosed organelles.
4. Their cell walls almost always contain the complex polysaccharide peptidoglycan.
5. They usually divide by **binary fission.** During this process, the DNA is copied, and the cell splits into two cells. Binary fission involves fewer structures and processes than eukaryotic cell division.

Eukaryotes (from the Greek words meaning true nucleus) have the following distinguishing characteristics:

1. Their DNA is found in the cell's nucleus, which is separated from the cytoplasm by a nuclear membrane, and the DNA is found in multiple chromosomes.
2. Their DNA is consistently associated with chromosomal proteins called histones and with nonhistones.
3. They have a number of membrane-enclosed organelles, including mitochondria, endoplasmic reticulum, Golgi complex, lysosomes, and sometimes chloroplasts.
4. Their cell walls, when present, are chemically simple.
5. Cell division usually involves mitosis, in which chromosomes replicate and an identical set is distributed into each of two nuclei. This process is guided by the mitotic spindle, a football-shaped assembly of microtubules. Division of the cytoplasm and other organelles follows so that the two cells produced are identical to each other.

Additional differences between prokaryotic and eukaryotic cells are listed in Table 4.2, page 100. Next we describe, in detail, the parts of the prokaryotic cell.

CHECK YOUR UNDERSTANDING

✓ What is the main feature that distinguishes prokaryotes from eukaryotes? **4-1**

The Prokaryotic Cell

The members of the prokaryotic world make up a vast heterogeneous group of very small unicellular organisms. Prokaryotes include bacteria and archaea. The majority of prokaryotes, including the photosynthesizing cyanobacteria, are bacteria. Although bacteria and archaea look similar, their chemical composition is different, as will be described later. The thousands of species of bacteria are differentiated by many factors, including morphology (shape), chemical composition (often detected by staining reactions), nutritional requirements, biochemical activities, and sources of energy (sunlight or chemicals). It is estimated that 99% of the bacteria in nature exist in biofilms (see pages 56 and 160).

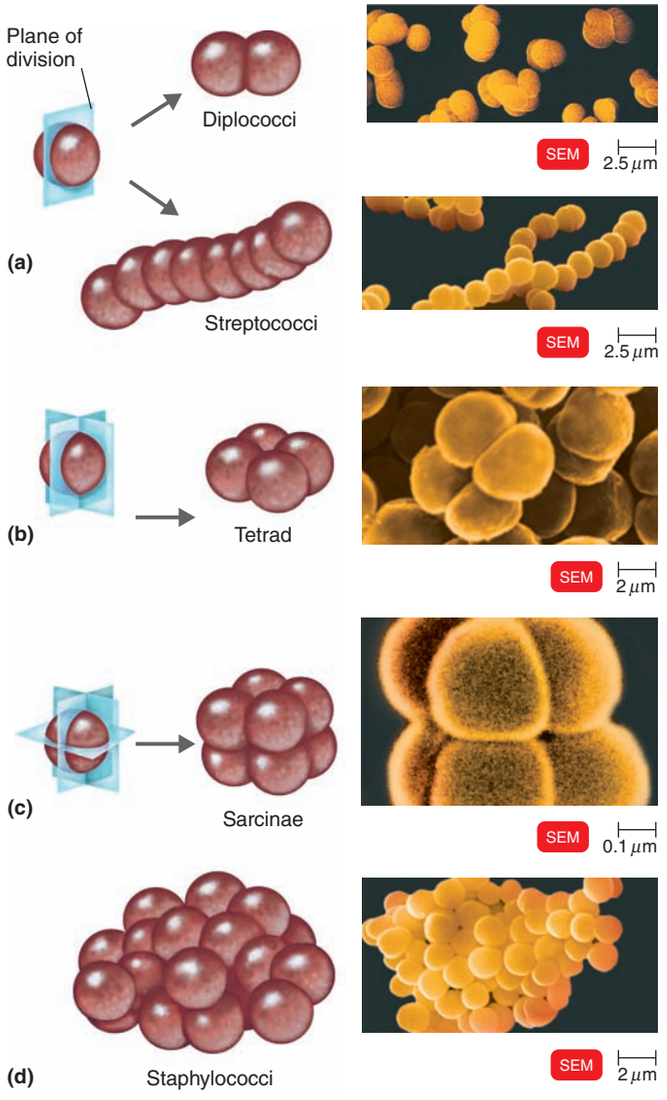


Figure 4.1 Arrangements of cocci. (**a**) Division in one plane produces diplococci and streptococci. (**b**) Division in two planes produces tetrads. (**c**) Division in three planes produces sarcinae, and (**d**) division in multiple planes produces staphylococci.

Q How do the planes of division determine the arrangement of cells?

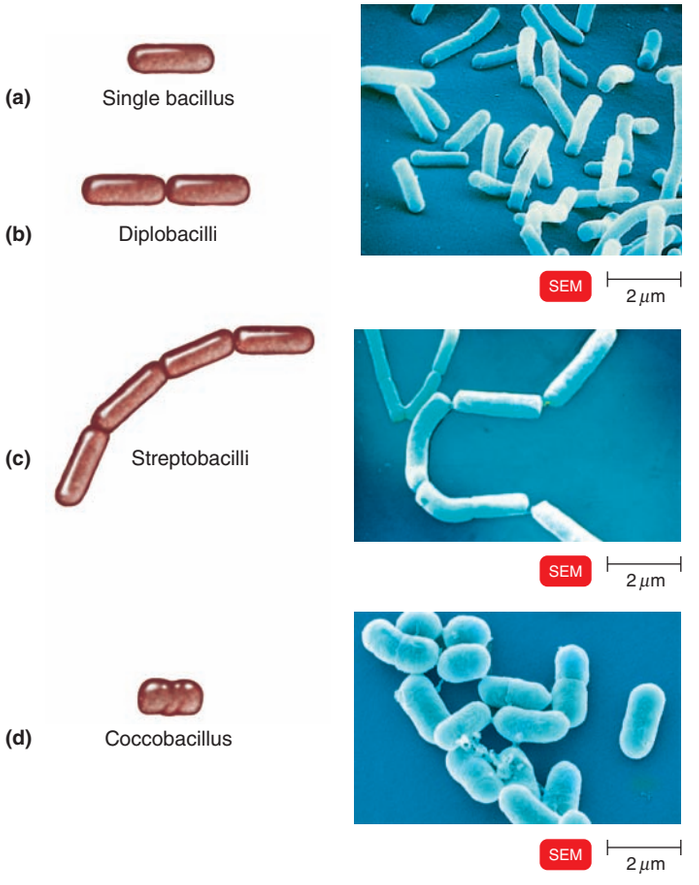


Figure 4.2 Bacilli. (**a**) Single bacilli. (**b**) Diplobacilli. In the top micrograph, a few joined pairs of bacilli could serve as examples of diplobacilli. (**c**) Streptobacilli. (**d**) Coccobacilli.

Q Why don't bacilli form tetrads or clusters?

The Size, Shape, and Arrangement of Bacterial Cells

LEARNING OBJECTIVE

4-2 Identify the three basic shapes of bacteria.

Bacteria come in a great many sizes and several shapes. Most bacteria range from 0.2 to 2.0 μm in diameter and from 2 to 8 μm in length. They have a few basic shapes: spherical **coccus** (plural: **cocci,** meaning berries), rod-shaped **bacillus** (plural: **bacilli,** meaning little staffs), and **spiral.**

Cocci are usually round but can be oval, elongated, or flattened on one side. When cocci divide to reproduce, the cells can remain attached to one another. Cocci that remain in pairs after dividing are called **diplococci;** those that divide and remain attached in chainlike patterns are called **streptococci** (Figure 4.1a). Those that divide in two planes and remain in groups of four are known as **tetrads** (Figure 4.1b). Those that divide in three planes and remain attached in cubelike groups of eight are called **sarcinae** (Figure 4.1c). Those that divide in multiple planes and form grapelike clusters or broad sheets are called **staphylococci** (Figure 4.1d). These group characteristics are frequently helpful in identifying certain cocci.

Bacilli divide only across their short axis, so there are fewer groupings of bacilli than of cocci. Most bacilli appear as single rods, called **single bacilli.** (Figure 4.2a). **Diplobacilli** appear in pairs after division (Figure 4.2b), and **streptobacilli** occur in chains (Figure 4.2c). Some bacilli look like straws. Others have tapered ends, like cigars. Still others are oval and look so much like cocci that they are called **coccobacilli** (Figure 4.2d).

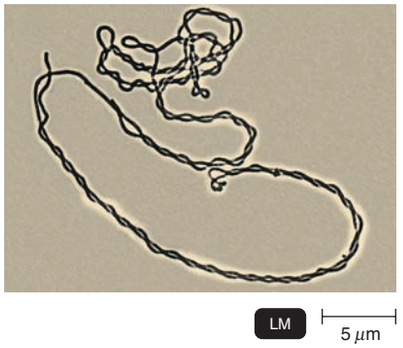


Figure 4.3 **A double-stranded helix formed by *Bacillus subtilis*.**

Q What is the difference between the term bacillus and *Bacillus*?

"Bacillus" has two meanings in microbiology. As we have just used it, bacillus refers to a bacterial shape. When capitalized and italicized, it refers to a specific genus. For example, the bacterium *Bacillus anthracis* is the causative agent of anthrax. Bacillus cells often form long, twisted chains of cells (Figure 4.3).

Spiral bacteria have one or more twists; they are never straight. Bacteria that look like curved rods are called **vibrios** (Figure 4.4a). Others, called **spirilla,** have a helical shape, like a corkscrew, and fairly rigid bodies (Figure 4.4b). Yet another group of spirals are helical and flexible; they are called **spirochetes** (Figure 4.4c). Unlike the spirilla, which use propeller-like external appendages called flagella to move, spirochetes move by means of axial filaments, which resemble flagella but are contained within a flexible external sheath.

In addition to the three basic shapes, there are star-shaped cells (genus *Stella;* Figure 4.5a); rectangular, flat cells (halophilic archaea) of the genus *Haloarcula* (Figure 4.5b); and triangular cells.

The shape of a bacterium is determined by heredity. Genetically, most bacteria are **monomorphic;** that is, they maintain a single shape. However, a number of environmental conditions can alter that shape. If the shape is altered, identification becomes difficult. Moreover, some bacteria, such as *Rhizobium* (rī-zō′bē-um) and *Corynebacterium* (kō-rī-nē-bak-ti′rē-um), are genetically **pleomorphic,** which means they can have many shapes, not just one.

The structure of a typical prokaryotic cell is shown in Figure 4.6. We will discuss its components according to the following organization: (1) structures external to the cell wall, (2) the cell wall itself, and (3) structures internal to the cell wall.

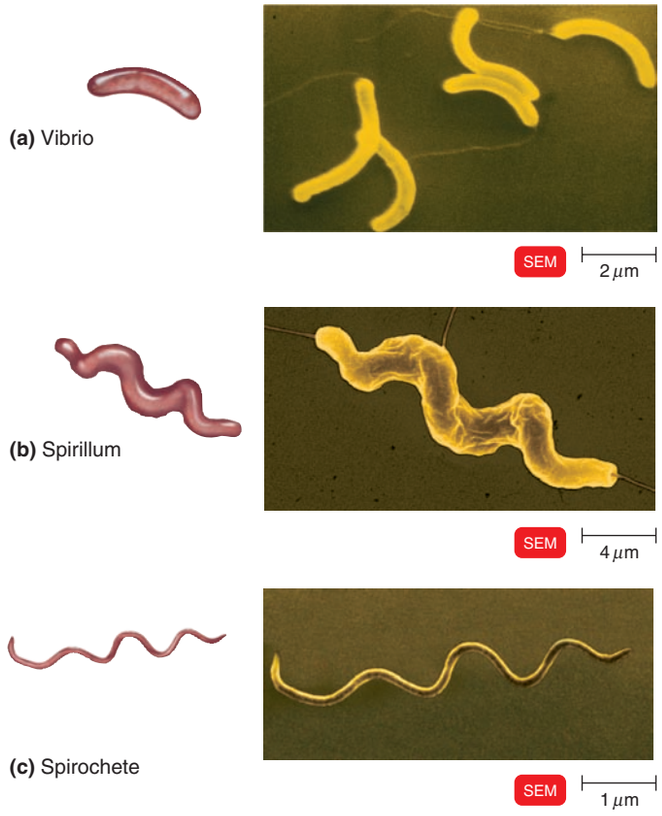


Figure 4.4 **Spiral bacteria.** (**a**) Vibrios. (**b**) Spirillum. (**c**) Spirochete.

Q What is the distinguishing feature of spirochete bacteria?

CHECK YOUR UNDERSTANDING

✓ How would you be able to identify streptococci through a microscope? 4-2

Structures External to the Cell Wall

LEARNING OBJECTIVES

4-3 Describe the structure and function of the glycocalyx

4-4 Differentiate flagella, axial filaments, fimbriae, and pili.

Among the possible structures external to the prokaryotic cell wall are the glycocalyx, flagella, axial filaments, fimbriae, and pili.

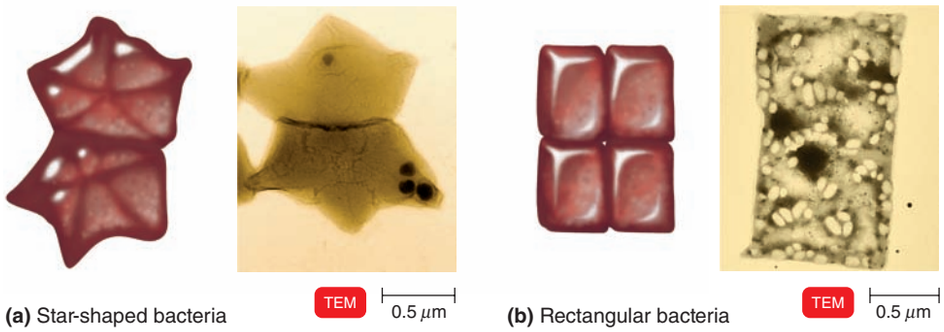


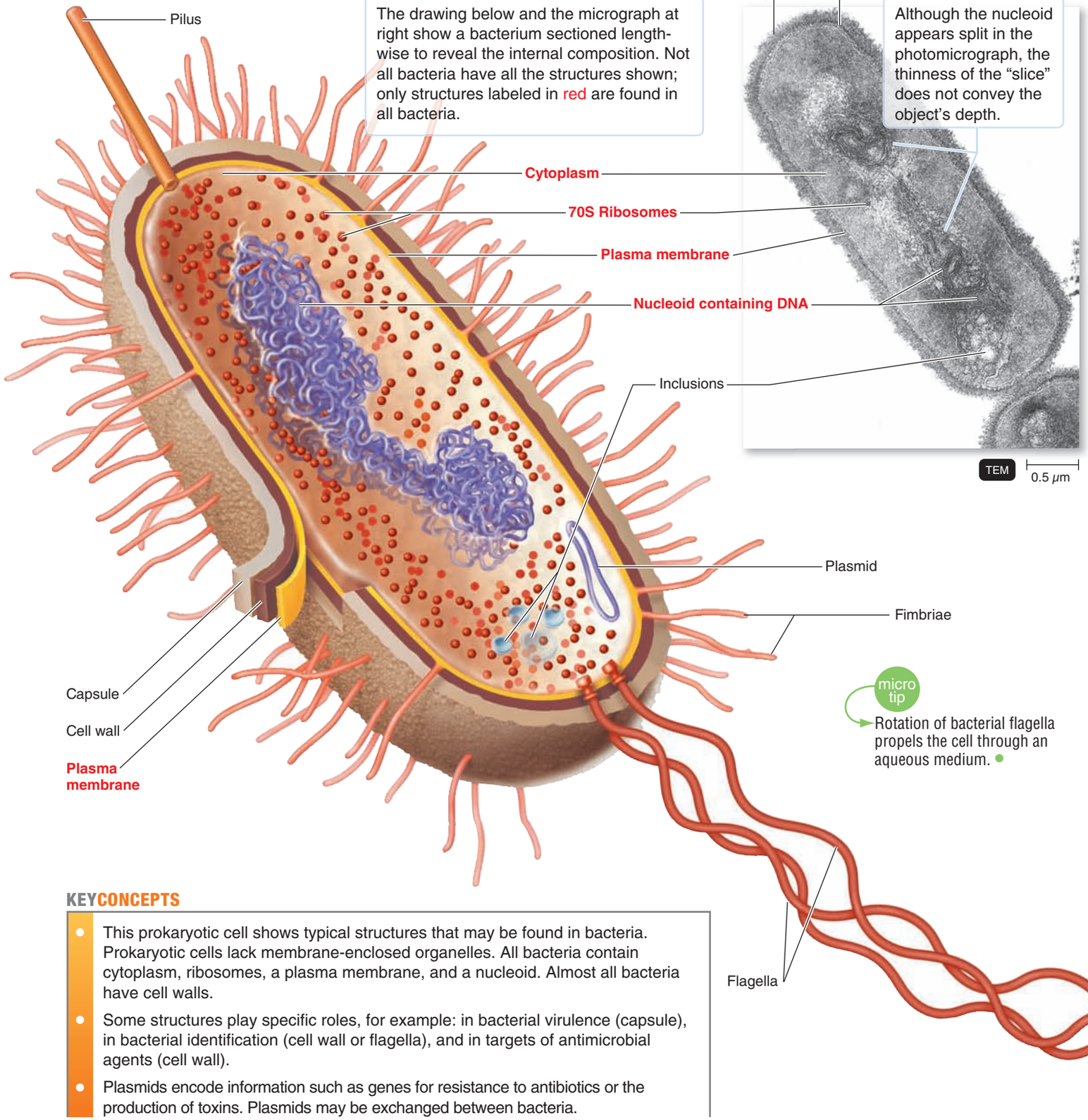
Figure 4.5 **Star-shaped and rectangular prokaryotes.** (**a**) *Stella* (star-shaped). (**b**) *Haloarcula,* a genus of halophilic archaea (rectangular cells).

Q What are the common bacterial shapes?

FOUNDATION FIGURE **4.6**

The Structure of a Prokaryotic Cell

The drawing below and the micrograph at right show a bacterium sectioned lengthwise to reveal the internal composition. Not all bacteria have all the structures shown; only structures labeled in red are found in all bacteria.



Although the nucleoid appears split in the photomicrograph, the thinness of the "slice" does not convey the object's depth.

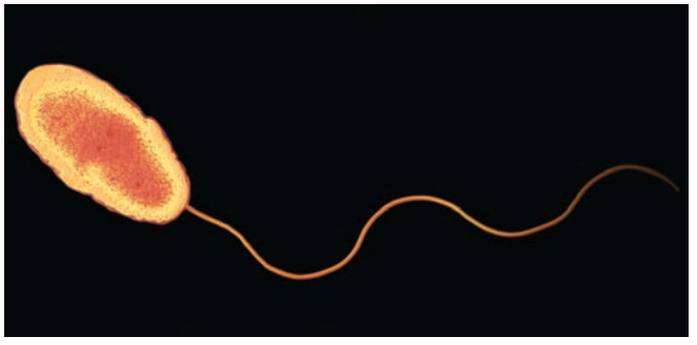
micro tip

Rotation of bacterial flagella propels the cell through an aqueous medium.

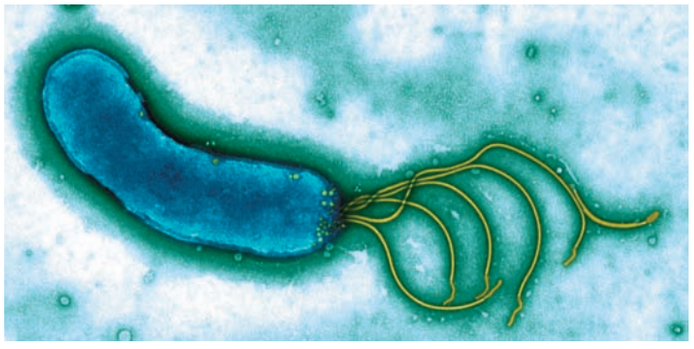
KEY CONCEPTS

- This prokaryotic cell shows typical structures that may be found in bacteria. Prokaryotic cells lack membrane-enclosed organelles. All bacteria contain cytoplasm, ribosomes, a plasma membrane, and a nucleoid. Almost all bacteria have cell walls.
- Some structures play specific roles, for example: in bacterial virulence (capsule), in bacterial identification (cell wall or flagella), and in targets of antimicrobial agents (cell wall).
- Plasmids encode information such as genes for resistance to antibiotics or the production of toxins. Plasmids may be exchanged between bacteria.

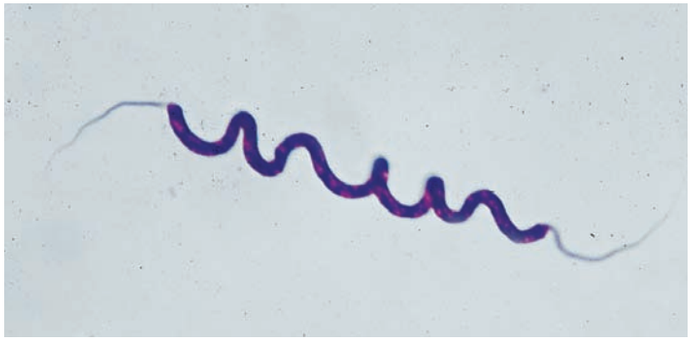
(a) Peritrichous SEM 0.6 μm



(b) Monotrichous and polar SEM 1 μm



(c) Lophotrichous and polar SEM 1 μm



(d) Amphitrichous and polar SEM 10 μm

Figure 4.7 Arrangements of bacterial flagella. (**a**) Peritrichous. (**b**)–(**d**) Polar.

Not all prokaryotic cells have flagella. What are bacteria without flagella called?

Glycocalyx

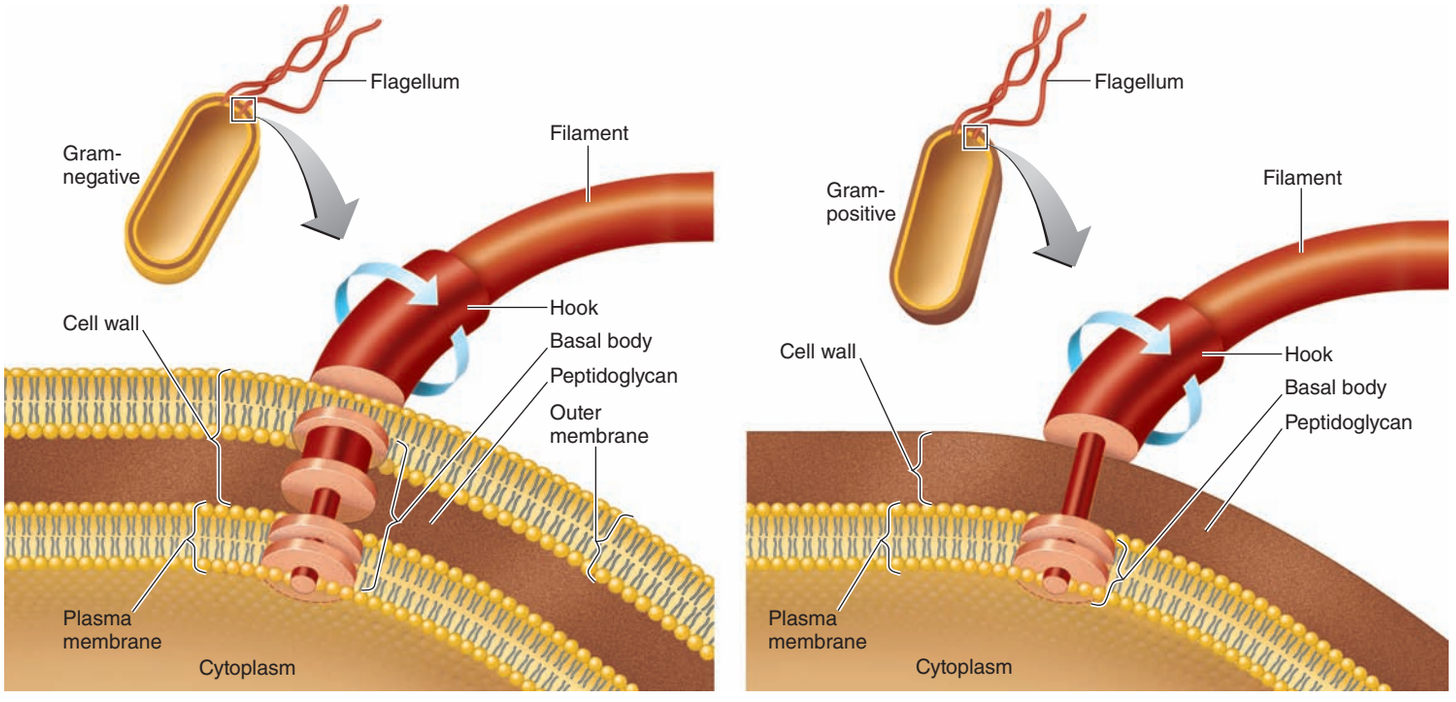
Many prokaryotes secrete on their surface a substance called glycocalyx. **Glycocalyx** (meaning sugar coat) is the general term used for substances that surround cells. The bacterial glycocalyx is a viscous (sticky), gelatinous polymer that is external to the cell wall and composed of polysaccharide, polypeptide, or both. Its chemical composition varies widely with the species. For the most part, it is made inside the cell and secreted to the cell surface. If the substance is organized and is firmly attached to the cell wall, the glycocalyx is described as a **capsule.** The presence of a capsule can be determined by using negative staining, described in Chapter 3 (see Figure 3.14a, page 70). If the substance is unorganized and only loosely attached to the cell wall, the glycocalyx is described as a **slime layer.**

In certain species, capsules are important in contributing to bacterial virulence (the degree to which a pathogen causes disease). Capsules often protect pathogenic bacteria from phagocytosis by the cells of the host. (As you will see later, phagocytosis is the ingestion and digestion of microorganisms and other solid particles.) For example, *Bacillus anthracis* produces a capsule of D-glutamic acid. (Recall from Chapter 2 that the D forms of amino acids are unusual.) Because only encapsulated *B. anthracis* causes anthrax, it is speculated that the capsule may prevent its being destroyed by phagocytosis.

Another example involves *Streptococcus pneumoniae* (streptō-kok′kus nü-mō′nē-ī), which causes pneumonia only when the cells are protected by a polysaccharide capsule. Unencapsulated *S. pneumoniae* cells cannot cause pneumonia and are readily phagocytized. The polysaccharide capsule of *Klebsiella* (kleb-sē-el′lä) also prevents phagocytosis and allows the bacterium to adhere to and colonize the respiratory tract.

The glycocalyx is a very important component of biofilms (see page 160). A glycocalyx that helps cells in a biofilm attach to their target environment and to each other is called an **extracellular polymeric substance (EPS).** The EPS protects the cells within it, facilitates communication among them, and enables the cells to survive by attaching to various surfaces in their natural environment.

Through attachment, bacteria can grow on diverse surfaces such as rocks in fast-moving streams, plant roots, human teeth, medical implants, water pipes, and even other bacteria. *Streptococcus mutans* (mū′tans), an important cause of dental caries, attaches itself to the surface of teeth by a glycocalyx. *S. mutans* may use its capsule as a source of nutrition by breaking it down and utilizing the sugars when energy stores are low. *Vibrio cholerae* (vib′-rē-o kol′-er-ī), the cause of cholera, produces a glycocalyx that helps it attach to the cells of the small intestine. A glycocalyx also can protect a cell against dehydration, and its viscosity may inhibit the movement of nutrients out of the cell.



(a) Parts and attachment of a flagellum of a gram-negative bacterium

(b) Parts and attachment of a flagellum of a gram-positive bacterium

Figure 4.8 The structure of a prokaryotic flagellum. The parts and attachment of a flagellum of a gram-negative bacterium and gram-positive bacterium are shown in these highly schematic diagrams.

Q How do the basal bodies of gram-negative and gram-positive bacteria differ?

Flagella

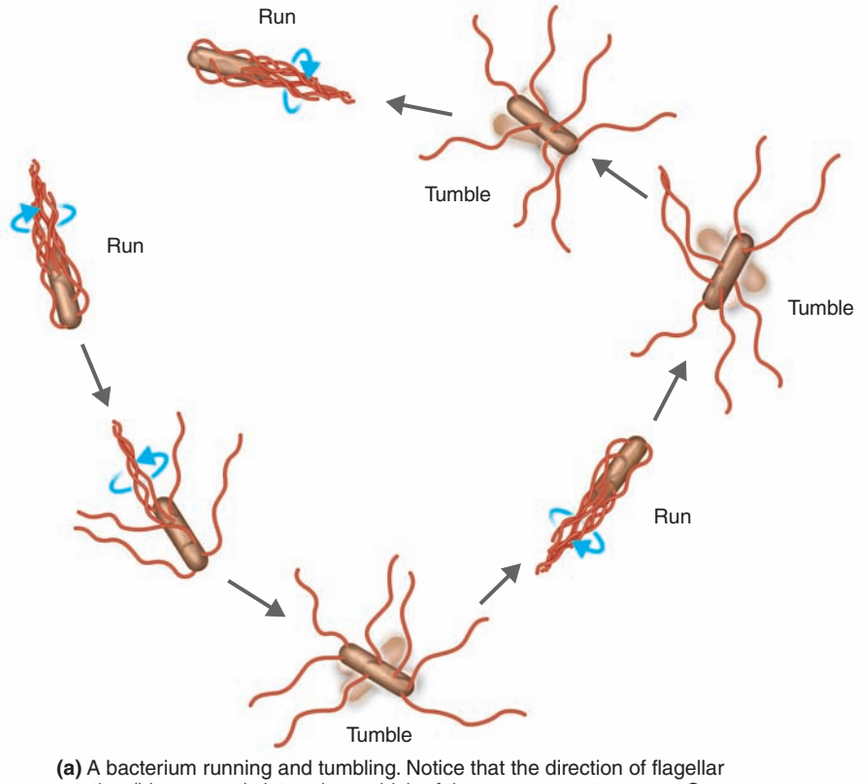
Some prokaryotic cells have **flagella** (singular: **flagellum**), which are long filamentous appendages that propel bacteria. Bacteria that lack flagella are referred to as **atrichous** (without projections). Flagella may be **peritrichous** (distributed over the entire cell; Figure 4.7a) or **polar** (at one or both poles or ends of the cell). If polar, flagella may be **monotrichous** (a single flagellum at one pole; Figure 4.7b), **lophotrichous** (a tuft of flagella coming from one pole; Figure 4.7c), or **amphitrichous** (flagella at both poles of the cell; Figure 4.7d).

A flagellum has three basic parts (Figure 4.8). The long outermost region, the *filament,* is constant in diameter and contains the globular (roughly spherical) protein *flagellin* arranged in several chains that intertwine and form a helix around a hollow core. In most bacteria, filaments are not covered by a membrane or sheath, as in eukaryotic cells. The filament is attached to a slightly wider *hook,* consisting of a different protein. The third portion of a flagellum is the *basal body,* which anchors the flagellum to the cell wall and plasma membrane.

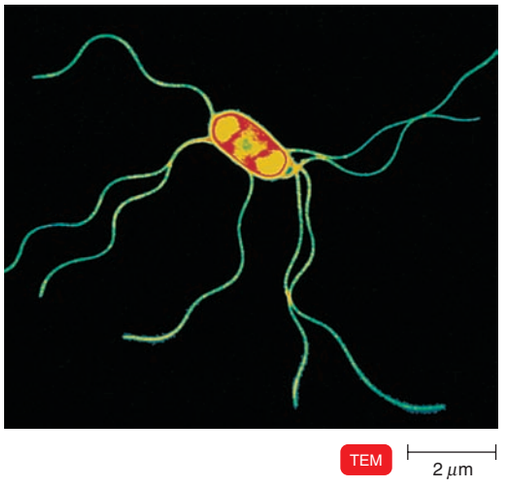
The basal body is composed of a small central rod inserted into a series of rings. Gram-negative bacteria contain two pairs of rings; the outer pair of rings is anchored to various portions of the cell wall, and the inner pair of rings is anchored to the plasma membrane. In gram-positive bacteria, only the inner pair is present. As you will see later, the flagella (and cilia) of eukaryotic cells are more complex than those of prokaryotic cells.

Each prokaryotic flagellum is a semirigid, helical structure that moves the cell by rotating from the basal body. The rotation of a flagellum is either clockwise or counterclockwise around its long axis. (Eukaryotic flagella, by contrast, undulate in a wavelike motion.) The movement of a prokaryotic flagellum results from rotation of its basal body and is similar to the movement of the shaft of an electric motor. As the flagella rotate, they form a bundle that pushes against the surrounding liquid and propels the bacterium. Flagellar rotation depends on the cell's continuous generation of energy.

Bacterial cells can alter the speed and direction of rotation of flagella and thus are capable of various patterns of **motility,** the ability of an organism to move by itself. When a bacterium moves in one direction for a length of time, the movement is called a "run" or "swim." "Runs" are interrupted by periodic, abrupt, random changes in direction called "tumbles." Then, a "run" resumes. "Tumbles" are caused by a reversal of flagellar rotation (Figure 4.9a). Some species of bacteria endowed with many flagella—*Proteus* (prō′tē-us), for example (Figure 4.9b)—can "swarm," or show rapid wavelike movement across a solid culture medium.



(a) A bacterium running and tumbling. Notice that the direction of flagellar rotation (blue arrows) determines which of these movements occurs. Gray arrows indicate direction of movement of the microbe.



(b) A *Proteus* cell in the swarming stage may have more than 1000 peritrichous flagella.

Figure 4.9 Flagella and bacterial motility.

Do bacterial flagella push or pull a cell?

One advantage of motility is that it enables a bacterium to move toward a favorable environment or away from an adverse one. The movement of a bacterium toward or away from a particular stimulus is called **taxis.** Such stimuli include chemicals (**chemotaxis**) and light (**phototaxis**). Motile bacteria contain receptors in various locations, such as in or just under the cell wall. These receptors pick up chemical stimuli, such as oxygen, ribose, and galactose. In response to the stimuli, information is passed to the flagella. If the chemotactic signal is positive, called an *attractant,* the bacteria move toward the stimulus with many runs and few tumbles. If the chemotactic signal is negative, called a *repellent,* the frequency of tumbles increases as the bacteria move away from the stimulus.

The flagellar protein called **H antigen** is useful for distinguishing among **serovars,** or variations within a species, of gram-negative bacteria (see page 310). For example, there are at least 50 different H antigens for *E. coli.* Those serovars identified as *E. coli* O157:H7 are associated with foodborne epidemics (see Chapter 1, page 19). **Animations** Motility; Flagella: Structure, Movement, Arrangement

Axial Filaments

Spirochetes are a group of bacteria that have unique structure and motility. One of the best-known spirochetes is *Treponema pallidum* (tre-pō-nē′mä pal′li-dum), the causative agent of syphilis. Another spirochete is *Borrelia burgdorferi* (bôr′-rel-ē-a burg-dor′fer-ē), the causative agent of Lyme disease. Spirochetes move by means of **axial filaments,** or **endoflagella,** bundles of fibrils that arise at the ends of the cell beneath an outer sheath and spiral around the cell (**Figure 4.10**).

Axial filaments, which are anchored at one end of the spirochete, have a structure similar to that of flagella. The rotation of the filaments produces a movement of the outer sheath that propels the spirochetes in a spiral motion. This type of movement is similar to the way a corkscrew moves through a cork. This corkscrew motion probably enables a bacterium such as *T. pallidum* to move effectively through body fluids. **Animation** Spirochetes

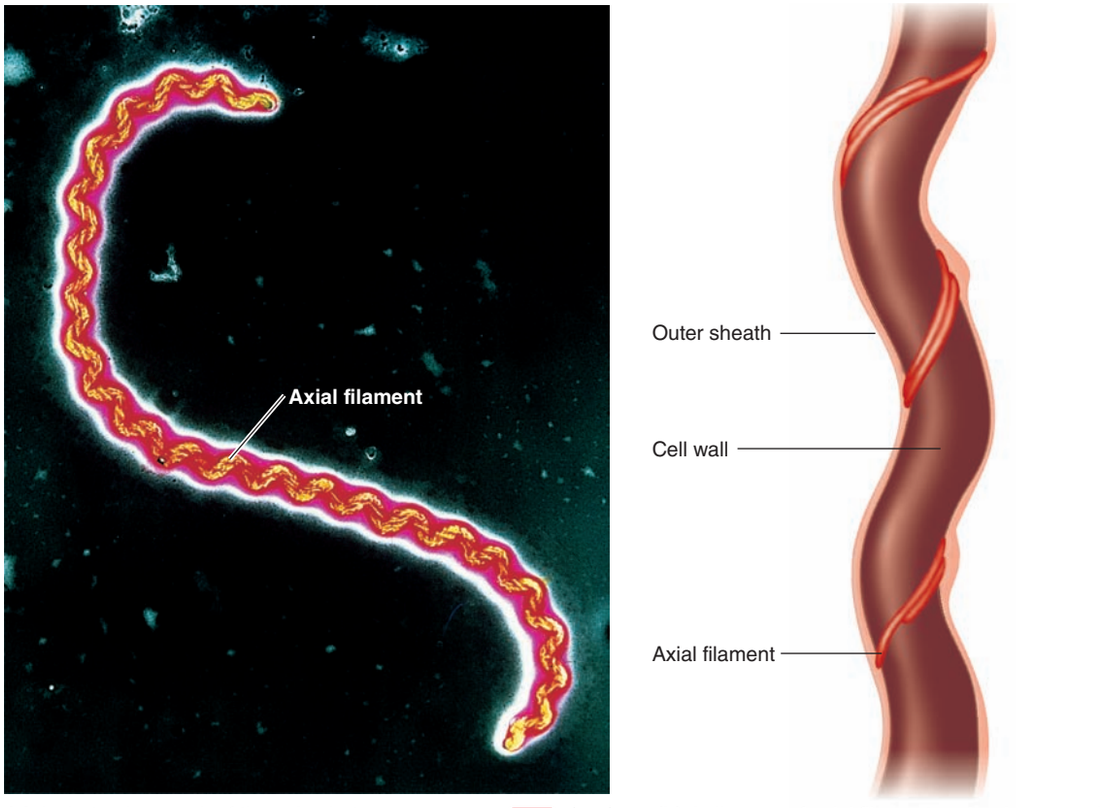
Fimbriae and Pili

Many gram-negative bacteria contain hairlike appendages that are shorter, straighter, and thinner than flagella and are used for attachment and transfer of DNA rather than for motility. These structures, which consist of a protein called *pilin* arranged helically around a central core, are divided into two types, fimbriae and pili, having very different functions. (Some microbiologists use the two terms interchangeably to refer to all such structures, but we distinguish between them.)

Fimbriae (singular: **fimbria**) can occur at the poles of the bacterial cell or can be evenly distributed over the entire surface of the cell. They can number anywhere from a few to several

Figure 4.10 **Axial filaments.**

Q How are endoflagella different from flagella?



(a) A photomicrograph of the spirochete *Leptospira,* showing an axial filament

(b) A diagram of axial filaments wrapping around part of a spirochete (see Figure 11.26a for a cross section of axial filaments)

hundred per cell (**Figure 4.11**). Fimbriae have a tendency to adhere to each other and to surfaces. As a result, they are involved in forming biofilms and other aggregations on the surfaces of liquids, glass, and rocks. Fimbriae can also help bacteria adhere to epithelial surfaces in the body. For example, fimbriae on the bacterium *Neisseria gonorrhoeae* (nī-se′rē-ä go-nôr-rē′ī), the causative agent of gonorrhea, help the microbe colonize mucous membranes. Once colonization occurs, the bacteria can cause disease. The fimbriae of *E. coli* O157 enable this bacterium to adhere to the lining of the small intestine, where it causes a severe watery diarrhea. When fimbriae are absent (because of genetic mutation), colonization cannot happen, and no disease ensues.

Pili (singular: **pilus**) are usually longer than fimbriae and number only one or two per cell. Pili are involved in motility and DNA transfer. In one type of motility, called **twitching motility,** a pilus extends by the addition of subunits of pilin, makes contact with a surface or another cell, and then retracts (powerstroke) as the pilin subunits are disassembled. This is called the *grappling hook model* of twitching motility and results in short, jerky, intermittent movements. Twitching motility has been observed in *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, and some strains of *E. coli.* The other type of motility associated with pili is **gliding motility,** the smooth gliding movement of myxobacteria. Although the exact mechanism is unknown for most myxobacteria, some utilize pilus retraction. Gliding motility provides a means for microbes to travel in environments with a low water content, such as biofilms and soil.

Figure 4.11 **Fimbriae.** The fimbriae seem to bristle from this *E. coli* cell, which is beginning to divide.

Q Why are fimbriae necessary for colonization?

Some pili are used to bring bacteria together allowing the transfer of DNA from one cell to another, a process called conjugation. Such pili are called **conjugation (sex) pili** (see page 234). In this process, the conjugation pilus of one bacterium called an F^+ cell connects to receptors on the surface of another bacterium of its own species or a different species. The two cells make physical contact, and DNA from the F^+ cell is transferred to the other cell. The exchanged DNA can add a new function to the recipient cell, such as antibiotic resistance or the ability to digest its medium more efficiently.

CHECK YOUR UNDERSTANDING

- Why are bacterial capsules medically important? **4-3**
- How do bacteria move? **4-4**

The Cell Wall

LEARNING OBJECTIVES

4-5 Compare and contrast the cell walls of gram-positive bacteria, gram-negative bacteria, acid-fast bacteria, archaea, and mycoplasmas.

4-6 Compare and contrast archaea and mycoplasmas.

4-7 Differentiate *protoplast, spheroplast,* and *L form.*

The **cell wall** of the bacterial cell is a complex, semirigid structure responsible for the shape of the cell. The cell wall surrounds the underlying, fragile plasma (cytoplasmic) membrane and protects it and the interior of the cell from adverse changes in the outside environment (see Figure 4.6). Almost all prokaryotes have cell walls.

The major function of the cell wall is to prevent bacterial cells from rupturing when the water pressure inside the cell is greater than that outside the cell (see Figure 4.18d, page 92). It also helps maintain the shape of a bacterium and serves as a point of anchorage for flagella. As the volume of a bacterial cell increases, its plasma membrane and cell wall extend as needed. Clinically, the cell wall is important because it contributes to the ability of some species to cause disease and is the site of action of some antibiotics. In addition, the chemical composition of the cell wall is used to differentiate major types of bacteria.

Although the cells of some eukaryotes, including plants, algae, and fungi, have cell walls, their walls differ chemically from those of prokaryotes, are simpler in structure, and are less rigid.

Composition and Characteristics

The bacterial cell wall is composed of a macromolecular network called **peptidoglycan** (also known as *murein*), which is present either alone or in combination with other substances. Peptidoglycan consists of a repeating disaccharide attached by polypeptides to form a lattice that surrounds and protects the entire cell. The disaccharide portion is made up of monosaccharides called N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) (from *murus,* meaning wall), which are related to glucose. The structural formulas for NAG and NAM are shown in **Figure 4.12**.

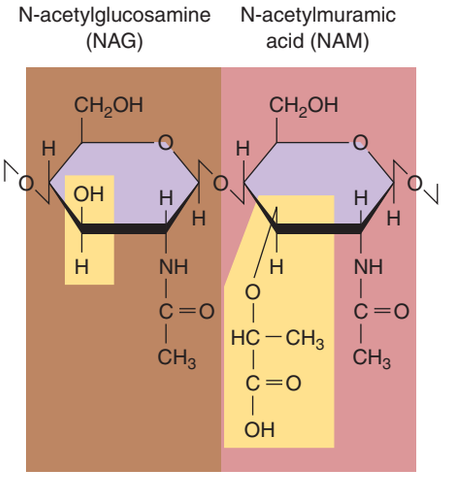


Figure 4.12 N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) joined as in a peptidoglycan. The gold areas show the differences between the two molecules. The linkage between them is called a β-1,4 linkage.

Q What kind of molecules are these: carbohydrates, lipids, or proteins?

The various components of peptidoglycan are assembled in the cell wall (**Figure 4.13a**). Alternating NAM and NAG molecules are linked in rows of 10 to 65 sugars to form a carbohydrate "backbone" (the glycan portion of peptidoglycan). Adjacent rows are linked by **polypeptides** (the peptide portion of peptidoglycan). Although the structure of the polypeptide link varies, it always includes *tetrapeptide side chains,* which consist of four amino acids attached to NAMs in the backbone. The amino acids occur in an alternating pattern of D and L forms (see Figure 2.13, page 43). This is unique because the amino acids found in other proteins are L forms. Parallel tetrapeptide side chains may be directly bonded to each other or linked by a *peptide cross-bridge,* consisting of a short chain of amino acids.

Penicillin interferes with the final linking of the peptidoglycan rows by peptide cross-bridges (see Figure 4.13a). As a result, the cell wall is greatly weakened and the cell undergoes **lysis,** destruction caused by rupture of the plasma membrane and the loss of cytoplasm.

Gram-Positive Cell Walls

In most gram-positive bacteria, the cell wall consists of many layers of peptidoglycan, forming a thick, rigid structure (**Figure 4.13b**). By contrast, gram-negative cell walls contain only a thin layer of peptidoglycan (**Figure 4.13c**).

In addition, the cell walls of gram-positive bacteria contain *teichoic acids,* which consist primarily of an alcohol (such as glycerol or ribitol) and phosphate. There are two classes of teichoic acids: *lipoteichoic acid,* which spans the peptidoglycan layer and is linked

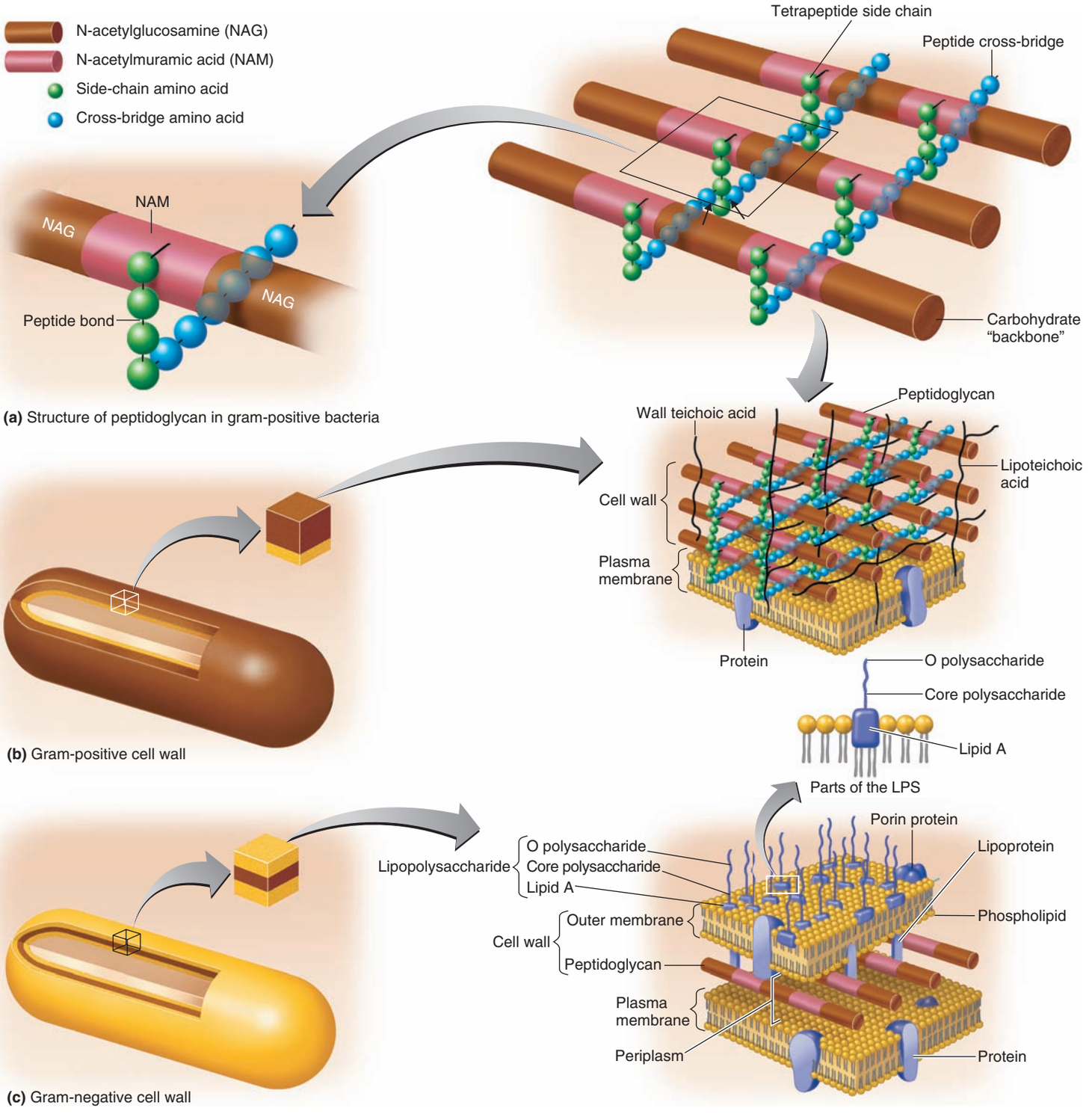


Figure 4.13 **Bacterial cell walls.** (**a**) The structure of peptidoglycan in gram-positive bacteria. Together the carbohydrate backbone (glycan portion) and tetrapeptide side chains (peptide portion) make up peptidoglycan. The frequency of peptide cross-bridges and the number of amino acids in these bridges vary with species of bacteria. The small arrows indicate where penicillin interferes with the linkage of peptidoglycan rows by peptide cross-bridges. (**b**) A gram-positive cell wall. (**c**) A gram-negative cell wall.

Q What are the major structural differences between gram-positive and gram-negative cell walls?

to the plasma membrane, and *wall teichoic acid,* which is linked to the peptidoglycan layer. Because of their negative charge (from the phosphate groups), teichoic acids may bind and regulate the movement of cations (positive ions) into and out of the cell. They may also assume a role in cell growth, preventing extensive wall breakdown and possible cell lysis. Finally, teichoic acids provide much of the wall's antigenic specificity and thus make it possible to identify gram-positive bacteria by certain laboratory tests (see Chapter 10). Similarly, the cell walls of gram-positive streptococci are covered with various polysaccharides that allow them to be grouped into medically significant types.

Gram-Negative Cell Walls

The cell walls of gram-negative bacteria consist of one or a very few layers of peptidoglycan and an outer membrane (see Figure 4.13c). The peptidoglycan is bonded to lipoproteins (lipids covalently linked to proteins) in the outer membrane and is in the *periplasm,* a gel-like fluid between the outer membrane and the plasma membrane. The periplasm contains a high concentration of degradative enzymes and transport proteins. Gram-negative cell walls do not contain teichoic acids. Because the cell walls of gram-negative bacteria contain only a small amount of peptidoglycan, they are more susceptible to mechanical breakage.

The *outer membrane* of the gram-negative cell consists of lipopolysaccharides (LPS), lipoproteins, and phospholipids (see Figure 4.13c). The outer membrane has several specialized functions. Its strong negative charge is an important factor in evading phagocytosis and the actions of complement (lyses cells and promotes phagocytosis), two components of the defenses of the host (discussed in detail in Chapter 16). The outer membrane also provides a barrier to certain antibiotics (for example, penicillin), digestive enzymes such as lysozyme, detergents, heavy metals, bile salts, and certain dyes.

However, the outer membrane does not provide a barrier to all substances in the environment because nutrients must pass through to sustain the metabolism of the cell. Part of the permeability of the outer membrane is due to proteins in the membrane, called **porins,** that form channels. Porins permit the passage of molecules such as nucleotides, disaccharides, peptides, amino acids, vitamin B_{12}, and iron.

The **lipopolysaccharide (LPS)** of the outer membrane is a large complex molecule that contains lipids and carbohydrates and consists of three components: (1) lipid A, (2) a core polysaccharide, and (3) an O polysaccharide. **Lipid A** is the lipid portion of the LPS and is embedded in the top layer of the outer membrane. When gram-negative bacteria die, they release lipid A, which functions as an endotoxin (Chapter 15). Lipid A is responsible for the symptoms associated with infections by gram-negative bacteria such as fever, dilation of blood vessels, shock, and blood clotting. The **core polysaccharide** is attached to lipid A and contains unusual sugars. Its role is structural—to provide stability. The **O polysaccharide** extends outward from the core polysaccharide and is composed of sugar molecules. The O polysaccharide functions as an antigen and is useful for distinguishing species of gram-negative bacteria. For example, the foodborne pathogen *E. coli* O157:H7 is distinguished from other serovars by certain laboratory tests that test for these specific antigens. This role is comparable to that of teichoic acids in gram-positive cells.

Clinical Case

Irene reviews what she knows about gram-negative *K. pneumoniae* bacteria. Although this bacterium is part of normal intestinal microbiota, outside its typical environment it can cause serious infection. *K. pneumoniae* accounts for about 8% of all health care–associated infections. Irene surmises that the bacteria had to come from the hospital somewhere.

What is causing the patients' fever and low blood pressure?

76 **86** 88 95 97

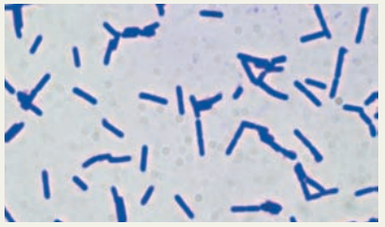
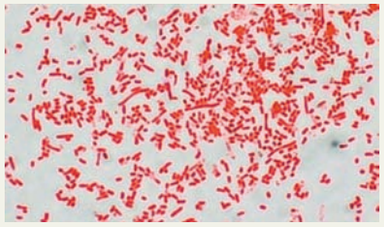
Cell Walls and the Gram Stain Mechanism

Now that you have studied the Gram stain (in Chapter 3, page 68) and the chemistry of the bacterial cell wall (in the previous section), it is easier to understand the mechanism of the Gram stain. The mechanism is based on differences in the structure of the cell walls of gram-positive and gram-negative bacteria and how each reacts to the various reagents (substances used for producing a chemical reaction). Crystal violet, the primary stain, stains both gram-positive and gram-negative cells purple because the dye enters the cytoplasm of both types of cells. When iodine (the mordant) is applied, it forms large crystals with the dye that are too large to escape through the cell wall. The application of alcohol dehydrates the peptidoglycan of gram-positive cells to make it more impermeable to the crystal violet-iodine. The effect on gram-negative cells is quite different; alcohol dissolves the outer membrane of gram-negative cells and even leaves small holes in the thin peptidoglycan layer through which crystal violet-iodine diffuse. Because gram-negative bacteria are colorless after the alcohol wash, the addition of safranin (the counterstain) turns the cells pink or red. Safranin provides a contrasting color to the primary stain (crystal violet). Although gram-positive and gram-negative cells both absorb safranin, the pink or red color of safranin is masked by the darker purple dye previously absorbed by gram-positive cells.

In any population of cells, some gram-positive cells will give a gram-negative response. These cells are usually dead. However, there are a few gram-positive genera that show an increasing number of gram-negative cells as the culture ages. *Bacillus* and *Clostridium* are examples and are often described as *gram-variable.*

A comparison of some of the characteristics of gram-positive and gram-negative bacteria is presented in **Table 4.1**.

TABLE 4.1 Some Comparative Characteristics of Gram-Positive and Gram-Negative Bacteria

Characteristic	Gram-Positive	Gram-Negative
	 LM 6 μm	 LM 15 μm
Gram Reaction	Retain crystal violet dye and stain blue or purple	Can be decolorized to accept counterstain (safranin) and stain pink or red
Peptidoglycan Layer	Thick (multilayered)	Thin (single-layered)
Teichoic Acids	Present in many	Absent
Periplasmic Space	Absent	Present
Outer Membrane	Absent	Present
Lipopolysaccharide (LPS) Content	Virtually none	High
Lipid and Lipoprotein Content	Low (acid-fast bacteria have lipids linked to peptidoglycan)	High (because of presence of outer membrane)
Flagellar Structure	2 rings in basal body	4 rings in basal body
Toxins Produced	Exotoxins	Endotoxins and exotoxins
Resistance to Physical Disruption	High	Low
Cell Wall Disruption by Lysozyme	High	Low (requires pretreatment to destabilize outer membrane)
Susceptibility to Penicillin and Sulfonamide	High	Low
Susceptibility to Streptomycin, Chloramphenicol, and Tetracycline	Low	High
Inhibition by Basic Dyes	High	Low
Susceptibility to Anionic Detergents	High	Low
Resistance to Sodium Azide	High	Low
Resistance to Drying	High	Low

Atypical Cell Walls

Among prokaryotes, certain types of cells have no walls or have very little wall material. These include members of the genus *Mycoplasma* (mī-kō-plaz′mä) and related organisms (see Figure 11.20, page 320). Mycoplasmas are the smallest known bacteria that can grow and reproduce outside living host cells. Because of their size and because they have no cell walls, they pass through most bacterial filters and were first mistaken for viruses. Their plasma membranes are unique among bacteria in having lipids called *sterols*, which are thought to help protect them from lysis (rupture).

Archaea may lack walls or may have unusual walls composed of polysaccharides and proteins but not peptidoglycan. These walls do, however, contain a substance similar to peptidoglycan called *pseudomurein*. Pseudomurein contains N-acetyltalosaminuronic acid instead of NAM and lacks the D-amino acids found in bacterial cell walls. Archaea generally cannot be Gram-stained but appear gram-negative because they do not contain peptidoglycan.

Acid-Fast Cell Walls

Recall from Chapter 3 that the acid-fast stain is used to identify all bacteria of the genus *Mycobacterium* and pathogenic species of

Nocardia. These bacteria contain high concentrations (60%) of a hydrophobic waxy lipid (**mycolic acid**) in their cell wall that prevents the uptake of dyes, including those used in the Gram stain. The mycolic acid forms a layer outside of a thin layer of peptidoglycan. The mycolic acid and peptidoglycan are held together by a polysaccharide. The hydrophobic waxy cell wall causes both cultures of *Mycobacterium* to clump and to stick to the walls of the flask. Acid-fast bacteria can be stained with carbolfuchsin; heating enhances penetration of the stain. The carbolfuchsin penetrates the cell wall, binds to cytoplasm, and resists removal by washing with acid-alcohol. Acid-fast bacteria retain the red color of carbolfuchsin because it is more soluble in the cell wall mycolic acid than in the acid-alcohol. If the mycolic acid layer is removed from the cell wall of acid-fast bacteria, they will stain gram-positive with the Gram stain.

Damage to the Cell Wall

Chemicals that damage bacterial cell walls, or interfere with their synthesis, often do not harm the cells of an animal host because the bacterial cell wall is made of chemicals unlike those in eukaryotic cells. Thus, cell wall synthesis is the target for some antimicrobial drugs. One way the cell wall can be damaged is by exposure to the digestive enzyme *lysozyme*. This enzyme occurs naturally in some eukaryotic cells and is a constituent of perspiration, tears, mucus, and saliva. Lysozyme is particularly active on the major cell wall components of most gram-positive bacteria, making them vulnerable to lysis. Lysozyme catalyzes hydrolysis of the bonds between the sugars in the repeating disaccharide "backbone" of peptidoglycan. This act is analogous to cutting the steel supports of a bridge with a cutting torch: the gram-positive cell wall is almost completely destroyed by lysozyme. The cellular contents that remain surrounded by the plasma membrane may remain intact if lysis does not occur; this wall-less cell is termed a **protoplast.** Typically, a protoplast is spherical and is still capable of carrying on metabolism.

Some members of the genus *Proteus,* as well as other genera, can lose their cell walls and swell into irregularly shaped cells called **L forms,** named for the Lister Institute, where they were discovered. They may form spontaneously or develop in response to penicillin (which inhibits cell wall formation) or lysozyme (which removes the cell wall). L forms can live and divide repeatedly or return to the walled state.

When lysozyme is applied to gram-negative cells, usually the wall is not destroyed to the same extent as in gram-positive cells; some of the outer membrane also remains. In this case, the cellular contents, plasma membrane, and remaining outer wall layer are called a **spheroplast,** also a spherical structure. For lysozyme to exert its effect on gram-negative cells, the cells are first treated with EDTA (ethylenediaminetetraacetic acid). EDTA weakens ionic bonds in the outer membrane and thereby damages it, giving the lysozyme access to the peptidoglycan layer.

Protoplasts and spheroplasts burst in pure water or very dilute salt or sugar solutions because the water molecules from the surrounding fluid rapidly move into and enlarge the cell, which has a much lower internal concentration of water. This rupturing, called **osmotic lysis,** will be discussed in detail shortly.

As noted earlier, certain antibiotics, such as penicillin, destroy bacteria by interfering with the formation of the peptide cross-bridges of peptidoglycan, thus preventing the formation of a functional cell wall. Most gram-negative bacteria are not as susceptible to penicillin as gram-positive bacteria are because the outer membrane of gram-negative bacteria forms a barrier that inhibits the entry of this and other substances, and gram-negative bacteria have fewer peptide cross-bridges. However, gram-negative bacteria are quite susceptible to some β-lactam antibiotics that penetrate the outer membrane better than penicillin. Antibiotics will be discussed in more detail in Chapter 20.

Clinical Case

The outer membrane of *K. pneumoniae*'s gram-negative cell wall contains the endotoxin, lipid A, which causes fever and capillary dilation.

Irene works with Joe's, Jessie's , and Maureen's physicians to combat this potentially deadly infection. Irene is particularly concerned about Jessie because of her already weakened respiratory condition. All three patients are treated with a β-lactam antibiotic, imipenem. *Klebsiella* bacteria are resistant to many antibiotics, but imipenem seems to be working for Joe and Maureen. Jessie, however, is getting worse.

Why are Jessie's symptoms worsening if the bacteria are being killed?

76 86 **88** 95 97

CHECK YOUR UNDERSTANDING

- Why are drugs that target cell wall synthesis useful? **4-5**
- Why are mycoplasmas resistant to antibiotics that interfere with cell wall synthesis? **4-6**
- How do protoplasts differ from L forms? **4-7**

Structures Internal to the Cell Wall

LEARNING OBJECTIVES

4-8 Describe the structure, chemistry, and functions of the prokaryotic plasma membrane.

4-9 Define *simple diffusion*, *facilitated diffusion*, *osmosis*, *active transport*, and *group translocation.*

4-10 Identify the functions of the nucleoid and ribosomes.

4-11 Identify the functions of four inclusions.

4-12 Describe the functions of endospores, sporulation, and endospore germination.

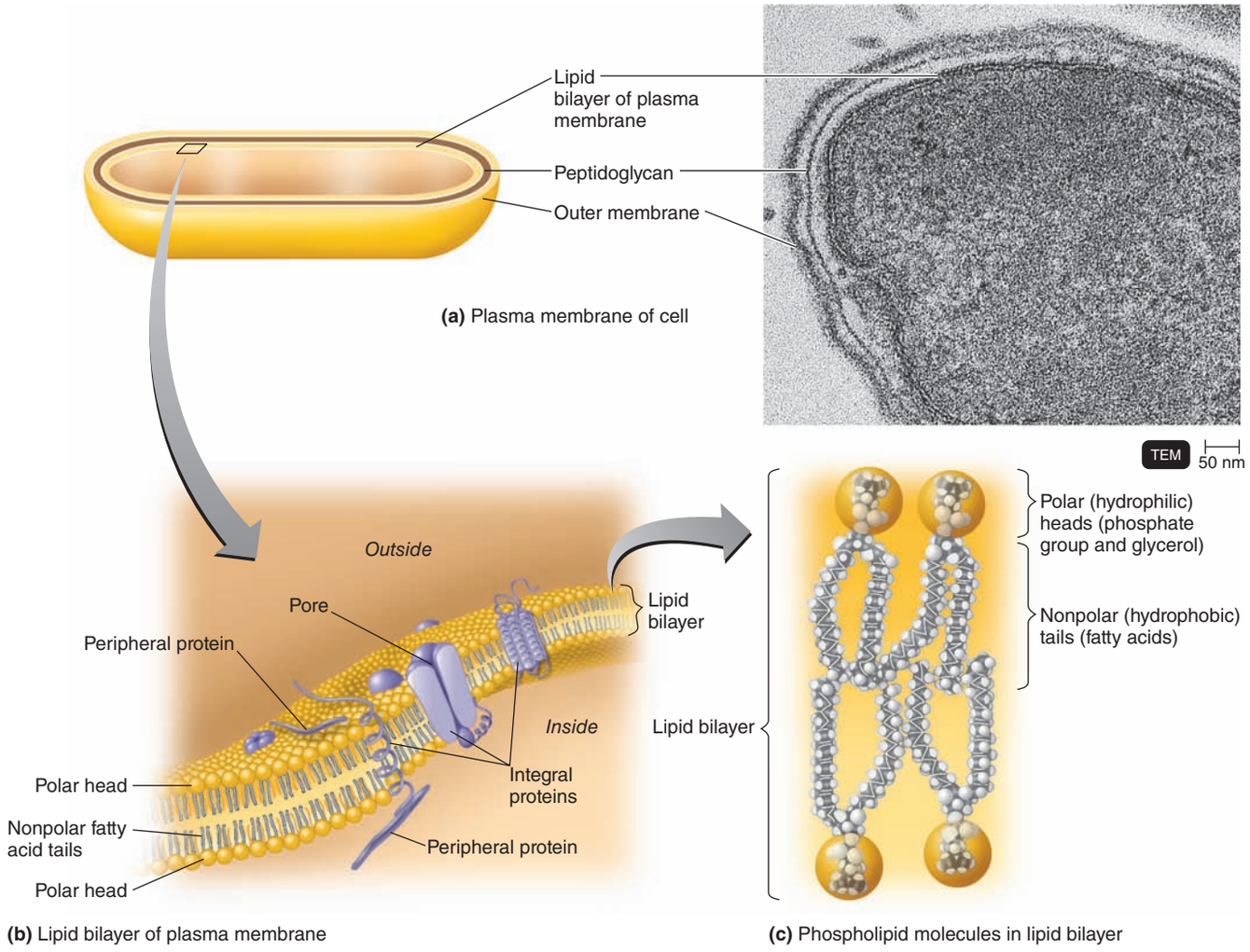


Figure 4.14 Plasma membrane. (**a**) A diagram and micrograph showing the lipid bilayer forming the inner plasma membrane of the gram-negative bacterium *Aquaspirillum serpens*. Layers of the cell wall, including the outer membrane, can be seen outside the inner membrane. (**b**) A portion of the inner membrane showing the lipid bilayer and proteins. The outer membrane of gram-negative bacteria is also a lipid bilayer. (**c**) Space-filling models of several phospholipid molecules as they are arranged in the lipid bilayer.

Q What is the difference between a peripheral and an integral protein?

Thus far, we have discussed the prokaryotic cell wall and structures external to it. We will now look inside the prokaryotic cell and discuss the structures and functions of the plasma membrane and components within the cytoplasm of the cell.

The Plasma (Cytoplasmic) Membrane

The **plasma (cytoplasmic) membrane** (or *inner membrane*) is a thin structure lying inside the cell wall and enclosing the cytoplasm of the cell (see Figure 4.6). The plasma membrane of prokaryotes consists primarily of phospholipids (see Figure 2.10, page 40), which are the most abundant chemicals in the membrane, and proteins. Eukaryotic plasma membranes also contain carbohydrates and sterols, such as cholesterol. Because they lack sterols, prokaryotic plasma membranes are less rigid than eukaryotic membranes. One exception is the wall-less prokaryote *Mycoplasma*, which contains membrane sterols.

Structure

In electron micrographs, prokaryotic and eukaryotic plasma membranes (and the outer membranes of gram-negative bacteria) look like two-layered structures; there are two dark lines with a light space between the lines (Figure 4.14a). The phospholipid molecules are arranged in two parallel rows, called a *lipid bilayer* (Figure 4.14b). As introduced in Chapter 2, each phospholipid molecule contains a polar head, composed of a phosphate group and glycerol that is hydrophilic (water-loving) and soluble in water, and nonpolar tails, composed of fatty acids that are hydrophobic (water-fearing) and insoluble in water (Figure 4.14c). The polar heads are on the two surfaces of the lipid bilayer, and the nonpolar tails are in the interior of the bilayer.

The protein molecules in the membrane can be arranged in a variety of ways. Some, called *peripheral proteins*, are easily removed from the membrane by mild treatments and lie at

Figure 4.15 Chromatophores. In this micrograph of *Rhodospirillum rubrum,* a purple (nonsulfur) bacterium, the chromatophores are clearly visible.

Q What is the function of chromatophores?

the inner or outer surface of the membrane. They may function as enzymes that catalyze chemical reactions, as a "scaffold" for support, and as mediators of changes in membrane shape during movement. Other proteins, called *integral proteins,* can be removed from the membrane only after disrupting the lipid bilayer (by using detergents, for example). Most integral proteins penetrate the membrane completely and are called *transmembrane proteins.* Some integral proteins are channels that have a pore, or hole, through which substances enter and exit the cell.

Many of the proteins and some of the lipids on the outer surface of the plasma membrane have carbohydrates attached to them. Proteins attached to carbohydrates are called **glycoproteins;** lipids attached to carbohydrates are called **glycolipids.** Both glycoproteins and glycolipids help protect and lubricate the cell and are involved in cell-to-cell interactions. For example, glycoproteins play a role in certain infectious diseases. The influenza virus and the toxins that cause cholera and botulism enter their target cells by first binding to glycoproteins on their plasma membranes.

Studies have demonstrated that the phospholipid and protein molecules in membranes are not static but move quite freely within the membrane surface. This movement is most probably associated with the many functions performed by the plasma membrane. Because the fatty acid tails cling together, phospholipids in the presence of water form a self-sealing bilayer; as a result, breaks and tears in the membrane heal themselves. The membrane must be about as viscous as olive oil, which allows membrane proteins to move freely enough to perform their functions without destroying the structure of the membrane. This dynamic arrangement of phospholipids and proteins is referred to as the **fluid mosaic model.**

Functions

The most important function of the plasma membrane is to serve as a selective barrier through which materials enter and exit the cell. In this function, plasma membranes have **selective permeability** (sometimes called *semipermeability*). This term indicates that certain molecules and ions pass through the membrane, but that others are prevented from passing through it. The permeability of the membrane depends on several factors. Large molecules (such as proteins) cannot pass through the plasma membrane, possibly because these molecules are larger than the pores in integral proteins that function as channels. But smaller molecules (such as water, oxygen, carbon dioxide, and some simple sugars) usually pass through easily. Ions penetrate the membrane very slowly. Substances that dissolve easily in lipids (such as oxygen, carbon dioxide, and nonpolar organic molecules) enter and exit more easily than other substances because the membrane consists mostly of phospholipids. The movement of materials across plasma membranes also depends on transporter molecules, which will be described shortly.

Plasma membranes are also important to the breakdown of nutrients and the production of energy. The plasma membranes of bacteria contain enzymes capable of catalyzing the chemical reactions that break down nutrients and produce ATP. In some bacteria, pigments and enzymes involved in photosynthesis are found in infoldings of the plasma membrane that extend into the cytoplasm. These membranous structures are called **chromatophores** or **thylakoids** (Figure 4.15).

When viewed with an electron microscope, bacterial plasma membranes often appear to contain one or more large, irregular folds called **mesosomes.** Many functions have been proposed for mesosomes. However, it is now known that they are artifacts, not true cell structures. Mesosomes are believed to be folds in the plasma membrane that develop by the process used for preparing specimens for electron microscopy. **Animations** Membrane Structure; Membrane Permeability

Destruction of the Plasma Membrane by Antimicrobial Agents

Because the plasma membrane is vital to the bacterial cell, it is not surprising that several antimicrobial agents exert their effects at this site. In addition to the chemicals that damage the cell wall and thereby indirectly expose the membrane to injury, many compounds specifically damage plasma membranes. These compounds include certain alcohols and quaternary ammonium compounds, which are used as disinfectants. By disrupting the membrane's phospholipids, a group of antibiotics known as the *polymyxins* cause leakage of intracellular contents and subsequent cell death. This mechanism will be discussed in Chapter 20.

The Movement of Materials across Membranes

Materials move across plasma membranes of both prokaryotic and eukaryotic cells by two kinds of processes: passive and active. In *passive processes,* substances cross the membrane from an area of high concentration to an area of low concentration (move with the concentration gradient, or difference), without any expenditure of energy (ATP) by the cell. In *active processes,* the cell must use energy (ATP) to move substances from areas of low concentration to areas of high concentration (against the concentration gradient).

Passive Processes

Passive processes include simple diffusion, facilitated diffusion, and osmosis.

Simple diffusion is the net (overall) movement of molecules or ions from an area of high concentration to an area of low concentration (Figure 4.16 and Figure 4.17a). The movement continues until the molecules or ions are evenly distributed. The point of even distribution is called *equilibrium.* Cells rely on simple diffusion to transport certain small molecules, such as oxygen and carbon dioxide, across their cell membranes.

In **facilitated diffusion,** integral membrane proteins function as channels or carriers that facilitate the movement of ions or large molecules across the plasma membrane. Such integral proteins are called *transporters* or *permeases.* Facilitated diffusion is similar to simple diffusion in that the cell *does not* expend energy, because the substance moves from a high to a low concentration. The process differs from simple diffusion in its use of transporters. Some transporters permit the passage of mostly small, inorganic ions that are too hydrophilic to penetrate the nonpolar interior of the lipid bilayer (Figure 4.17b). These transporters, which are common in prokaryotes, are nonspecific and allow the passage of a wide variety of ions (or even small molecules). Other transporters, which are common in eukaryotes, are specific and transport only specific, usually larger, molecules, such as simple sugars (glucose, fructose, and galactose) and vitamins. In this process, the transported substance binds to a specific transporter on the outer surface of the plasma membrane, which undergoes a change of shape; then the transporter releases the substance on the other side of the membrane (Figure 4.17c).

Figure 4.16 **The principle of simple diffusion.** (**a**) After a dye pellet is put into a beaker of water, the molecules of dye in the pellet diffuse into the water from an area of high dye concentration to areas of low dye concentration. (**b**) The dye potassium permanganate in the process of diffusing.

Q Why are passive processes important to a cell?

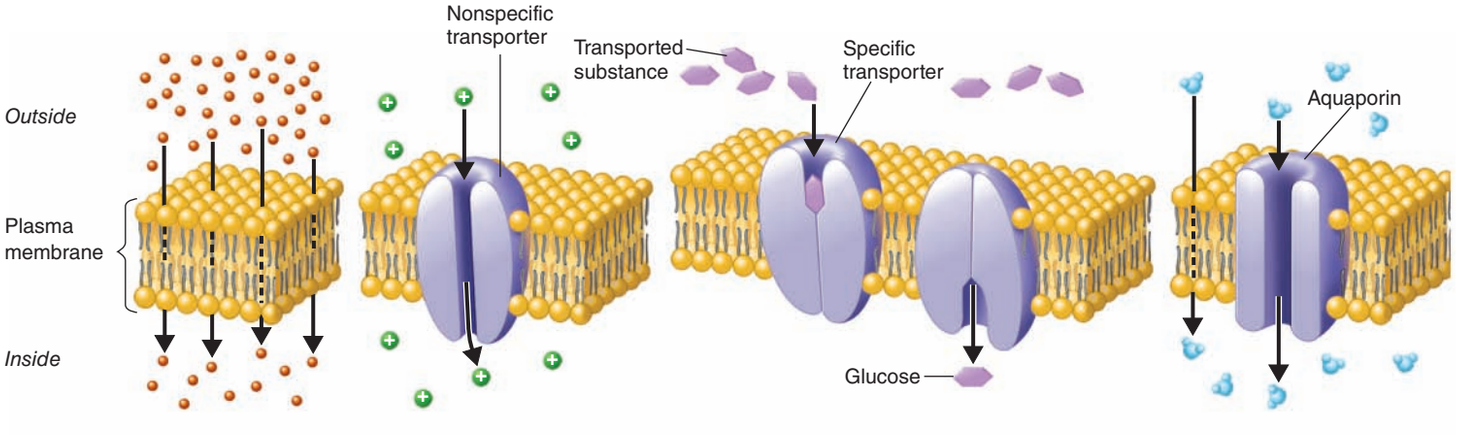


Figure 4.17 **Passive processes.**

Q How does simple diffusion differ from facilitated diffusion?

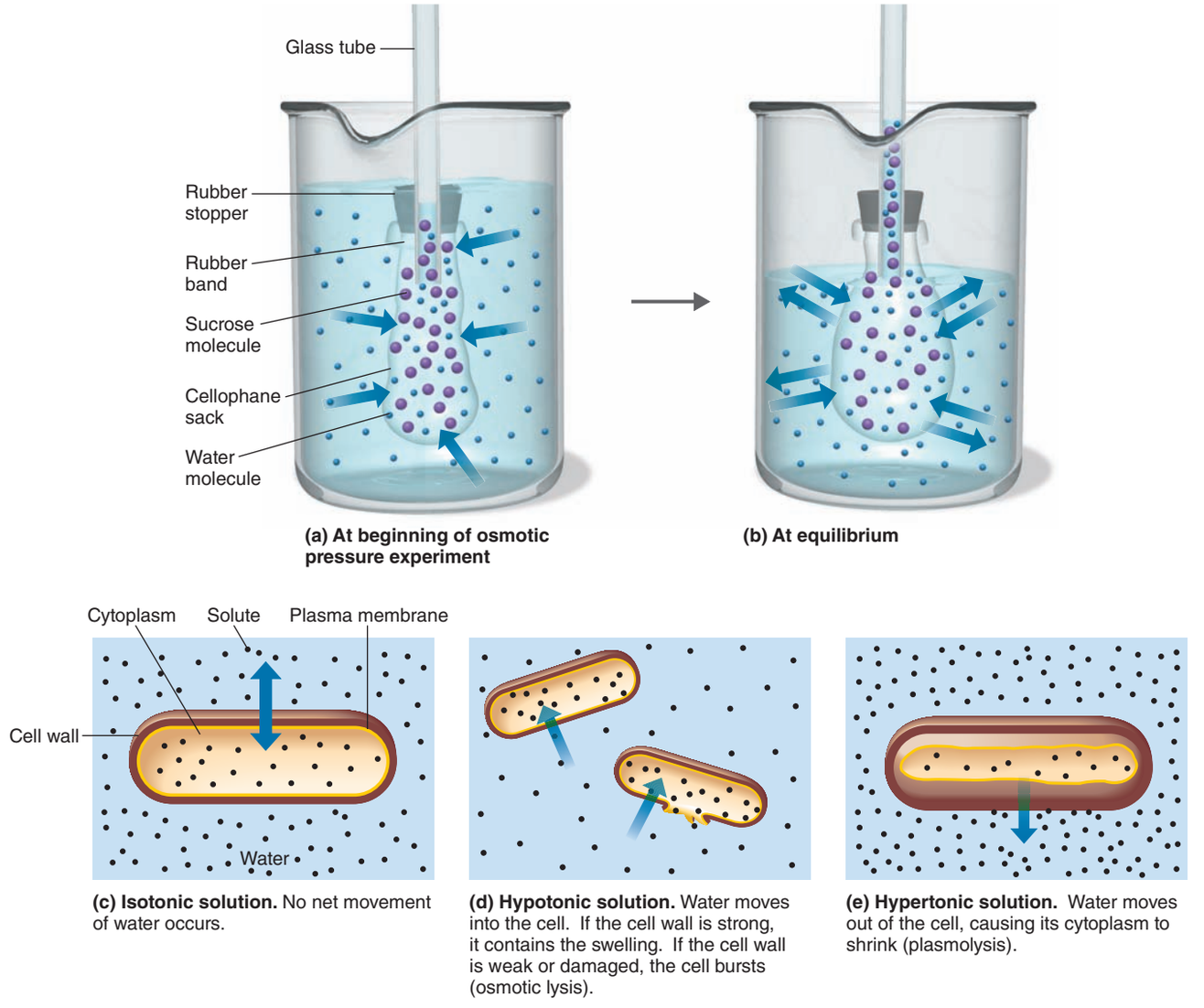


Figure 4.18 The principle of osmosis. (**a**) Setup at the beginning of an osmotic pressure experiment. Water molecules start to move from the beaker into the sack along the concentration gradient. (**b**) Setup at equilibrium. The osmotic pressure exerted by the solution in the sack pushes water molecules from the sack back into the beaker to balance the rate of water entry into the sack. The height of the solution in the glass tube at equilibrium is a measure of the osmotic pressure. (**c**)–(**e**) The effects of various solutions on bacterial cells.

Q Why is osmosis important?

In some cases, molecules that bacteria need are too large to be transported into the cells by these methods. Most bacteria, however, produce enzymes that can break down large molecules into simpler ones (such as proteins into amino acids, or polysaccharides into simple sugars). Such enzymes, which are released by the bacteria into the surrounding medium, are appropriately called *extracellular enzymes*. Once the enzymes degrade the large molecules, the subunits move into the cell with the help of transporters. For example, specific carriers retrieve DNA bases, such as the purine guanine, from extracellular media and bring them into the cell's cytoplasm.

Osmosis is the net movement of solvent molecules across a selectively permeable membrane from an area with a high concentration of solvent molecules (low concentration of solute molecules) to an area of low concentration of solvent molecules (high concentration of solute molecules). In living systems, the chief solvent is water. Water molecules may pass through plasma membranes by moving through the lipid bilayer by simple diffusion or through integral membrane proteins, called *aquaporins*, that function as water channels (Figure 4.17d).

Osmosis may be demonstrated with the apparatus shown in Figure 4.18a. A sack constructed from cellophane, which is a selectively permeable membrane, is filled with a solution of 20% sucrose (table sugar). The cellophane sack is placed into a beaker containing distilled water. Initially, the concentrations of water on either side of the membrane are different. Because of the

sucrose molecules, the concentration of water is lower inside the cellophane sack. Therefore, water moves from the beaker (where its concentration is higher) into the cellophane sack (where its concentration is lower).

There is no movement of sugar out of the cellophane sack into the beaker, however, because the cellophane is impermeable to molecules of sugar—the sugar molecules are too large to go through the pores of the membrane. As water moves into the cellophane sack, the sugar solution becomes increasingly dilute, and, because the cellophane sack has expanded to its limit as a result of an increased volume of water, water begins to move up the glass tube. In time, the water that has accumulated in the cellophane sack and the glass tube exerts a downward pressure that forces water molecules out of the cellophane sack and back into the beaker. This movement of water through a selectively permeable membrane produces osmotic pressure. **Osmotic pressure** is the pressure required to prevent the movement of pure water (water with no solutes) into a solution containing some solutes. In other words, osmotic pressure is the pressure needed to stop the flow of water across the selectively permeable membrane (cellophane). When water molecules leave and enter the cellophane sack at the same rate, equilibrium is reached (**Figure 4.18b**).

A bacterial cell may be subjected to any of three kinds of osmotic solutions: isotonic, hypotonic, or hypertonic. An **isotonic solution** is a medium in which the overall concentration of solutes equals that found inside a cell (*iso* means equal). Water leaves and enters the cell at the same rate (no net change); the cell's contents are in equilibrium with the solution outside the cell wall (**Figure 4.18c**).

Earlier we mentioned that lysozyme and certain antibiotics (such as penicillin) damage bacterial cell walls, causing the cells to rupture, or lyse. Such rupturing occurs because bacterial cytoplasm usually contains such a high concentration of solutes that, when the wall is weakened or removed, additional water enters the cell by osmosis. The damaged (or removed) cell wall cannot constrain the swelling of the cytoplasmic membrane, and the membrane bursts. This is an example of osmotic lysis caused by immersion in a hypotonic solution. A **hypotonic solution** outside the cell is a medium whose concentration of solutes is lower than that inside the cell (*hypo* means under or less). Most bacteria live in hypotonic solutions, and the cell wall resists further osmosis and protects cells from lysis. Cells with weak cell walls, such as gram-negative bacteria, may burst or undergo osmotic lysis as a result of excessive water intake (**Figure 4.18d**).

A **hypertonic solution** is a medium having a higher concentration of solutes than inside the cell has (*hyper* means above or more). Most bacterial cells placed in a hypertonic solution shrink and collapse or *plasmolyze* because water leaves the cells by osmosis (**Figure 4.18e**). Keep in mind that the terms *isotonic, hypotonic,* and *hypertonic* describe the concentration of solutions outside the cell *relative to* the concentration inside the cell. **Animations** Passive Transport: Principles of Diffusion, Special Types of Diffusion

Active Processes

Simple diffusion and facilitated diffusion are useful mechanisms for transporting substances into cells when the concentrations of the substances are greater outside the cell. However, when a bacterial cell is in an environment in which nutrients are in low concentration, the cell must use active processes, such as active transport and group translocation, to accumulate the needed substances.

In performing **active transport,** the cell *uses energy* in the form of ATP to move substances across the plasma membrane. Among the substances actively transported are ions (for example Na^+, K^+, H^+, Ca^{2+}, and Cl^-), amino acids, and simple sugars. Although these substances can also be moved into cells by passive processes, their movement by active processes can go against the concentration gradient, allowing a cell to accumulate needed materials. The movement of a substance in active transport is usually from outside to inside, even though the concentration might be much higher inside the cell. Like facilitated diffusion, active transport depends on transporter proteins in the plasma membrane (see Figure 4.17b, c). There appears to be a different transporter for each transported substance or group of closely related transported substances. Active transport enables microbes to move substances across the plasma membrane at a constant rate, even if they are in short supply.

In active transport, the substance that crosses the membrane is not altered by transport across the membrane. In **group translocation,** a special form of active transport that occurs exclusively in prokaryotes, the substance is chemically altered during transport across the membrane. Once the substance is altered and inside the cell, the plasma membrane is impermeable to it, so it remains inside the cell. This important mechanism enables a cell to accumulate various substances even though they may be in low concentrations outside the cell. Group translocation requires energy supplied by high-energy phosphate compounds, such as phosphoenolpyruvic acid (PEP).

One example of group translocation is the transport of the sugar glucose, which is often used in growth media for bacteria. While a specific carrier protein is transporting the glucose molecule across the membrane, a phosphate group is added to the sugar. This phosphorylated form of glucose, which cannot be transported out, can then be used in the cell's metabolic pathways.

Some eukaryotic cells (those without cell walls) can use two additional active transport processes called phagocytosis and pinocytosis. These processes, which do not occur in bacteria, are explained on page 100. **Animations** Active Transport: Types, Overview

CHECK YOUR UNDERSTANDING

- Which agents can cause injury to the bacterial plasma membrane? **4-8**
- How are simple diffusion and facilitated diffusion similar? How are they different? **4-9**

Cytoplasm

For a prokaryotic cell, the term **cytoplasm** refers to the substance of the cell inside the plasma membrane (see Figure 4.6). Cytoplasm is about 80% water and contains primarily proteins (enzymes), carbohydrates, lipids, inorganic ions, and many low-molecular-weight compounds. Inorganic ions are present in much higher concentrations in cytoplasm than in most media. Cytoplasm is thick, aqueous, semitransparent, and elastic. The major structures in the cytoplasm of prokaryotes are a nucleoid (containing DNA), particles called ribosomes, and reserve deposits called inclusions. Protein filaments in the cytoplasm are most likely responsible for the rod and helical cell shapes of bacteria.

Prokaryotic cytoplasm lacks certain features of eukaryotic cytoplasm, such as a cytoskeleton and cytoplasmic streaming. These features will be described later.

The Nucleoid

The **nucleoid** of a bacterial cell (see Figure 4.6) usually contains a single long, continuous, and frequently circularly arranged thread of double-stranded DNA called the **bacterial chromosome.** This is the cell's genetic information, which carries all the information required for the cell's structures and functions. Unlike the chromosomes of eukaryotic cells, bacterial chromosomes are not surrounded by a nuclear envelope (membrane) and do not include histones. The nucleoid can be spherical, elongated, or dumbbell-shaped. In actively growing bacteria, as much as 20% of the cell volume is occupied by DNA because such cells presynthesize nuclear material for future cells. The chromosome is attached to the plasma membrane. Proteins in the plasma membrane are believed to be responsible for replication of the DNA and segregation of the new chromosomes to daughter cells during cell division.

In addition to the bacterial chromosome, bacteria often contain small usually circular, double-stranded DNA molecules called **plasmids** (see the F factor in Figure 8.26a, page 234). These molecules are extrachromosomal genetic elements; that is, they are not connected to the main bacterial chromosome, and they replicate independently of chromosomal DNA. Research indicates that plasmids are associated with plasma membrane proteins. Plasmids usually contain from 5 to 100 genes that are generally not crucial for the survival of the bacterium under normal environmental conditions; plasmids may be gained or lost without harming the cell. Under certain conditions, however, plasmids are an advantage to cells. Plasmids may carry genes for such activities as antibiotic resistance, tolerance to toxic metals, the production of toxins, and the synthesis of enzymes. Plasmids can be transferred from one bacterium to another. In fact, plasmid DNA is used for gene manipulation in biotechnology.

Ribosomes

All eukaryotic and prokaryotic cells contain **ribosomes,** which function as the sites of protein synthesis. Cells that have high rates of protein synthesis, such as those that are actively growing, have a large number of ribosomes. The cytoplasm of a prokaryotic cell contains tens of thousands of these very small structures, which give the cytoplasm a granular appearance (see Figure 4.6).

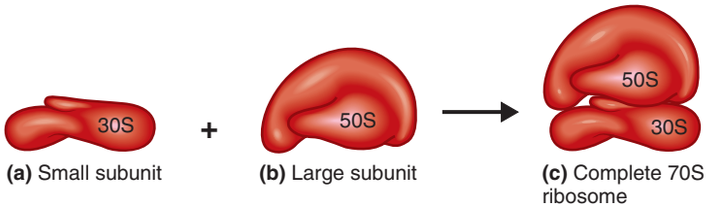


Figure 4.19 The prokaryotic ribosome. (**a**) A small 30S subunit and (**b**) a large 50S subunit make up (**c**) the complete 70S prokaryotic ribosome.

Q What is the importance of the differences between prokaryotic and eukaryotic ribosomes with regard to antibiotic therapy?

Ribosomes are composed of two subunits, each of which consists of protein and a type of RNA called *ribosomal RNA (rRNA).* Prokaryotic ribosomes differ from eukaryotic ribosomes in the number of proteins and rRNA molecules they contain; they are also somewhat smaller and less dense than ribosomes of eukaryotic cells. Accordingly, prokaryotic ribosomes are called 70S ribosomes (**Figure 4.19**), and those of eukaryotic cells are known as 80S ribosomes. The letter S refers to Svedberg units, which indicate the relative rate of sedimentation during ultra-high-speed centrifugation. Sedimentation rate is a function of the size, weight, and shape of a particle. The subunits of a 70S ribosome are a small 30S subunit containing one molecule of rRNA and a larger 50S subunit containing two molecules of rRNA.

Several antibiotics work by inhibiting protein synthesis on prokaryotic ribosomes. Antibiotics such as streptomycin and gentamicin attach to the 30S subunit and interfere with protein synthesis. Other antibiotics, such as erythromycin and chloramphenicol, interfere with protein synthesis by attaching to the 50S subunit. Because of differences in prokaryotic and eukaryotic ribosomes, the microbial cell can be killed by the antibiotic while the eukaryotic host cell remains unaffected.

Inclusions

Within the cytoplasm of prokaryotic cells are several kinds of reserve deposits, known as **inclusions.** Cells may accumulate certain nutrients when they are plentiful and use them when the environment is deficient. Evidence suggests that macromolecules concentrated in inclusions avoid the increase in osmotic pressure that would result if the molecules were dispersed in the cytoplasm. Some inclusions are common to a wide variety of bacteria, whereas others are limited to a small number of species and therefore serve as a basis for identification.

Clinical Case

The antibiotic killed the bacteria, but endotoxin is released when the cells die, causing Jessie's condition to worsen. Jessie's physician prescribes polymyxin, an antibiotic primarily used for imipenem-resistant gram-negative infections, to which Jessie responds favorably.

As Irene sits with Jessie, she notices another patient being fed ice chips by a relative. On a hunch, Irene hurries back to her office to find out whether the ice machines had been swabbed. They have not. She immediately orders the machines to be swabbed and cultured. Her hunch turns out to be correct: the samples are positive for *K. pneumoniae*. Bacteria growing in the hospital's water pipes entered the ice machine with incoming water.

How can *K. pneumoniae* grow in water pipes?

76 86 88 **95** 97

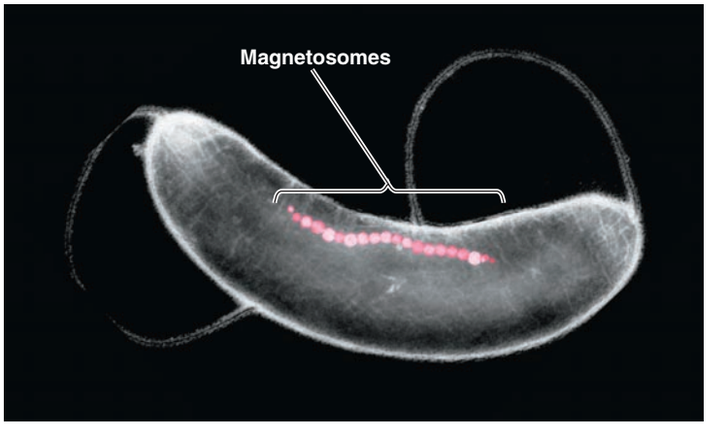


Figure 4.20 Magnetosomes. This micrograph of *Magnetospirillum magnetotacticum* shows a chain of magnetosomes. This bacterium is usually found in shallow freshwater mud.

How do magnetosomes behave like magnets?

Metachromatic Granules

Metachromatic granules are large inclusions that take their name from the fact that they sometimes stain red with certain blue dyes such as methylene blue. Collectively they are known as **volutin.** Volutin represents a reserve of inorganic phosphate (polyphosphate) that can be used in the synthesis of ATP. It is generally formed by cells that grow in phosphate-rich environments. Metachromatic granules are found in algae, fungi, and protozoa, as well as in bacteria. These granules are characteristic of *Corynebacterium diphtheriae* (kô-rī-nē-bak-ti′ rē-um dif-thi′ rē-ī), the causative agent of diphtheria; thus, they have diagnostic significance.

Polysaccharide Granules

Inclusions known as **polysaccharide granules** typically consist of glycogen and starch, and their presence can be demonstrated when iodine is applied to the cells. In the presence of iodine, glycogen granules appear reddish brown and starch granules appear blue.

Lipid Inclusions

Lipid inclusions appear in various species of *Mycobacterium, Bacillus, Azotobacter* (ä-zō-tō-bak′ tér), *Spirillum* (spī-ril′ lum), and other genera. A common lipid-storage material, one unique to bacteria, is the polymer *poly-β-hydroxybutyric acid.* Lipid inclusions are revealed by staining cells with fat-soluble dyes, such as Sudan dyes.

Sulfur Granules

Certain bacteria—for example, the "sulfur bacteria" that belong to the genus *Thiobacillus*—derive energy by oxidizing sulfur and sulfur-containing compounds. These bacteria may deposit **sulfur granules** in the cell, where they serve as an energy reserve.

Carboxysomes

Carboxysomes are inclusions that contain the enzyme ribulose 1,5-diphosphate carboxylase. Photosynthetic bacteria use carbon dioxide as their sole source of carbon and require this enzyme for carbon dioxide fixation. Among the bacteria containing carboxysomes are nitrifying bacteria, cyanobacteria, and thiobacilli.

Gas Vacuoles

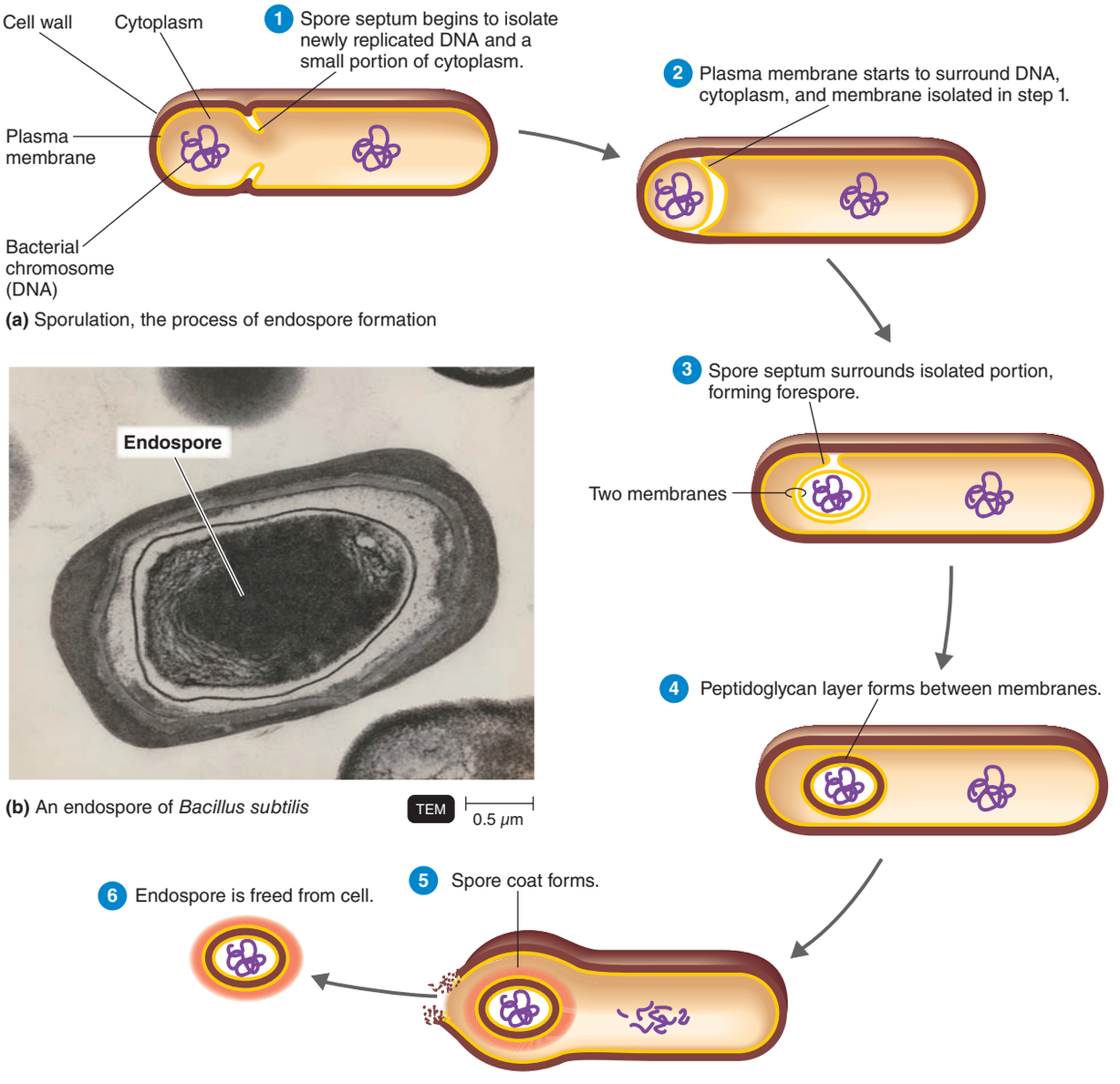
Hollow cavities found in many aquatic prokaryotes, including cyanobacteria, anoxygenic photosynthetic bacteria, and halobacteria are called **gas vacuoles.** Each vacuole consists of rows of several individual *gas vesicles,* which are hollow cylinders covered by protein. Gas vacuoles maintain buoyancy so that the cells can remain at the depth in the water appropriate for them to receive sufficient amounts of oxygen, light, and nutrients.

Magnetosomes

Magnetosomes are inclusions of iron oxide (Fe_3O_4) surrounded by invaginations of the plasma membrane. Magnetosomes are formed by several gram-negative bacteria such as *Magnetospirillum magnetotacticum* and act like magnets (**Figure 4.20**). Bacteria may use magnetosomes to move downward until they reach a suitable attachment site. In vitro, magnetosomes can decompose hydrogen peroxide, which forms in cells in the presence of oxygen. Researchers speculate that magnetosomes may protect the cell against hydrogen peroxide accumulation.

Endospores

When essential nutrients are depleted, certain gram-positive bacteria, such as those of the genera *Clostridium* and *Bacillus,* form specialized "resting" cells called **endospores** (**Figure 4.21**). As



(a) Sporulation, the process of endospore formation

(b) An endospore of *Bacillus subtilis*

Figure 4.21 **Formation of endospores by sporulation.**

Q What properties make endospores resistant to processes that normally kill vegetative cells?

you will see later, some members of the genus *Clostridium* cause diseases such as gangrene, tetanus, botulism, and food poisoning. Some members of the genus *Bacillus* cause anthrax and food poisoning. Unique to bacteria, endospores are highly durable dehydrated cells with thick walls and additional layers. They are formed internal to the bacterial cell membrane.

When released into the environment, they can survive extreme heat, lack of water, and exposure to many toxic chemicals and radiation. For example, 7500-year-old endospores of *Thermoactinomyces vulgaris* (thėr-mō-ak-tin-ō-mī′sēs vul-ga′-ris) from the freezing muds of Elk Lake in Minnesota have germinated when rewarmed and placed in a nutrient medium, and 25- to 40-million-year-old endospores found in the gut of a stingless bee entombed in amber (hardened tree resin) in the Dominican Republic are reported to have germinated when placed in nutrient media. Although true endospores are found in gram-positive bacteria, one gram-negative species, *Coxiella burnetii* (käks-ē-el′lä bėr-ne′tē-ē), the cause of Q fever, forms endosporelike structures that resist heat and chemicals and can be stained with endospore stains (see Figure 24.14, page 696).

The process of endospore formation within a vegetative cell takes several hours and is known as **sporulation** or **sporogenesis** (Figure 4.21a). Vegetative cells of endospore-forming bacteria begin sporulation when a key nutrient, such as the carbon or

nitrogen source, becomes scarce or unavailable. In the first observable stage of sporulation, a newly replicated bacterial chromosome and a small portion of cytoplasm are isolated by an ingrowth of the plasma membrane called a *spore septum*. The spore septum becomes a double-layered membrane that surrounds the chromosome and cytoplasm. This structure, entirely enclosed within the original cell, is called a *forespore*. Thick layers of peptidoglycan are laid down between the two membrane layers. Then a thick *spore coat* of protein forms around the outside membrane; this coat is responsible for the resistance of endospores to many harsh chemicals. The original cell is degraded, and the endospore is released.

The diameter of the endospore may be the same as, smaller than, or larger than the diameter of the vegetative cell. Depending on the species, the endospore might be located *terminally* (at one end), *subterminally* (near one end; **Figure 4.21b**), or *centrally* inside the vegetative cell. When the endospore matures, the vegetative cell wall ruptures (lyses), killing the cell, and the endospore is freed.

Most of the water present in the forespore cytoplasm is eliminated by the time sporulation is complete, and endospores do not carry out metabolic reactions. The endospore contains a large amount of an organic acid called *dipicolinic acid* (DPA), which is accompanied by a large number of calcium ions. Evidence indicates that DPA protects the endospore DNA against damage. The highly dehydrated endospore core contains only DNA, small amounts of RNA, ribosomes, enzymes, and a few important small molecules. These cellular components are essential for resuming metabolism later.

Endospores can remain dormant for thousands of years. An endospore returns to its vegetative state by a process called **germination.** Germination is triggered by physical or chemical damage to the endospore's coat. The endospore's enzymes then break down the extra layers surrounding the endospore, water enters, and metabolism resumes. Because one vegetative cell forms a single endospore, which, after germination, remains one cell, sporulation in bacteria is *not* a means of reproduction. This process does not increase the number of cells. Bacterial endospores differ from spores formed by (prokaryotic) actinomycetes and the eukaryotic fungi and algae, which detach from the parent and develop into another organism and, therefore, represent reproduction.

Endospores are important from a clinical viewpoint and in the food industry because they are resistant to processes that normally kill vegetative cells. Such processes include heating, freezing, desiccation, use of chemicals, and radiation. Whereas most vegetative cells are killed by temperatures above 70°C, endospores can survive in boiling water for several hours or more. Endospores of thermophilic (heat-loving) bacteria can survive in boiling water for 19 hours. Endospore-forming bacteria are a problem in the food industry because they are likely to survive underprocessing, and, if conditions for growth occur, some species produce toxins and disease. Special methods for controlling organisms that produce endospores are discussed in Chapter 7.

Clinical Case Resolved

It is the glycocalyx that enables bacteria in water to stick inside a pipe. The bacteria grow slowly in the nutrient-poor tap water but do not get washed away by the flowing water. A slimy layer of bacteria can accumulate in a pipe. Irene discovers that the disinfectant in the hospital's water supply was inadequate to prevent bacterial growth. Some bacteria can get dislodged by flowing water, and even normally harmless bacteria can infect a surgical incision or weakened host.

76 86 88 95 **97**

CHECK YOUR UNDERSTANDING

- Where is the DNA located in a prokaryotic cell? **4-10**
- What is the general function of inclusions? **4-11**
- Under what conditions do endospores form? **4-12**

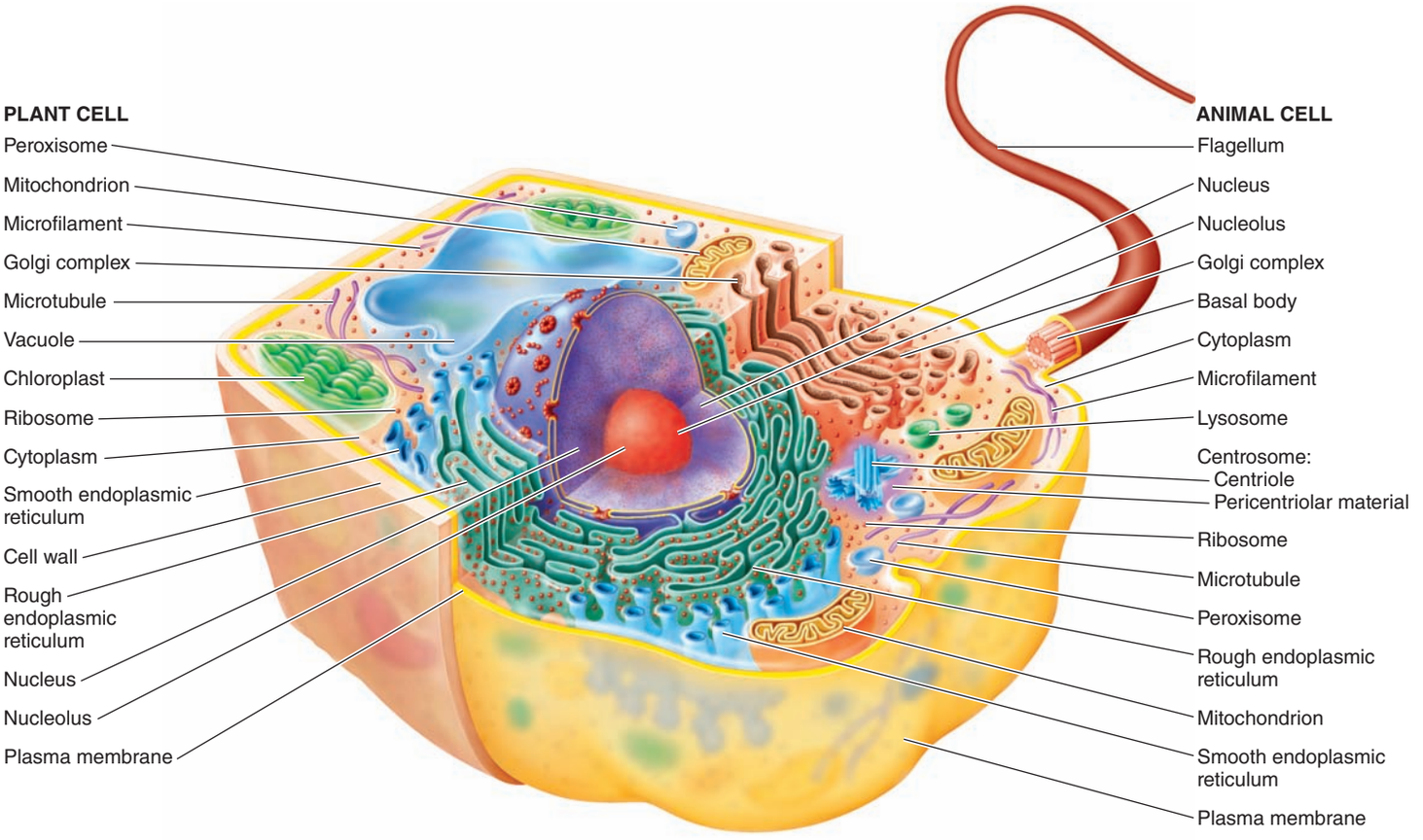
* * *

Having examined the functional anatomy of the prokaryotic cell, we will now look at the functional anatomy of the eukaryotic cell.

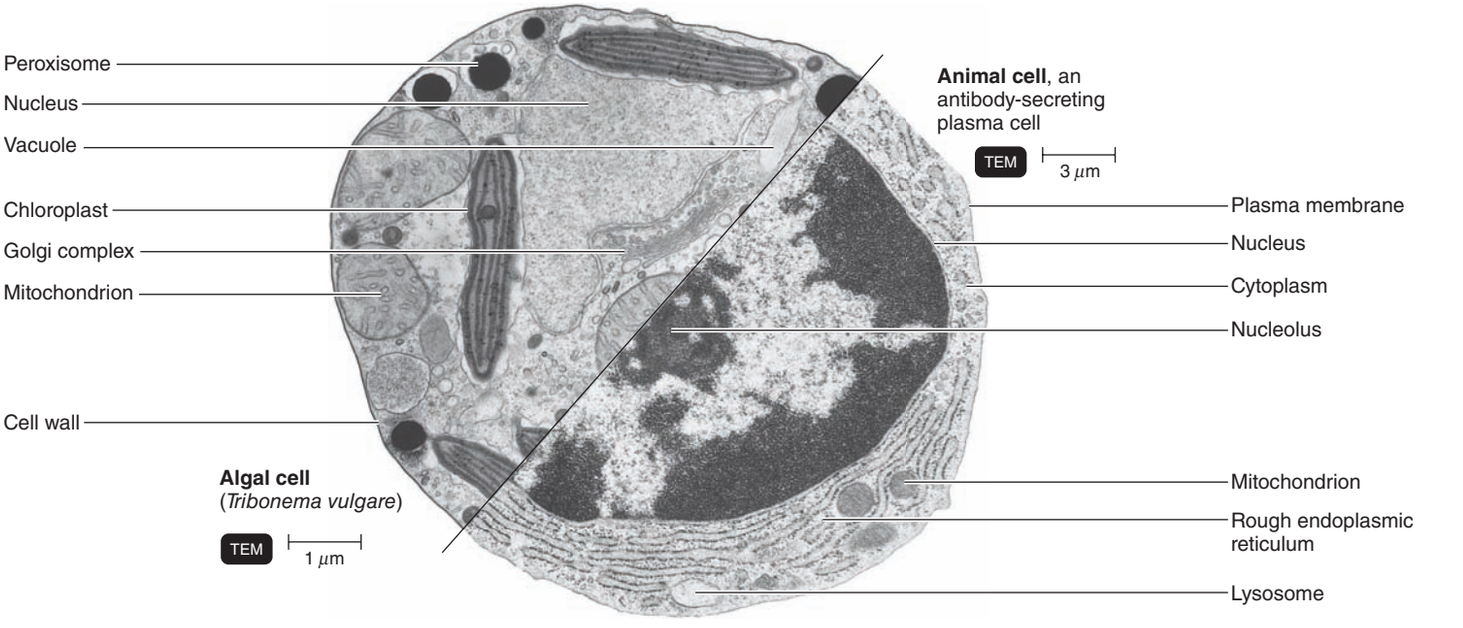
The Eukaryotic Cell

As mentioned earlier, eukaryotic organisms include algae, protozoa, fungi, plants, and animals. The eukaryotic cell is typically larger and structurally more complex than the prokaryotic cell (**Figure 4.22**). When the structure of the prokaryotic cell in Figure 4.6 is compared with that of the eukaryotic cell, the differences between the two types of cells become apparent. The principal differences between prokaryotic and eukaryotic cells are summarized in **Table 4.2** page 100.

The following discussion of eukaryotic cells will parallel our discussion of prokaryotic cells by starting with structures that extend to the outside of the cell.



(a) Highly schematic diagram of a composite eukaryotic cell, half plant and half animal



(b) Transmission electron micrographs of plant and animal cells.

Figure 4.22 **Eukaryotic cells showing typical structures.**

Q What kingdoms contain eukaryotic organisms?

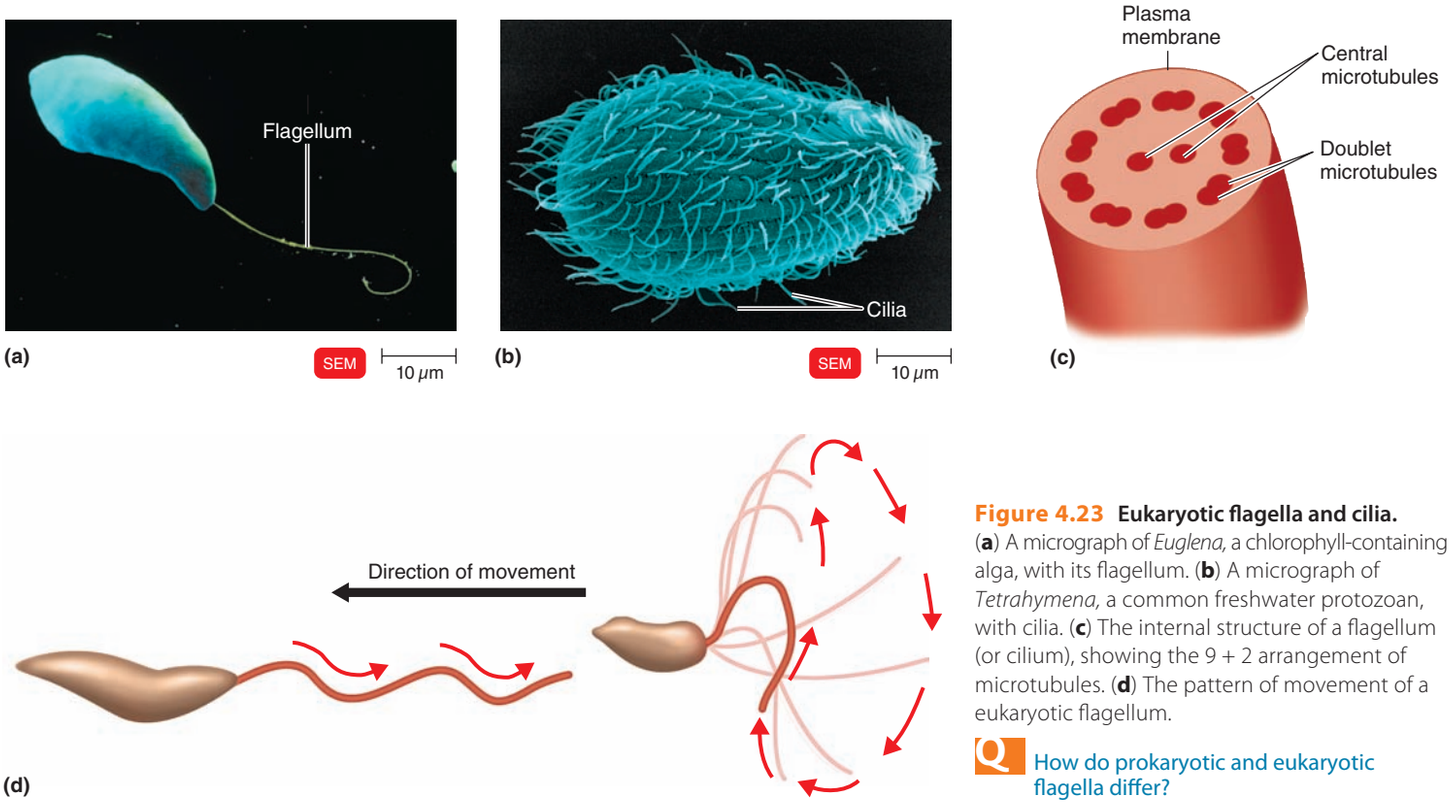


Figure 4.23 Eukaryotic flagella and cilia. (**a**) A micrograph of *Euglena,* a chlorophyll-containing alga, with its flagellum. (**b**) A micrograph of *Tetrahymena,* a common freshwater protozoan, with cilia. (**c**) The internal structure of a flagellum (or cilium), showing the 9 + 2 arrangement of microtubules. (**d**) The pattern of movement of a eukaryotic flagellum.

Q How do prokaryotic and eukaryotic flagella differ?

Flagella and Cilia

LEARNING OBJECTIVE

4-13 Differentiate prokaryotic and eukaryotic flagella.

Many types of eukaryotic cells have projections that are used for cellular locomotion or for moving substances along the surface of the cell. These projections contain cytoplasm and are enclosed by the plasma membrane. If the projections are few and are long in relation to the size of the cell, they are called **flagella.** If the projections are numerous and short, they are called **cilia** (singular: **cilium**).

Algae of the genus *Euglena* (ū-glē′na) use a flagellum for locomotion, whereas protozoa, such as *Tetrahymena* (tet-rä-hī′me-nä), use cilia for locomotion (Figure 4.23a and Figure 4.23b). Both flagella and cilia are anchored to the plasma membrane by a basal body, and both consist of nine pairs of microtubules (doublets) arranged in a ring, plus another two microtubules in the center of the ring, an arrangement called a *9 + 2 array* (Figure 4.23c). **Microtubules** are long, hollow tubes made up of a protein called *tubulin.* A prokaryotic flagellum rotates, but a eukaryotic flagellum moves in a wavelike manner (Figure 4.23d). To help keep foreign material out of the lungs, ciliated cells of the human respiratory system move the material along the surface of the cells in the bronchial tubes and trachea toward the throat and mouth (see Figure 16.4, page 454).

The Cell Wall and Glycocalyx

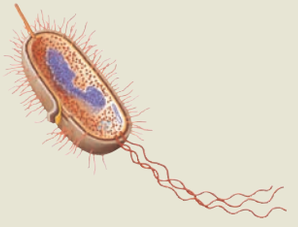
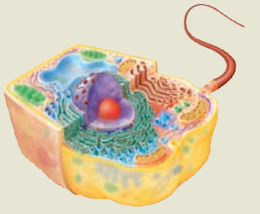
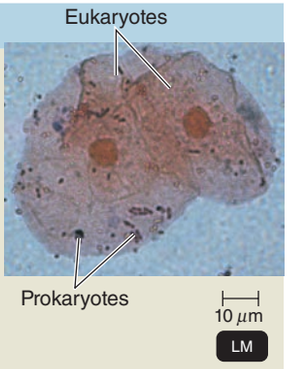
LEARNING OBJECTIVE

4-14 Compare and contrast prokaryotic and eukaryotic cell walls and glycocalyxes.

Most eukaryotic cells have cell walls, although they are generally much simpler than those of prokaryotic cells. Many algae have cell walls consisting of the polysaccharide *cellulose* (as do all plants); other chemicals may be present as well. Cell walls of some fungi also contain cellulose, but in most fungi the principal structural component of the cell wall is the polysaccharide *chitin,* a polymer of N-acetylglucosamine (NAG) units. (Chitin is also the main structural component of the exoskeleton of crustaceans and insects.) The cell walls of yeasts contain the polysaccharides *glucan* and *mannan.* In eukaryotes that lack a cell wall, the plasma membrane may be the outer covering; however, cells that have direct contact with the environment may have coatings outside the plasma membrane. Protozoa do not have a typical cell wall; instead, they have a flexible outer protein covering called a *pellicle.*

In other eukaryotic cells, including animal cells, the plasma membrane is covered by a **glycocalyx,** a layer of material containing substantial amounts of sticky carbohydrates. Some of these carbohydrates are covalently bonded to proteins and lipids in the plasma membrane, forming glycoproteins and glycolipids that anchor the glycocalyx to the cell. The glycocalyx strengthens the cell surface, helps attach cells together, and may contribute to cell–cell recognition.

TABLE 4.2 Principal Differences between Prokaryotic and Eukaryotic Cells

Characteristic	Prokaryotic	Eukaryotic
		 
Size of Cell	Typically 0.2–2.0 μm in diameter	Typically 10–100 μm in diameter
Nucleus	No nuclear membrane or nucleoli	True nucleus, consisting of nuclear membrane and nucleoli
Membrane-Enclosed Organelles	Absent	Present; examples include lysosomes, Golgi complex, endoplasmic reticulum, mitochondria, and chloroplasts
Flagella	Consist of two protein building blocks	Complex; consist of multiple microtubules
Glycocalyx	Present as a capsule or slime layer	Present in some cells that lack a cell wall
Cell Wall	Usually present; chemically complex (typical bacterial cell wall includes peptidoglycan)	When present, chemically simple (includes cellulose and chitin)
Plasma Membrane	No carbohydrates and generally lacks sterols	Sterols and carbohydrates that serve as receptors
Cytoplasm	No cytoskeleton or cytoplasmic streaming	Cytoskeleton; cytoplasmic streaming
Ribosomes	Smaller size (70S)	Larger size (80S); smaller size (70S) in organelles
Chromosome (DNA)	Usually single circular chromosome; typically lacks histones	Multiple linear chromosomes with histones
Cell Division	Binary fission	Involves mitosis
Sexual Recombination	None; transfer of DNA only	Involves meiosis

Eukaryotic cells do not contain peptidoglycan, the framework of the prokaryotic cell wall. This is significant medically because antibiotics, such as penicillins and cephalosporins, act against peptidoglycan and therefore do not affect human eukaryotic cells.

The Plasma (Cytoplasmic) Membrane

LEARNING OBJECTIVE

4-15 Compare and contrast prokaryotic and eukaryotic plasma membranes.

The **plasma (cytoplasmic) membrane** of eukaryotic and prokaryotic cells is very similar in function and basic structure. There are, however, differences in the types of proteins found in the membranes. Eukaryotic membranes also contain carbohydrates, which serve as attachment sites for bacteria and as receptor sites that assume a role in such functions as cell–cell recognition. Eukaryotic plasma membranes also contain *sterols,* complex lipids not found in prokaryotic plasma membranes (with the exception of *Mycoplasma* cells). Sterols seem to be associated with the ability of the membranes to resist lysis resulting from increased osmotic pressure.

Substances can cross eukaryotic and prokaryotic plasma membranes by simple diffusion, facilitated diffusion, osmosis, or active transport. Group translocation does not occur in eukaryotic cells. However, eukaryotic cells can use a mechanism called **endocytosis.** This occurs when a segment of the plasma membrane surrounds a particle or large molecule, encloses it, and brings it into the cell.

The three types of endocytosis are phagocytosis, pinocytosis, and receptor-mediated endocytosis. During *phagocytosis,* cellular projections called pseudopods engulf particles and bring them into the cell. Phagocytosis is used by white blood cells to destroy bacteria and foreign substances (see Figure 16.8, page 464, and further discussion in Chapter 16). In *pinocytosis,* the plasma membrane folds inward, bringing extracellular fluid into the cell, along with whatever substances are dissolved in the fluid. In *receptor-mediated endocytosis*, substances (ligands)

bind to receptors in the membrane. When binding occurs, the membrane folds inward. Receptor-mediated endocytosis is one of the ways viruses can enter animal cells (see Figure 13.14a, page 386).

Cytoplasm

LEARNING OBJECTIVE

4-16 Compare and contrast prokaryotic and eukaryotic cytoplasms.

The **cytoplasm** of eukaryotic cells encompasses the substance inside the plasma membrane and outside the nucleus (see Figure 4.22). The cytoplasm is the substance in which various cellular components are found. (The term **cytosol** refers to the fluid portion of cytoplasm.) A major difference between eukaryotic and prokaryotic cytoplasm is that eukaryotic cytoplasm has a complex internal structure, consisting of exceedingly small rods (*microfilaments* and *intermediate filaments*) and cylinders (*microtubules*). Together, they form the **cytoskeleton.** The cytoskeleton provides support and shape and assists in transporting substances through the cell (and even in moving the entire cell, as in phagocytosis). The movement of eukaryotic cytoplasm from one part of the cell to another, which helps distribute nutrients and move the cell over a surface, is called **cytoplasmic streaming.** Another difference between prokaryotic and eukaryotic cytoplasm is that many of the important enzymes found in the cytoplasmic fluid of prokaryotes are sequestered in the organelles of eukaryotes.

Ribosomes

LEARNING OBJECTIVE

4-17 Compare the structure and function of eukaryotic and prokaryotic ribosomes.

Attached to the outer surface of rough endoplasmic reticulum (discussed on page 102) are **ribosomes** (see Figure 4.25), which are also found free in the cytoplasm. As in prokaryotes, ribosomes are the sites of protein synthesis in the cell.

The ribosomes of eukaryotic endoplasmic reticulum and cytoplasm are somewhat larger and denser than those of prokaryotic cells. These eukaryotic ribosomes are 80S ribosomes, each of which consists of a large 60S subunit containing three molecules of rRNA and a smaller 40S subunit with one molecule of rRNA. The subunits are made separately in the nucleolus and, once produced, exit the nucleus and join together in the cytosol. Chloroplasts and mitochondria contain 70S ribosomes, which may indicate their evolution from prokaryotes. (This theory is discussed on page 105.) The role of ribosomes in protein synthesis will be discussed in more detail in Chapter 8.

Some ribosomes, called *free ribosomes,* are unattached to any structure in the cytoplasm. Primarily, free ribosomes synthesize proteins used *inside* the cell. Other ribosomes, called *membrane-bound ribosomes,* attach to the nuclear membrane and the endoplasmic reticulum. These ribosomes synthesize proteins destined for insertion in the plasma membrane or for export from the cell. Ribosomes located within mitochondria synthesize mitochondrial proteins. Sometimes 10 to 20 ribosomes join together in a stringlike arrangement called a *polyribosome.*

CHECK YOUR UNDERSTANDING

- Identify at least one significant difference between eukaryotic and prokaryotic flagella and cilia, cell walls, plasma membranes, and cytoplasm. **4-13–4-16**
- The antibiotic erythromycin binds with the 50S portion of a ribosome. What effect does this have on a prokaryotic cell? On a eukaryotic cell? **4–17**

Organelles

LEARNING OBJECTIVES

4-18 Define *organelle.*

4-19 Describe the functions of the nucleus, endoplasmic reticulum, Golgi complex, lysosomes, vacuoles, mitochondria, chloroplasts, peroxisomes, and centrosomes.

Organelles are structures with specific shapes and specialized functions and are characteristic of eukaryotic cells. They include the nucleus, endoplasmic reticulum, Golgi complex, lysosomes, vacuoles, mitochondria, chloroplasts, peroxisomes, and centrosomes. Not all of the organelles described are found in all cells. Certain cells have their own type and distribution of organelles based on specialization, age, and level of activity.

The Nucleus

The most characteristic eukaryotic organelle is the nucleus (see Figure 4.22). The **nucleus** (Figure 4.24) is usually spherical or oval, is frequently the largest structure in the cell, and contains almost all of the cell's hereditary information (DNA). Some DNA is also found in mitochondria and in the chloroplasts of photosynthetic organisms.

The nucleus is surrounded by a double membrane called the **nuclear envelope.** Both membranes resemble the plasma membrane in structure. Tiny channels in the membrane called **nuclear pores** allow the nucleus to communicate with the cytoplasm (Figure 4.24b). Nuclear pores control the movement of substances between the nucleus and cytoplasm. Within the nuclear envelope are one or more spherical bodies called **nucleoli** (singular: **nucleolus**). Nucleoli are actually condensed regions of chromosomes where ribosomal RNA is being synthesized. Ribosomal RNA is an essential component of ribosomes.

The nucleus also contains most of the cell's DNA, which is combined with several proteins, including some basic proteins called **histones** and nonhistones. The combination of about 165 base pairs of DNA and 9 molecules of histones is referred to as a *nucleosome.* When the cell is not reproducing, the DNA

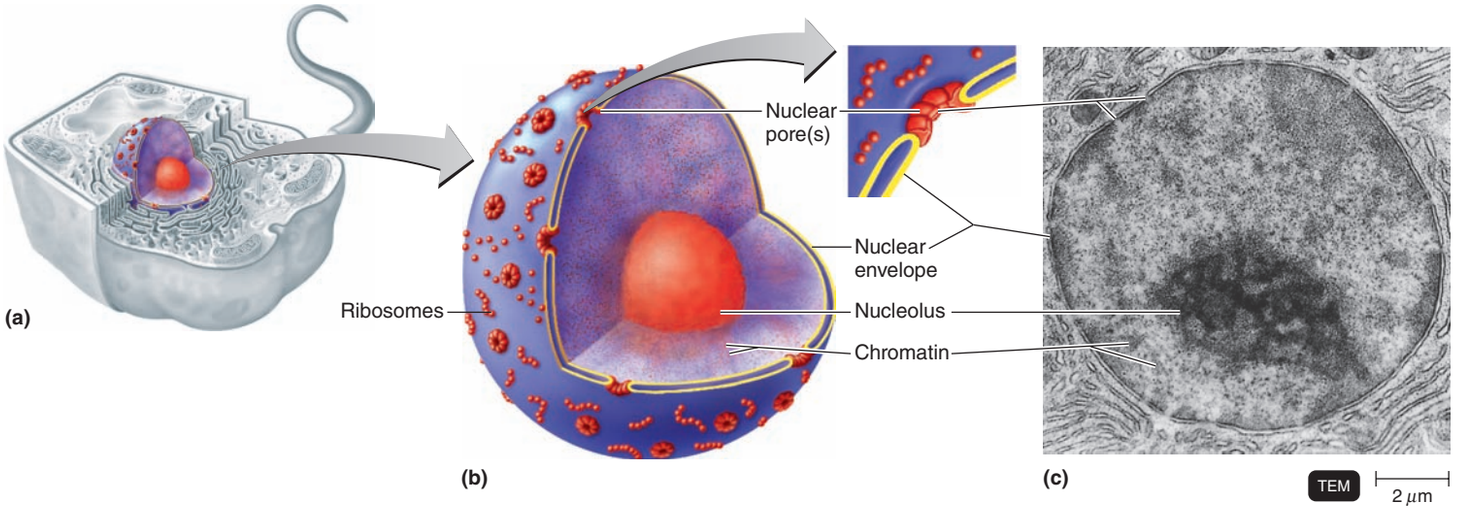


Figure 4.24 The eukaryotic nucleus. (**a, b**) Drawings of details of a nucleus. (**c**) A micrograph of a nucleus.

Q What keeps the nucleus suspended in the cell?

and its associated proteins appear as a threadlike mass called **chromatin.** During nuclear division, the chromatin coils into shorter and thicker rodlike bodies called **chromosomes.** Prokaryotic chromosomes do not undergo this process, do not have histones, and are not enclosed in a nuclear envelope.

Eukaryotic cells require two elaborate mechanisms: mitosis and meiosis to segregate chromosomes prior to cell division. Neither process occurs in prokaryotic cells.

Endoplasmic Reticulum

Within the cytoplasm of eukaryotic cells is the **endoplasmic reticulum,** or **ER,** an extensive network of flattened membranous sacs or tubules called **cisternae** (Figure 4.25). The ER network is continuous with the nuclear envelope (see Figure 4.22a).

Most eukaryotic cells contain two distinct, but interrelated, forms of ER that differ in structure and function. The membrane of **rough ER** is continuous with the nuclear membrane and usually unfolds into a series of flattened sacs. The outer surface of rough ER is studded with ribosomes, the sites of protein synthesis. Proteins synthesized by ribosomes that are attached to rough ER enter cisternae within the ER for processing and sorting. In some cases, enzymes within the cisternae attach the proteins to carbohydrates to form glycoproteins. In other cases, enzymes attach the proteins to phospholipids, also synthesized by rough ER. These molecules may be incorporated into organelle membranes or the plasma membrane. Thus, rough ER is a factory for synthesizing secretory proteins and membrane molecules.

Smooth ER extends from the rough ER to form a network of membrane tubules (see Figure 4.25). Unlike rough ER, smooth ER does not have ribosomes on the outer surface of its membrane. However, smooth ER contains unique enzymes that make it functionally more diverse than rough ER. Although it does not synthesize proteins, smooth ER does synthesize phospholipids, as does rough ER. Smooth ER also synthesizes fats and steroids, such as estrogens and testosterone. In liver cells, enzymes of the smooth ER help release glucose into the bloodstream and inactivate or detoxify drugs and other potentially harmful substances (for example, alcohol). In muscle cells, calcium ions released from the sarcoplasmic reticulum, a form of smooth ER, trigger the contraction process.

Golgi Complex

Most of the proteins synthesized by ribosomes attached to rough ER are ultimately transported to other regions of the cell. The first step in the transport pathway is through an organelle called the **Golgi complex.** It consists of 3 to 20 cisternae that resemble a stack of pita bread (Figure 4.26). The cisternae are often curved, giving the Golgi complex a cuplike shape.

Proteins synthesized by ribosomes on the rough ER are surrounded by a portion of the ER membrane, which eventually buds from the membrane surface to form a **transport vesicle.** The transport vesicle fuses with a cistern of the Golgi complex, releasing proteins into the cistern. The proteins are modified and move from one cistern to another via **transfer vesicles** that bud from the edges of the cisternae. Enzymes in the cisternae modify the proteins to form glycoproteins, glycolipids, and lipoproteins. Some of the processed proteins leave the cisternae in **secretory vesicles,** which detach from the cistern and deliver the proteins to the plasma membrane, where they are discharged by exocytosis. Other processed proteins leave the cisternae in vesicles that deliver their contents to the plasma membrane for incorporation into the membrane. Finally, some processed proteins leave the cisternae in vesicles that are called

storage vesicles. The major storage vesicle is a lysosome, whose structure and functions are discussed next.

Lysosomes

Lysosomes are formed from Golgi complexes and look like membrane-enclosed spheres. Unlike mitochondria, lysosomes have only a single membrane and lack internal structure (see Figure 4.22). But they contain as many as 40 different kinds of powerful digestive enzymes capable of breaking down various molecules. Moreover, these enzymes can also digest bacteria that enter the cell. Human white blood cells, which use phagocytosis to ingest bacteria, contain large numbers of lysosomes.

Vacuoles

A **vacuole** (see Figure 4.22) is a space or cavity in the cytoplasm of a cell that is enclosed by a membrane called a *tonoplast*. In plant cells, vacuoles may occupy 5–90% of the cell volume, depending on the type of cell. Vacuoles are derived from the Golgi complex and have several diverse functions. Some vacuoles serve as temporary storage organelles for substances such as proteins, sugars, organic acids, and inorganic ions. Other vacuoles form during endocytosis to help bring food into the cell. Many plant cells also store metabolic wastes and poisons that would otherwise be injurious if they accumulated in the cytoplasm. Finally, vacuoles may take up water, enabling plant cells to increase in size and also providing rigidity to leaves and stems.

Mitochondria

Spherical or rod-shaped organelles called **mitochondria** (singular: **mitochondrion**) appear throughout the cytoplasm of most eukaryotic cells (see Figure 4.22). The number of mitochondria per cell varies greatly among different types of cells. For example, the protozoan *Giardia* has no mitochondria, whereas liver cells contain 1000 to 2000 per cell. A mitochondrion consists of a double membrane similar in structure to the plasma membrane (Figure 4.27). The outer mitochondrial membrane is smooth, but the inner mitochondrial membrane is arranged in a series of folds called **cristae** (singular: **crista**). The center of the mitochondrion is a semifluid substance called the **matrix.** Because of the nature and arrangement of the cristae, the inner membrane provides an enormous surface area on which chemical reactions can occur. Some proteins that function in cellular respiration, including the enzyme that makes ATP, are located on the cristae of the inner mitochondrial membrane, and many of the metabolic steps involved in cellular respiration are concentrated in the matrix (see Chapter 5). Mitochondria are often called the "powerhouses of the cell" because of their central role in ATP production.

Mitochondria contain 70S ribosomes and some DNA of their own, as well as the machinery necessary to replicate, transcribe, and translate the information encoded by their DNA. In addition, mitochondria can reproduce more or less on their own by growing and dividing in two.

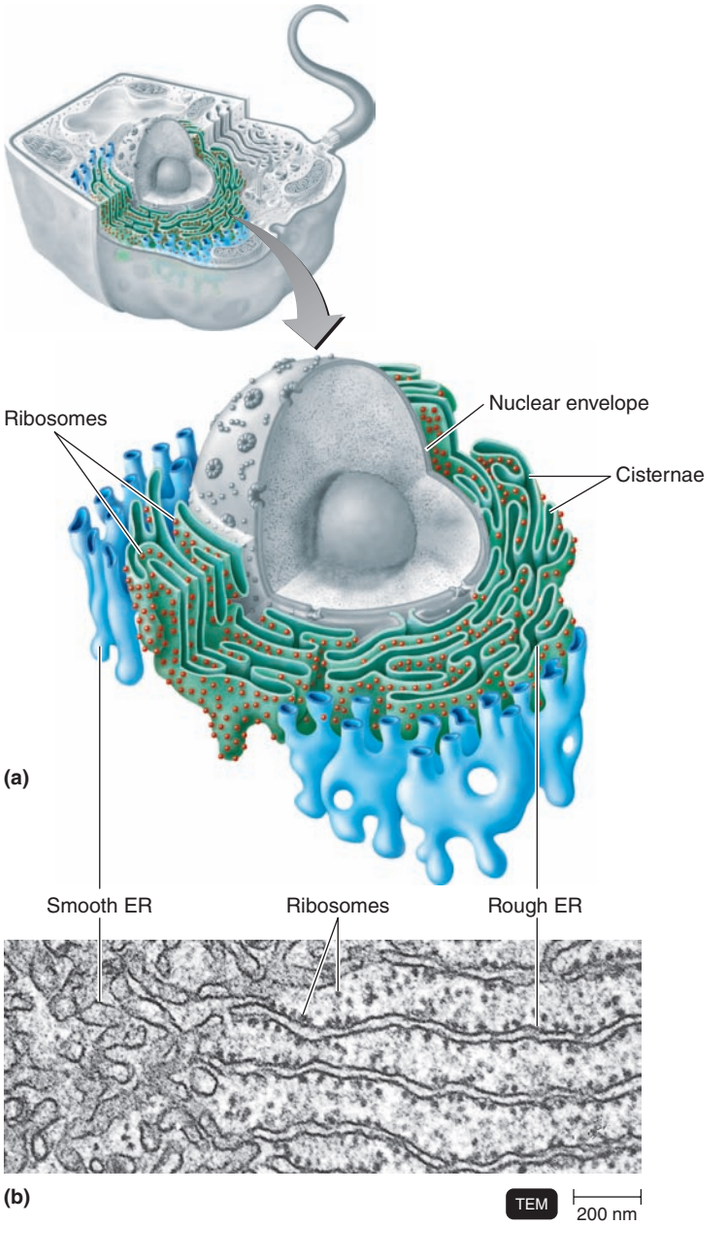


Figure 4.25 Rough endoplasmic reticulum and ribosomes. (**a**) A drawing of details of the endoplasmic reticulum. (**b**) A micrograph of the endoplasmic reticulum and ribosomes.

Q What functions of the smooth ER and rough ER are similar?

Chloroplasts

Algae and green plants contain a unique organelle called a **chloroplast** (Figure 4.28), a membrane-enclosed structure that contains both the pigment chlorophyll and the enzymes required for the light-gathering phases of photosynthesis (see Chapter 5). The chlorophyll is contained in flattened membrane sacs called **thylakoids;** stacks of thylakoids are called *grana* (singular: **granum**) (see Figure 4.28).

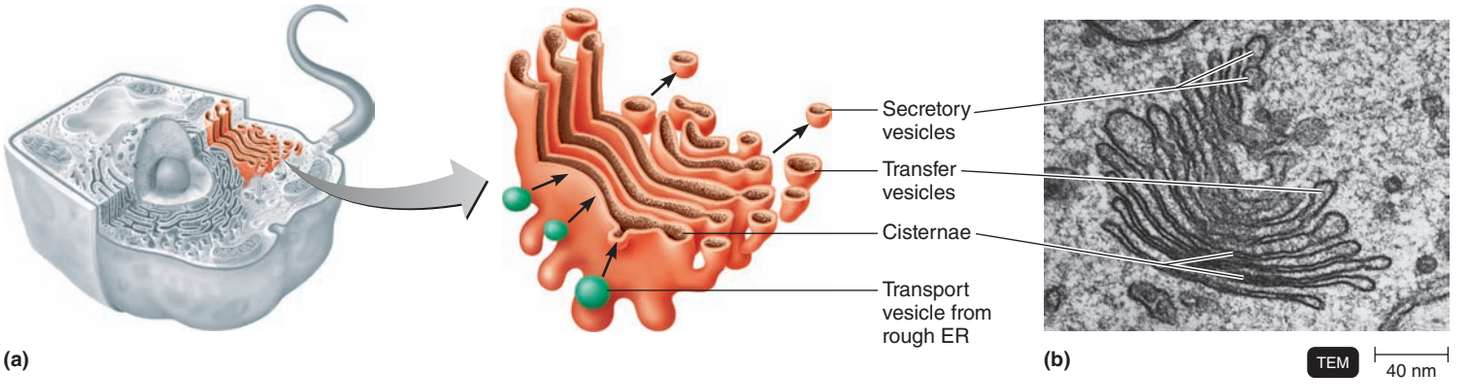


Figure 4.26 Golgi complex. (**a**) A drawing of details of a Golgi complex. (**b**) A micrograph of a Golgi complex.

Q What is the function of the Golgi complex?

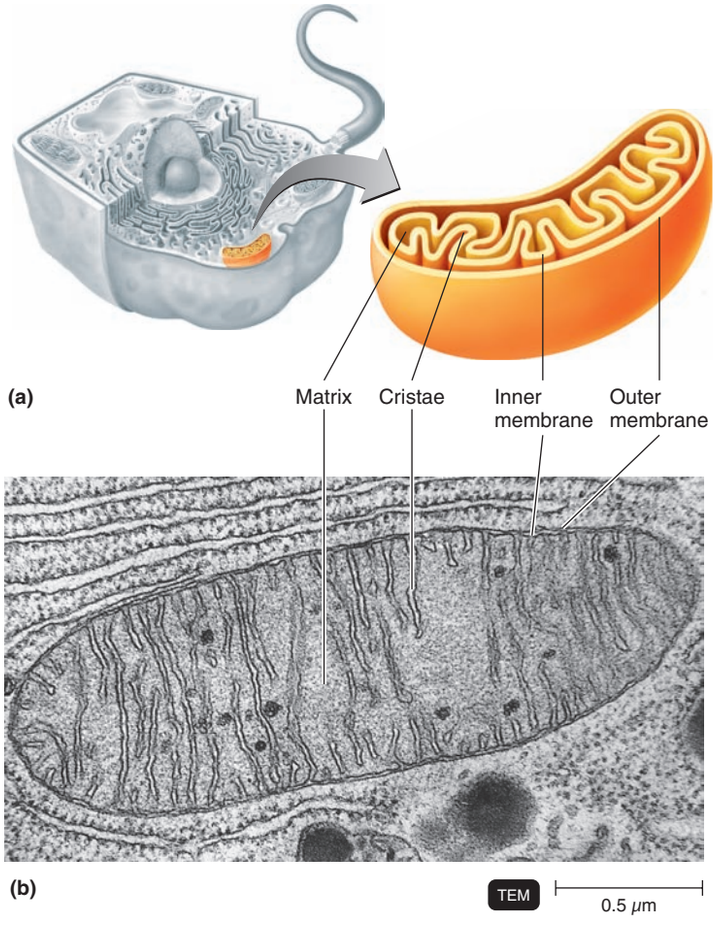


Figure 4.27 Mitochondria. (**a**) A drawing of details of a mitochondrion. (**b**) A micrograph of a mitochondrion from a rat pancreas cell.

Q How are mitochondria similar to prokaryotic cells?

Like mitochondria, chloroplasts contain 70S ribosomes, DNA, and enzymes involved in protein synthesis. They are capable of multiplying on their own within the cell. The way both chloroplasts and mitochondria multiply—by increasing in size and then dividing in two—is strikingly reminiscent of bacterial multiplication.

Peroxisomes

Organelles similar in structure to lysosomes, but smaller, are called **peroxisomes** (see Figure 4.22). Although peroxisomes were once thought to form by budding off the ER, it is now generally agreed that they form by the division of preexisting peroxisomes.

Peroxisomes contain one or more enzymes that can oxidize various organic substances. For example, substances such as amino acids and fatty acids are oxidized in peroxisomes as part of normal metabolism. In addition, enzymes in peroxisomes oxidize toxic substances, such as alcohol. A by-product of the oxidation reactions is hydrogen peroxide (H_2O_2), a potentially toxic compound. However, peroxisomes also contain the enzyme *catalase,* which decomposes H_2O_2 (see Chapter 6, page 160). Because the generation and degradation of H_2O_2 occurs within the same organelle, peroxisomes protect other parts of the cell from the toxic effects of H_2O_2.

Centrosome

The **centrosome,** located near the nucleus, consists of two components: the pericentriolar area and centrioles (see Figure 4.22). The *pericentriolar material* is a region of the cytosol composed of a dense network of small protein fibers. This area is the organizing center for the mitotic spindle, which plays a critical role in cell division, and for microtubule formation in nondividing cells. Within the pericentriolar material is a pair of cylindrical structures called *centrioles,* each of which is composed of nine clusters of three microtubules (triplets) arranged in a circular

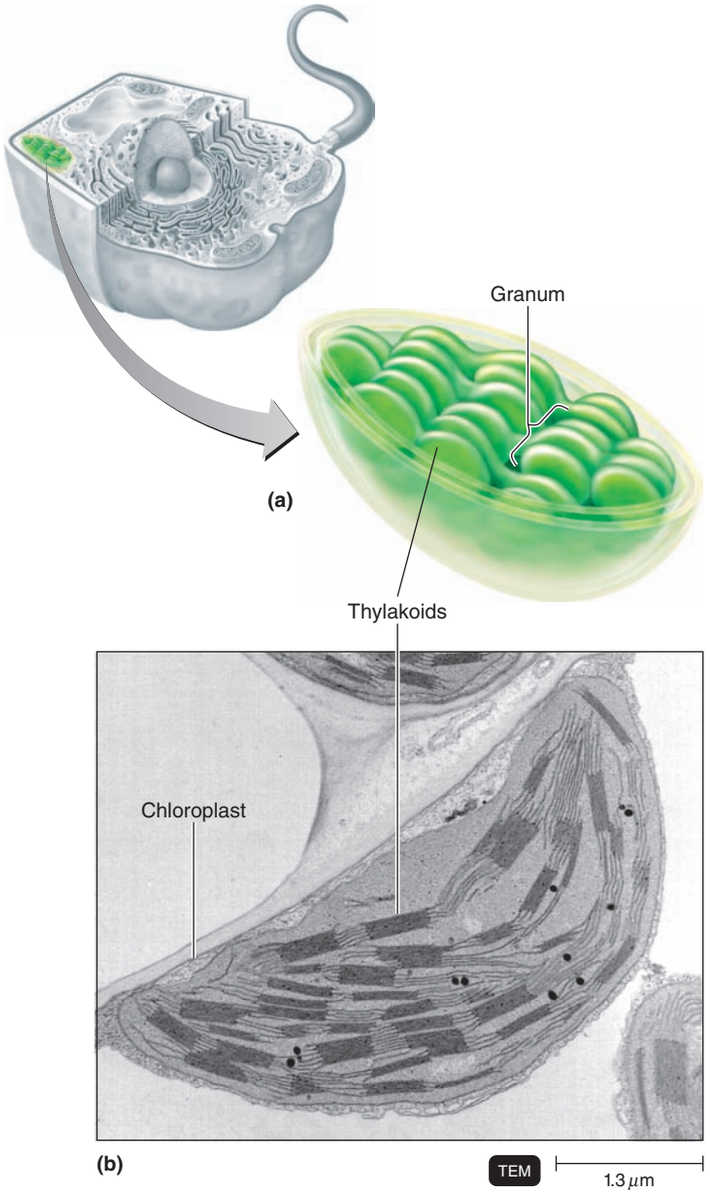


Figure 4.28 Chloroplasts. Photosynthesis occurs in chloroplasts; the light-trapping pigments are located on the thylakoids. (**a**) A drawing of details of a chloroplast, showing grana. (**b**) A micrograph of chloroplasts in a plant cell.

Q What are the similarities between chloroplasts and prokaryotic cells?

pattern, an arrangement called a *9 + 0 array*. The 9 refers to the nine clusters of microtubules, and the 0 refers to the absence of microtubules in the center. The long axis of one centriole is at a right angle to the long axis of the other.

CHECK YOUR UNDERSTANDING

- Compare the structure of the nucleus of a eukaryote and the nucleoid of a prokaryote. **4-18**
- How do rough and smooth ER compare structurally and functionally? **4-19**

The Evolution of Eukaryotes

LEARNING OBJECTIVE

4-20 Discuss evidence that supports the endosymbiotic theory of eukaryotic evolution.

Biologists generally believe that life arose on Earth in the form of very simple organisms, similar to prokaryotic cells, about 3.5 to 4 billion years ago. About 2.5 billion years ago, the first eukaryotic cells evolved from prokaryotic cells. Recall that prokaryotes and eukaryotes differ mainly in that eukaryotes contain highly specialized organelles. The theory explaining the origin of eukaryotes from prokaryotes, pioneered by Lynn Margulis, is the **endosymbiotic theory.** According to this theory, larger bacterial cells lost their cell walls and engulfed smaller bacterial cells. This relationship, in which one organism lives within another, is called *endosymbiosis* (*symbiosis* = living together).

According to the endosymbiotic theory, the ancestral eukaryote developed a rudimentary nucleus when the plasma membrane folded around the chromosome (see Figure 10.2, page 275). This cell, called a nucleoplasm, may have ingested aerobic bacteria. Some ingested bacteria lived inside the host nucleoplasm. This arrangement evolved into a symbiotic relationship in which the host nucleoplasm supplied nutrients and the endosymbiotic bacterium produced energy that could be used by the nucleoplasm. Similarly, chloroplasts may be descendants of photosynthetic prokaryotes ingested by this early nucleoplasm. Eukaryotic flagella and cilia are believed to have originated from symbiotic associations between the plasma membrane of early eukaryotes and motile spiral bacteria called spirochetes. A living example that suggests how flagella developed is described in the box on the next page.

Studies comparing prokaryotic and eukaryotic cells provide evidence for the endosymbiotic theory. For example, both mitochondria and chloroplasts resemble bacteria in size and shape. Further, these organelles contain circular DNA, which is typical of prokaryotes, and the organelles can reproduce independently of their host cell. Moreover, mitochondrial and chloroplast ribosomes resemble those of prokaryotes, and their mechanism of protein synthesis is more similar to that found in bacteria than eukaryotes. Also, the same antibiotics that inhibit protein synthesis on ribosomes in bacteria also inhibit protein synthesis on ribosomes in mitochondria and chloroplasts.

CHECK YOUR UNDERSTANDING

- Which three organelles are not associated with the Golgi complex? What does this suggest about their origin? **4-20**

* * *

Our next concern is to examine microbial metabolism. In Chapter 5, you will learn about the importance of enzymes to microorganisms and the ways microbes produce and use energy.

Why Microbiologists Study Termites

Although termites are famous for their ability to eat wood, causing damage to wooden structures and recycling cellulose in the soil, they are unable to digest the wood that they eat. To break down the cellulose, termites enlist the help of a variety of microorganisms. Some termites, for example, dig tunnels in the wood, then inoculate the tunnels with fungi that grow on the wood. These termites then eat the fungi, not the wood itself.

What microbiologists find more interesting are the termites that contain, within their digestive tracts, symbiotic microorganisms that digest the cellulose that the termites chew and swallow. In fact, these symbiotic microorganisms can survive only because of even smaller symbionts that live on and within them, without which they would not even be able to move. By studying how a single termite survives, microbiologists have begun to gain an entirely new understanding of symbiosis.

The termite's dependence on nitrogen-fixing bacteria to supply its nitrogen and on protozoans such as *Trichonympha sphaerica* to digest cellulose are examples of endosymbiosis, a symbiotic relationship with an organism that lives inside the body of the host organism (in this case, within the hindgut of the termite).

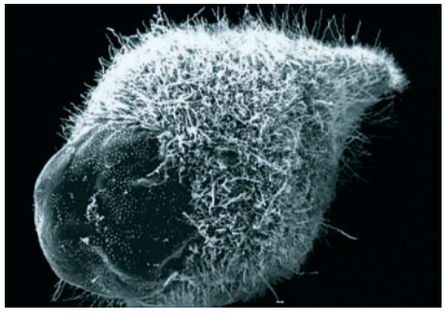
The picture is more complicated than this, however, for *T. sphaerica* is unable to digest cellulose without the aid of bacteria that live within its body: in other words, the protozoan has its own endosymbionts.

Certain hindgut flagellates such as *T. sphaerica* also demonstrate another form of symbiosis—ectosymbiosis, a symbiotic relationship with organisms that live outside its body. Advances in microscopy have shown that these flagellates are covered by precise rows consisting of thousands of bacteria, either rods or spirochetes. If these bacteria are killed, the protozoan is unable to move. Instead of using its own flagella, the protozoan relies on the rows of bacteria to row it about like oarsmen in a boat.

The protozoan *Mixotricha,* for example, has rows of spirochetes on its surface (see photo, upper right). The end of each spirochete abuts against a swelling known as a bracket; see part a of the figure. The spirochetes undulate in unison, thereby creating waves of motion along *Mixotricha's* surface.

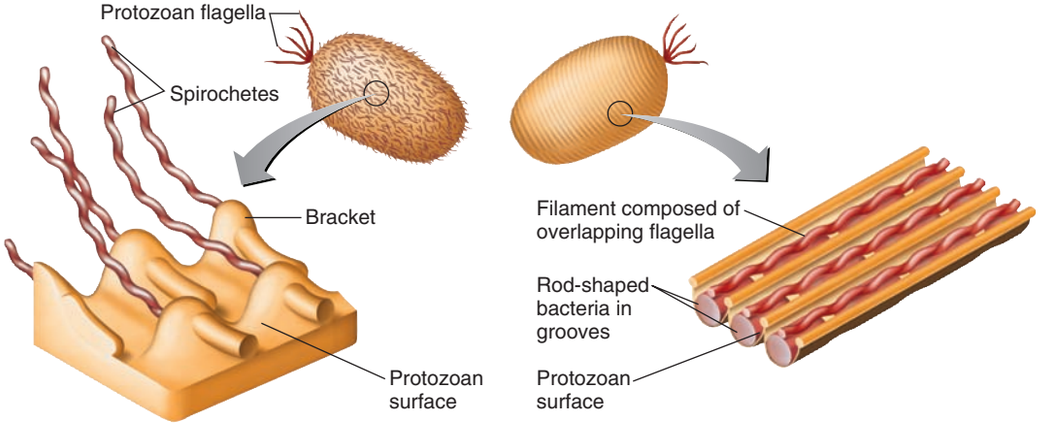
Rod-shaped bacteria align in grooves that cover the surface of devescovinids, another group of termite-hindgut protozoans. Each rod has 12 flagella that overlap the flagella of the adjacent bacteria to form a continuous filament along the groove (see part b). The bacteria rotate their flagella, thus creating coordinated waves along all these rows of filaments, which propel the protozoan.

Sid Tamm and his colleagues at Boston University have found that the protozoa cannot control motility of the ectosymbiotics. *Mixotricha* uses its flagella to steer, and the bacteria push the protozoa forward—shoving and being shoved by its neighbors, much like bumper cars.



***Mixotricha,* a protozoan that lives in the termite gut.**

Arrangements of bacteria on the surfaces of two protozoans.



(a) Spirochetes attached to brackets over the surface of a *Mixotricha* protozoan align themselves and move in unison.

(b) On this devescovinid protozoan, the flagella from one rod-shaped bacterium overlap the next to form a continuous filament.

Study Outline

MasteringMICROBIOLOGY™

Test your understanding with quizzes, microbe review, and a chapter post-test at www.masteringmicrobiology.com.

Comparing Prokaryotic and Eukaryotic Cells: An Overview (p. 76)

1. Prokaryotic and eukaryotic cells are similar in their chemical composition and chemical reactions.

2. Prokaryotic cells lack membrane-enclosed organelles (including a nucleus).
3. Peptidoglycan is found in prokaryotic cell walls but not in eukaryotic cell walls.
4. Eukaryotic cells have a membrane-bound nucleus and other or ganelles.

The Prokaryotic Cell (pp. 76–97)

1. Bacteria are unicellular, and most of them multiply by binary fission.
2. Bacterial species are differentiated by morphology, chemical composition, nutritional requirements, biochemical activities, and source of energy.

The Size, Shape, and Arrangement of Bacterial Cells (pp. 77–78)

1. Most bacteria are 0.2 to 2.0 μm in diameter and 2 to 8 μm in length.
2. The three basic bacterial shapes are coccus (spherical), bacillus (rod-shaped), and spiral (twisted).
3. Pleomorphic bacteria can assume several shapes.

Structures External to the Cell Wall (pp. 78–84)

Glycocalyx (p. 80)

1. The glycocalyx (capsule, slime layer, or extracellular polysaccharide) is a gelatinous polysaccharide and/or polypeptide covering.
2. Capsules may protect pathogens from phagocytosis.
3. Capsules enable adherence to surfaces, prevent desiccation, and may provide nutrients.

Flagella (pp. 81–82)

4. Flagella are relatively long filamentous appendages consisting of a filament, hook, and basal body.
5. Prokaryotic flagella rotate to push the cell.
6. Motile bacteria exhibit taxis; positive taxis is movement toward an attractant, and negative taxis is movement away from a repellent.
7. Flagellar (H) protein is an antigen.

Axial Filaments (p. 82)

8. Spiral cells that move by means of an axial filament (endoflagellum) are called spirochetes.
9. Axial filaments are similar to flagella, except that they wrap around the cell.

Fimbriae and Pili (pp. 82–84)

10. Fimbriae help cells adhere to surfaces.
11. Pili are involved in twitching motility and DNA transfer.

The Cell Wall (pp. 84–88)

Composition and Characteristics (pp. 84–86)

1. The cell wall surrounds the plasma membrane and protects the cell from changes in water pressure.
2. The bacterial cell wall consists of peptidoglycan, a polymer consisting of NAG and NAM and short chains of amino acids.
3. Penicillin interferes with peptidoglycan synthesis.
4. Gram-positive cell walls consist of many layers of peptidoglycan and also contain teichoic acids.
5. Gram-negative bacteria have a lipopolysaccharide-lipoprotein-phospholipid outer membrane surrounding a thin peptidoglycan layer.
6. The outer membrane protects the cell from phagocytosis and from penicillin, lysozyme, and other chemicals.
7. Porins are proteins that permit small molecules to pass through the outer membrane; specific channel proteins allow other molecules to move through the outer membrane.
8. The lipopolysaccharide component of the outer membrane consists of sugars (O polysaccharides), which function as antigens, and lipid A, which is an endotoxin.

Cell Walls and the Gram Stain Mechanism (pp. 86–87)

9. The crystal violet–iodine complex combines with peptidoglycan.
10. The decolorizer removes the lipid outer membrane of gram-negative bacteria and washes out the crystal violet.

Atypical Cell Walls (pp. 87–88)

11. *Mycoplasma* is a bacterial genus that naturally lacks cell walls.
12. Archaea have pseudomurein; they lack peptidoglycan.
13. Acid-fast cell walls have a layer of mycolic acid outside a thin peptidoglycan layer.

Damage to the Cell Wall (p. 88)

14. In the presence of lysozyme, gram-positive cell walls are destroyed, and the remaining cellular contents are referred to as a protoplast.
15. In the presence of lysozyme, gram-negative cell walls are not completely destroyed, and the remaining cellular contents are referred to as a spheroplast.
16. L forms are gram-positive or gram-negative bacteria that do not make a cell wall.
17. Antibiotics such as penicillin interfere with cell wall synthesis.

Structures Internal to the Cell Wall (pp. 88–97)

The Plasma (Cytoplasmic) Membrane (pp. 89–90)

1. The plasma membrane encloses the cytoplasm and is a lipid bilayer with peripheral and integral proteins (the fluid mosaic model).
2. The plasma membrane is selectively permeable.
3. Plasma membranes contain enzymes for metabolic reactions, such as nutrient breakdown, energy production, and photosynthesis.
4. Mesosomes, irregular infoldings of the plasma membrane, are artifacts, not true cell structures.
5. Plasma membranes can be destroyed by alcohols and polymyxins.

The Movement of Materials across Membranes (pp. 91–93)

6. Movement across the membrane may be by passive processes, in which materials move from areas of higher to lower concentration and no energy is expended by the cell.
7. In simple diffusion, molecules and ions move until equilibrium is reached.
8. In facilitated diffusion, substances are transported by transporter proteins across membranes from areas of high to low concentration.
9. Osmosis is the movement of water from areas of high to low concentration across a selectively permeable membrane until equilibrium is reached.

10. In active transport, materials move from areas of low to high concentration by transporter proteins, and the cell must expend energy.
11. In group translocation, energy is expended to modify chemicals and transport them across the membrane.

Cytoplasm (p. 94)

12. Cytoplasm is the fluid component inside the plasma membrane.
13. The cytoplasm is mostly water, with inorganic and organic molecules, DNA, ribosomes, and inclusions.

The Nucleoid (p. 94)

14. The nucleoid contains the DNA of the bacterial chromosome.
15. Bacteria can also contain plasmids, which are circular, extrachromosomal DNA molecules.

Ribosomes (p. 94)

16. The cytoplasm of a prokaryote contains numerous 70S ribosomes; ribosomes consist of rRNA and protein.
17. Protein synthesis occurs at ribosomes; it can be inhibited by certain antibiotics.

Inclusions (pp. 94–95)

18. Inclusions are reserve deposits found in prokaryotic and eukaryotic cells.
19. Among the inclusions found in bacteria are metachromatic granules (inorganic phosphate), polysaccharide granules (usually glycogen or starch), lipid inclusions, sulfur granules, carboxysomes (ribulose 1,5-diphosphate carboxylase), magnetosomes (Fe_3O_4), and gas vacuoles.

Endospores (pp. 95–97)

20. Endospores are resting structures formed by some bacteria; they allow survival during adverse environmental conditions.
21. The process of endospore formation is called sporulation; the return of an endospore to its vegetative state is called germination.

The Eukaryotic Cell (pp. 97–106)

Flagella and Cilia (pp. 97–99)

1. Flagella are few and long in relation to cell size; cilia are numerous and short.
2. Flagella and cilia are used for motility, and cilia also move substances along the surface of the cells.
3. Both flagella and cilia consist of an arrangement of nine pairs and two single microtubules.

The Cell Wall and Glycocalyx (p. 99)

1. The cell walls of many algae and some fungi contain cellulose.
2. The main material of fungal cell walls is chitin.
3. Yeast cell walls consist of glucan and mannan.
4. Animal cells are surrounded by a glycocalyx, which strengthens the cell and provides a means of attachment to other cells.

The Plasma (Cytoplasmic) Membrane (p. 100)

1. Like the prokaryotic plasma membrane, the eukaryotic plasma membrane is a phospholipid bilayer containing proteins.
2. Eukaryotic plasma membranes contain carbohydrates attached to the proteins and sterols not found in prokaryotic cells (except *Mycoplasma* bacteria).
3. Eukaryotic cells can move materials across the plasma membrane by the passive processes used by prokaryotes and by active transport and endocytosis (phagocytosis, pinocytosis, and receptor-mediated endocytosis).

Cytoplasm (p. 101)

1. The cytoplasm of eukaryotic cells includes everything inside the plasma membrane and external to the nucleus.
2. The chemical characteristics of the cytoplasm of eukaryotic cells resemble those of the cytoplasm of prokaryotic cells.
3. Eukaryotic cytoplasm has a cytoskeleton and exhibits cytoplasmic streaming.

Ribosomes (p. 101)

1. 80S ribosomes are found in the cytoplasm or attached to the rough endoplasmic reticulum.

Organelles (pp. 101–105)

1. Organelles are specialized membrane-enclosed structures in the cytoplasm of eukaryotic cells.
2. The nucleus, which contains DNA in the form of chromosomes, is the most characteristic eukaryotic organelle.
3. The nuclear envelope is connected to a system of membranes in the cytoplasm called the endoplasmic reticulum (ER).
4. The ER provides a surface for chemical reactions and serves as a transport network. Protein synthesis and transport occur on the rough ER; lipid synthesis occurs on the smooth ER.
5. The Golgi complex consists of flattened sacs called cisterns. It functions in membrane formation and protein secretion.
6. Lysosomes are formed from Golgi complexes. They store digestive enzymes.
7. Vacuoles are membrane-enclosed cavities derived from the Golgi complex or endocytosis. They are usually found in plant cells that store various substances and provide rigidity to leaves and stems.
8. Mitochondria are the primary sites of ATP production. They contain 70S ribosomes and DNA, and they multiply by binary fission.
9. Chloroplasts contain chlorophyll and enzymes for photosynthesis. Like mitochondria, they contain 70S ribosomes and DNA and multiply by binary fission.
10. A variety of organic compounds are oxidized in peroxisomes. Catalase in peroxisomes destroys H_2O_2.
11. The centrosome consists of the pericentriolar material and centrioles. Centrioles are 9 triplet microtubules involved in formation of the mitotic spindle and microtubules.

The Evolution of Eukaryotes (p. 105)

1. According to the endosymbiotic theory, eukaryotic cells evolved from symbiotic prokaryotes living inside other prokaryotic cells.

Study Questions

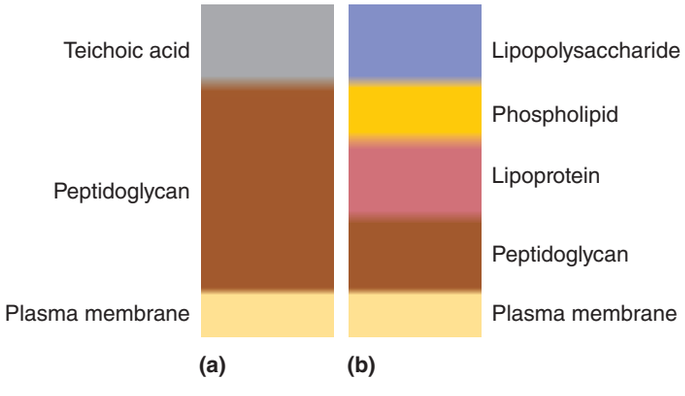
Answers to the Review and Multiple Choice questions can be found by turning to the Answers tab at the back of the textbook.

Review

1. DRAW IT Diagram each of the following flagellar arrangements:
   a. lophotrichous **d.** amphitrichous
   b. monotrichous **e.** polar
   c. peritrichous
2. Endospore formation is called (a) _____. It is initiated by (b) _____. Formation of a new cell from an endospore is called (c) _____. This process is triggered by (d) _____.
3. DRAW IT Draw the bacterial shapes listed in (a), (b), and (c). Then draw the shapes in (d), (e), and (f), showing how they are special conditions of a, b, and c, respectively.
   a. spiral **d.** spirochetes
   b. bacillus **e.** streptobacilli
   c. coccus **f.** staphylococci
4. Match the structures in column A to their functions in column B.

Column A	Column B
_____ **a.** Cell wall	**1.** Attachment to surfaces
_____ **b.** Endospore	**2.** Cell wall formation
_____ **c.** Fimbriae	**3.** Motility
_____ **d.** Flagella	**4.** Protection from osmotic lysis
_____ **e.** Glycocalyx	**5.** Protection from phagocytes
_____ **f.** Pili	**6.** Resting
_____ **g.** Plasma membrane	**7.** Protein synthesis
_____ **h.** Ribosomes	**8.** Selective permeability
	9. Transfer of genetic material

5. Why is an endospore called a resting structure? Of what advantage is an endospore to a bacterial cell?
6. Compare and contrast the following:
   a. simple diffusion and facilitated diffusion
   b. active transport and facilitated diffusion
   c. active transport and group translocation
7. Answer the following questions using the diagrams provided, which represent cross sections of bacterial cell walls.
   a. Which diagram represents a gram-positive bacterium? How can you tell?



   b. Explain how the Gram stain works to distinguish these two types of cell walls.
   c. Why does penicillin have no effect on most gram-negative cells?
   d. How do essential molecules enter cells through each wall?
   e. Which cell wall is toxic to humans?
8. Starch is readily metabolized by many cells, but a starch molecule is too large to cross the plasma membrane. How does a cell obtain the glucose molecules from a starch polymer? How does the cell transport these glucose molecules across the plasma membrane?
9. Match the characteristics of eukaryotic cells in column A with their functions in column B.

Column A	Column B
_____ **a.** Pericentriolar material	**1.** Digestive enzyme storage
_____ **b.** Chloroplasts	**2.** Oxidation of fatty acids
_____ **c.** Golgi complex	**3.** Microtubule formation
_____ **d.** Lysosomes	**4.** Photosynthesis
_____ **e.** Mitochondria	**5.** Protein synthesis
_____ **f.** Peroxisomes	**6.** Respiration
_____ **g.** Rough ER	**7.** Secretion

10. NAME IT What group of microbes is characterized by cells that form filaments, reproduce by spores, and have peptidoglycan in their cell walls?

Multiple Choice

1. Which of the following is *not* a distinguishing characteristic of prokaryotic cells?
   a. They usually have a single, circular chromosome.
   b. They lack membrane-enclosed organelles.
   c. They have cell walls containing peptidoglycan.
   d. Their DNA is not associated with histones.
   e. They lack a plasma membrane.

Use the following choices to answer questions 2–4.
   a. No change will result; the solution is isotonic.
   b. Water will move into the cell.
   c. Water will move out of the cell.
   d. The cell will undergo osmotic lysis.
   e. Sucrose will move into the cell from an area of higher concentration to one of lower concentration.

2. Which statement best describes what happens when a gram-positive bacterium is placed in distilled water and penicillin?
3. Which statement best describes what happens when a gram-negative bacterium is placed in distilled water and penicillin?
4. Which statement best describes what happens when a gram-positive bacterium is placed in an aqueous solution of lysozyme and 10% sucrose?
5. Which of the following statements best describes what happens to a cell exposed to polymyxins that destroy phospholipids?
   a. In an isotonic solution, nothing will happen.
   b. In a hypotonic solution, the cell will lyse.
   c. Water will move into the cell.
   d. Intracellular contents will leak from the cell.
   e. Any of the above might happen.

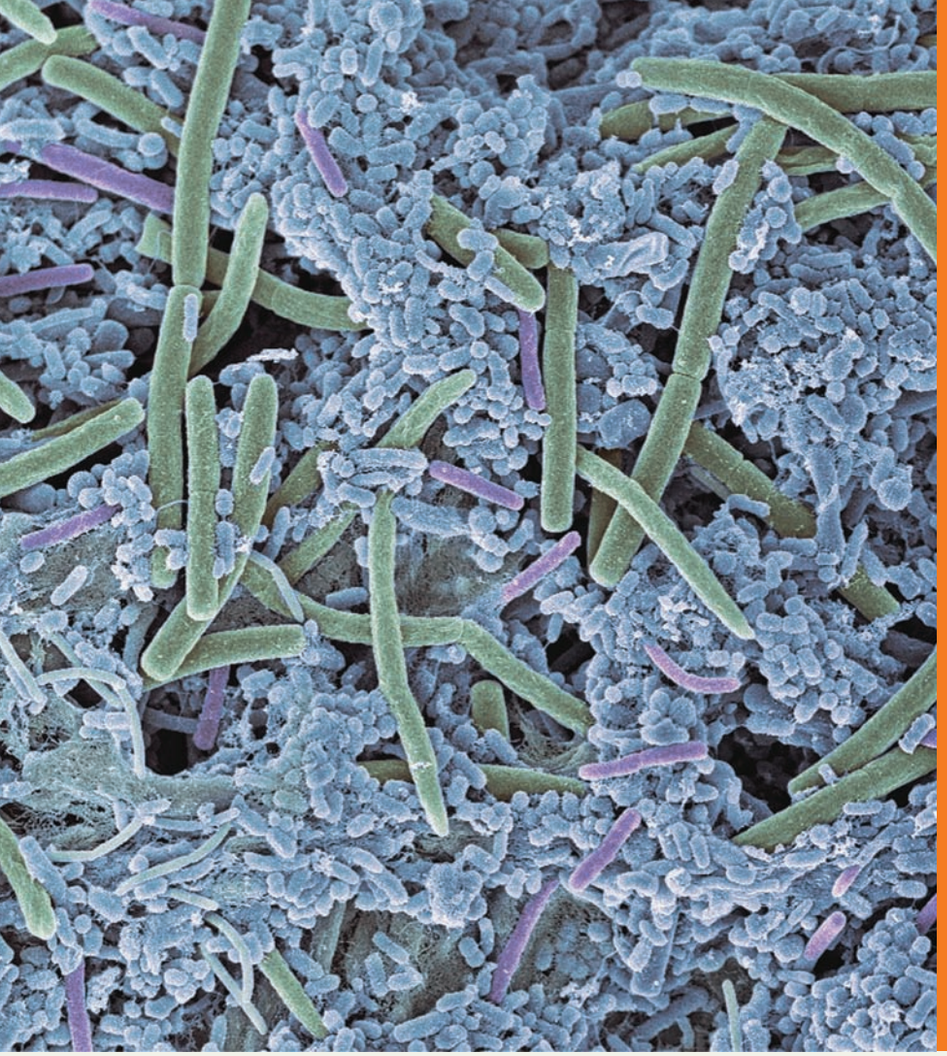
6. Which of the following is *false* about fimbriae?
   a. They are composed of protein.
   b. They may be used for attachment.
   c. They are found on gram-negative cells.
   d. They are composed of pilin.
   e. They may be used for motility.
7. Which of the following pairs is *mismatched*?
   a. glycocalyx—adherence
   b. pili—reproduction
   c. cell wall—toxin
   d. cell wall—protection
   e. plasma membrane—transport
8. Which of the following pairs is *mismatched*?
   a. metachromatic granules—stored phosphates
   b. polysaccharide granules—stored starch
   c. lipid inclusions—poly-β-hydroxybutyric acid
   d. sulfur granules—energy reserve
   e. ribosomes—protein storage
9. You have isolated a motile, gram-positive cell with no visible nucleus. You can assume this cell has
   a. ribosomes.
   b. mitochondria.
   c. an endoplasmic reticulum.
   d. a Golgi complex.
   e. all of the above
10. The antibiotic amphothericin B disrupts plasma membranes by combining with sterols; it will affect all of the following cells *except*
    a. animal cells.
    b. gram-negative bacterial cells.
    c. fungal cells.
    d. *Mycoplasma* cells.
    e. plant cells.

Critical Thinking

1. How can prokaryotic cells be smaller than eukaryotic cells and still carry on all the functions of life?
2. The smallest eukaryotic cell is the motile alga *Micromonas.* What is the minimum number of organelles this alga must have?
3. Two types of prokaryotic cells have been distinguished: bacteria and archaea. How do these cells differ from each other? How are they similar?
4. In 1985, a 0.5-mm cell was discovered in surgeonfish and named *Epulopiscium fishelsoni* (see Figure 11.14 page 315). It was presumed to be a protozoan. In 1993, researchers determined that *Epulopiscium* was actually a gram-positive bacterium. Why do you suppose this organism was initially identified as a protozoan? What evidence would change the classification to bacterium?
5. When *E. coli* cells are exposed to a hypertonic solution, the bacteria produce a transporter protein that can move K^+ (potassium ions) into the cell. Of what value is the active transport of K^+, which requires ATP?

Clinical Applications

1. *Clostridium botulinum* is a strict anaerobe; that is, it is killed by the molecular oxygen (O_2) present in air. Humans can die of botulism from eating foods in which *C. botulinum* is growing. How does this bacterium survive on plants picked for human consumption? Why are home-canned foods most often the source of botulism?
2. A South San Francisco child enjoyed bath time at his home because of the colorful orange and red water. The water did not have this rusty color at its source, and the water department could not culture the *Thiobacillus* bacteria responsible for the rusty color from the source. How were the bacteria getting into the household water? What bacterial structures make this possible?
3. Live cultures of *Bacillus thuringiensis* (Dipel) and *B. subtilis* (Kodiak) are sold as pesticides. What bacterial structures make it possible to package and sell these bacteria? For what purpose is each product used? (*Hint:* Refer to Chapter 11.)



5

Microbial Metabolism

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Now that you are familiar with the structure of prokaryotic cells, we can discuss the activities that enable these microbes to thrive. The life-support processes of even the most structurally simple organism involve a large number of complex biochemical reactions. Most, although not all, of the biochemical processes of bacteria also occur in eukaryotic microbes and in the cells of multicellular organisms, including humans. However, the reactions that are unique to bacteria are fascinating because they allow microorganisms to do things we cannot do. For example, some bacteria can live on cellulose, whereas others can live on petroleum. Through their metabolism, bacteria recycle elements after other organisms have used them. Still other bacteria can live on diets of such inorganic substances as carbon dioxide, iron, sulfur, hydrogen gas, and ammonia. Microbial metabolism allows some microorganisms to grow in or on the human body as shown in dental plaque in the photograph. An example of the bacterial metabolism that contributes to dental caries is discussed in the Clinical Case.

This chapter examines some representative chemical reactions that either produce energy (the catabolic reactions) or use energy (the anabolic reactions) in microorganisms. We will also look at how these various reactions are integrated within the cell.

Catabolic and Anabolic Reactions

LEARNING OBJECTIVES

5-1 Define *metabolism*, and describe the fundamental differences between anabolism and catabolism.

5-2 Identify the role of ATP as an intermediate between catabolism and anabolism.

We use the term **metabolism** to refer to the sum of all chemical reactions within a living organism. Because chemical reactions either release or require energy, metabolism can be viewed as an energy-balancing act. Accordingly, metabolism can be divided into two classes of chemical reactions: those that release energy and those that require energy.

In living cells, the enzyme-regulated chemical reactions that release energy are generally the ones involved in **catabolism,** the breakdown of complex organic compounds into simpler ones. These reactions are called *catabolic,* or *degradative,* reactions. Catabolic reactions are generally *hydrolytic reactions* (reactions which use water and in which chemical bonds are broken), and they are *exergonic* (produce more energy than they consume). An example of catabolism occurs when cells break down sugars into carbon dioxide and water.

The enzyme-regulated energy-requiring reactions are mostly involved in **anabolism,** the building of complex organic molecules from simpler ones. These reactions are called *anabolic,* or *biosynthetic,* reactions. Anabolic processes often involve *dehydration synthesis* reactions (reactions that release water), and they are *endergonic* (consume more energy than they produce). Examples of anabolic processes are the formation of proteins from amino acids, nucleic acids from nucleotides, and polysaccharides from simple sugars. These biosynthetic reactions generate the materials for cell growth.

Clinical Case: More Than a Sweet Tooth

Dr. Antonia Rivera is a pediatric dentist in St. Louis, Missouri. Her latest patient, 7-year-old Micah Thompson, has just left the office with strict instructions about brushing and flossing regularly. What most worries Dr. Rivera, however, is that Micah is her seventh patient this week to present with multiple dental caries, or cavities. Dr. Rivera is used to seeing some increase in tooth decay after Halloween and Easter, but why are all these children getting cavities in the middle of the summer? When possible, she has been speaking to each of the patient's parents or grandparents, but no one has noticed anything out of the ordinary in the children's diets.

Why do so many of Dr. Rivera's patients have multiple dental caries? Read on to find out.

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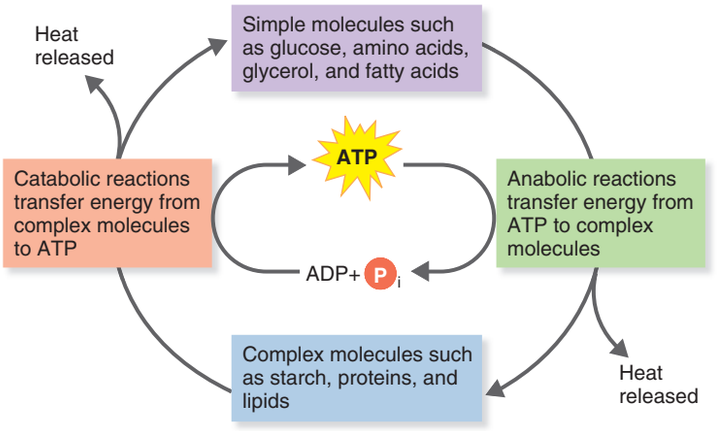


Figure 5.1 The role of ATP in coupling anabolic and catabolic reactions. When complex molecules are split apart (catabolism), some of the energy is transferred to and trapped in ATP, and the rest is given off as heat. When simple molecules are combined to form complex molecules (anabolism), ATP provides the energy for synthesis, and again some energy is given off as heat.

Q How does ATP provide the energy for synthesis?

Catabolic reactions provide building blocks for anabolic reactions and furnish the energy needed to drive anabolic reactions. This coupling of energy-requiring and energy-releasing reactions is made possible through the molecule adenosine triphosphate (ATP). (You can review its structure in Figure 2.18, page 48.) ATP stores energy derived from catabolic reactions and releases it later to drive anabolic reactions and perform other cellular work. Recall from Chapter 2 that a molecule of ATP consists of an adenine, a ribose, and three phosphate groups. When the terminal phosphate group is split from ATP, adenosine diphosphate (ADP) is formed, and energy is released to drive anabolic reactions. Using P to represent a phosphate group (P_i represents inorganic phosphate, which is not bound to any other molecule), we write this reaction as follows:

$$ATP \rightarrow ADP + P_i + \text{energy}$$

Then, the energy from catabolic reactions is used to combine ADP and a P_i to resynthesize ATP:

$$ADP + P_i + \text{energy} \rightarrow ATP$$

Thus, anabolic reactions are coupled to ATP breakdown, and catabolic reactions are coupled to ATP synthesis. This concept of coupled reactions is very important; you will see why by the end of this chapter. For now, you should know that the chemical composition of a living cell is constantly changing: some molecules are broken down while others are being synthesized. This balanced flow of chemicals and energy maintains the life of a cell.

The role of ATP in coupling anabolic and catabolic reactions is shown in Figure 5.1. Only part of the energy released in catabolism is actually available for cellular functions because part

of the energy is lost to the environment as heat. Because the cell must use energy to maintain life, it has a continuous need for new external sources of energy.

Before we discuss how cells produce energy, let's first consider the principal properties of a group of proteins involved in almost all biologically important chemical reactions: enzymes. A cell's **metabolic pathways** (sequences of chemical reactions) are determined by its enzymes, which are in turn determined by the cell's genetic makeup. **Animation** Metabolism: Overview

CHECK YOUR UNDERSTANDING

- Distinguish catabolism from anabolism. **5-1**
- How is ATP an intermediate between catabolism and anabolism? **5-2**

Enzymes

LEARNING OBJECTIVES

5-3 Identify the components of an enzyme.
5-4 Describe the mechanism of enzymatic action.
5-5 List the factors that influence enzymatic activity.
5-6 Distinguish competitive and noncompetitive inhibition.
5-7 Define *ribozyme*.

Collision Theory

We indicated in Chapter 2 that chemical reactions occur when chemical bonds are formed or broken. For reactions to take place, atoms, ions, or molecules must collide. The **collision theory** explains how chemical reactions occur and how certain factors affect the rates of those reactions. The basis of the collision theory is that all atoms, ions, and molecules are continuously moving and are thus continuously colliding with one another. The energy transferred by the particles in the collision can disrupt their electron structures enough to break chemical bonds or form new bonds.

Several factors determine whether a collision will cause a chemical reaction: the velocities of the colliding particles, their energy, and their specific chemical configurations. Up to a point, the higher the particles' velocities, the more probable that their collision will cause a reaction. Also, each chemical reaction requires a specific level of energy. But even if colliding particles possess the minimum energy needed for reaction, no reaction will take place unless the particles are properly oriented toward each other.

Let's assume that molecules of substance AB (the reactant) are to be converted to molecules of substances A and B (the products). In a given population of molecules of substance AB, at a specific temperature, some molecules possess relatively little energy; the majority of the population possesses an average amount of energy; and a small portion of the population has high energy. If only the high-energy AB molecules are able to react and be converted to A and B molecules, then only relatively few molecules at any one time possess enough energy to react in a collision. The collision energy required for a chemical reaction is its **activation energy,** which is the amount of energy needed to disrupt the stable electronic configuration of any specific molecule so that the electrons can be rearranged.

The **reaction rate**—the frequency of collisions containing sufficient energy to bring about a reaction—depends on the number of reactant molecules at or above the activation energy level. One way to increase the reaction rate of a substance is to raise its temperature. By causing the molecules to move faster, heat increases both the frequency of collisions and the number of molecules that attain activation energy. The number of collisions also increases when pressure is increased or when the reactants are more concentrated (because the distance between molecules is thereby decreased). In living systems, enzymes increase the reaction rate without raising the temperature.

Enzymes and Chemical Reactions

Substances that can speed up a chemical reaction without being permanently altered themselves are called **catalysts.** In living cells, **enzymes** serve as biological catalysts. As catalysts, enzymes are specific. Each acts on a specific substance, called the enzyme's **substrate** (or substrates, when there are two or more reactants), and each catalyzes only one reaction. For example, sucrose (table sugar) is the substrate of the enzyme sucrase, which catalyzes the hydrolysis of sucrose to glucose and fructose.

As catalysts, enzymes typically accelerate chemical reactions. The three-dimensional enzyme molecule has an *active site,* a region that interacts with a specific chemical substance (see Figure 5.4).

The enzyme orients the substrate into a position that increases the probability of a reaction. The **enzyme–substrate complex** formed by the temporary binding of enzyme and reactants enables the collisions to be more effective and lowers the activation energy of the reaction (**Figure 5.2**). The enzyme therefore speeds up the reaction by increasing the number of AB molecules that attain sufficient activation energy to react.

An enzyme's ability to accelerate a reaction without the need for an increase in temperature is crucial to living systems because a significant temperature increase would destroy cellular proteins. The crucial function of enzymes, therefore, is to speed up biochemical reactions at a temperature that is compatible with the normal functioning of the cell.

Enzyme Specificity and Efficiency

The specificity of enzymes is made possible by their structures. Enzymes are generally large globular proteins that range in molecular weight from about 10,000 to several million. Each of the thousands of known enzymes has a characteristic three-dimensional shape with a specific surface configuration as a result of its primary, secondary, and tertiary structures (see Figure 2.15, page 45). The unique configuration of each enzyme enables it to "find" the correct substrate from among the large number of diverse molecules in the cell.

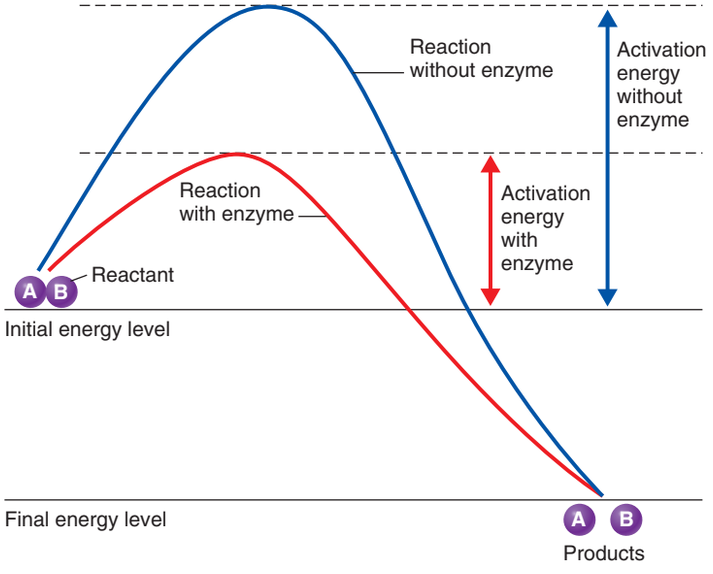


Figure 5.2 Energy requirements of a chemical reaction. This graph shows the progress of the reaction AB → A + B both without (blue line) and with (red line) an enzyme. The presence of an enzyme lowers the activation energy of the reaction (see arrows). Thus, more molecules of reactant AB are converted to products A and B because more molecules of reactant AB possess the activation energy needed for the reaction.

Q Why does a chemical reaction require increased activation energy without an enzyme as a biological catalyst?

Enzymes are extremely efficient. Under optimum conditions, they can catalyze reactions at rates 10^8 to 10^{10} times (up to 10 billion times) higher than those of comparable reactions without enzymes. The **turnover number** (maximum number of substrate molecules an enzyme molecule converts to product each second) is generally between 1 and 10,000 and can be as high as 500,000. For example, the enzyme DNA polymerase I, which participates in the synthesis of DNA, has a turnover number of 15, whereas the enzyme lactate dehydrogenase, which removes hydrogen atoms from lactic acid, has a turnover number of 1000.

Many enzymes exist in the cell in both active and inactive forms. The rate at which enzymes switch between these two forms is determined by the cellular environment.

Naming Enzymes

The names of enzymes usually end in *-ase.* All enzymes can be grouped into six classes, according to the type of chemical reaction they catalyze (**Table 5.1**). Enzymes within each of the major classes are named according to the more specific types of reactions they assist. For example, the class called *oxidoreductases* is involved with oxidation-reduction reactions (described shortly). Enzymes in the oxidoreductase class that remove hydrogen from a substrate are called *dehydrogenases;* those that add molecular oxygen (O_2) are called *oxidases.* As you will see later, dehydrogenase and oxidase enzymes have even more specific names, such as lactate dehydrogenase and cytochrome oxidase, depending on the specific substrates on which they act.

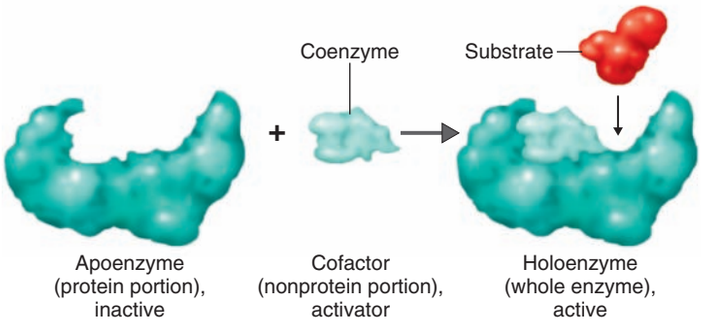


Figure 5.3 Components of a holoenzyme. Many enzymes require both an apoenzyme (protein portion) and a cofactor (nonprotein portion) to become active. The cofactor can be a metal ion, or if it is an organic molecule, it is called a coenzyme (as shown here). The apoenzyme and cofactor together make up the holoenzyme, or whole enzyme. The substrate is the reactant acted upon by the enzyme.

Q How does the enzyme–substrate complex lower the activation energy of the reaction?

Enzyme Components

Although some enzymes consist entirely of proteins, most consist of both a protein portion, called an **apoenzyme,** and a nonprotein component, called a **cofactor.** Ions of iron, zinc, magnesium, or calcium are examples of cofactors. If the cofactor is an organic molecule, it is called a **coenzyme.** Apoenzymes are inactive by themselves; they must be activated by cofactors. Together, the apoenzyme and cofactor form a **holoenzyme,** or whole, active enzyme (**Figure 5.3**). If the cofactor is removed, the apoenzyme will not function.

Coenzymes may assist the enzyme by accepting atoms removed from the substrate or by donating atoms required by the substrate. Some coenzymes act as electron carriers, removing electrons from the substrate and donating them to other molecules in subsequent reactions. Many coenzymes are derived from vitamins (**Table 5.2**). Two of the most important coenzymes in cellular metabolism are **nicotinamide adenine dinucleotide (NAD^+)** and **nicotinamide adenine dinucleotide phosphate ($NADP^+$).** Both compounds contain derivatives of the B vitamin niacin (nicotinic acid), and both function as electron carriers. Whereas NAD^+ is primarily involved in catabolic (energy-yielding) reactions, $NADP^+$ is primarily involved in anabolic (energy-requiring) reactions. The flavin coenzymes, such as **flavin mononucleotide (FMN)** and **flavin adenine dinucleotide (FAD),** contain derivatives of the B vitamin riboflavin and are also electron carriers. Another important coenzyme, **coenzyme A (CoA),** contains a derivative of pantothenic acid, another B vitamin. This coenzyme plays an important role in the synthesis and breakdown of fats and in a series of oxidizing reactions called the Krebs cycle.

TABLE 5.1 Enzyme Classification Based on Type of Chemical Reaction Catalyzed

Class	Type of Chemical Reaction Catalyzed	Examples
Oxidoreductase	Oxidation-reduction, in which oxygen and hydrogen are gained or lost	Cytochrome oxidase, lactate dehydrogenase
Transferase	Transfer of functional groups, such as an amino group, acetyl group, or phosphate group	Acetate kinase, alanine deaminase
Hydrolase	Hydrolysis (addition of water)	Lipase, sucrase
Lyase	Removal of groups of atoms without hydrolysis	Oxalate decarboxylase, isocitrate lyase
Isomerase	Rearrangement of atoms within a molecule	Glucose-phosphate isomerase, alanine racemase
Ligase	Joining of two molecules (using energy usually derived from the breakdown of ATP)	Acetyl-CoA synthetase, DNA ligase

We will come across all of these coenzymes in our discussion of metabolism later in the chapter.

As noted earlier, some cofactors are metal ions, including iron, copper, magnesium, manganese, zinc, calcium, and cobalt. Such cofactors may help catalyze a reaction by forming a bridge between the enzyme and a substrate. For example, magnesium (Mg^{2+}) is required by many phosphorylating enzymes (enzymes that transfer a phosphate group from ATP to another substrate). The Mg^{2+} can form a link between the enzyme and the ATP molecule. Most trace elements required by living cells are probably used in some such way to activate cellular enzymes.

The Mechanism of Enzymatic Action

Enzymes lower the activation energy of chemical reactions. The general sequence of events in enzyme action is as follows (Figure 5.4a):

1. The surface of the substrate contacts a specific region of the surface of the enzyme molecule, called the **active site.**
2. A temporary intermediate compound forms, called an **enzyme–substrate complex.**
3. The substrate molecule is transformed by the rearrangement of existing atoms, the breakdown of the substrate molecule, or in combination with another substrate molecule.
4. The transformed substrate molecules—the products of the reaction—are released from the enzyme molecule because they no longer fit in the active site of the enzyme.
5. The unchanged enzyme is now free to react with other substrate molecules.

As a result of these events, an enzyme speeds up a chemical reaction.

TABLE 5.2 Selected Vitamins and Their Coenzymatic Functions

Vitamin	Function
Vitamin B_1 (Thiamine)	Part of coenzyme cocarboxylase; has many functions, including the metabolism of pyruvic acid
Vitamin B_2 (Riboflavin)	Coenzyme in flavoproteins; active in electron transfers
Niacin (Nicotinic Acid)	Part of NAD molecule*; active in electron transfers
Vitamin B_6 (Pyridoxine)	Coenzyme in amino acid metabolism
Vitamin B_{12} (Cyanocobalamin)	Coenzyme (methyl cyanocobalamide) involved in the transfer of methyl groups; active in amino acid metabolism
Pantothenic Acid	Part of coenzyme A molecule; involved in the metabolism of pyruvic acid and lipids
Biotin	Involved in carbon dioxide fixation reactions and fatty acid synthesis
Folic Acid	Coenzyme used in the synthesis of purines and pyrimidines
Vitamin E	Needed for cellular and macromolecular syntheses
Vitamin K	Coenzyme used in electron transport (naphthoquinones and quinones)

*NAD = nicotinamide adenine dinucleotide

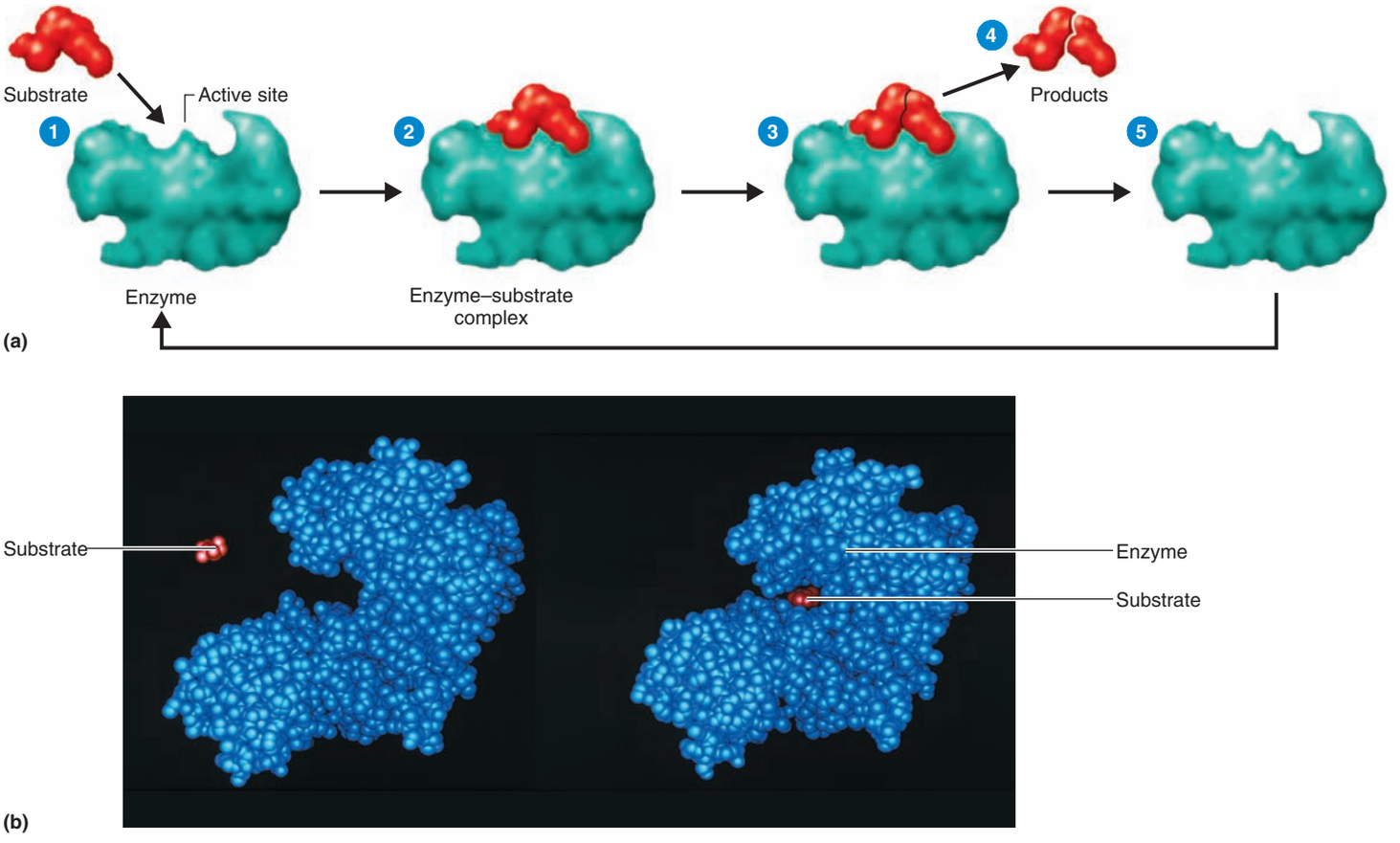


Figure 5.4 The mechanism of enzymatic action. (**a**) 1 The substrate contacts the active site on the enzyme to form 2 an enzyme–substrate complex. 3 The substrate is then transformed into products, 4 the products are released, and 5 the enzyme is recovered unchanged. In the example shown, the transformation into products involves a breakdown of the substrate into two products. Other transformations, however, may occur. (**b**) Left: A molecular model of the enzyme in step 1 of part (a). The active site of the enzyme can be seen here as a groove on the surface of the protein. Right: As the enzyme and substrate meet in step 2 of part (a), the enzyme changes shape slightly to fit together more tightly with the substrate.

Give an example of enzymatic specifity.

As mentioned earlier, enzymes have *specificity* for particular substrates. For example, a specific enzyme may be able to hydrolyze a peptide bond only between two specific amino acids. Other enzymes can hydrolyze starch but not cellulose; even though both starch and cellulose are polysaccharides composed of glucose subunits, the orientations of the subunits in the two polysaccharides differ. Enzymes have this specificity because the three-dimensional shape of the active site fits the substrate somewhat as a lock fits with its key (**Figure 5.4b**). However, the active site and substrate are flexible, and they change shape somewhat as they meet to fit together more tightly. The substrate is usually much smaller than the enzyme, and relatively few of the enzyme's amino acids make up the active site.

A certain compound can be a substrate for several different enzymes that catalyze different reactions, so the fate of a compound depends on the enzyme that acts on it. At least four different enzymes can act on glucose 6-phosphate, a molecule important in cell metabolism, and each reaction will yield a different product. **Animations** Enzymes: Overview, Steps in a Reaction

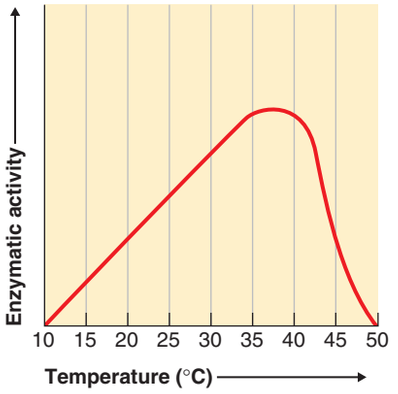
Factors Influencing Enzymatic Activity

Enzymes are subject to various cellular controls. Two primary types are the control of enzyme *synthesis* (see Chapter 8) and the control of enzyme *activity* (how much enzyme is present versus how active it is).

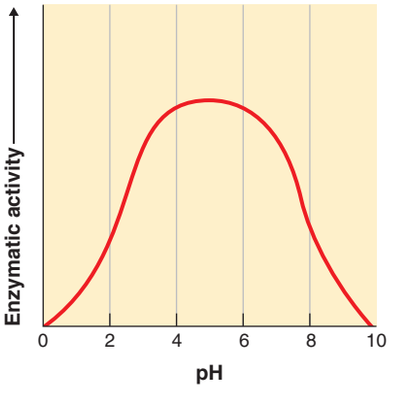
Several factors influence the activity of an enzyme. Among the more important are temperature, pH, substrate concentration, and the presence or absence of inhibitors.

Temperature

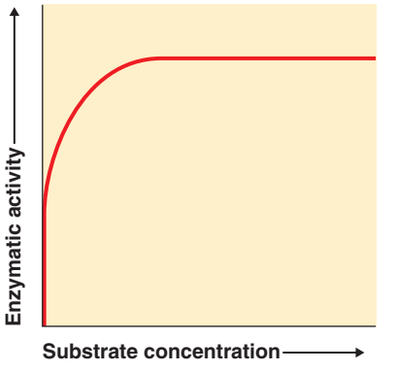
The rate of most chemical reactions increases as the temperature increases. Molecules move more slowly at lower temperatures than at higher temperatures and so may not have enough energy



(a) Temperature. The enzymatic activity (rate of reaction catalyzed by the enzyme) increases with increasing temperature until the enzyme, a protein, is denatured by heat and inactivated. At this point, the reaction rate falls steeply.



(b) pH. The enzyme illustrated is most active at about pH 5.0.



(c) Substrate concentration. With increasing concentration of substrate molecules, the rate of reaction increases until the active sites on all the enzyme molecules are filled, at which point the maximum rate of reaction is reached.

Figure 5.5 **Factors that influence enzymatic activity, plotted for a hypothetical enzyme.**

Q How will this enzyme act at 25°C? At 45°C? At pH 7?

to cause a chemical reaction. For enzymatic reactions, however, elevation beyond a certain temperature (the optimal temperature) drastically reduces the rate of reaction (Figure 5.5a). The optimal temperature for most disease-producing bacteria in the human body is between 35°C and 40°C. The rate of reaction declines beyond the optimal temperature because of the enzyme's **denaturation,** the loss of its characteristic three-dimensional structure (tertiary configuration) (Figure 5.6). Denaturation of a protein involves the breakage of hydrogen bonds and other noncovalent bonds; a common example is the transformation of uncooked egg white (a protein called albumin) to a hardened state by heat.

Denaturation of an enzyme changes the arrangement of the amino acids in the active site, altering its shape and causing the enzyme to lose its catalytic ability. In some cases, denaturation is partially or fully reversible. However, if denaturation continues until the enzyme has lost its solubility and coagulates, the enzyme cannot regain its original properties. Enzymes can also be denatured by concentrated acids, bases, heavy-metal ions (such as lead, arsenic, or mercury), alcohol, and ultraviolet radiation.

pH

Most enzymes have an optimum pH at which their activity is characteristically maximal. Above or below this pH value, enzyme activity, and therefore the reaction rate, decline (Figure 5.5b). When the H^+ concentration (pH) in the medium is changed drastically, the protein's three-dimensional structure is altered. Extreme changes in pH can cause denaturation. Acids (and bases) alter a protein's three-dimensional structure because the H^+ (and OH^-) compete with hydrogen and ionic bonds in an enzyme, resulting in the enzyme's denaturation.

Substrate Concentration

There is a maximum rate at which a certain amount of enzyme can catalyze a specific reaction. Only when the concentration of substrate(s) is extremely high can this maximum rate be attained. Under conditions of high substrate concentration, the enzyme is said to be in **saturation;** that is, its active site is always occupied by substrate or product molecules. In this condition, a further increase in substrate concentration will not affect the reaction rate because all active sites are already in use (Figure 5.5c). Under normal cellular conditions, enzymes are not saturated with substrate(s). At any given time, many of the enzyme molecules are inactive for lack of substrate; thus, the substrate concentration is likely to influence the rate of reaction.

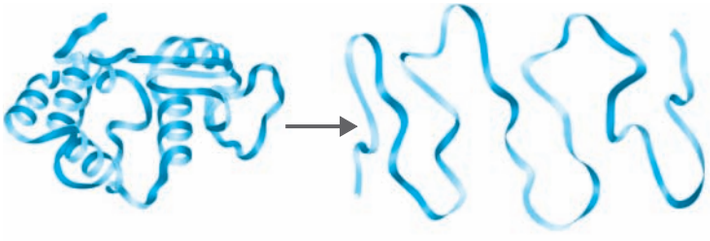


Figure 5.6 **Denaturation of a protein.** Breakage of the noncovalent bonds (such as hydrogen bonds) that hold the active protein in its three-dimensional shape renders the denatured protein nonfunctional.

Q When is denaturation irreversible?

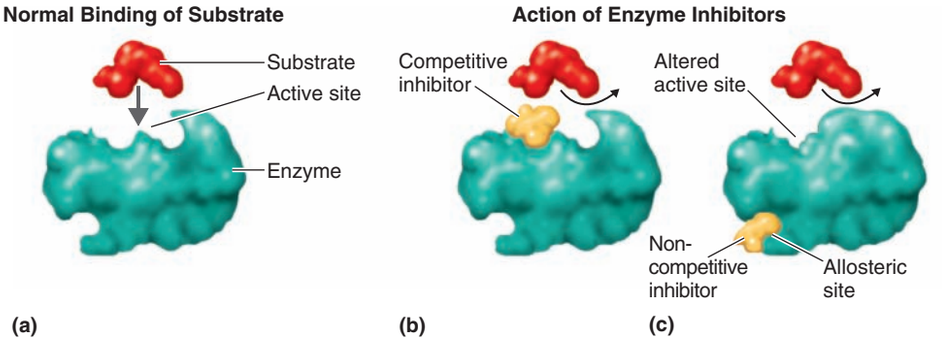


Figure 5.7 Enzyme inhibitors. (**a**) An uninhibited enzyme and its normal substrate. (**b**) A competitive inhibitor. (**c**) One type of noncompetitive inhibitor, causing allosteric inhibition.

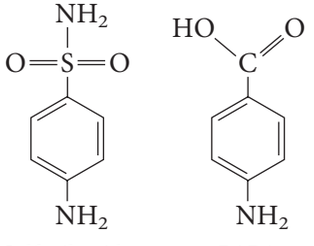
Q How do competitive inhibitors operate in comparison to noncompetitive inhibitors?

Inhibitors

An effective way to control the growth of bacteria is to control their enzymes. Certain poisons, such as cyanide, arsenic, and mercury, combine with enzymes and prevent them from functioning. As a result, the cells stop functioning and die.

Enzyme inhibitors are classified as either competitive or noncompetitive inhibitors (Figure 5.7). **Competitive inhibitors** fill the active site of an enzyme and compete with the normal substrate for the active site. A competitive inhibitor can do this because its shape and chemical structure are similar to those of the normal substrate (Figure 5.7b). However, unlike the substrate, it does not undergo any reaction to form products. Some competitive inhibitors bind irreversibly to amino acids in the active site, preventing any further interactions with the substrate. Others bind reversibly, alternately occupying and leaving the active site; these slow the enzyme's interaction with the substrate. Increasing the substrate concentration can overcome reversible competitive inhibition. As active sites become available, more substrate molecules than competitive inhibitor molecules are available to attach to the active sites of enzymes.

One good example of a competitive inhibitor is sulfanilamide (a sulfa drug), which inhibits the enzyme whose normal substrate is *para*-aminobenzoic acid (PABA):



Sulfanilamide PABA

PABA is an essential nutrient used by many bacteria in the synthesis of folic acid, a vitamin that functions as a coenzyme. When sulfanilamide is administered to bacteria, the enzyme that normally converts PABA to folic acid combines instead with the sulfanilamide. Folic acid is not synthesized, and the bacteria cannot grow. Because human cells do not use PABA to make their folic acid, sulfanilamide can kill bacteria but does not harm human cells.

Noncompetitive inhibitors do not compete with the substrate for the enzyme's active site; instead, they interact with another part of the enzyme (Figure 5.7c). In this process, called **allosteric** ("other space") **inhibition,** the inhibitor binds to a site on the enzyme other than the substrate's binding site, called the **allosteric site.** This binding causes the active site to change its shape, making it nonfunctional. As a result, the enzyme's activity is reduced. This effect can be either reversible or irreversible, depending on whether the active site can return to its original shape. In some cases, allosteric interactions can activate an enzyme rather than inhibit it. Another type of noncompetitive inhibition can operate on enzymes that require metal ions for their activity. Certain chemicals can bind or tie up the metal ion activators and thus prevent an enzymatic reaction. Cyanide can bind the iron in iron-containing enzymes, and fluoride can bind calcium or magnesium. Substances such as cyanide and fluoride are sometimes called *enzyme poisons* because they permanently inactivate enzymes. MM **Animations** Enzymes: Competitive Inhibition, Noncompetitive Inhibition

Feedback Inhibition

Allosteric inhibitors play a role in a kind of biochemical control called **feedback inhibition,** or **end-product inhibition.** This control mechanism stops the cell from making more of a substance than it needs and thereby wasting chemical resources. In some metabolic reactions, several steps are required for the synthesis of a particular chemical compound, called the *end-product.* The process is similar to an assembly line, with each step catalyzed by a separate enzyme (Figure 5.8). In many anabolic pathways, the final product can allosterically inhibit the activity of one of the enzymes earlier in the pathway. This phenomenon is feedback inhibition.

Feedback inhibition generally acts on the first enzyme in a metabolic pathway (similar to shutting down an assembly line by stopping the first worker). Because the enzyme is inhibited, the product of the first enzymatic reaction in the pathway is not synthesized. Because that unsynthesized product would

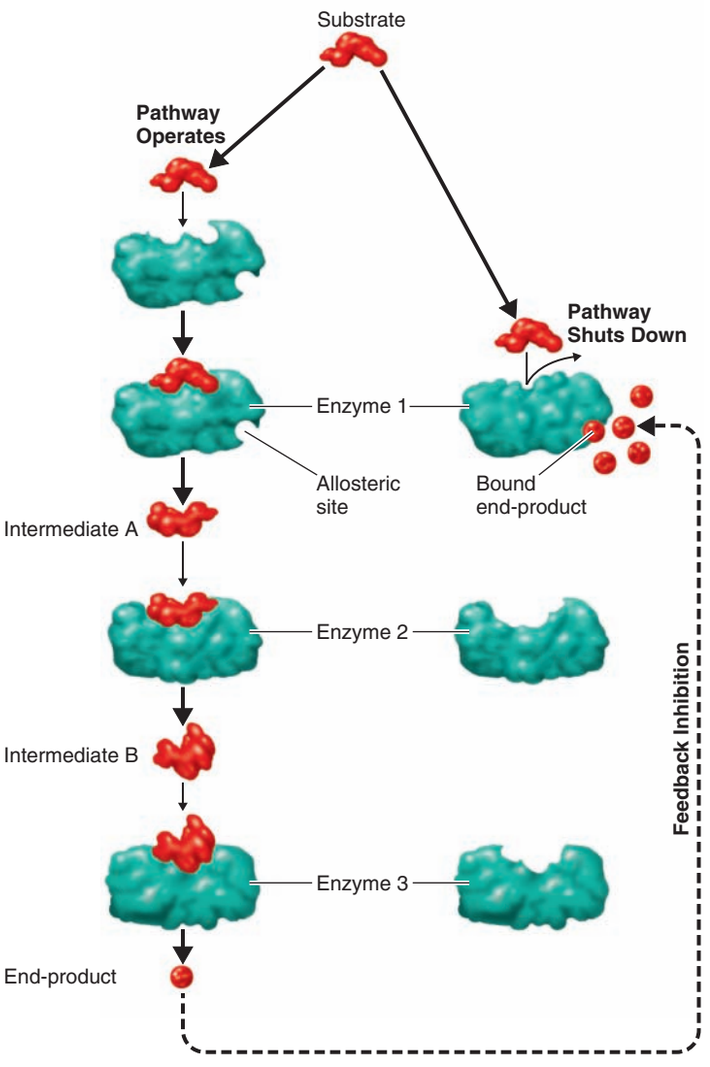


Figure 5.8 **Feedback inhibition.**

Q Explain the differences between competitive inhibition and feedback inhibition.

normally be the substrate for the second enzyme in the pathway, the second reaction stops immediately as well. Thus, even though only the first enzyme in the pathway is inhibited, the entire pathway shuts down, and no new end-product is formed. By inhibiting the first enzyme in the pathway, the cell also keeps metabolic intermediates from accumulating. As the cell uses up the existing end-product, the first enzyme's allosteric site more often remains unbound, and the pathway resumes activity.

The bacterium *E. coli* can be used to demonstrate feedback inhibition in the synthesis of the amino acid isoleucine, which is required for the cell's growth. In this metabolic pathway, the amino acid threonine is enzymatically converted to isoleucine in five steps. If isoleucine is added to the growth medium for *E. coli,* it inhibits the first enzyme in the pathway, and the bacteria stop synthesizing isoleucine. This condition is maintained until the supply of isoleucine is depleted. This type of feedback inhibition is also involved in regulating the cells' production of other amino acids, as well as vitamins, purines, and pyrimidines.

Ribozymes

Prior to 1982, it was believed that only protein molecules had enzymatic activity. Researchers working on microbes discovered a unique type of RNA called a **ribozyme.** Like protein enzymes, ribozymes function as catalysts, have active sites that bind to substrates, and are not used up in a chemical reaction. Ribozymes specifically act on strands of RNA by removing sections and splicing together the remaining pieces. In this respect, ribozymes are more restricted than protein enzymes in terms of the diversity of substrates with which they interact.

CHECK YOUR UNDERSTANDING

- What is a coenzyme? **5-3**
- Why is enzyme specificity important? **5-4**
- What happens to an enzyme below its optimal temperature? Above its optimal temperature? **5-5**
- Why is feedback inhibition noncompetitive inhibition? **5-6**
- What is a ribozyme? **5-7**

Energy Production

LEARNING OBJECTIVES

5-8 Explain the term *oxidation-reduction.*

5-9 List and provide examples of three types of phosphorylation reactions that generate ATP.

5-10 Explain the overall function of metabolic pathways.

Nutrient molecules, like all molecules, have energy associated with the electrons that form bonds between their atoms. When it is spread throughout the molecule, this energy is difficult for the cell to use. Various reactions in catabolic pathways, however, concentrate the energy into the bonds of ATP, which serves as a convenient energy carrier. ATP is generally referred to as having "high-energy" bonds. Actually, a better term is probably *unstable bonds.* Although the amount of energy in these bonds is not exceptionally large, it can be released quickly and easily. In a sense, ATP is similar to a highly flammable liquid such as kerosene. Although a large log might eventually burn to produce more heat than a cup of kerosene, the kerosene is easier to ignite and provides heat more quickly and conveniently. In a similar way, the "high-energy" unstable bonds of ATP provide the cell with readily available energy for anabolic reactions.

Before discussing the catabolic pathways, we will consider two general aspects of energy production: the concept of oxidation-reduction and the mechanisms of ATP generation.

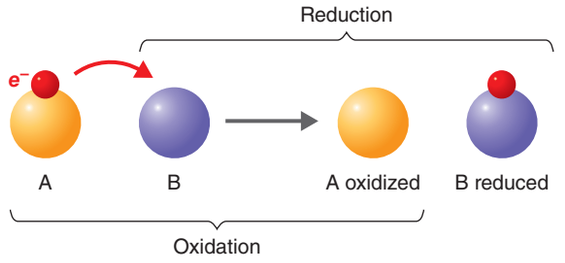


Figure 5.9 Oxidation-reduction. An electron is transferred from molecule A to molecule B. In the process, molecule A is oxidized, and molecule B is reduced.

Q How do oxidation and reduction differ?

Oxidation-Reduction Reactions

Oxidation is the removal of electrons (e^-) from an atom or molecule, a reaction that often produces energy. Figure 5.9 shows an example of an oxidation in which molecule A loses an electron to molecule B. Molecule A has undergone oxidation (meaning that it has lost one or more electrons), whereas molecule B has undergone **reduction** (meaning that it has gained one or more electrons).* Oxidation and reduction reactions are always coupled; in other words, each time one substance is oxidized, another is simultaneously reduced. The pairing of these reactions is called **oxidation-reduction** or a **redox reaction.**

In many cellular oxidations, electrons and protons (hydrogen ions, H^+) are removed at the same time; this is equivalent to the removal of hydrogen atoms, because a hydrogen atom is made up of one proton and one electron (see Table 2.2, page 28). Because most biological oxidations involve the loss of hydrogen atoms, they are also called **dehydrogenation** reactions. Figure 5.10 shows an example of a biological oxidation. An organic molecule is oxidized by the loss of two hydrogen atoms, and a molecule of NAD^+ is reduced. Recall from our earlier discussion of coenzymes that NAD^+ assists enzymes by accepting hydrogen atoms removed from the substrate, in this case the organic molecule. As shown in Figure 5.10, NAD^+ accepts two electrons and one proton. One proton (H^+) is left over and is released into the surrounding medium. The reduced coenzyme, NADH, contains more energy than NAD^+. This energy can be used to generate ATP in later reactions.

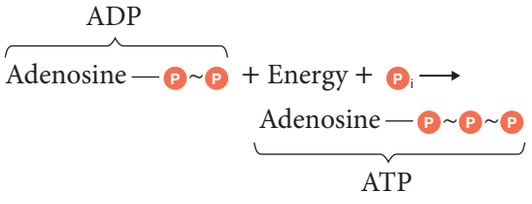
An important point to remember about biological oxidation-reduction reactions is that cells use them in catabolism to extract energy from nutrient molecules. Cells take nutrients, some of which serve as energy sources, and degrade them from highly reduced compounds (with many hydrogen atoms) to highly oxidized compounds. For example, when a cell oxidizes a molecule of glucose ($C_6H_{12}O_6$) to CO_2 and H_2O, the energy in the glucose molecule is removed in a stepwise manner and ultimately is trapped by ATP, which can then serve as an energy source for energy-requiring reactions. Compounds such as glucose that have many hydrogen atoms are highly reduced compounds, containing a large amount of potential energy. Thus, glucose is a valuable nutrient for organisms. MM **Animation** Oxidation-Reduction Reactions

CHECK YOUR UNDERSTANDING

✓ Why is glucose such an important molecule for organisms? 5-8

The Generation of ATP

Much of the energy released during oxidation-reduction reactions is trapped within the cell by the formation of ATP. Specifically, an inorganic phosphate group, P_i, is added to ADP with the input of energy to form ATP:



The symbol ~ designates a "high-energy" bond—that is, one that can readily be broken to release usable energy. The high-energy bond that attaches the third P in a sense contains the energy stored in this reaction. When this P is removed, usable energy is released. The addition of P to a chemical compound is called **phosphorylation.** Organisms use three mechanisms of phosphorylation to generate ATP from ADP.

Substrate-Level Phosphorylation

In **substrate-level phosphorylation,** ATP is usually generated when a high-energy P is directly transferred from a phosphorylated compound (a substrate) to ADP. Generally, the P has acquired its energy during an earlier reaction in which the substrate itself was oxidized. The following example shows only the carbon skeleton and the P of a typical substrate:

Oxidative Phosphorylation

In **oxidative phosphorylation,** electrons are transferred from organic compounds to one group of electron carriers (usually to NAD^+ and FAD). Then, the electrons are passed through a

*The terms do not seem logical until one considers the history of the discovery of these reactions. When mercury is heated, it gains weight as mercuric oxide is formed; this was called *oxidation*. Later it was determined that the mercury actually *lost* electrons, and the observed *gain* in oxygen was a direct result of this. Oxidation, therefore, is a *loss* of electrons, and reduction is a *gain* of electrons, but the gain and loss of electrons is not usually apparent as chemical-reaction equations are usually written. For example, in the equations for aerobic respiration on page 130, notice that each carbon in glucose had only one oxygen originally, and later, as carbon dioxide, each carbon now has two oxygens. However, the gain or loss of electrons actually responsible for this is not apparent.

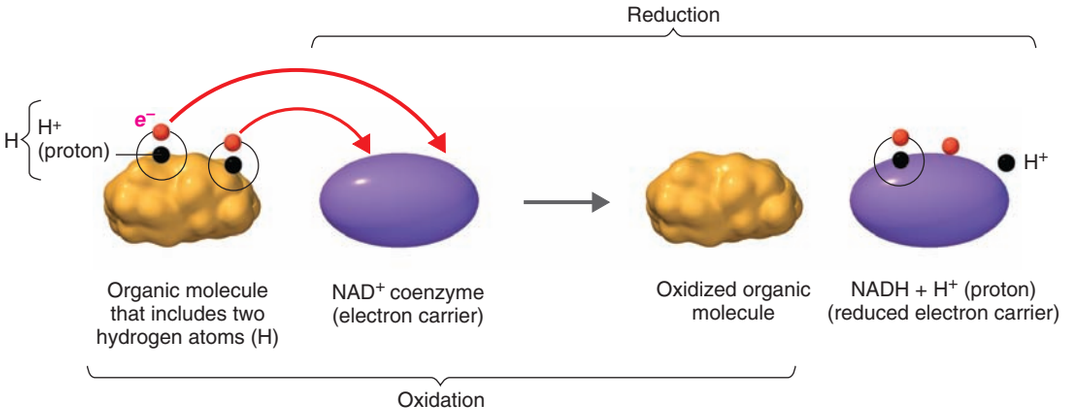


Figure 5.10 Representative biological oxidation. Two electrons and two protons (altogether equivalent to two hydrogen atoms) are transferred from an organic substrate molecule to a coenzyme, NAD^+. NAD^+ actually receives one hydrogen atom and one electron, and one proton is released into the medium. NAD^+ is reduced to NADH, which is a more energy-rich molecule.

Q How do organisms use oxidation-reduction reactions?

series of different electron carriers to molecules of oxygen (O_2) or other oxidized inorganic and organic molecules. This process occurs in the plasma membrane of prokaryotes and in the inner mitochondrial membrane of eukaryotes. The sequence of electron carriers used in oxidative phosphorylation is called an **electron transport chain (system)** (see Figure 5.14). The transfer of electrons from one electron carrier to the next releases energy, some of which is used to generate ATP from ADP through a process called *chemiosmosis,* to be described on page 128.

Photophosphorylation

The third mechanism of phosphorylation, **photophosphorylation,** occurs only in photosynthetic cells, which contain light-trapping pigments such as chlorophylls. In photosynthesis, organic molecules, especially sugars, are synthesized with the energy of light from the energy-poor building blocks carbon dioxide and water. Photophosphorylation starts this process by converting light energy to the chemical energy of ATP and NADPH, which, in turn, are used to synthesize organic molecules. As in oxidative phosphorylation, an electron transport chain is involved.

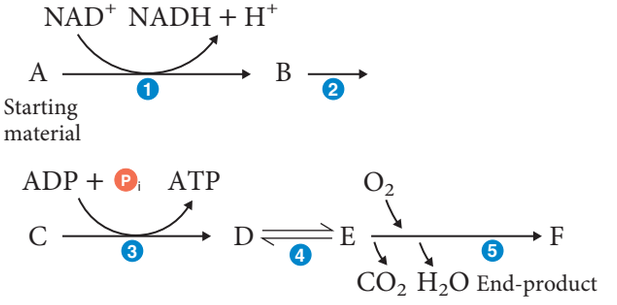
CHECK YOUR UNDERSTANDING

Outline the three ways that ATP is generated. **5-9**

Metabolic Pathways of Energy Production

Organisms release and store energy from organic molecules by a series of controlled reactions rather than in a single burst. If the energy were released all at once, as a large amount of heat, it could not be readily used to drive chemical reactions and would, in fact, damage the cell. To extract energy from organic compounds and store it in chemical form, organisms pass electrons from one compound to another through a series of oxidation-reduction reactions.

As noted earlier, a sequence of enzymatically catalyzed chemical reactions occurring in a cell is called a metabolic pathway. Below is a hypothetical metabolic pathway that converts starting material A to end-product F in a series of five steps:



The first step is the conversion of molecule A to molecule B. The curved arrow indicates that the reduction of coenzyme NAD^+ to NADH is coupled to that reaction; the electrons and protons come from molecule A. Similarly, the two arrows in 3 show a coupling of two reactions. As C is converted to D, ADP is converted to ATP; the energy needed comes from C as it transforms into D. The reaction converting D to E is readily reversible, as indicated by the double arrow. In the fifth step, the curved arrow leading from O_2 indicates that O_2 is a reactant. The curved arrows leading to CO_2 and H_2O indicate that these substances are secondary products produced in the reaction, in addition to F, the end-product that (presumably) interests us the most. Secondary products such as CO_2 and H_2O shown here are sometimes called *by-products* or *waste products.* Keep in mind that almost every reaction in a metabolic pathway is catalyzed by a specific enzyme; sometimes the name of the enzyme is printed near the arrow.

CHECK YOUR UNDERSTANDING

What is the purpose of metabolic pathways? **5-10**

Carbohydrate Catabolism

LEARNING OBJECTIVES

5-11 Describe the chemical reactions of glycolysis.

5-12 Identify the functions of the pentose phosphate and Entner-Doudoroff pathways.

5-13 Explain the products of the Krebs cycle.

5-14 Describe the chemiosmotic model for ATP generation.

5-15 Compare and contrast aerobic and anaerobic respiration.

5-16 Describe the chemical reactions of, and list some products of, fermentation.

Most microorganisms oxidize carbohydrates as their primary source of cellular energy. **Carbohydrate catabolism,** the breakdown of carbohydrate molecules to produce energy, is therefore of great importance in cell metabolism. Glucose is the most common carbohydrate energy source used by cells. Microorganisms can also catabolize various lipids and proteins for energy production (page 133).

To produce energy from glucose, microorganisms use two general processes: *cellular respiration* and *fermentation*. (In discussing cellular respiration, we frequently refer to the process simply as respiration, but it should not be confused with breathing.) Both cellular respiration and fermentation usually start with the same first step, glycolysis, but follow different subsequent pathways (**Figure 5.11**). Before examining the details of glycolysis, respiration, and fermentation, we will first look at a general overview of the processes.

As shown in Figure 5.11, the respiration of glucose typically occurs in three principal stages: glycolysis, the Krebs cycle, and the electron transport chain (system).

1. Glycolysis is the oxidation of glucose to pyruvic acid with the production of some ATP and energy-containing NADH.
2. The Krebs cycle is the oxidation of acetyl CoA (a derivative of pyruvic acid) to carbon dioxide, with the production of some ATP, energy-containing NADH, and another reduced electron carrier, $FADH_2$ (the reduced form of flavin adenine dinucleotide).
3. In the electron transport chain (system), NADH and $FADH_2$ are oxidized, contributing the electrons they have carried from the substrates to a "cascade" of oxidation-reduction reactions involving a series of additional electron carriers. Energy from these reactions is used to generate a considerable amount of ATP. In respiration, most of the ATP is generated in the third step.

Because respiration involves a long series of oxidation-reduction reactions, the entire process can be thought of as involving a flow of electrons from the energy-rich glucose molecule to the relatively energy-poor CO_2 and H_2O molecules. The coupling of ATP production to this flow is somewhat analogous to producing electrical power by using energy from a flowing stream. Carrying the analogy further, you could imagine a stream flowing down a gentle slope during glycolysis and the Krebs cycle, supplying energy to turn two old-fashioned waterwheels. Then the stream rushes down a steep slope in the electron transport chain, supplying energy for a large modern power plant. In a similar way, glycolysis and the Krebs cycle generate a small amount of ATP and also supply the electrons that generate a great deal of ATP at the electron transport chain stage.

Typically, the initial stage of fermentation is also glycolysis (Figure 5.11). However, once glycolysis has taken place, the pyruvic acid is converted into one or more different products, depending on the type of cell. These products might include alcohol (ethanol) and lactic acid. Unlike respiration, there is no Krebs cycle or electron transport chain in fermentation. Accordingly, the ATP yield, which comes only from glycolysis, is much lower.

Glycolysis

Glycolysis, the oxidation of glucose to pyruvic acid, is usually the first stage in carbohydrate catabolism. Most microorganisms use this pathway; in fact, it occurs in most living cells.

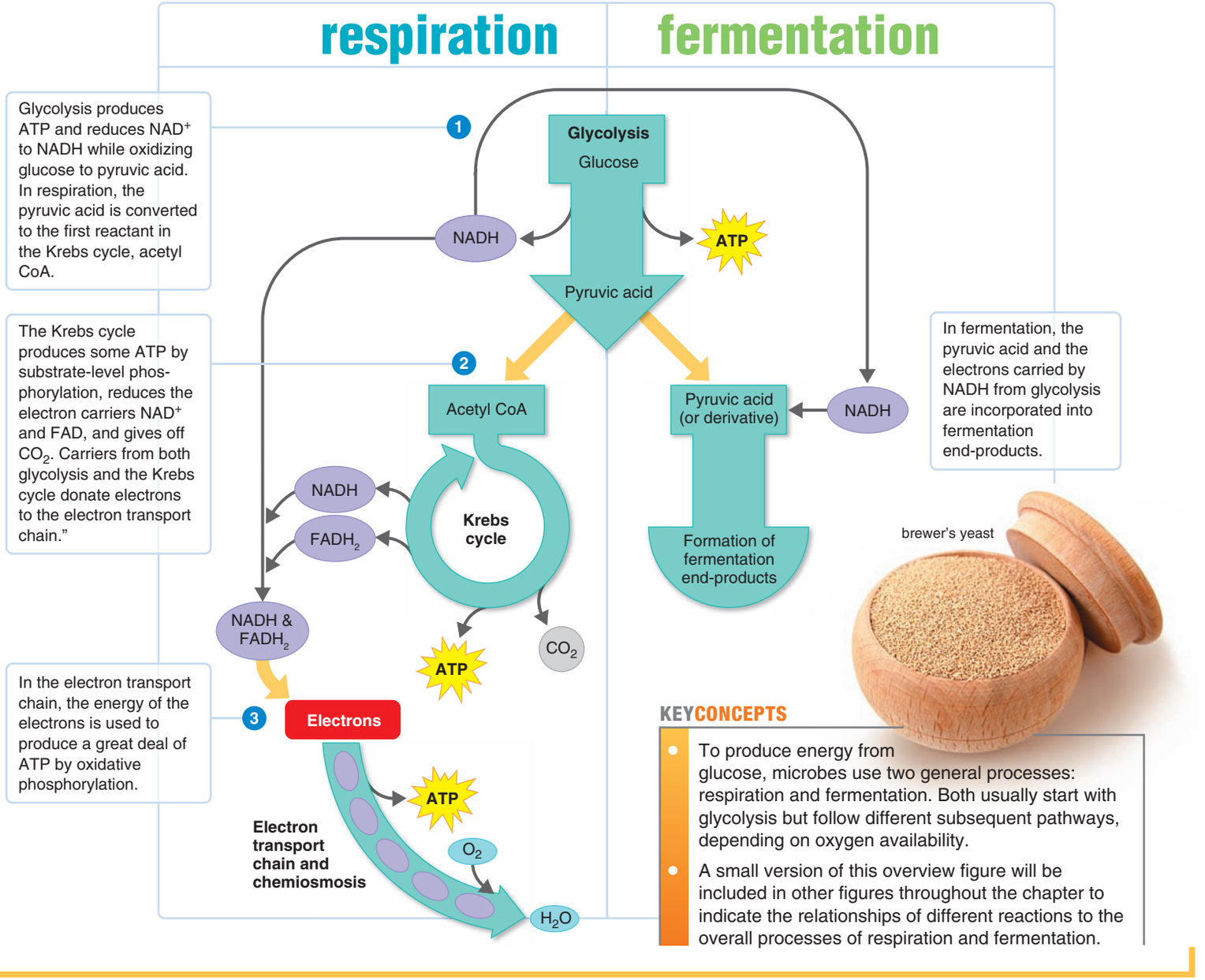
Glycolysis is also called the *Embden-Meyerhof pathway*. The word *glycolysis* means splitting of sugar, and this is exactly what happens. The enzymes of glycolysis catalyze the splitting of glucose, a six-carbon sugar, into two three-carbon sugars. These sugars are then oxidized, releasing energy, and their atoms are rearranged to form two molecules of pyruvic acid. During glycolysis NAD^+ is reduced to NADH, and there is a net production of two ATP molecules by substrate-level phosphorylation. Glycolysis does not require oxygen; it can occur whether oxygen is present or not. This pathway is a series of ten chemical reactions, each catalyzed by a different enzyme. The steps are outlined in **Figure 5.12**; see also Figure A.2 in Appendix A for a more detailed representation of glycolysis.

To summarize the process, glycolysis consists of two basic stages, a preparatory stage and an energy-conserving stage:

1. First, in the preparatory stage (steps 1–4 in Figure 5.12), two molecules of ATP are used as a six-carbon glucose molecule is phosphorylated, restructured, and split into two three-carbon compounds: glyceraldehyde 3-phosphate (GP) and dihydroxyacetone phosphate (DHAP). 5 DHAP is readily converted to GP. (The reverse reaction may also occur.) The conversion of DHAP into GP means that from this point on in glycolysis, two molecules of GP are fed into the remaining chemical reactions.
2. In the energy-conserving stage (steps 6–10), the two three-carbon molecules are oxidized in several steps to two molecules of pyruvic acid. In these reactions, two molecules of NAD^+ are reduced to NADH, and four molecules of ATP are formed by substrate-level phosphorylation.

FOUNDATION FIGURE **5.11**

An Overview of Respiration and Fermentation



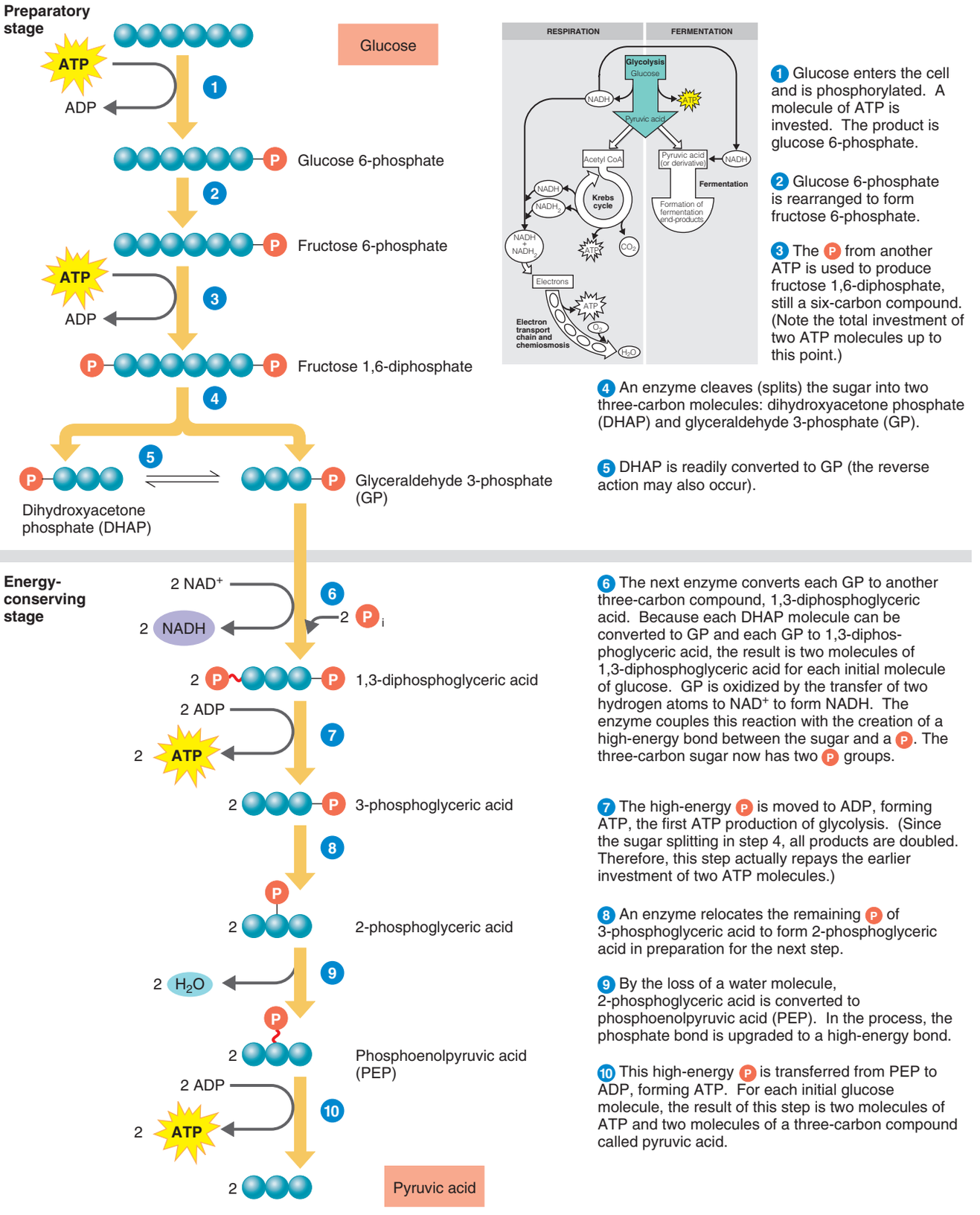
Because two molecules of ATP were needed to get glycolysis started and four molecules of ATP are generated by the process, *there is a net gain of two molecules of ATP for each molecule of glucose that is oxidized.* **Animations** Glycolysis: Overview, Steps

Alternatives to Glycolysis

Many bacteria have another pathway in addition to glycolysis for the oxidation of glucose. The most common alternative is the pentose phosphate pathway; another alternative is the Entner-Doudoroff pathway.

The Pentose Phosphate Pathway

The **pentose phosphate pathway** (or *hexose monophosphate shunt*) operates simultaneously with glycolysis and provides a means for the breakdown of five-carbon sugars (pentoses) as well as glucose (see Figure A.3 in Appendix A for a more



1 Glucose enters the cell and is phosphorylated. A molecule of ATP is invested. The product is glucose 6-phosphate.

2 Glucose 6-phosphate is rearranged to form fructose 6-phosphate.

3 The P from another ATP is used to produce fructose 1,6-diphosphate, still a six-carbon compound. (Note the total investment of two ATP molecules up to this point.)

4 An enzyme cleaves (splits) the sugar into two three-carbon molecules: dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GP).

5 DHAP is readily converted to GP (the reverse action may also occur).

6 The next enzyme converts each GP to another three-carbon compound, 1,3-diphosphoglyceric acid. Because each DHAP molecule can be converted to GP and each GP to 1,3-diphosphoglyceric acid, the result is two molecules of 1,3-diphosphoglyceric acid for each initial molecule of glucose. GP is oxidized by the transfer of two hydrogen atoms to NAD^+ to form NADH. The enzyme couples this reaction with the creation of a high-energy bond between the sugar and a P. The three-carbon sugar now has two P groups.

7 The high-energy P is moved to ADP, forming ATP, the first ATP production of glycolysis. (Since the sugar splitting in step 4, all products are doubled. Therefore, this step actually repays the earlier investment of two ATP molecules.)

8 An enzyme relocates the remaining P of 3-phosphoglyceric acid to form 2-phosphoglyceric acid in preparation for the next step.

9 By the loss of a water molecule, 2-phosphoglyceric acid is converted to phosphoenolpyruvic acid (PEP). In the process, the phosphate bond is upgraded to a high-energy bond.

10 This high-energy P is transferred from PEP to ADP, forming ATP. For each initial glucose molecule, the result of this step is two molecules of ATP and two molecules of a three-carbon compound called pyruvic acid.

Figure 5.12 An outline of the reactions of glycolysis (Embden-Meyerhof pathway). The inset indicates the relationship of glycolysis to the overall processes of respiration and fermentation. A more detailed version of glycolysis is presented in Figure A.2 in Appendix A.

Q What is glycolysis?

detailed representation of the pentose phosphate pathway). A key feature of this pathway is that it produces important intermediate pentoses used in the synthesis of (1) nucleic acids, (2) glucose from carbon dioxide in photosynthesis, and (3) certain amino acids. The pathway is an important producer of the reduced coenzyme NADPH from $NADP^+$. The pentose phosphate pathway yields a net gain of only one molecule of ATP for each molecule of glucose oxidized. Bacteria that use the pentose phosphate pathway include *Bacillus subtilis* (sub′ til-us), *E. coli, Leuconostoc mesenteroides* (lü-kō-nos′ tok mes-en-ter-oi′ dēz), and *Enterococcus faecalis* (fē-kāl′ is).

The Entner-Doudoroff Pathway

From each molecule of glucose, the **Entner-Doudoroff pathway** produces two molecules of NADPH and one molecule of ATP for use in cellular biosynthetic reactions (see Figure A.4 in Appendix A for a more detailed representation). Bacteria that have the enzymes for the Entner-Doudoroff pathway can metabolize glucose without either glycolysis or the pentose phosphate pathway. The Entner-Doudoroff pathway is found in some gram-negative bacteria, including *Rhizobium, Pseudomonas* (sū-dō-mō′ nas), and *Agrobacterium* (ag-rō-bak-ti′ rē-um); it is generally not found among gram-positive bacteria. Tests for the ability to oxidize glucose by this pathway are sometimes used to identify *Pseudomonas* in the clinical laboratory.

CHECK YOUR UNDERSTANDING

- What happens during the preparatory and energy-conserving stages of glycolysis? **5-11**
- What is the value of the pentose phosphate and Entner-Doudoroff pathways if they produce only one ATP molecule? **5-12**

Cellular Respiration

After glucose has been broken down to pyruvic acid, the pyruvic acid can be channeled into the next step of either fermentation (page 130) or cellular respiration (see Figure 5.11). **Cellular respiration,** or simply **respiration,** is defined as an ATP-generating process in which molecules are oxidized and the final electron acceptor is (almost always) an inorganic molecule. An essential feature of respiration is the operation of an electron transport chain.

There are two types of respiration, depending on whether an organism is an **aerobe,** which uses oxygen, or an **anaerobe,** which does not use oxygen and may even be killed by it. In **aerobic respiration,** the final electron acceptor is O_2; in **anaerobic respiration,** the final electron acceptor is an inorganic molecule other than O_2 or, rarely, an organic molecule. First we will describe respiration as it typically occurs in an aerobic cell.

Aerobic Respiration

The Krebs Cycle The **Krebs cycle,** also called the *tricarboxylic acid (TCA) cycle* or *citric acid cycle,* is a series of biochemical reactions in which the large amount of potential chemical energy stored in acetyl CoA is released step by step (see Figure 5.11). In this cycle, a series of oxidations and reductions transfer that potential energy, in the form of electrons, to electron carrier coenzymes, chiefly NAD^+. The pyruvic acid derivatives are oxidized; the coenzymes are reduced.

Pyruvic acid, the product of glycolysis, cannot enter the Krebs cycle directly. In a preparatory step, it must lose one molecule of CO_2 and become a two-carbon compound (**Figure 5.13**, at top). This process is called **decarboxylation.** The two-carbon compound, called an *acetyl group,* attaches to coenzyme A through a high-energy bond; the resulting complex is known as *acetyl coenzyme A (acetyl CoA).* During this reaction, pyruvic acid is also oxidized, and NAD^+ is reduced to NADH.

Remember that the oxidation of one glucose molecule produces two molecules of pyruvic acid, so for each molecule of glucose, two molecules of CO_2 are released in this preparatory step, two molecules of NADH are produced, and two molecules of acetyl CoA are formed. Once the pyruvic acid has undergone decarboxylation and its derivative (the acetyl group) has attached to CoA, the resulting acetyl CoA is ready to enter the Krebs cycle.

As acetyl CoA enters the Krebs cycle, CoA detaches from the acetyl group. The two-carbon acetyl group combines with a four-carbon compound called oxaloacetic acid to form the six-carbon citric acid. This synthesis reaction requires energy, which is provided by the cleavage of the high-energy bond between the acetyl group and CoA. The formation of citric acid is thus the first step in the Krebs cycle. The major chemical reactions of this cycle are outlined in Figure 5.13; a more detailed representation of the Krebs cycle is provided in Figure A.5 in Appendix A. Keep in mind that each reaction is catalyzed by a specific enzyme.

The chemical reactions of the Krebs cycle fall into several general categories; one of these is decarboxylation. For example, in step 3 isocitric acid, a six-carbon compound, is decarboxylated to the five-carbon compound called α-ketoglutaric acid. Another decarboxylation takes place in step 4. Because one decarboxylation has taken place in the preparatory step and two in the Krebs cycle, all three carbon atoms in pyruvic acid are eventually released as CO_2 by the Krebs cycle. This represents the conversion to CO_2 of all six carbon atoms contained in the original glucose molecule.

Another general category of Krebs cycle chemical reactions is oxidation-reduction. For example, in step 3, two hydrogen atoms are lost during the conversion of the six-carbon isocitric acid to a five-carbon compound. In other words, the six-carbon compound is oxidized. Hydrogen atoms are also released in the Krebs cycle in steps 4, 6, and 8 and are picked up by the coenzymes NAD^+ and FAD. Because NAD^+ picks up two electrons but only one additional proton, its reduced form is represented as NADH; however, FAD picks up two complete hydrogen atoms and is reduced to $FADH_2$.

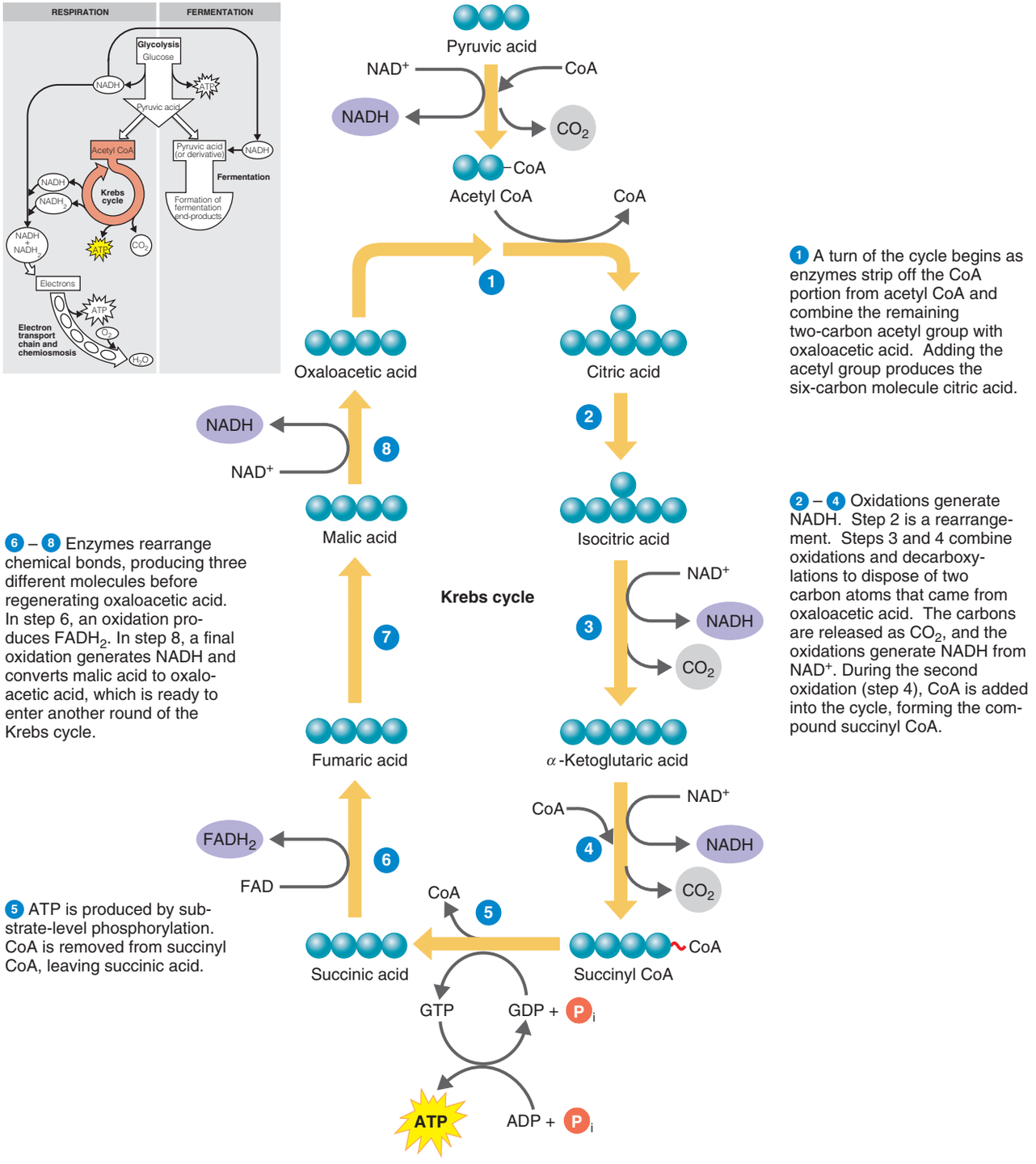


Figure 5.13 The Krebs cycle. The inset indicates the relationship of the Krebs cycle to the overall process of respiration. A more detailed version of the Krebs cycle is presented in Figure A.5 in Appendix A.

Q What are the products of the Krebs cycle?

If we look at the Krebs cycle as a whole, we see that for every two molecules of acetyl CoA that enter the cycle, four molecules of CO_2 are liberated by decarboxylation, six molecules of NADH and two molecules of $FADH_2$ are produced by oxidation-reduction reactions, and two molecules of ATP are generated by substrate-level phosphorylation. A molecule of guanosine triphosphate (GTP), formed from guanosine diphosphate (GDP + P_i), is similar to ATP and serves as an intermediary at this point in the cycle. Many of the intermediates in the Krebs cycle also play a role in other pathways, especially in amino acid biosynthesis (page 144).

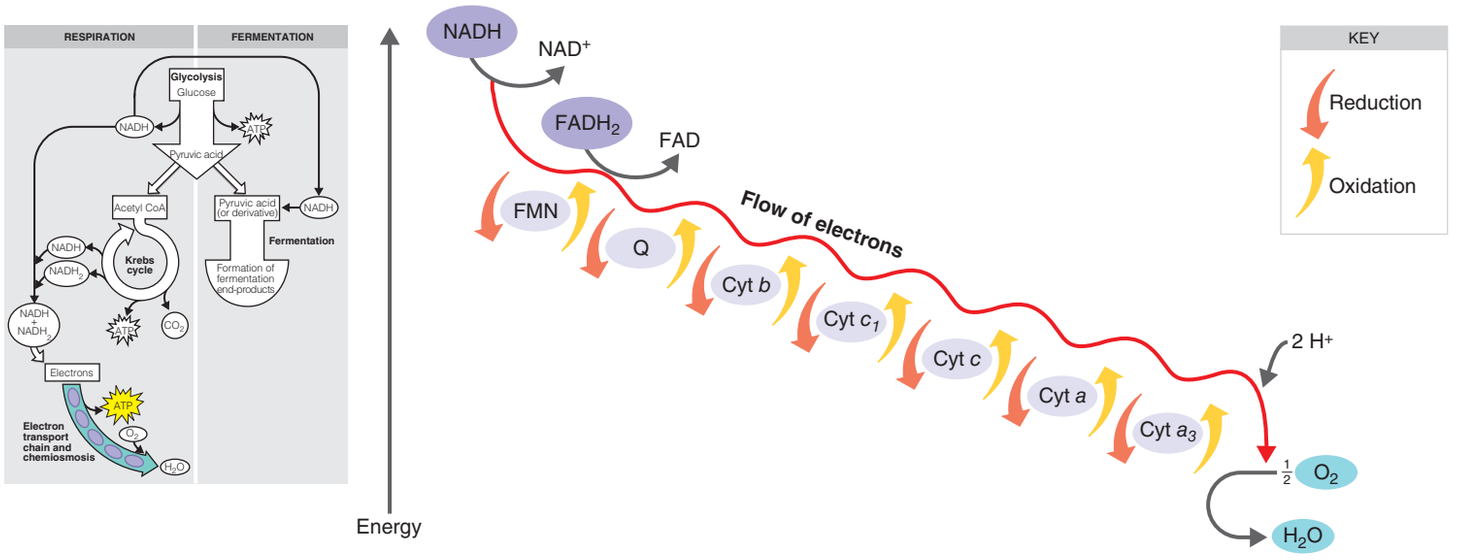


Figure 5.14 **An electron transport chain (system).** The inset indicates the relationship of the electron transport chain to the overall process of respiration. In the mitochondrial electron transport chain shown, the electrons pass along the chain in a gradual and stepwise fashion, so energy is released in manageable quantities. To learn where ATP is formed, see Figure 5.16.

What are the functions of the electron transport chain?

The CO_2 produced in the Krebs cycle is ultimately liberated into the atmosphere as a gaseous by-product of aerobic respiration. (Humans produce CO_2 from the Krebs cycle in most cells of the body and discharge it through the lungs during exhalation.) The reduced coenzymes NADH and $FADH_2$ are the most important products of the Krebs cycle because they contain most of the energy originally stored in glucose. During the next phase of respiration, a series of reductions indirectly transfers the energy stored in those coenzymes to ATP. These reactions are collectively called the electron transport chain. **Animations** Krebs Cycle: Overview, Steps

The Electron Transport Chain (System) An **electron transport chain (system)** consists of a sequence of carrier molecules that are capable of oxidation and reduction. As electrons are passed through the chain, there occurs a stepwise release of energy, which is used to drive the chemiosmotic generation of ATP, to be described shortly. The final oxidation is irreversible. In eukaryotic cells, the electron transport chain is contained in the inner membrane of mitochondria; in prokaryotic cells, it is found in the plasma membrane.

There are three classes of carrier molecules in electron transport chains. The first are **flavoproteins.** These proteins contain flavin, a coenzyme derived from riboflavin (vitamin B_2), and are capable of performing alternating oxidations and reductions. One important flavin coenzyme is flavin mononucleotide (FMN). The second class of carrier molecules are **cytochromes,** proteins with an iron-containing group (heme) capable of existing alternately as a reduced form (Fe^{2+}) and an oxidized form (Fe^{3+}). The cytochromes involved in electron transport chains include cytochrome *b* (cyt *b*), cytochrome c_1 (cyt c_1), cytochrome *c* (cyt *c*), cytochrome *a* (cyt *a*), and cytochrome a_3 (cyt a_3). The third class is known as **ubiquinones,** or **coenzyme Q,** symbolized Q; these are small nonprotein carriers.

The electron transport chains of bacteria are somewhat diverse, in that the particular carriers used by a bacterium and the order in which they function may differ from those of other bacteria and from those of eukaryotic mitochondrial systems. Even a single bacterium may have several types of electron transport chains. However, keep in mind that all electron transport chains achieve the same basic goal: to release energy as electrons are transferred from higher-energy compounds to lower-energy compounds. Much is known about the electron transport chain in the mitochondria of eukaryotic cells, so this is the chain we will describe.

The first step in the mitochondrial electron transport chain involves the transfer of high-energy electrons from NADH to FMN, the first carrier in the chain (**Figure 5.14**). This transfer actually involves the passage of a hydrogen atom with two electrons to FMN, which then picks up an additional H^+ from the surrounding aqueous medium. As a result of the first transfer, NADH is oxidized to NAD^+, and FMN is reduced to $FMNH_2$. In the second step in the electron transport chain, $FMNH_2$ passes $2H^+$ to the other side of the mitochondrial membrane (see Figure 5.16) and passes two electrons to Q. As a result, $FMNH_2$ is oxidized to FMN. Q also picks up an additional $2H^+$ from the surrounding aqueous medium and releases it on the other side of the membrane.

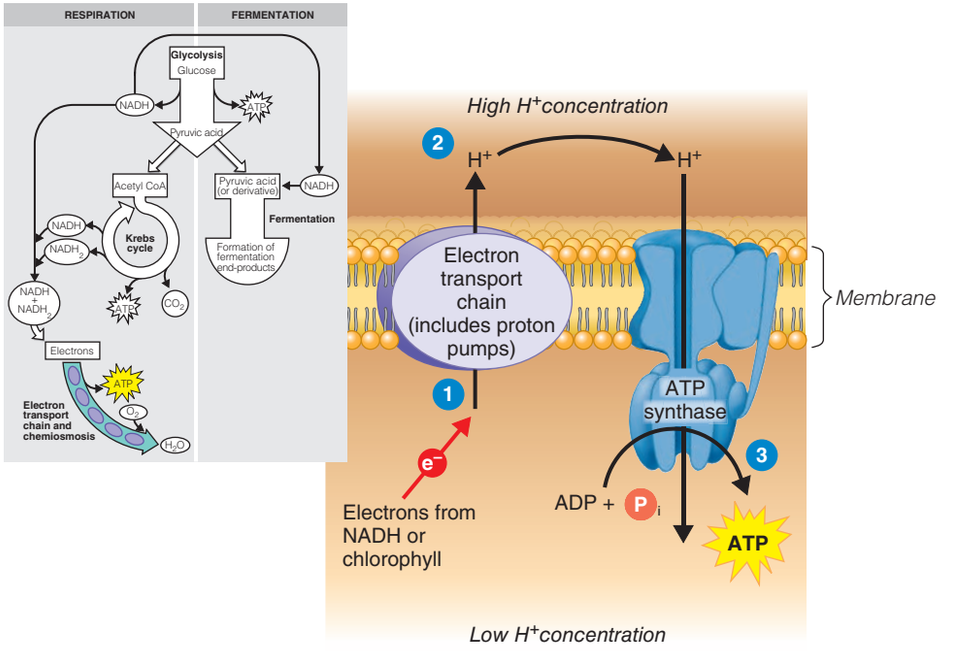


Figure 5.15 Chemiosmosis. An overview of the mechanism of chemiosmosis. The membrane shown could be a prokaryotic plasma membrane, a eukaryotic mitochondrial membrane, or a photosynthetic thylakoid. The numbered steps are described in the text.

Q What is the proton motive force?

The next part of the electron transport chain involves the cytochromes. Electrons are passed successively from Q to cyt *b*, cyt c_1, cyt *c*, cyt *a*, and cyt a_3. Each cytochrome in the chain is reduced as it picks up electrons and is oxidized as it gives up electrons. The last cytochrome, cyt a_3, passes its electrons to molecular oxygen (O_2), which becomes negatively charged and then picks up protons from the surrounding medium to form H_2O.

Notice that Figure 5.14 shows $FADH_2$, which is derived from the Krebs cycle, as another source of electrons. However, $FADH_2$ adds its electrons to the electron transport chain at a lower level than NADH. Because of this, the electron transport chain produces about one-third less energy for ATP generation when $FADH_2$ donates electrons than when NADH is involved.

An important feature of the electron transport chain is the presence of some carriers, such as FMN and Q, that accept and release protons as well as electrons, and other carriers, such as cytochromes, that transfer electrons only. Electron flow down the chain is accompanied at several points by the active transport (pumping) of protons from the matrix side of the inner mitochondrial membrane to the opposite side of the membrane. The result is a buildup of protons on one side of the membrane. Just as water behind a dam stores energy that can be used to generate electricity, this buildup of protons provides energy for the generation of ATP by the chemiosmotic mechanism.

The Chemiosmotic Mechanism of ATP Generation The mechanism of ATP synthesis using the electron transport chain is called **chemiosmosis.** To understand chemiosmosis, we need to recall several concepts that were introduced in Chapter 4 as part of the section on the movement of materials across membranes (page 91). Recall that substances diffuse passively across membranes from areas of high concentration to areas of low concentration; this diffusion yields energy. Recall also that the movement of substances *against* such a concentration gradient *requires* energy and that, in such an active transport of molecules or ions across biological membranes, the required energy is usually provided by ATP. In chemiosmosis, the energy released when a substance moves along a gradient is used to *synthesize* ATP. The "substance" in this case refers to protons. In respiration, chemiosmosis is responsible for most of the ATP that is generated. The steps of chemiosmosis are as follows (**Figure 5.15** and **Figure 5.16**):

1. As energetic electrons from NADH (or chlorophyll) pass down the electron transport chain, some of the carriers in the chain pump—actively transport—protons across the membrane. Such carrier molecules are called *proton pumps.*
2. The phospholipid membrane is normally impermeable to protons, so this one-directional pumping establishes a proton gradient (a difference in the concentrations of protons on the two sides of the membrane). In addition to a concentration gradient, there is an electrical charge gradient. The excess H^+ on one side of the membrane makes that side positively charged compared with the other side. The resulting electrochemical gradient has potential energy, called the *proton motive force.*
3. The protons on the side of the membrane with the higher proton concentration can diffuse across the membrane only through special protein channels that contain an enzyme called *ATP synthase.* When this flow occurs, energy is released and is used by the enzyme to synthesize ATP from ADP and P_i.

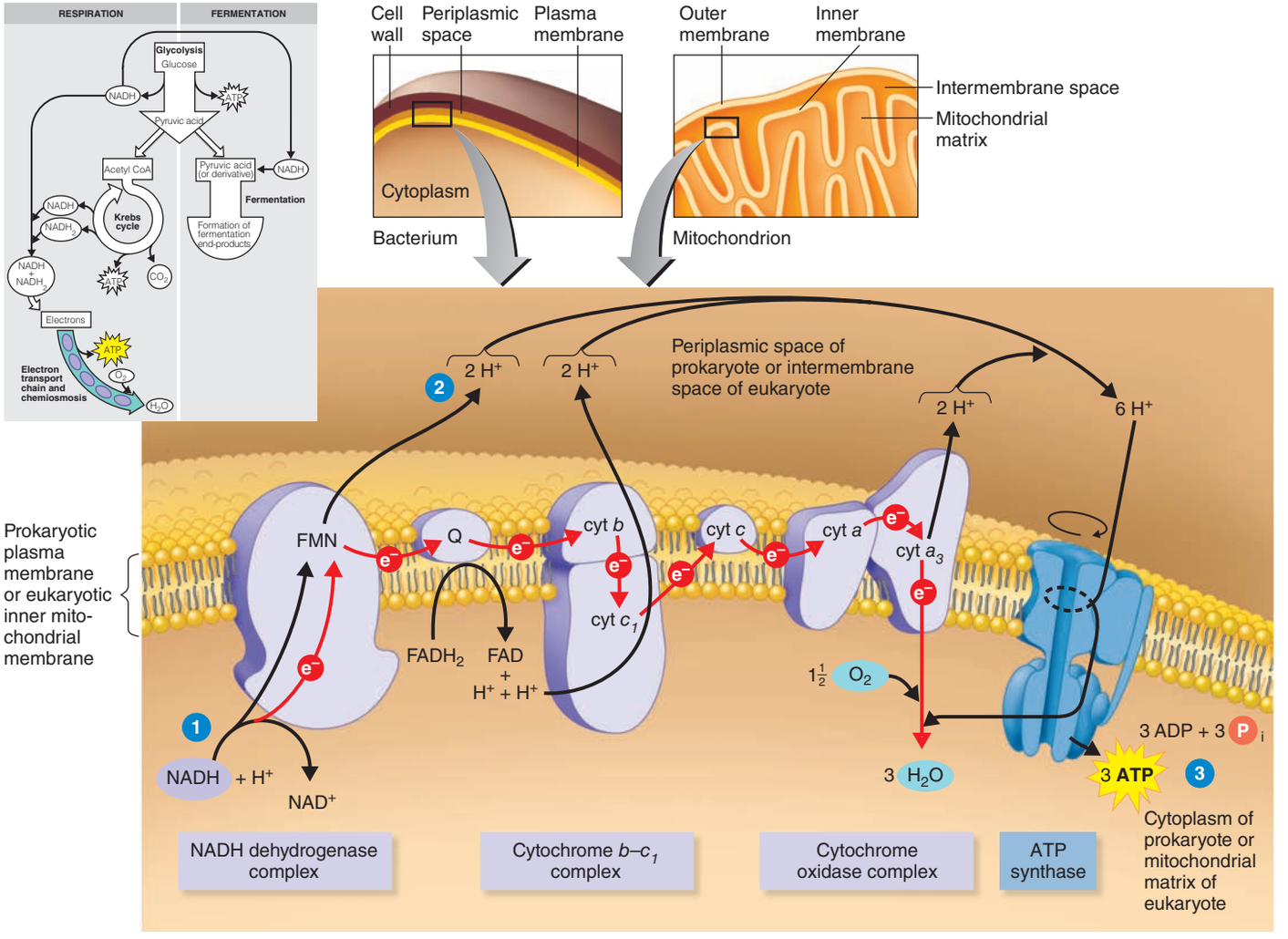


Figure 5.16 Electron transport and the chemiosmotic generation of ATP. Electron carriers are organized into three complexes, and protons (H^+) are pumped across the membrane at three points. In a prokaryotic cell, protons are pumped across the plasma membrane from the cytoplasmic side. In a eukaryotic cell, they are pumped from the matrix side of the mitochondrial membrane to the opposite side. The flow of electrons is indicated with red arrows.

Q Where does chemiosmosis occur in eukaryotes? In prokaryotes?

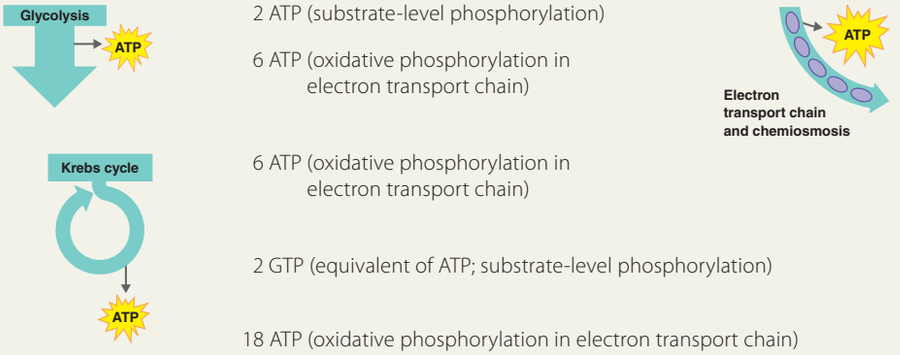
Figure 5.16 shows in detail how the electron transport chain operates in eukaryotes to drive the chemiosmotic mechanism. 1 Energetic electrons from NADH pass down the electron transport chains. Within the inner mitochondrial membrane, the carriers of the electron transport chain are organized into three complexes, with Q transporting electrons between the first and second complexes, and cyt *c* transporting them between the second and third complexes. 2 Three components of the system pump protons: the first and third complexes and Q. At the end of the chain, electrons join with protons and oxygen (O_2) in the matrix fluid to form water (H_2O). Thus, O_2 is the final electron acceptor.

Both prokaryotic and eukaryotic cells use the chemiosmotic mechanism to generate energy for ATP production. However, in eukaryotic cells, 3 the inner mitochondrial membrane contains the electron transport carriers and ATP synthase, whereas in most prokaryotic cells, the plasma membrane does so. An electron transport chain also operates in photophosphorylation and is located in the thylakoid membrane of cyanobacteria and eukaryotic chloroplasts.

A Summary of Aerobic Respiration The electron transport chain regenerates NAD^+ and FAD, which can be used again in glycolysis and the Krebs cycle. The various electron transfers in the electron transport chain generate about 34 molecules of ATP from each molecule of glucose oxidized: approximately three from each of the ten molecules of NADH (a total of 30), and approximately two from each of the two molecules of $FADH_2$ (a total of four). To arrive at the total number of ATP molecules generated for each molecule of glucose, the 34 from chemiosmosis

TABLE 5.3 ATP Yield during Prokaryotic Aerobic Respiration of One Glucose Molecule

Source	ATP Yield (Method)
Glycolysis	
1. Oxidation of glucose to pyruvic acid	2 ATP (substrate-level phosphorylation)
2. Production of 2 NADH	6 ATP (oxidative phosphorylation in electron transport chain)
Preparatory Step	
1. Formation of acetyl CoA produces 2 NADH	6 ATP (oxidative phosphorylation in electron transport chain)
Krebs Cycle	
1. Oxidation of succinyl CoA to succinic acid	2 GTP (equivalent of ATP; substrate-level phosphorylation)
2. Production of 6 NADH	18 ATP (oxidative phosphorylation in electron transport chain)
3. Production of 2 FADH	4 ATP (oxidative phosphorylation in electron transport chain)
	Total: 38 ATP



are added to those generated by oxidation in glycolysis and the Krebs cycle. In aerobic respiration among prokaryotes, a total of 38 molecules of ATP can be generated from one molecule of glucose. Note that four of those ATPs come from substrate-level phosphorylation in glycolysis and the Krebs cycle. **Table 5.3** provides a detailed accounting of the ATP yield during prokaryotic aerobic respiration.

Aerobic respiration among eukaryotes produces a total of only 36 molecules of ATP. There are fewer ATPs than in prokaryotes because some energy is lost when electrons are shuttled across the mitochondrial membranes that separate glycolysis (in the cytoplasm) from the electron transport chain. No such separation exists in prokaryotes. We can now summarize the overall reaction for aerobic respiration in prokaryotes as follows:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Oxygen}}{6\,O_2} + 38\,ADP + 38\,P_i \longrightarrow \underset{\text{Carbon dioxide}}{6\,CO_2} + \underset{\text{Water}}{6\,H_2O} + 38\,ATP$$

A summary of the various stages of aerobic respiration in prokaryotes is presented in **Figure 5.17**.

Anaerobic Respiration

In anaerobic respiration, the final electron acceptor is an inorganic substance other than oxygen (O_2). Some bacteria, such as *Pseudomonas* and *Bacillus,* can use a nitrate ion (NO_3^-) as a final electron acceptor; the nitrate ion is reduced to a nitrite ion (NO_2^-), nitrous oxide (N_2O), or nitrogen gas (N_2). Other bacteria, such as *Desulfovibrio* (dē-sul-fō-vib′ rē-ō), use sulfate (SO_4^{2-}) as the final electron acceptor to form hydrogen sulfide (H_2S). Still other bacteria use carbonate (CO_3^{2-}) to form methane (CH_4). Anaerobic respiration by bacteria using nitrate and sulfate as final acceptors is essential for the nitrogen and sulfur cycles that occur in nature. The amount of ATP generated in anaerobic respiration varies with the organism and the pathway. Because only part of the Krebs cycle operates under anaerobic conditions, and because not all the carriers in the electron transport chain participate in anaerobic respiration, the ATP yield is never as high as in aerobic respiration. Accordingly, anaerobes tend to grow more slowly than aerobes. MM **Animations** Electron Transport Chain: Overview, The Process, Factors Affecting ATP Yield

CHECK YOUR UNDERSTANDING

- What are the principal products of the Krebs cycle? **5-13**
- How do carrier molecules function in the electron transport chain? **5-14**
- Compare the energy yield (ATP) of aerobic and anaerobic respiration. **5-15**

Fermentation

After glucose has been broken down into pyruvic acid, the pyruvic acid can be completely broken down in respiration, as previously described, or it can be converted to an organic product in fermentation, whereupon NAD^+ and $NADP^+$ are regenerated and can enter another round of glycolysis (see Figure 5.11). **Fermentation** can be defined in several ways (see the box, page 134), but we define it here as a process that

1. releases energy from sugars or other organic molecules, such as amino acids, organic acids, purines, and pyrimidines;
2. does not require oxygen (but sometimes can occur in its presence);
3. does not require the use of the Krebs cycle or an electron transport chain;

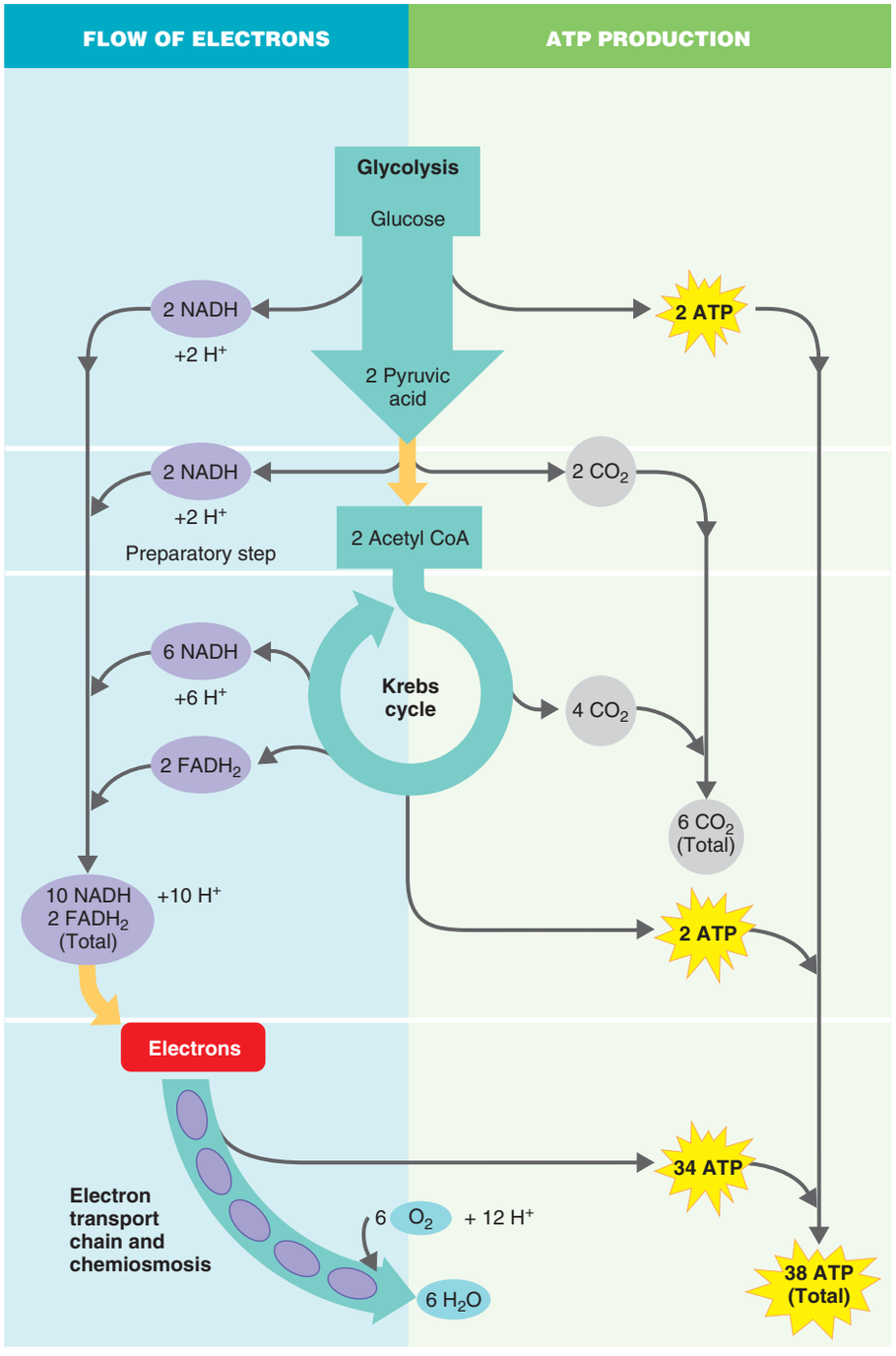


Figure 5.17 A summary of aerobic respiration in prokaryotes. Glucose is broken down completely to carbon dioxide and water, and ATP is generated. This process has three major phases: glycolysis, the Krebs cycle, and the electron transport chain. The preparatory step is between glycolysis and the Krebs cycle. The key event in aerobic respiration is that electrons are picked up from intermediates of glycolysis and the Krebs cycle by NAD^+ or FAD and are carried by NADH or $FADH_2$ to the electron transport chain. NADH is also produced during the conversion of pyruvic acid to acetyl CoA. Most of the ATP generated by aerobic respiration is made by the chemiosmotic mechanism during the electron transport chain phase; this is called oxidative phosphorylation.

Q How do aerobic and anaerobic respiration differ?

4. uses an organic molecule as the final electron acceptor;
5. produces only small amounts of ATP (only one or two ATP molecules for each molecule of starting material) because much of the original energy in glucose remains in the chemical bonds of the organic end-products, such as lactic acid or ethanol.

During fermentation, electrons are transferred (along with protons) from reduced coenzymes (NADH, NADPH) to pyruvic acid or its derivatives (Figure 5.18a). Those final electron acceptors are reduced to the end-products shown in Figure 5.18b. An essential function of the second stage of fermentation is to ensure a steady supply of NAD^+ and $NADP^+$ so that glycolysis can continue. In fermentation, ATP is generated only during glycolysis.

Microorganisms can ferment various substrates; the end-products depend on the particular microorganism, the substrate, and the enzymes that are present and active. Chemical analyses of these end-products are useful in identifying microorganisms.

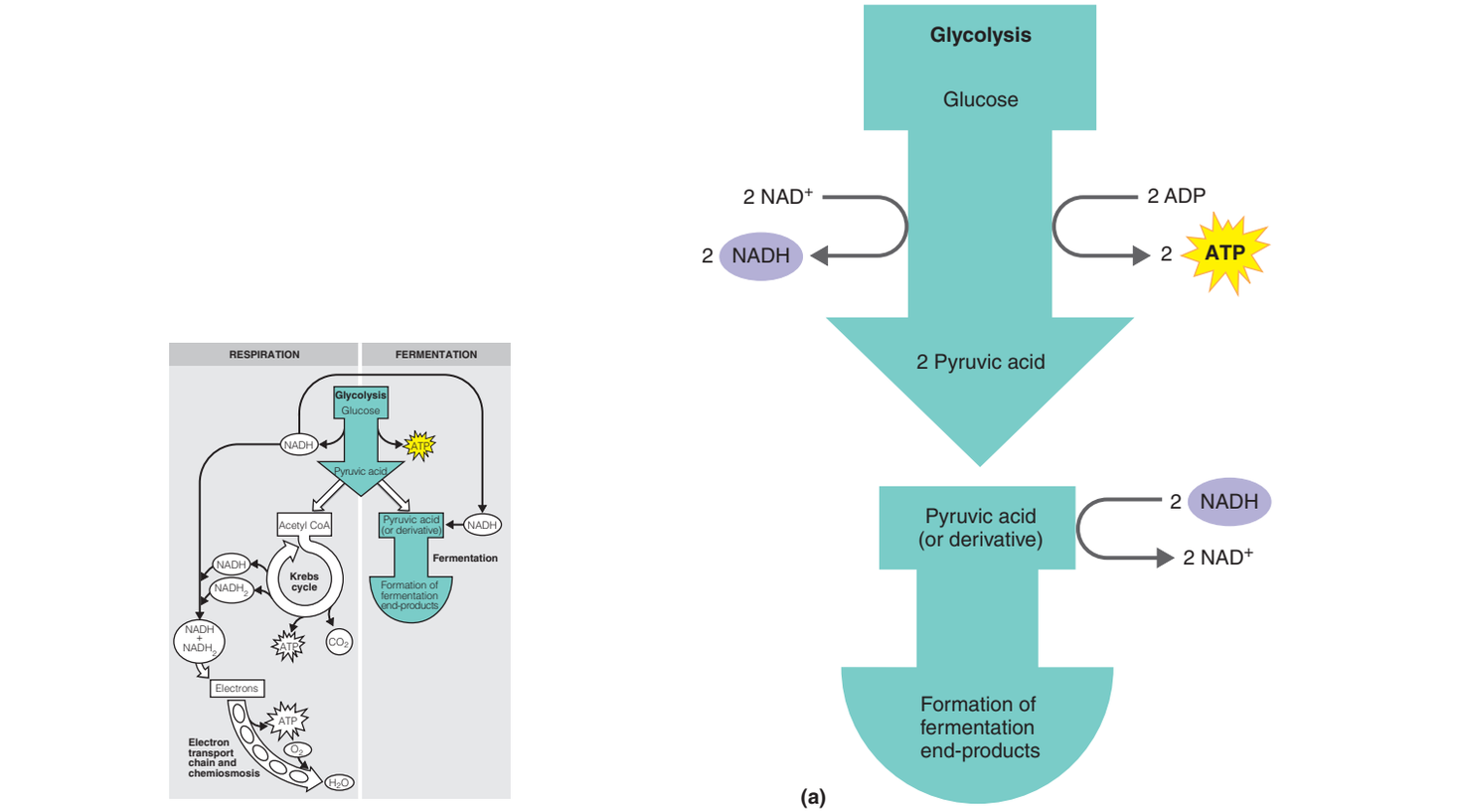


Figure 5.18 Fermentation. The inset indicates the relationship of fermentation to the overall energy-producing processes. (**a**) An overview of fermentation. The first step is glycolysis, the conversion of glucose to pyruvic acid. In the second step, the reduced coenzymes from glycolysis or its alternatives (NADH, NADPH) donate their electrons and hydrogen ions to pyruvic acid or a derivative to form a fermentation end-product. (**b**) End-products of various microbial fermentations.

Q During which phase of fermentation is ATP generated?

We next consider two of the more important processes: lactic acid fermentation and alcohol fermentation.

Lactic Acid Fermentation

During glycolysis, which is the first phase of **lactic acid fermentation,** a molecule of glucose is oxidized to two molecules of pyruvic acid (Figure 5.19; see also Figure 5.10). This oxidation generates the energy that is used to form the two molecules of ATP. In the next step, the two molecules of pyruvic acid are reduced by two molecules of NADH to form two molecules of lactic acid (Figure 5.19a). Because lactic acid is the end-product of the reaction, it undergoes no further oxidation, and most of the

energy produced by the reaction remains stored in the lactic acid. Thus, this fermentation yields only a small amount of energy.

Two important genera of lactic acid bacteria are *Streptococcus* and *Lactobacillus* (lak-tō-bä-sil′lus). Because these microbes produce only lactic acid, they are referred to as **homolactic** (or *homofermentative*). Lactic acid fermentation can result in food spoilage. However, the process can also produce yogurt from milk, sauerkraut from fresh cabbage, and pickles from cucumbers.

Clinical Case

Feeling certain that there must be some connection between the increase in dental caries and the activities of her patients, Dr. Rivera starts to ask more questions about the children's activities. She finds out that they all attend a summer program at the same church in a nearby neighborhood. She also discovers that the culprit isn't candy, but bubblegum. The camp counselors have been giving out bubblegum as an incentive for attendance and good behavior. Although Dr. Rivera is pleased to hear that her patients have all been behaving themselves, she is concerned about the amount of bubblegum they have been chewing on a daily basis. The sucrose in gum causes a decrease in the pH of saliva, and the acid erodes the tooth enamel, thus exposing the tooth to bacterial decay.

If the pH of gum and sucrose is 7, what lowers the salivary pH?

112 **133** 135 137

Alcohol Fermentation

Alcohol fermentation also begins with the glycolysis of a molecule of glucose to yield two molecules of pyruvic acid and two molecules of ATP. In the next reaction, the two molecules of pyruvic acid are converted to two molecules of acetaldehyde and two molecules of CO_2 (**Figure 5.19b**). The two molecules of acetaldehyde are next reduced by two molecules of NADH to form two molecules of ethanol. Again, alcohol fermentation is a low-energy-yield process because most of the energy contained in the initial glucose molecule remains in the ethanol, the end-product.

Alcohol fermentation is carried out by a number of bacteria and yeasts. The ethanol and carbon dioxide produced by the yeast *Saccharomyces* (sak-ä-rō-mī′sēs) are waste products for yeast cells but are useful to humans. Ethanol made by yeasts is the alcohol in alcoholic beverages, and carbon dioxide made by yeasts causes bread dough to rise.

Organisms that produce lactic acid as well as other acids or alcohols are known as **heterolactic** (or *heterofermentative*) and often use the pentose phosphate pathway.

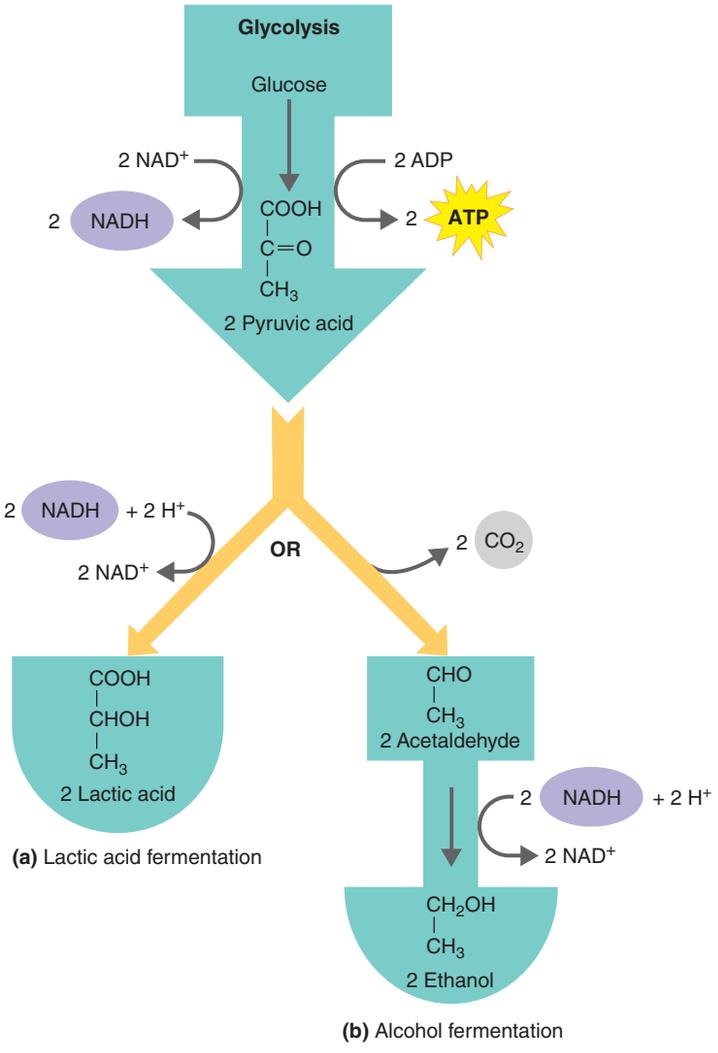


Figure 5.19 **Types of fermentation.**

Q What is the difference between homolactic and heterolactic fermentation?

Table 5.4 lists some of the various microbial fermentations used by industry to convert inexpensive raw materials into useful end-products. **Table 5.5** provides a summary comparison of aerobic respiration, anaerobic respiration, and fermentation.

MM **Animation** Fermentation

CHECK YOUR UNDERSTANDING

List four compounds that can be made from pyruvic acid by an organism that uses fermentation. **5-16**

Lipid and Protein Catabolism

LEARNING OBJECTIVE

5-17 Describe how lipids and proteins undergo catabolism.

Our discussion of energy production has emphasized the oxidation of glucose, the main energy-supplying carbohydrate.

What Is Fermentation?

To many people, *fermentation* simply means the production of alcohol: grains and fruits are fermented to produce beer and wine. If a food soured, you might say it was "off," or fermented. Here are some definitions of *fermentation*. They range from informal, general usage to more scientific definitions.

1. Any spoilage of food by microorganisms (general use)
2. Any process that produces alcoholic beverages or acidic dairy products (general use)
3. Any large-scale microbial process occurring with or without air (common definition used in industry)
4. Any energy-releasing metabolic process that takes place only under anaerobic conditions (becoming more scientific)
5. Any metabolic process that releases energy from a sugar or other organic molecule, does not require oxygen or an electron transport system, and uses an organic molecule as the final electron acceptor (the definition we use in this book).

However, microbes also oxidize lipids and proteins, and the oxidations of all these nutrients are related.

Recall that fats are lipids consisting of fatty acids and glycerol. Microbes produce extracellular enzymes called *lipases* that break fats down into their fatty acid and glycerol components. Each component is then metabolized separately (Figure 5.20). The Krebs cycle functions in the oxidation of glycerol and fatty acids. Many bacteria that hydrolyze fatty acids can use the same enzymes to degrade petroleum products. Although these bacteria are a nuisance when they grow in a fuel storage tank, they are beneficial when they grow in oil spills. Beta-oxidation (the oxidation of fatty acids) of petroleum is illustrated in the box in Chapter 2 (page 32).

Proteins are too large to pass unaided through plasma membranes. Microbes produce extracellular *proteases* and *peptidases*,

TABLE 5.4 Some Industrial Uses for Different Types of Fermentations*

Fermentation End-Product(s)	Industrial or Commercial Use	Starting Material	Microorganism
Ethanol	Beer, wine	Starch, sugar	*Saccharomyces cerevisiae* (yeast, a fungus)
	Fuel	Agricultural wastes	*Saccharomyces cerevisiae* (yeast)
Acetic Acid	Vinegar	Ethanol	*Acetobacter*
Lactic Acid	Cheese, yogurt	Milk	*Lactobacillus, Streptococcus*
	Rye bread	Grain, sugar	*Lactobacillus delbrueckii*
	Sauerkraut	Cabbage	*Lactobacillus plantarum*
	Summer sausage	Meat	*Pediococcus*
Propionic Acid and Carbon Dioxide	Swiss cheese	Lactic acid	*Propionibacterium freudenreichii*
Acetone and Butanol	Pharmaceutical, industrial uses	Molasses	*Clostridium acetobutylicum*
Citric Acid	Flavoring	Molasses	*Aspergillus* (fungus)
Methane	Fuel	Acetic acid	*Methanosarcina*
Sorbose	Vitamin C (ascorbic acid)	Sorbitol	*Gluconobacter*

*Unless otherwise noted, the microorganisms listed are bacteria.

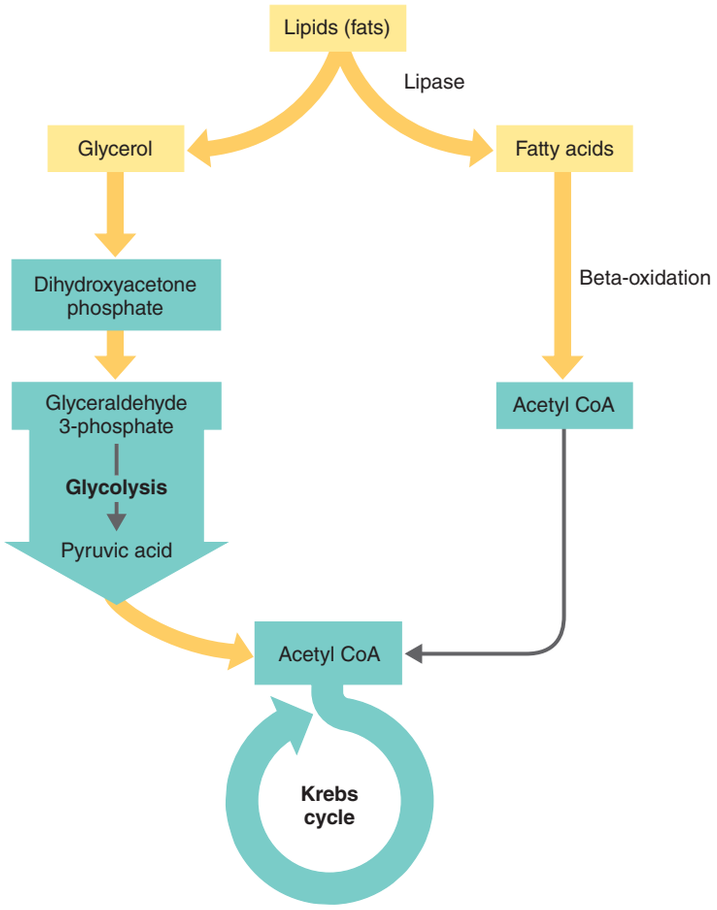


Figure 5.20 Lipid catabolism. Glycerol is converted into dihydroxyacetone phosphate (DHAP) and catabolized via glycolysis and the Krebs cycle. Fatty acids undergo beta-oxidation, in which carbon fragments are split off two at a time to form acetyl CoA, which is catabolized via the Krebs cycle.

Q What is the role of lipases?

enzymes that break down proteins into their component amino acids, which can cross the membranes. However, before amino acids can be catabolized, they must be enzymatically converted to other substances that can enter the Krebs cycle. In one such conversion, called **deamination,** the amino group of an amino acid is removed and converted to an ammonium ion (NH_4^+), which can be excreted from the cell. The remaining organic acid can enter the Krebs cycle. Other conversions involve **decarboxylation** (the removal of —COOH) and **dehydrogenation.**

A summary of the interrelationships of carbohydrate, lipid, and protein catabolism is shown in **Figure 5.21**.

CHECK YOUR UNDERSTANDING

What are the end-products of lipid and protein catabolism? **5-17**

Clinical Case

Dental caries are caused by oral streptococci, including *S. mutans*, *S. salivarius*, and *S. sobrinus*, that attach to tooth surfaces. Oral streptococci ferment sucrose and produce lactic acid, which lowers the salivary pH. Dr. Rivera decides to ask the camp counselors to substitute the bubblegum with a sugarless gum made with xylitol. A study has shown that chewing gum sweetened with xylitol, a naturally occuring sugar alcohol, can significantly lower the number of dental caries in children because it lowers the number of *S. mutans* in the mouth.

Why might xylitol reduce the number of *S. mutans*?

112 133 **135** 137

Biochemical Tests and Bacterial Identification

LEARNING OBJECTIVE

5-18 Provide two examples of the use of biochemical tests to identify bacteria in the laboratory.

Biochemical testing is frequently used to identify bacteria and yeasts because different species produce different enzymes. Such biochemical tests are designed to detect the presence of enzymes.

TABLE 5.5 Aerobic Respiration, Anaerobic Respiration, and Fermentation

Energy-Producing Process	Growth Conditions	Final Hydrogen (Electron) Acceptor	Type of Phosphorylation Used to Generate ATP	ATP Molecules Produced per Glucose Molecule
Aerobic Respiration	Aerobic	Molecular oxygen (O_2)	Substrate-level and oxidative	36 (eukaryotes) 38 (prokaryotes)
Anaerobic Respiration	Anaerobic	Usually an inorganic substance (such as NO_3^-, SO_4^{2-}, or CO_3^{2-}) but not molecular oxygen (O_2)	Substrate-level and oxidative	Variable (fewer than 38 but more than 2)
Fermentation	Aerobic or anaerobic	An organic molecule	Substrate-level	2

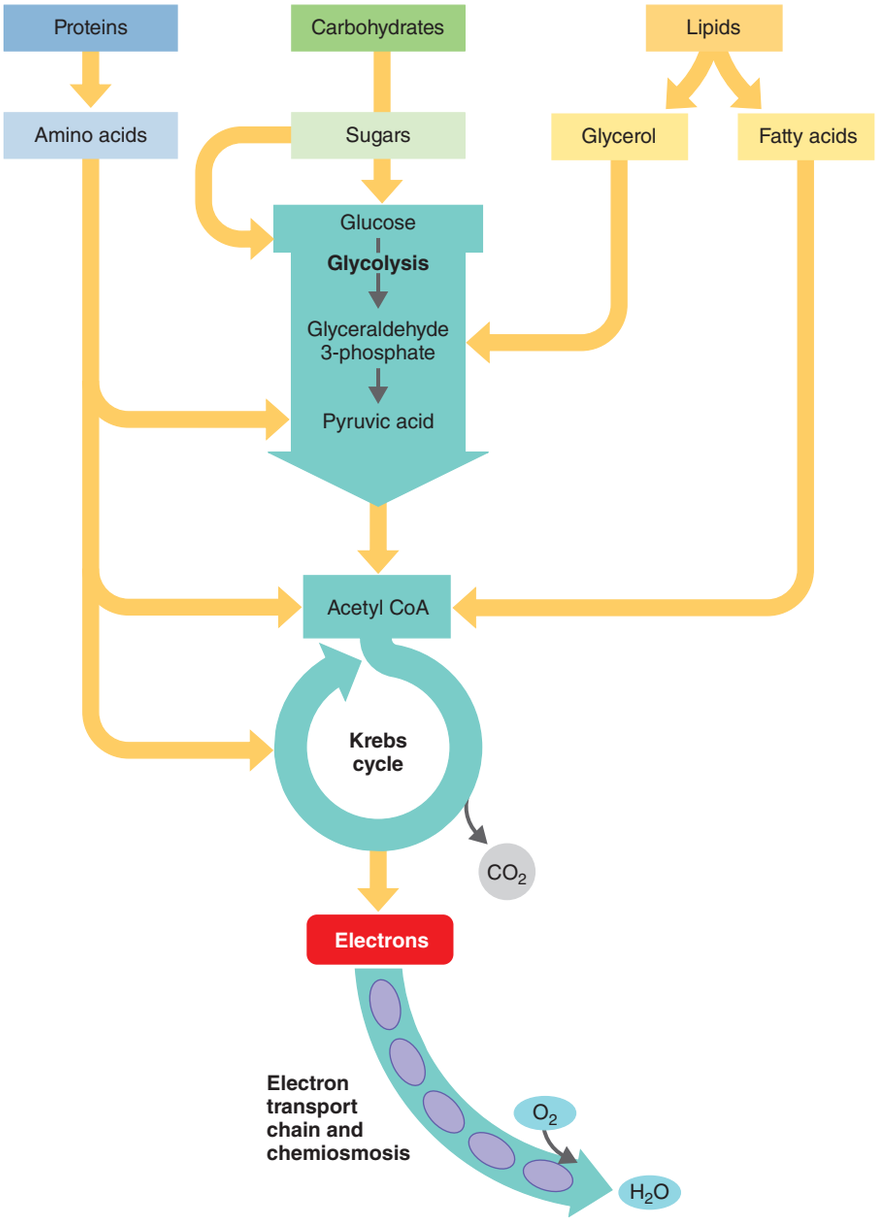


Figure 5.21 Catabolism of various organic food molecules. Proteins, carbohydrates, and lipids can all be sources of electrons and protons for respiration. These food molecules enter glycolysis or the Krebs cycle at various points.

Q What are the catabolic pathways through which high-energy electrons from all kinds of organic molecules flow on their energy-releasing pathways?

One type of biochemical test is the detection of amino acid catabolizing enzymes involved in decarboxylation and dehydrogenation (discussed on page 120; **Figure 5.22**).

Another biochemical test is a **fermentation test.** The test medium contains protein, a single carbohydrate, a pH indicator, and an inverted Durham tube, which is used to capture gas (**Figure 5.23a**). Bacteria inoculated into the tube can use the protein or carbohydrate as a carbon and energy source. If they catabolize the carbohydrate and produce acid, the pH indicator changes color. Some organisms produce gas as well as acid from carbohydrate catabolism. The presence of a bubble in the Durham tube indicates gas formation (**Figure 5.23b–d**).

E. coli ferments the carbohydrate sorbitol. The pathogenic *E. coli* O157 strain, however, does not ferment sorbitol, a characteristic that differentiates it from nonpathogenic, commensal *E. coli*.

Another example of the use of biochemical tests is shown in Figure 10.8 on page 284.

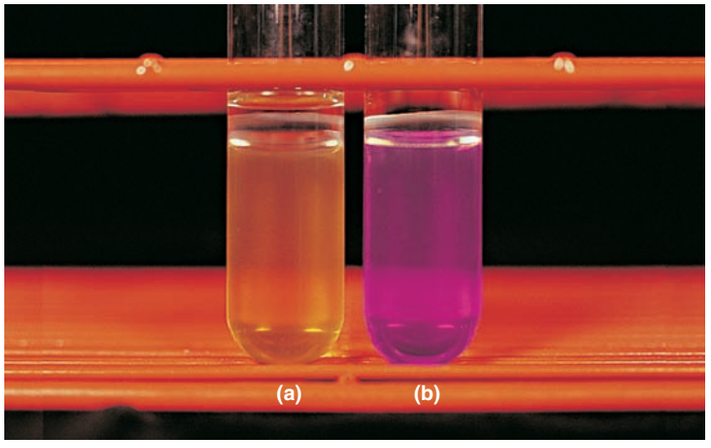


Figure 5.22 Detecting amino acid catabolizing enzymes in the lab. Bacteria are inoculated in tubes containing glucose, a pH indicator, and a specific amino acid. (**a**) The pH indicator turns to yellow when bacteria produce acid from glucose. (**b**) Alkaline products from decarboxylation turn the indicator to purple.

Q What is decarboxylation?

In some instances, the waste products of one microorganism can be used as a carbon and energy source by another species. *Acetobacter* (ä-sē-tō-bak′tėr) bacteria oxidize ethanol made by yeast. *Propionibacterium* (prō-pē-on-ē-bak-ti′re-um) can use lactic acid produced by other bacteria. Propionibacteria convert lactic acid to pyruvic acid in preparation for the Krebs cycle. During the Krebs cycle, propionic acid and CO_2 are made. The holes in Swiss cheese are formed by the accumulation of the CO_2 gas.

Biochemical tests are used to identify bacteria that cause disease. All aerobic bacteria use the electron transport chain (ETC), but not all their ETCs are identical. Some bacteria have cytochrome *c*, but others do not. In the former, *cytochrome c oxidase* is the last enzyme, which transfers electrons to oxygen. The oxidase test is routinely used to quickly identify *Neisseria gonorrhoeae*. *Neisseria* is positive for cytochrome oxidase. The oxidase test can also be used to distinguish some gram-negative rods: *Pseudomonas* is oxidase-positive, and *Escherichia* is oxidase-negative.

Shigella causes dysentery. *Shigella* is differentiated from *E. coli* by biochemical tests. Unlike *E. coli*, *Shigella* does not produce gas from lactose and does not produce the enzyme lactate dehydrogenase.

Salmonella bacteria are readily distinguishable from *E. coli* by the production of hydrogen sulfide (H_2S). Hydrogen sulfide is released when the bacteria remove sulfur from amino acids (**Figure 5.24**). The H_2S combines with iron to form a black precipitate in a culture medium.

The box on page 142 describes how biochemical tests were used to determine the cause of disease in a young child in Dallas, Texas.

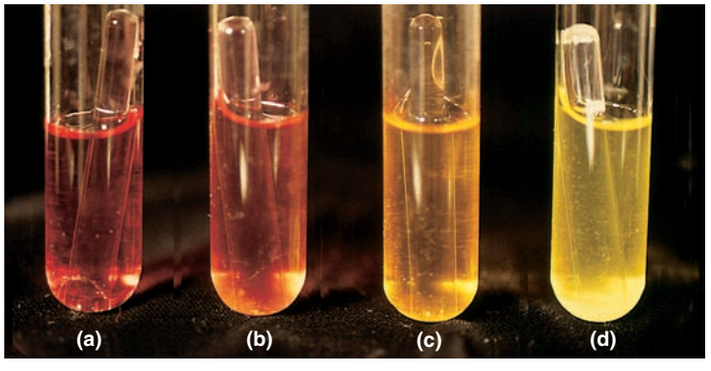


Figure 5.23 A fermentation test. (**a**) An uninoculated fermentation tube containing the carbohydrate mannitol. (**b**) *Staphylococcus epidermidis* grew on the protein but did not use the carbohydrate. This organism is described as mannitol −. (**c**) *Staphylococcus aureus* produced acid but not gas. This species is mannitol +. (**d**) *Escherichia coli* is also mannitol + and produced acid and gas from mannitol. The gas is trapped in the inverted Durham tube.

Q On what is the *S. epidermidis* growing?

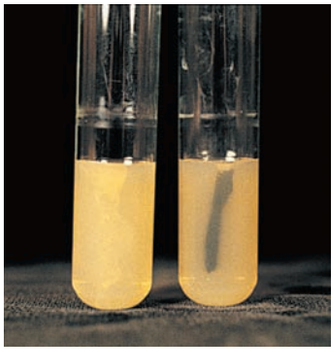


Figure 5.24 Use of peptone iron agar to detect the production of H_2S. H_2S produced in the tube precipitates with iron in the medium as ferrous sulfide.

Q What chemical reaction causes the release of H_2S?

Clinical Case Resolved

S. mutans cannot ferment xylitol; consequently, it doesn't grow and can't produce acid in the mouth. The camp counselors agree to switch to sugarless gum made with xylitol, and Dr. Rivera is pleased. She understands that there will be other sources of sucrose in the children's diets, but at least her patients are no longer going to be adversely affected by the camp's well-intentioned incentives. Researchers are still investigating ways that antimicrobials and vaccines can be used to reduce bacterial colonization. However, reducing consumption of sucrose-containing gum and candy may be an effective preventive measure.

112 133 135 137

CHECK YOUR UNDERSTANDING

- On what biochemical basis are *Pseudomonas* and *Escherichia* differentiated? **5-18**

Photosynthesis

LEARNING OBJECTIVES

5-19 Compare and contrast cyclic and noncyclic photophosphorylation.

5-20 Compare and contrast the light-dependent and light-independent reactions of photosynthesis.

5-21 Compare and contrast oxidative phosphorylation and photophosphorylation.

In all of the metabolic pathways just discussed, organisms obtain energy for cellular work by oxidizing organic compounds. But where do organisms obtain these organic compounds? Some, including animals and many microbes, feed on matter produced by other organisms. For example, bacteria may catabolize compounds from dead plants and animals, or they may obtain nourishment from a living host.

Other organisms synthesize complex organic compounds from simple inorganic substances. The major mechanism for such synthesis is a process called **photosynthesis,** which is carried out by plants and many microbes. Essentially, photosynthesis is the conversion of light energy from the sun into chemical energy. The chemical energy is then used to convert CO_2 from the atmosphere to more reduced carbon compounds, primarily sugars. The word *photosynthesis* summarizes the process: *photo* means light, and *synthesis* refers to the assembly of organic compounds. This synthesis of sugars by using carbon atoms from CO_2 gas is also called **carbon fixation.** Continuation of life as we know it on Earth depends on the recycling of carbon in this way (see Figure 27.3 on page 775). Cyanobacteria, algae, and green plants all contribute to this vital recycling with photosynthesis.

Photosynthesis can be summarized with the following equations:

1. Plants, algae, and cyanobacteria use water as a hydrogen donor, releasing O_2.

$$6\,CO_2 + 12\,H_2O + \text{Light energy} \rightarrow C_6H_{12}O_6 + 6\,H_2O + 6\,O_2$$

2. Purple sulfur and green sulfur bacteria use H_2S as a hydrogen donor, producing sulfur granules.

$$6\,CO_2 + 12\,H_2S + \text{Light energy} \rightarrow C_6H_{12}O_6 + 6\,H_2O + 12\,S$$

In the course of photosynthesis, electrons are taken from hydrogen atoms, an energy-poor molecule, and incorporated into sugar, an energy-rich molecule. The energy boost is supplied by light energy, although indirectly.

Photosynthesis takes place in two stages. In the first stage, called the **light-dependent (light) reactions,** light energy is used to convert ADP and P to ATP. In addition, in the predominant form of the light-dependent reactions, the electron carrier $NADP^+$ is reduced to NADPH. The coenzyme NADPH, like NADH, is an energy-rich carrier of electrons. In the second stage, the **light-independent (dark) reactions,** these electrons are used along with energy from ATP to reduce CO_2 to sugar.
MM **Animation** Photosynthesis: Overview

The Light-Dependent Reactions: Photophosphorylation

Photophosphorylation is one of the three ways ATP is formed, and it occurs only in photosynthetic cells. In this mechanism, light energy is absorbed by chlorophyll molecules in the photosynthetic cell, exciting some of the molecules' electrons. The chlorophyll principally used by green plants, algae, and cyanobacteria is *chlorophyll a*. It is located in the membranous thylakoids of chloroplasts in algae and green plants (see Figure 4.28, page 105) and in the thylakoids found in the photosynthetic structures of cyanobacteria. Other bacteria use *bacteriochlorophylls*.

The excited electrons jump from the chlorophyll to the first of a series of carrier molecules, an electron transport chain similar to that used in respiration. As electrons are passed along the series of carriers, protons are pumped across the membrane, and ADP is converted to ATP by chemiosmosis. Chlorophyll and other pigments are packed into thylakoids of chloroplasts (see Figure 4.28 on page 105) and are called **photosystems.** *Photosystem II* is so numbered because even though it was most likely the first photosystem to evolve, it was the second one discovered. It contains chlorophyll that is sensitive to wavelengths of light of 680 nm. *Photosystem I* contains chlorophyll that is sensitive to wavelengths of light of 700 nm. In **cyclic photophosphorylation,** the electrons released from chlorophyll in photosystem I eventually return to chlorophyll (**Figure 5.25a**). In **noncyclic photophosphorylation,** which is used in oxygenic organisms, the electrons released from the chlorophyll in photosystem II and photosystem I do not return to chlorophyll but become incorporated into NADPH (**Figure 5.25b**). The electrons lost from chlorophyll are replaced by electrons from H_2O. To summarize: the products of noncyclic photophosphorylation are ATP (formed by chemiosmosis using energy released in an electron transport chain), O_2 (from water molecules), and NADPH (in which the hydrogen electrons and protons were derived ultimately from water). MM **Animations** Light Reaction: Cyclic Photophosphorylation; Light Reaction: Noncyclic Photophosphorylation

The Light-Independent Reactions: The Calvin-Benson Cycle

The light-independent (dark) reactions are so named because they require no light directly. They include a complex cyclic pathway called the **Calvin-Benson cycle,** in which CO_2 is "fixed"—that is,

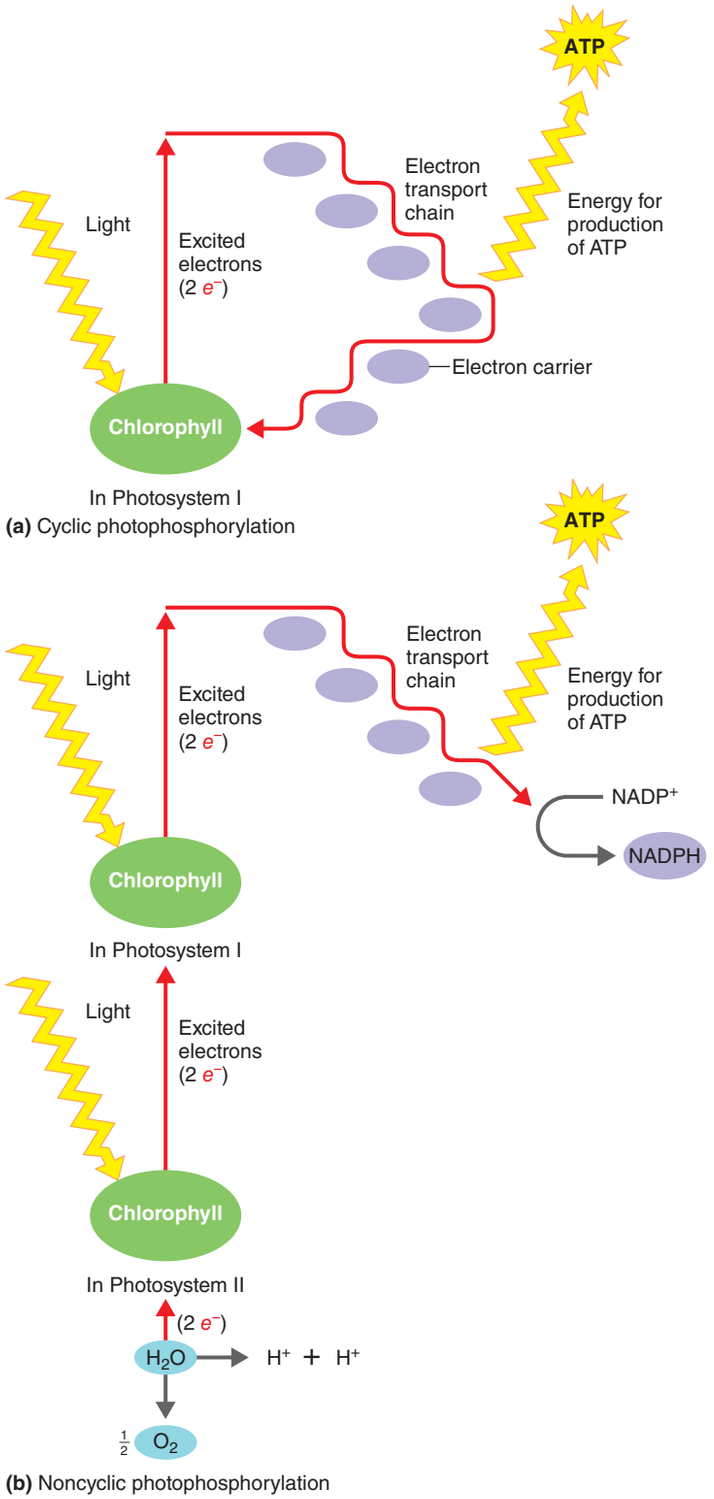


Figure 5.25 Photophosphorylation. (**a**) In cyclic photophosphorylation, electrons released from chlorophyll by light return to chlorophyll after passage along the electron transport chain. The energy from electron transfer is converted to ATP. (**b**) In noncyclic photophosphorylation, electrons released from chlorophyll in photosystem II are replaced by electrons from the hydrogen atoms in water. This process also releases hydrogen ions. Electrons from chlorophyll in photosystem I are passed along the electron transport chain to the electron acceptor $NADP^+$. $NADP^+$ combines with electrons and with hydrogen ions from water, forming NADPH.

Q How are oxidative phosphorylation and photophosphorylation similar?

used to synthesize sugars (**Figure 5.26**, see also Figure A.1 in Appendix A). MM **Animation** Light Independent Reactions

CHECK YOUR UNDERSTANDING

- How is photosynthesis important to catabolism? **5-19**
- What is made during the light-dependent reactions? **5-20**
- How are oxidative phosphorylation and photophosphorylation similar? **5-21**

A Summary of Energy Production Mechanisms

LEARNING OBJECTIVE

5-22 Write a sentence to summarize energy production in cells.

In the living world, energy passes from one organism to another in the form of the potential energy contained in the bonds of chemical compounds. Organisms obtain the energy from oxidation reactions. To obtain energy in a usable form, a cell must have an electron (or hydrogen) donor, which serves as an initial energy source within the cell. Electron donors are diverse and can include photosynthetic pigments, glucose or other organic compounds, elemental sulfur, ammonia, or hydrogen gas (**Figure 5.27**). Next, electrons removed from the chemical energy sources are transferred to electron carriers, such as the coenzymes NAD^+, $NADP^+$, and FAD. This transfer is an oxidation-reduction reaction; the initial energy source is oxidized as this first electron carrier is reduced. During this phase, some ATP is produced. In the third stage, electrons are transferred from electron carriers to their final electron acceptors in further oxidation-reduction reactions, producing more ATP.

In aerobic respiration, oxygen (O_2) serves as the final electron acceptor. In anaerobic respiration, inorganic substances other than oxygen, such as nitrate ions (NO_3^-) or sulfate ions (SO_4^{2-}), serve as the final electron acceptors. In fermentation, organic compounds serve as the final electron acceptors. In aerobic and anaerobic respiration, a series of electron carriers called an electron transport chain releases energy that is used by the mechanism of chemiosmosis to synthesize ATP. Regardless of their energy sources, all organisms use similar oxidation-reduction

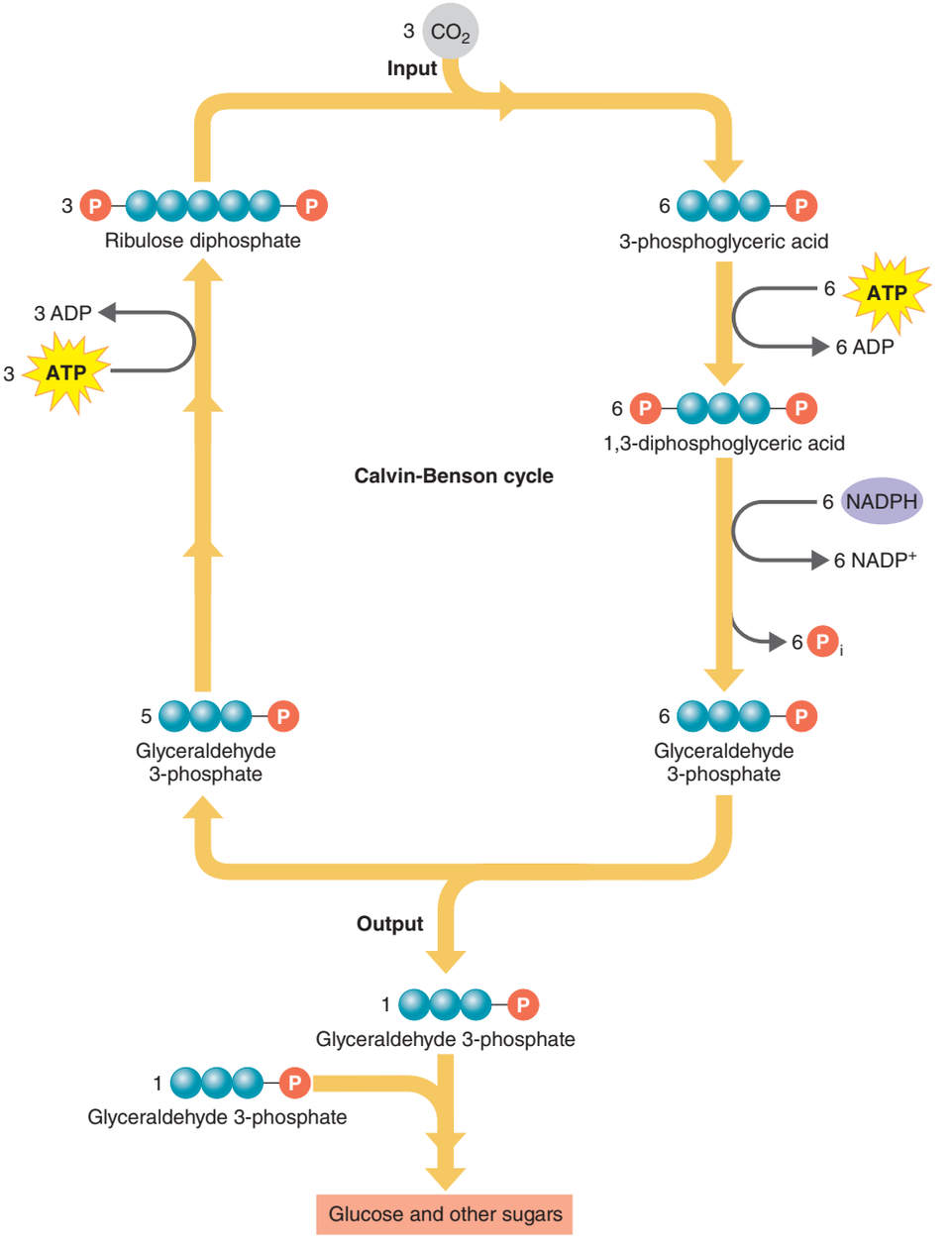


Figure 5.26 A simplified version of the Calvin-Benson cycle. This diagram shows three turns of the cycle, in which three molecules of CO_2 are fixed and one molecule of glyceraldehyde 3-phosphate is produced and leaves the cycle. Two molecules of glyceraldehyde 3-phosphate are needed to make one molecule of glucose. Therefore, the cycle must turn six times for each glucose molecule produced, requiring a total investment of 6 molecules of CO_2, 18 molecules of ATP, and 12 molecules of NADPH. A more detailed version of this cycle is presented in Figure A.1 in Appendix A.

Q In the Calvin-Benson cycle, which molecule is used to synthesize sugars?

reactions to transfer electrons and similar mechanisms to use the energy released to produce ATP.

CHECK YOUR UNDERSTANDING

✓ Summarize how oxidation enables organisms to get energy from glucose, sulfur, or sunlight. 5-22

Metabolic Diversity among Organisms

LEARNING OBJECTIVE

5-23 Categorize the various nutritional patterns among organisms according to carbon source and mechanisms of carbohydrate catabolism and ATP generation.

We have looked in detail at some of the energy-generating metabolic pathways that are used by animals and plants, as well as by many microbes. Microbes are distinguished by their great metabolic diversity, however, and some can sustain themselves on inorganic substances by using pathways that are unavailable to either plants or animals. All organisms, including microbes, can be classified metabolically according to their *nutritional pattern*—their source of energy and their source of carbon.

First considering the energy source, we can generally classify organisms as phototrophs or chemotrophs. **Phototrophs** use light as their primary energy source, whereas **chemotrophs** depend on oxidation-reduction reactions of inorganic or organic compounds for energy. For their principal carbon source, **autotrophs** (self-feeders) use carbon dioxide, and **heterotrophs**

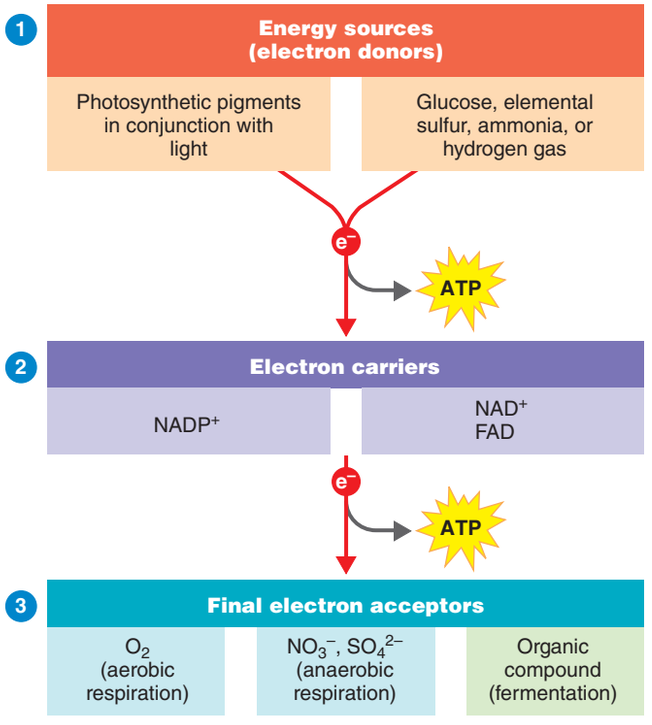


Figure 5.27 Requirements of ATP production. The production of ATP requires 1 an energy source (electron donor), 2 the transfer of electrons to an electron carrier during an oxidation-reduction reaction, and 3 the transfer of electrons to a final electron acceptor.

Q Are energy-generating reactions oxidations or reductions?

(feeders on others) require an organic carbon source. Autotrophs are also referred to as *lithotrophs* (rock eating), and heterotrophs are also referred to as *organotrophs*.

If we combine the energy and carbon sources, we derive the following nutritional classifications for organisms: *photoautotrophs, photoheterotrophs, chemoautotrophs,* and *chemoheterotrophs* (Figure 5.28). Almost all of the medically important microorganisms discussed in this book are chemoheterotrophs. Typically, infectious organisms catabolize substances obtained from the host.

Photoautotrophs

Photoautotrophs use light as a source of energy and carbon dioxide as their chief source of carbon. They include photosynthetic bacteria (green and purple bacteria and cyanobacteria), algae, and green plants. In the photosynthetic reactions of cyanobacteria, algae, and green plants, the hydrogen atoms of water are used to reduce carbon dioxide, and oxygen gas is given off. Because this photosynthetic process produces O_2, it is sometimes called **oxygenic.**

In addition to the cyanobacteria (see Figure 11.21, page 321), there are several other families of photosynthetic prokaryotes. Each is classified according to the way it reduces CO_2. These bacteria cannot use H_2O to reduce CO_2 and cannot carry on photosynthesis when oxygen is present (they must have an anaerobic environment). Consequently, their photosynthetic process does

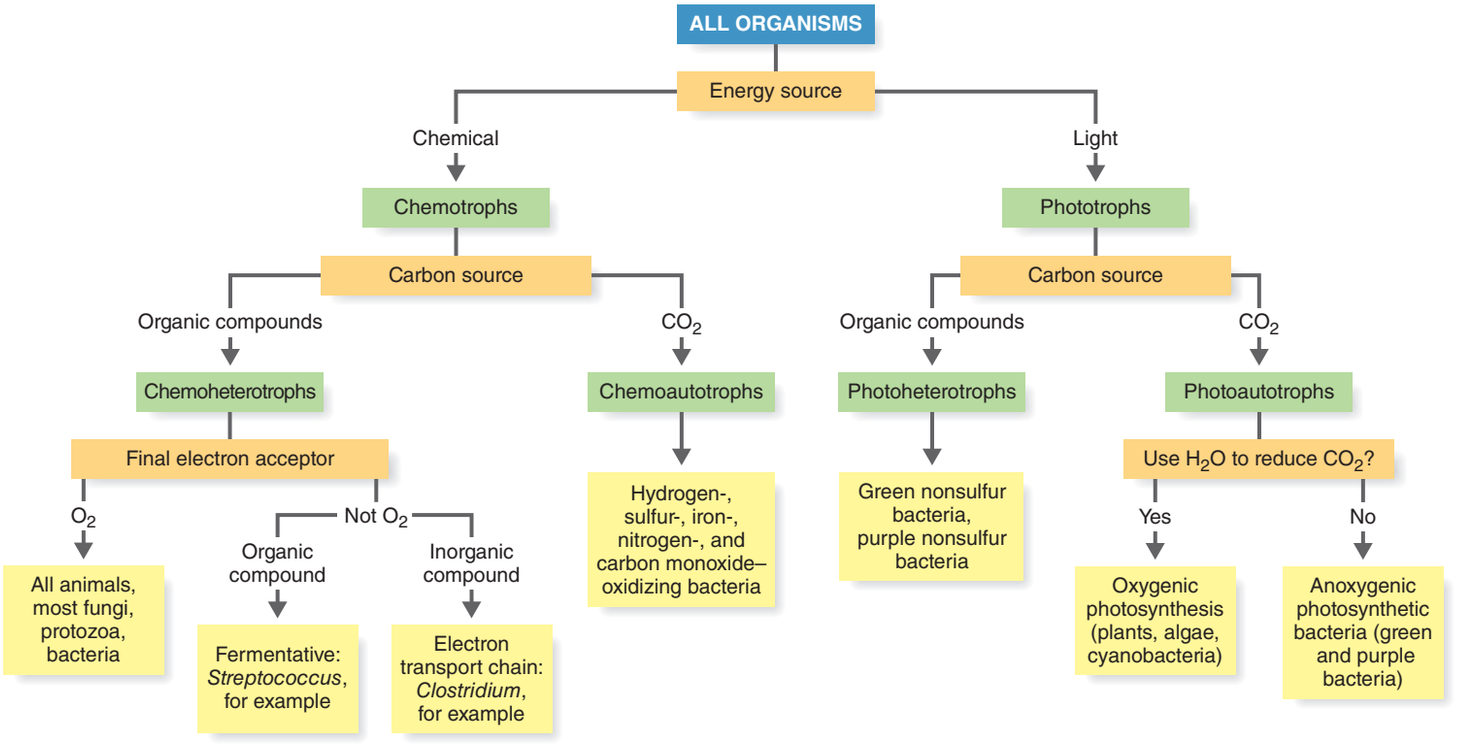


Figure 5.28 A nutritional classification of organisms.

Q What is the basic difference between chemotrophs and phototrophs?

Human Tuberculosis–Dallas, Texas

As you read through this box, you will encounter a series of questions that laboratory technicians ask themselves as they identify bacteria. Try to answer each question before going on to the next one.

1. Daria, a 12-month-old African American girl, is brought by her parents to the emergency department of a Dallas, Texas, hospital. She has a fever of 39°C, a distended abdomen, some abdominal pain, and watery diarrhea. Daria is admitted to the pediatric wing of the hospital, pending results of laboratory and radiologic tests. Test results suggest peritoneal tuberculosis. Caused by one of several closely related species in the *Mycobacterium tuberculosis* complex, TB is a reportable condition in the United States. Peritoneal TB is a disease of the intestines and abdominal cavity.
   What organ is usually associated with tuberculosis? How might someone get peritoneal TB?
2. Pulmonary TB is contracted by inhaling the bacteria; ingesting the bacteria can result in peritoneal TB. A laparoscopy reveals that nodules are present in Daria's abdominal cavity. A portion of a nodule is removed for biopsy so that it can be observed for the presence of acid-fast bacteria. Based on the presence of the abdominal nodules, Daria's physician begins conventional antituberculosis treatment. This long-term treatment can last up to 12 months.
   What is the next step?
3. The lab results confirm that acid-fast bacteria are indeed present in Daria's abdominal cavity. The laboratory now needs to identify the *Mycobacterium* species. Speciation of the *M. tuberculosis* complex is done by biochemical testing in reference laboratories (**Figure A**). The bacteria need to be grown in culture media. Slow-growing mycobacteria may take up to 6 weeks to form colonies.
   After colonies have been isolated, what is the next step?
4. Two weeks later, the laboratory results show that the bacteria are slow-growing. According to the identification scheme, the urease test should be performed.
   What is the result shown in Figure B?
5. Because the urease test is positive, the nitrate reduction test is performed. It shows that the bacteria do not produce the enzyme nitrate reductase. Daria's physician lets her parents know that they are very close to identifying the pathogen that is causing Daria's illness.
   What is the bacterium?
6. *M. bovis* is a pathogen that primarily infects cattle. However, humans can become infected by consuming unpasteurized dairy products or inhaling infectious droplets from cattle. Human-to-human transmission occurs only rarely. The clinical and pathologic characteristics of *M. bovis* TB are indistinguishable from *M. tuberculosis* TB, but identification of the bacterium is important for prevention and treatment. Children may be at higher risk. In one study, almost half of the culture-positive pediatric TB cases were caused by *M. bovis*.

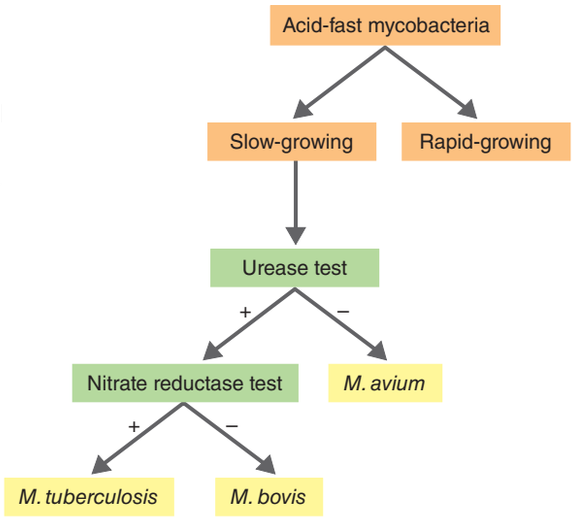


Figure A **An identification scheme for selected species of slow-growing mycobacteria.**

Figure B **The urease test. In a positive test, bacterial urease hydrolyzes urea, producing ammonia. The ammonia raises the pH, and the indicator in the medium turns to fuchsia.**

Unfortunately, Daria does not recover from her illness. Her cadiovascular system collapses, and she dies. The official cause of death is peritoneal tuberculosis caused by *M. bovis*. Everyone should avoid consuming products from unpasteurized cow's milk, which carry the risk of transmitting *M. bovis* if imported from countries where the bacterium is common in cattle.

Source: Adapted from Rodwell T.C., Moore M., Moser K.S., Brodine S.K., Strathdee S.A, "Mycobacterium bovis Tuberculosis in Binational Communities," Emerging Infectious Diseases, June 2008, Volume 14 (6), pp. 909–916. Available from http://www.cdc.gov/eid/content/14/6/909.htm.

not produce O_2 and is called **anoxygenic.** The anoxygenic photoautotrophs are the green and purple bacteria. The **green bacteria,** such as *Chlorobium* (klô-rō′bē-um), use sulfur (S), sulfur compounds (such as hydrogen sulfide, H_2S), or hydrogen gas (H_2) to reduce carbon dioxide and form organic compounds. Applying the energy from light and the appropriate enzymes, these bacteria oxidize sulfide (S^{2-}) or sulfur (S) to sulfate (SO_4^{2-}) or oxidize hydrogen gas to water (H_2O). The **purple bacteria,** such as *Chromatium* (krō-mā′tē-um), also use sulfur, sulfur compounds, or hydrogen gas to reduce carbon dioxide. They are distinguished from the green bacteria by their type of chlorophyll, location of stored sulfur, and ribosomal RNA.

TABLE 5.6 Photosynthesis Compared in Selected Eukaryotes and Prokaryotes

Characteristic	Eukaryotes	Prokaryotes		
	Algae, Plants	**Cyanobacteria**	**Green Bacteria**	**Purple Bacteria**
Substance That Reduces CO_2	H atoms of H_2O	H atoms of H_2O	Sulfur, sulfur compounds, H_2 gas	Sulfur, sulfur compounds, H_2 gas
Oxygen Production	Oxygenic	Oxygenic (and anoxygenic)	Anoxygenic	Anoxygenic
Type of Chlorophyll	Chlorophyll *a*	Chlorophyll *a*	Bacteriochlorophyll *a*	Bacteriochlorophyll *a* or *b*
Site of Photosynthesis	Chloroplasts with thylakoids	Thylakoids	Chlorosomes	Chromatophores
Environment	Aerobic	Aerobic (and anaerobic)	Anaerobic	Anaerobic

The chlorophylls used by these photosynthetic bacteria are called *bacteriochlorophylls,* and they absorb light at longer wavelengths than that absorbed by chlorophyll *a*. Bacteriochlorophylls of green sulfur bacteria are found in vesicles called *chlorosomes* (or *chlorobium vesicles*) underlying and attached to the plasma membrane. In the purple sulfur bacteria, the bacteriochlorophylls are located in invaginations of the plasma membrane (*chromatophores*).

Table 5.6 summarizes several characteristics that distinguish eukaryotic photosynthesis from prokaryotic photosynthesis.

Animation Comparing Prokaryotes and Eukaryotes

Photoheterotrophs

Photoheterotrophs use light as a source of energy but cannot convert carbon dioxide to sugar; rather, they use as sources of carbon organic compounds, such as alcohols, fatty acids, other organic acids, and carbohydrates. Photoheterotrophs are anoxygenic. The **green nonsulfur bacteria,** such as *Chloroflexus* (klô-rō-flex′us), and **purple nonsulfur bacteria,** such as *Rhodopseudomonas* (rō-dō-sū-dō-mō′nas), are photoheterotrophs (see also page 323).

Chemoautotrophs

Chemoautotrophs use the electrons from reduced inorganic compounds as a source of energy, and they use CO_2 as their principal source of carbon. They fix CO_2 in the Calvin-Benson Cycle (see Figure 5.26). Inorganic sources of energy for these organisms include hydrogen sulfide (H_2S) for *Beggiatoa* (bej-jē-ä-tō′ä); elemental sulfur (S) for *Thiobacillus thiooxidans;* ammonia (NH_3) for *Nitrosomonas* (nī-trō-sō-mō′näs); nitrite ions (NO_2^-) for *Nitrobacter* (nī-trō-bak′tėr); hydrogen gas (H_2) for *Cupriavidus* (kü′prē-ä-vid-us); ferrous iron (Fe^{2+}) for *Thiobacillus ferrooxidans;* and carbon monoxide (CO) for *Pseudomonas carboxydohydrogena* (kär′boks-i-dō-hi-drō-je-nä). The energy derived from the oxidation of these inorganic compounds is eventually stored in ATP, which is produced by oxidative phosphorylation.

Chemoheterotrophs

When we discuss photoautotrophs, photoheterotrophs, and chemoautotrophs, it is easy to categorize the energy source and carbon source because they occur as separate entities. However, in chemoheterotrophs, the distinction is not as clear because the energy source and carbon source are usually the same organic compound—glucose, for example. **Chemoheterotrophs** specifically use the electrons from hydrogen atoms in organic compounds as their energy source.

Heterotrophs are further classified according to their source of organic molecules. **Saprophytes** live on dead organic matter, and **parasites** derive nutrients from a living host. Most bacteria, and all fungi, protozoa, and animals, are chemoheterotrophs.

Bacteria and fungi can use a wide variety of organic compounds for carbon and energy sources. This is why they can live in diverse environments. Understanding microbial diversity is scientifically interesting and economically important. In some situations microbial growth is undesirable, such as when rubber-degrading bacteria destroy a gasket or shoe sole. However, these same bacteria might be beneficial if they decomposed discarded rubber products, such as used tires. *Rhodococcus erythropolis* (rō-dō-kok′kus er-i-throp′ō-lis) is widely distributed in soil and can cause disease in humans and other animals. However, this same species is able to replace sulfur atoms in petroleum with atoms of oxygen. A Texas company is currently using *R. erythropolis* to produce desulfurized oil.

CHECK YOUR UNDERSTANDING

Almost all medically important microbes belong to which of the four aforementioned groups? **5-23**

* * *

We will next consider how cells use ATP pathways for the synthesis of organic compounds such as carbohydrates, lipids, proteins, and nucleic acids.

Metabolic Pathways of Energy Use

LEARNING OBJECTIVE

5-24 Describe the major types of anabolism and their relationship to catabolism.

Up to now we have been considering energy production. Through the oxidation of organic molecules, organisms produce energy by aerobic respiration, anaerobic respiration, and fermentation. Much of this energy is given off as heat. The complete metabolic oxidation of glucose to carbon dioxide and water is considered a very efficient process, but about 45% of the energy of glucose is lost as heat. Cells use the remaining energy, which is trapped in the bonds of ATP, in a variety of ways. Microbes use ATP to provide energy for the transport of substances across plasma membranes—the process called active transport that we discussed in Chapter 4. Microbes also use some of their energy for flagellar motion (also discussed in Chapter 4). Most of the ATP, however, is used in the production of new cellular components. This production is a continuous process in cells and, in general, is faster in prokaryotic cells than in eukaryotic cells.

Autotrophs build their organic compounds by fixing carbon dioxide in the Calvin-Benson cycle (see Figure 5.26). This requires both energy (ATP) and electrons (from the oxidation of NADPH). Heterotrophs, by contrast, must have a ready source of organic compounds for biosynthesis—the production of needed cellular components, usually from simpler molecules. The cells use these compounds as both the carbon source and the energy source. We will next consider the biosynthesis of a few representative classes of biological molecules: carbohydrates, lipids, amino acids, purines, and pyrimidines. As we do so, keep in mind that synthesis reactions require a net input of energy.

Polysaccharide Biosynthesis

Microorganisms synthesize sugars and polysaccharides. The carbon atoms required to synthesize glucose are derived from the intermediates produced during processes such as glycolysis and the Krebs cycle and from lipids or amino acids. After synthesizing glucose (or other simple sugars), bacteria may assemble it into more complex polysaccharides, such as glycogen. For bacteria to build glucose into glycogen, glucose units must be phosphorylated and linked. The product of glucose phosphorylation is glucose 6-phosphate. Such a process involves the expenditure of energy, usually in the form of ATP. In order for bacteria to synthesize glycogen, a molecule of ATP is added to glucose 6-phosphate to form *adenosine diphosphoglucose (ADPG)* (**Figure 5.29**). Once ADPG is synthesized, it is linked with similar units to form glycogen.

Using a nucleotide called uridine triphosphate (UTP) as a source of energy and glucose 6-phosphate, animals synthesize glycogen (and many other carbohydrates) from *uridine diphosphoglucose, UDPG* (see Figure 5.29). A compound related to UDPG, called *UDP-N-acetylglucosamine (UDPNAc),* is a key starting material in the biosynthesis of peptidoglycan, the substance that forms bacterial cell walls. UDPNAc is formed from fructose 6-phosphate, and the reaction also uses UTP.

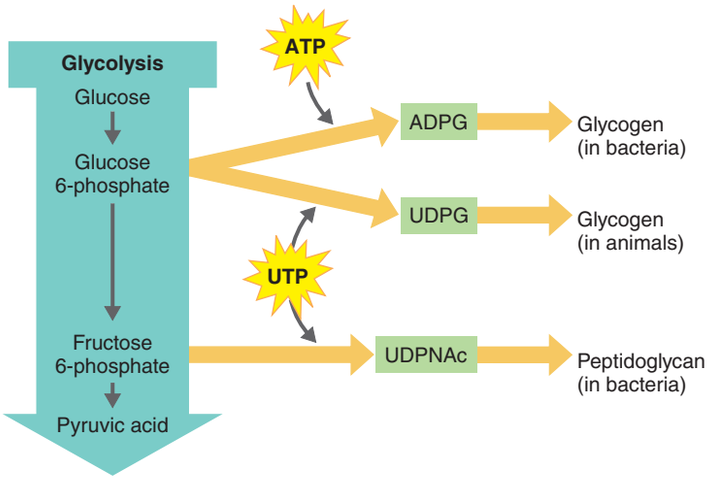


Figure 5.29 **The biosynthesis of polysaccharides.**

Q How are polysaccharides used in cells?

Lipid Biosynthesis

Because lipids vary considerably in chemical composition, they are synthesized by a variety of routes. Cells synthesize fats by joining glycerol and fatty acids. The glycerol portion of the fat is derived from dihydroxyacetone phosphate, an intermediate formed during glycolysis. Fatty acids, which are long-chain hydrocarbons (hydrogen linked to carbon), are built up when two-carbon fragments of acetyl CoA are successively added to each other (**Figure 5.30**). As with polysaccharide synthesis, the building units of fats and other lipids are linked via dehydration synthesis reactions that require energy, not always in the form of ATP.

The most important role of lipids is to serve as structural components of biological membranes, and most membrane lipids are phospholipids. A lipid of a very different structure, cholesterol, is also found in plasma membranes of eukaryotic cells. Waxes are lipids that are important components of the cell wall of acid-fast bacteria. Other lipids, such as carotenoids, provide the red, orange, and yellow pigments of some microorganisms. Some lipids form portions of chlorophyll molecules. Lipids also function in energy storage. Recall that the breakdown products of lipids after biological oxidation feed into the Krebs cycle.

Amino Acid and Protein Biosynthesis

Amino acids are required for protein biosynthesis. Some microbes, such as *E. coli,* contain the enzymes necessary to use starting materials, such as glucose and inorganic salts, for the synthesis of all the amino acids they need. Organisms with the necessary enzymes can synthesize all amino acids directly or indirectly from intermediates of carbohydrate metabolism (**Figure 5.31a**). Other microbes require that the environment provide some preformed amino acids.

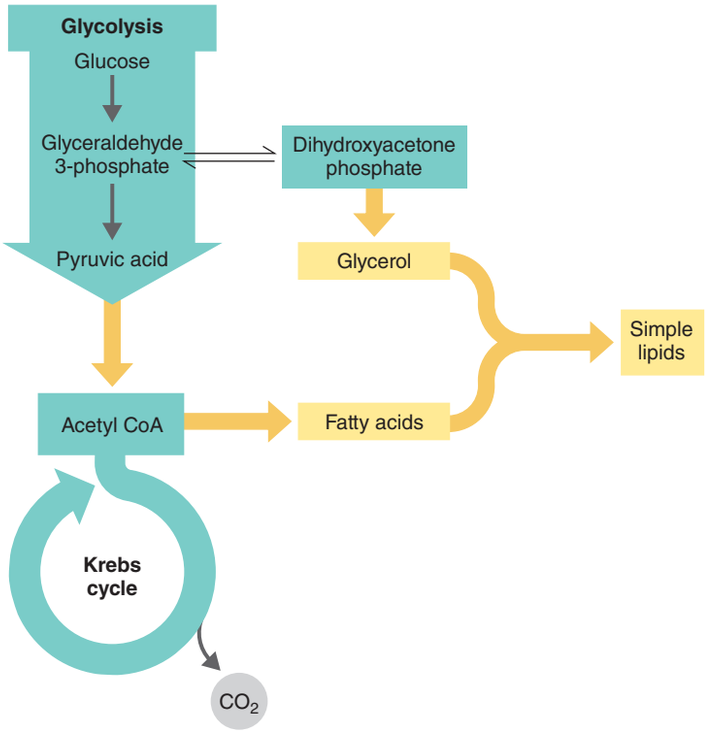


Figure 5.30 **The biosynthesis of simple lipids.**

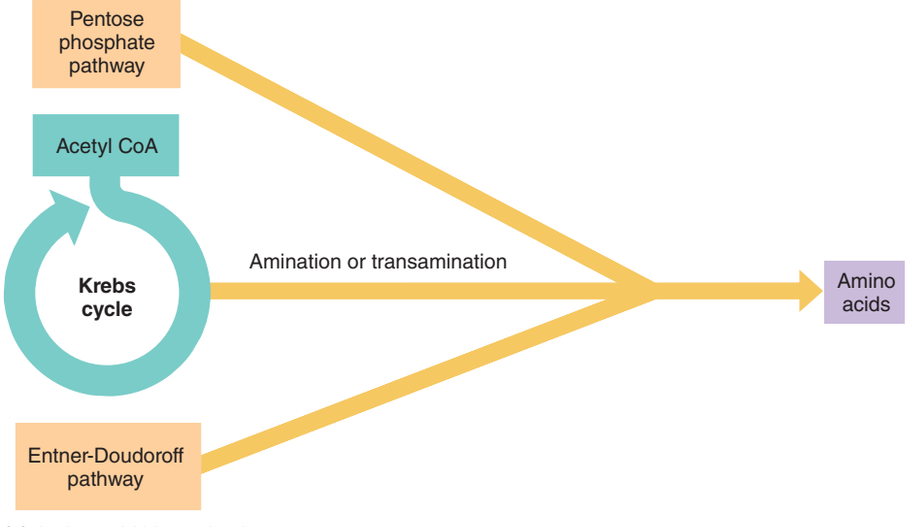
What is the primary use of lipids in cells?

One important source of the *precursors* (intermediates) used in amino acid synthesis is the Krebs cycle. Adding an amine group to pyruvic acid or to an appropriate organic acid of the Krebs cycle converts the acid into an amino acid. This process is called **amination.** If the amine group comes from a preexisting amino acid, the process is called **transamination** (Figure 5.31b).

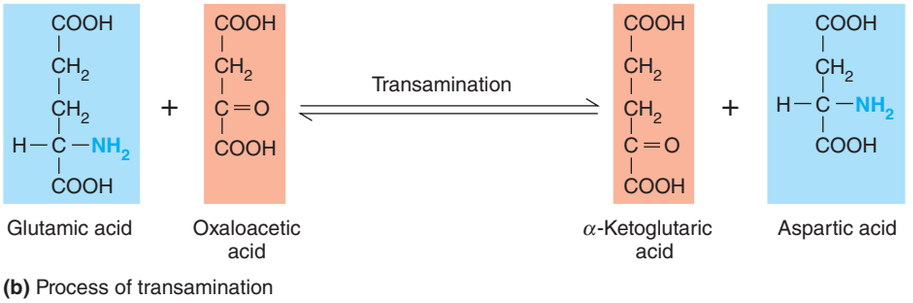
Most amino acids within cells are destined to be building blocks for protein synthesis. Proteins play major roles in the cell as enzymes, structural components, and toxins, to name just a few uses. The joining of amino acids to form proteins involves dehydration synthesis and requires energy in the form of ATP. The mechanism of protein synthesis involves genes and is discussed in Chapter 8.

Purine and Pyrimidine Biosynthesis

Recall from Chapter 2 that the informational molecules DNA and RNA consist of repeating units called *nucleotides,* each of which consists of a purine or pyrimidine, a pentose (five-carbon sugar), and a phosphate group. The five-carbon sugars of nucleotides are derived from either the pentose phosphate pathway or the Entner-Doudoroff pathway. Certain amino acids—aspartic acid, glycine, and glutamine—made from intermediates produced during glycolysis and in the Krebs cycle participate in the biosyntheses of purines and pyrimidines (Figure 5.32). The carbon and



(a) Amino acid biosynthesis



(b) Process of transamination

Figure 5.31 **The biosynthesis of amino acids.** (**a**) Pathways of amino acid biosynthesis through amination or transamination of intermediates of carbohydrate metabolism from the Krebs cycle, pentose phosphate pathway, and Entner-Doudoroff pathway. (**b**) Transamination, a process by which new amino acids are made with the amine groups from old amino acids. Glutamic acid and aspartic acid are both amino acids; the other two compounds are intermediates in the Krebs cycle.

What is the function of amino acids in cells?

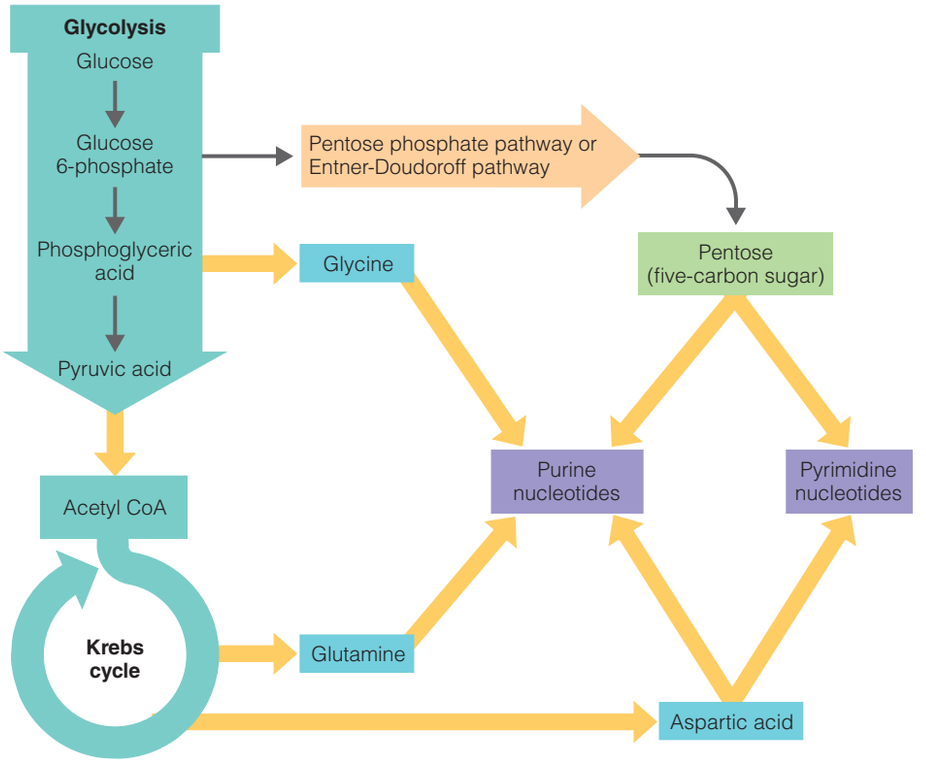


Figure 5.32 **The biosynthesis of purine and pyrimidine nucleotides.**

Q What are the functions of nucleotides in a cell?

nitrogen atoms derived from these amino acids form the purine and pyrimidine rings, and the energy for synthesis is provided by ATP. DNA contains all the information necessary to determine the specific structures and functions of cells. Both RNA and DNA are required for protein synthesis. In addition, such nucleotides as ATP, NAD^+, and $NADP^+$ assume roles in stimulating and inhibiting the rate of cellular metabolism. The synthesis of DNA and RNA from nucleotides will be discussed in Chapter 8.

CHECK YOUR UNDERSTANDING

✓ Where do amino acids required for protein synthesis come from? **5-24**

The Integration of Metabolism

LEARNING OBJECTIVE

5-25 Define *amphibolic pathways.*

We have seen thus far that the metabolic processes of microbes produce energy from light, inorganic compounds, and organic compounds. Reactions also occur in which energy is used for biosynthesis. With such a variety of activity, you might imagine that anabolic and catabolic reactions occur independently of each other in space and time. Actually, anabolic and catabolic reactions are joined through a group of common intermediates (identified as key intermediates in **Figure 5.33**). Both anabolic and catabolic reactions also share some metabolic pathways, such as the Krebs cycle. For example, reactions in the Krebs cycle not only participate in the oxidation of glucose but also produce intermediates that can be converted to amino acids. Metabolic pathways that function in both anabolism and catabolism are called **amphibolic pathways,** meaning that they are dual-purpose.

Amphibolic pathways bridge the reactions that lead to the breakdown and synthesis of carbohydrates, lipids, proteins, and nucleotides. Such pathways enable simultaneous reactions to occur in which the breakdown product formed in one reaction is used in another reaction to synthesize a different compound, and vice versa. Because various intermediates are common to both anabolic and catabolic reactions, mechanisms exist that regulate synthesis and breakdown pathways and allow these reactions to occur simultaneously. One such mechanism involves the use of different coenzymes for opposite pathways. For example, NAD^+ is involved in catabolic reactions, whereas $NADP^+$ is involved in anabolic reactions. Enzymes can also coordinate anabolic and catabolic reactions by accelerating or inhibiting the rates of biochemical reactions.

The energy stores of a cell can also affect the rates of biochemical reactions. For example, if ATP begins to accumulate, an enzyme shuts down glycolysis; this control helps to synchronize the rates of glycolysis and the Krebs cycle. Thus, if citric acid consumption increases, either because of a demand for more ATP or because anabolic pathways are draining off intermediates of the citric acid cycle, glycolysis accelerates and meets the demand. MM **Animation** Metabolism: The Big picture

CHECK YOUR UNDERSTANDING

✓ Summarize the integration of metabolic pathways using peptidoglycan synthesis as an example. **5-25**

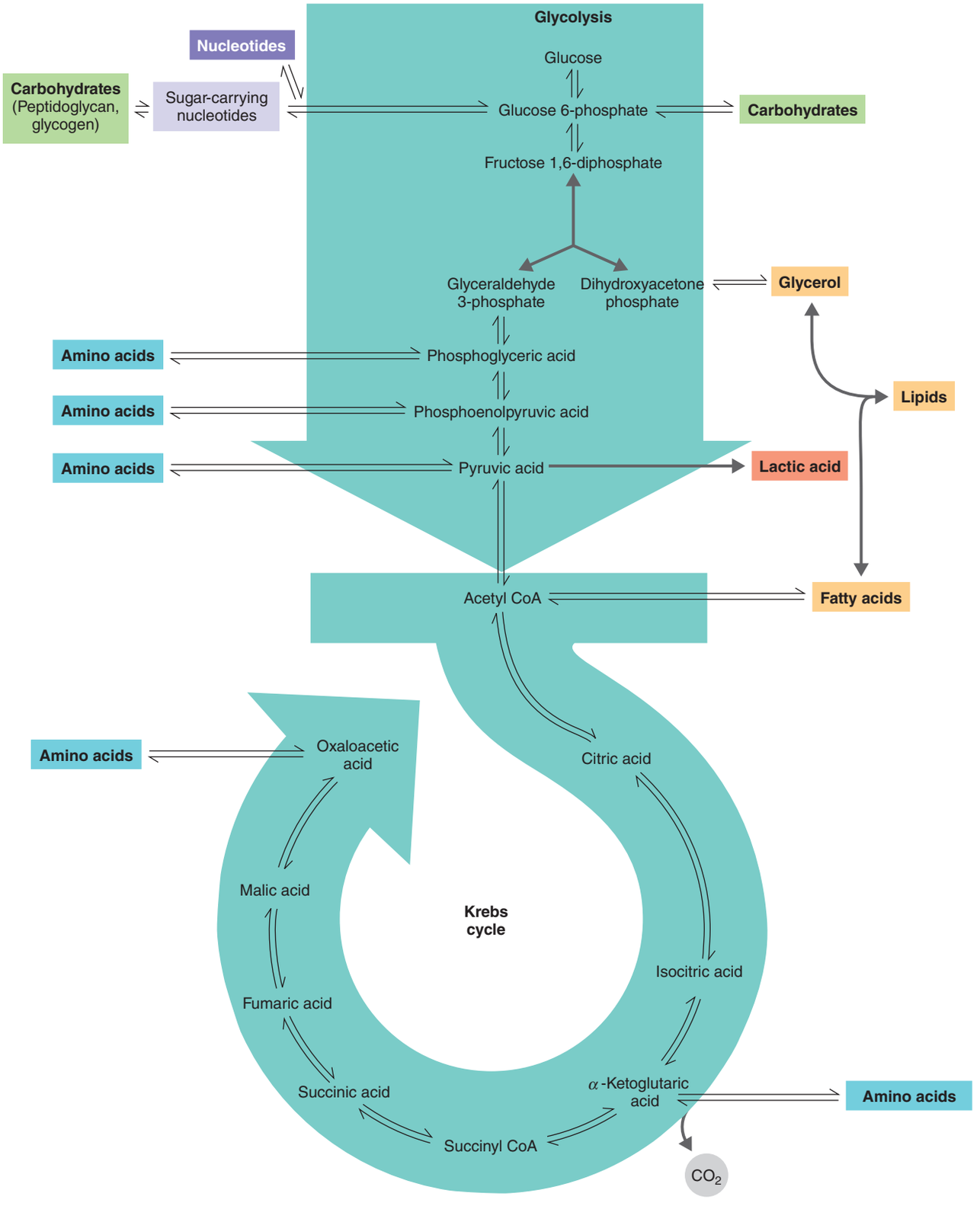


Figure 5.33 **The integration of metabolism.** Key intermediates are shown. Although not indicated in the figure, amino acids and ribose are used in the synthesis of purine and pyrimidine nucleotides (see Figure 5.32). The double arrows indicate amphibolic pathways.

Q What is the purpose of an amphibolic pathway?

Study Outline

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Catabolic and Anabolic Reactions (pp. 112–113)

1. The sum of all chemical reactions within a living organism is known as metabolism.
2. Catabolism refers to chemical reactions that result in the breakdown of more complex organic molecules into simpler substances. Catabolic reactions usually release energy.
3. Anabolism refers to chemical reactions in which simpler substances are combined to form more complex molecules. Anabolic reactions usually require energy.
4. The energy of catabolic reactions is used to drive anabolic reactions.
5. The energy for chemical reactions is stored in ATP.

Enzymes (pp. 113–119)

1. Enzymes are proteins, produced by living cells, that catalyze chemical reactions by lowering the activation energy.
2. Enzymes are generally globular proteins with characteristic three-dimensional shapes.
3. Enzymes are efficient, can operate at relatively low temperatures, and are subject to various cellular controls.

Naming Enzymes (p. 114)

4. Enzyme names usually end in *-ase*.
5. The six classes of enzymes are defined on the basis of the types of reactions they catalyze.

Enzyme Components (pp. 114–115)

6. Most enzymes are holoenzymes, consisting of a protein portion (apoenzyme) and a nonprotein portion (cofactor).
7. The cofactor can be a metal ion (iron, copper, magnesium, manganese, zinc, calcium, or cobalt) or a complex organic molecule known as a coenzyme (NAD^+, $NADP^+$, FMN, FAD, or coenzyme A).

The Mechanism of Enzymatic Action (pp. 115–116)

8. When an enzyme and substrate combine, the substrate is transformed, and the enzyme is recovered.
9. Enzymes are characterized by specificity, which is a function of their active sites.

Factors Influencing Enzymatic Activity (pp. 116–118)

10. At high temperatures, enzymes undergo denaturation and lose their catalytic properties; at low temperatures, the reaction rate decreases.
11. The pH at which enzymatic activity is maximal is known as the optimum pH.
12. Enzymatic activity increases as substrate concentration increases until the enzymes are saturated.
13. Competitive inhibitors compete with the normal substrate for the active site of the enzyme. Noncompetitive inhibitors act on other parts of the apoenzyme or on the cofactor and decrease the enzyme's ability to combine with the normal substrate.

Feedback Inhibition (pp. 118–119)

14. Feedback inhibition occurs when the end-product of a metabolic pathway inhibits an enzyme's activity near the start of the pathway.

Ribozymes (p. 119)

15. Ribozymes are enzymatic RNA molecules that cut and splice RNA in eukaryotic cells.

Energy Production (pp. 119–121)

Oxidation-Reduction Reactions (p. 120)

1. Oxidation is the removal of one or more electrons from a substrate. Protons (H^+) are often removed with the electrons.
2. Reduction of a substrate refers to its gain of one or more electrons.
3. Each time a substance is oxidized, another is simultaneously reduced.
4. NAD^+ is the oxidized form; NADH is the reduced form.
5. Glucose is a reduced molecule; energy is released during a cell's oxidation of glucose.

The Generation of ATP (pp. 120–121)

6. Energy released during certain metabolic reactions can be trapped to form ATP from ADP and P_i (phosphate). Addition of a P_i to a molecule is called phosphorylation.
7. During substrate-level phosphorylation, a high-energy P from an intermediate in catabolism is added to ADP.
8. During oxidative phosphorylation, energy is released as electrons are passed to a series of electron acceptors (an electron transport chain) and finally to O_2 or another inorganic compound.
9. During photophosphorylation, energy from light is trapped by chlorophyll, and electrons are passed through a series of electron acceptors. The electron transfer releases energy used for the synthesis of ATP.

Metabolic Pathways of Energy Production (p. 121)

10. A series of enzymatically catalyzed chemical reactions called metabolic pathways store energy in and release energy from organic molecules.

Carbohydrate Catabolism (pp. 122–133)

1. Most of a cell's energy is produced from the oxidation of carbohydrates.
2. Glucose is the most commonly used carbohydrate.
3. The two major types of glucose catabolism are respiration, in which glucose is completely broken down, and fermentation, in which it is partially broken down.

Glycolysis (pp. 122–123)

4. The most common pathway for the oxidation of glucose is glycolysis. Pyruvic acid is the end-product.

5. Two ATP and two NADH molecules are produced from one glucose molecule.

Alternatives to Glycolysis (pp. 123, 125)

6. The pentose phosphate pathway is used to metabolize five-carbon sugars; one ATP and 12 NADPH molecules are produced from one glucose molecule.
7. The Entner-Doudoroff pathway yields one ATP and two NADPH molecules from one glucose molecule.

Cellular Respiration (pp. 125–130)

8. During respiration, organic molecules are oxidized. Energy is generated from the electron transport chain.
9. In aerobic respiration, O_2 functions as the final electron acceptor.
10. In anaerobic respiration, the final electron acceptor is usually an inorganic molecule other than O_2.
11. Decarboxylation of pyruvic acid produces one CO_2 molecule and one acetyl group.
12. Two-carbon acetyl groups are oxidized in the Krebs cycle. Electrons are picked up by NAD^+ and FAD for the electron transport chain.
13. From one molecule of glucose, oxidation produces six molecules of NADH, two molecules of $FADH_2$, and two molecules of ATP.
14. Decarboxylation produces six molecules of CO_2.
15. Electrons are brought to the electron transport chain by NADH.
16. The electron transport chain consists of carriers, including flavoproteins, cytochromes, and ubiquinones.
17. Protons being pumped across the membrane generate a proton motive force as electrons move through a series of acceptors or carriers.
18. Energy produced from movement of the protons back across the membrane is used by ATP synthase to make ATP from ADP and P_i.
19. In eukaryotes, electron carriers are located in the inner mitochondrial membrane; in prokaryotes, electron carriers are in the plasma membrane.
20. In aerobic prokaryotes, 38 ATP molecules can be produced from complete oxidation of a glucose molecule in glycolysis, the Krebs cycle, and the electron transport chain.
21. In eukaryotes, 36 ATP molecules are produced from complete oxidation of a glucose molecule.
22. The final electron acceptors in anaerobic respiration include NO_3^-, SO_4^{2-}, and CO_3^{2-}.
23. The total ATP yield is less than in aerobic respiration because only part of the Krebs cycle operates under anaerobic conditions.

Fermentation (pp. 130–133)

24. Fermentation releases energy from sugars or other organic molecules by oxidation.
25. O_2 is not required in fermentation.
26. Two ATP molecules are produced by substrate-level phosphorylation.
27. Electrons removed from the substrate reduce NAD^+.
28. The final electron acceptor is an organic molecule.
29. In lactic acid fermentation, pyruvic acid is reduced by NADH to lactic acid.
30. In alcohol fermentation, acetaldehyde is reduced by NADH to produce ethanol.
31. Heterolactic fermenters can use the pentose phosphate pathway to produce lactic acid and ethanol.

Lipid and Protein Catabolism (pp. 133–135)

1. Lipases hydrolyze lipids into glycerol and fatty acids.
2. Fatty acids and other hydrocarbons are catabolized by beta-oxidation.
3. Catabolic products can be further broken down in glycolysis and the Krebs cycle.
4. Before amino acids can be catabolized, they must be converted to various substances that enter the Krebs cycle.
5. Transamination, decarboxylation, and dehydrogenation reactions convert the amino acids to be catabolized.

Biochemical Tests and Bacterial Identification (pp. 135–137)

1. Bacteria and yeast can be identified by detecting action of their enzymes.
2. Fermentation tests are used to determine whether an organism can ferment a carbohydrate to produce acid and gas.

Photosynthesis (pp. 137–139)

1. Photosynthesis is the conversion of light energy from the sun into chemical energy; the chemical energy is used for carbon fixation.

The Light-Dependent Reactions: Photophosphorylation (p. 138)

2. Chlorophyll *a* is used by green plants, algae, and cyanobacteria; it is found in thylakoid membranes.
3. Electrons from chlorophyll pass through an electron transport chain, from which ATP is produced by chemiosmosis.
4. Photosystems are made up of chlorophyll and other pigments packed into thylakoid membranes.
5. In cyclic photophosphorylation, the electrons return to the chlorophyll.
6. In noncyclic photophosphorylation, the electrons are used to reduce $NADP^+$. The electrons from H_2O or H_2S replace those lost from chlorophyll.
7. When H_2O is oxidized by green plants, algae, and cyanobacteria, O_2 is produced; when H_2S is oxidized by the sulfur bacteria, S^0 granules are produced.

The Light-Independent Reactions: The Calvin-Benson Cycle (pp. 138–139)

8. CO_2 is used to synthesize sugars in the Calvin-Benson cycle.

A Summary of Energy Production Mechanisms (pp. 139–140)

1. Sunlight is converted to chemical energy in oxidation-reduction reactions carried on by phototrophs. Chemotrophs can use this chemical energy.
2. In oxidation-reduction reactions, energy is derived from the transfer of electrons.
3. To produce energy, a cell needs an electron donor (organic or inorganic), a system of electron carriers, and a final electron acceptor (organic or inorganic).

Metabolic Diversity among Organisms

(pp. 140–143)

1. Photoautotrophs obtain energy by photophosphorylation and fix carbon from CO_2 via the Calvin-Benson cycle to synthesize organic compounds.
2. Cyanobacteria are oxygenic phototrophs. Green bacteria and purple bacteria are anoxygenic phototrophs.
3. Photoheterotrophs use light as an energy source and an organic compound for their carbon source and electron donor.
4. Chemoautotrophs use inorganic compounds as their energy source and carbon dioxide as their carbon source.
5. Chemoheterotrophs use complex organic molecules as their carbon and energy sources.

Metabolic Pathways of Energy Use (pp. 144–145)

Polysaccharide Biosynthesis (p. 144)

1. Glycogen is formed from ADPG.
2. UDPNAc is the starting material for the biosynthesis of peptidoglycan.

Lipid Biosynthesis (p. 144)

3. Lipids are synthesized from fatty acids and glycerol.
4. Glycerol is derived from dihydroxyacetone phosphate, and fatty acids are built from acetyl CoA.

Amino Acid and Protein Biosynthesis (pp. 144–145)

5. Amino acids are required for protein biosynthesis.
6. All amino acids can be synthesized either directly or indirectly from intermediates of carbohydrate metabolism, particularly from the Krebs cycle.

Purine and Pyrimidine Biosynthesis (p. 146)

7. The sugars composing nucleotides are derived from either the pentose phosphate pathway or the Entner-Doudoroff pathway.
8. Carbon and nitrogen atoms from certain amino acids form the backbones of the purines and pyrimidines.

The Integration of Metabolism (pp. 146–147)

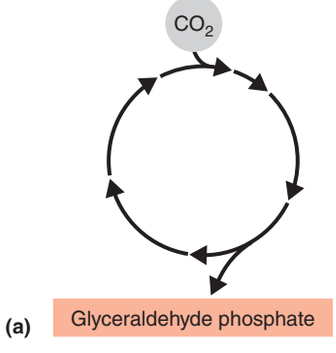
1. Anabolic and catabolic reactions are integrated through a group of common intermediates.
2. Such integrated metabolic pathways are referred to as amphibolic pathways.

Study Questions

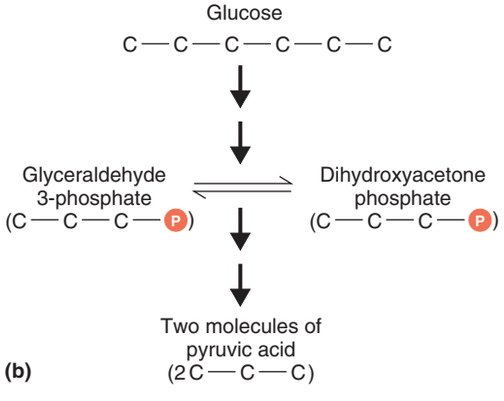
Answers to the Review and Multiple Choice questions can be found by turning to the Answers tab at the back of the textbook.

Review

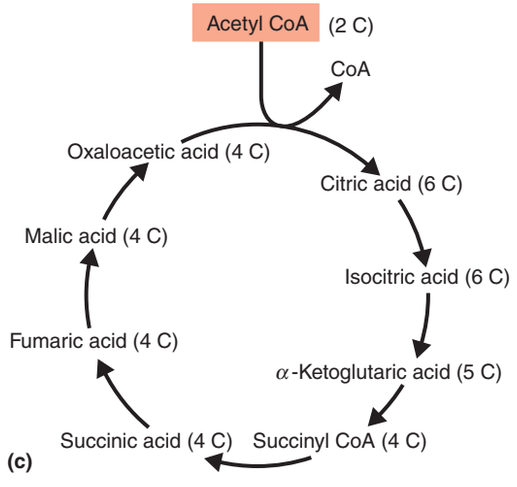
Use the following diagrams (a), (b), and (c) for question 1.



(a)

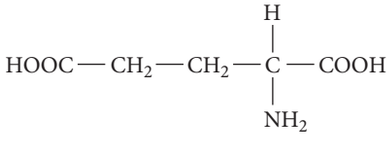


(b)



(c)

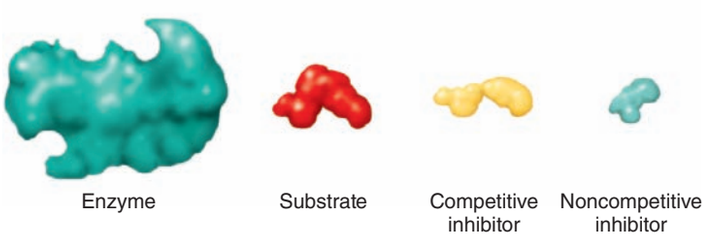
1. Name pathways diagrammed in parts (a), (b), and (c) of the figure.
   - **a.** Show where glycerol is catabolized and where fatty acids are catabolized.
   - **b.** Show where glutamic acid (an amino acid) is catabolized:



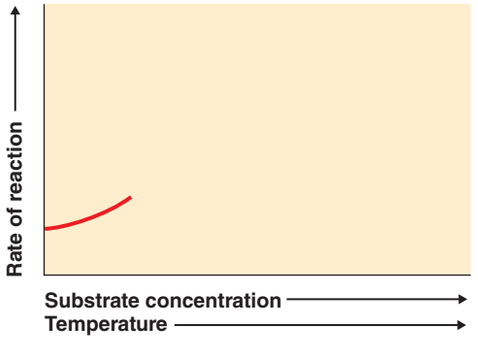
   - **c.** Show how these pathways are related.
   - **d.** Where is ATP required in pathways (a) and (b)?
   - **e.** Where is CO_2 released in pathways (b) and (c)?

f. Show where a long-chain hydrocarbon such as petroleum is catabolized.
g. Where is NADH (or $FADH_2$ or NADPH) used and produced in these pathways?
h. Identify four places where anabolic and catabolic pathways are integrated.

2. DRAW IT Using the diagrams below, show each of the following:
a. where the substrate will bind
b. where the competitive inhibitor will bind
c. where the noncompetitive inhibitor will bind
d. which of the four elements could be the inhibitor in feedback inhibition
e. What effect will the reactions in (a), (b), and (c) have?

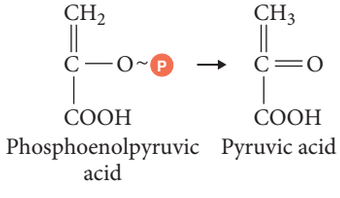


3. DRAW IT An enzyme and substrate are combined. The rate of reaction begins as shown in the following graph. To complete the graph, show the effect of increasing substrate concentration on a constant enzyme concentration. Show the effect of increasing temperature.



4. Define *oxidation-reduction*, and differentiate the following terms:
a. aerobic and anaerobic respiration
b. respiration and fermentation
c. cyclic and noncyclic photophosphorylation

5. There are three mechanisms for the phosphorylation of ADP to produce ATP. Write the name of the mechanism that describes each of the reactions in the following table.

ATP Generated by	Reaction
a. _____	An electron, liberated from chlorophyll by light, is passed down an electron transport chain.
b. _____	Cytochrome *c* passes two electrons to cytochrome *a*.
c. _____	

Phosphoenolpyruvic acid → Pyruvic acid

6. All of the energy-producing biochemical reactions that occur in cells, such as photophosphorylation and glycolysis, are _____ reactions.

7. Fill in the following table with the carbon source and energy source of each type of organism.

Organism	Carbon Source	Energy Source
Photoautotroph	a. _____	b. _____
Photoheterotroph	c. _____	d. _____
Chemoautotroph	e. _____	f. _____
Chemoheterotroph	g. _____	h. _____

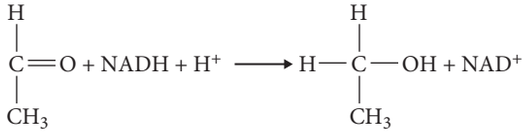
8. Write your own definition of the chemiosmotic mechanism of ATP generation. On Figure 5.16, mark the following using the appropriate letter:
a. the acidic side of the membrane
b. the side with a positive electrical charge
c. potential energy
d. kinetic energy

9. Why must NADH be reoxidized? How does this happen in an organism that uses respiration? Fermentation?

10. NAME IT What nutritional type is a colorless microbe that uses the Calvin cycle, uses H_2 as the electron donor to its ETC, and uses elemental S as the final electron acceptor in the ETC?

Multiple Choice

1. Which substance in the following reaction is being reduced?



Acetaldehyde → Ethanol

a. acetaldehyde
b. NADH
c. ethanol
d. NAD^+

2. Which of the following reactions produces the most molecules of ATP during aerobic metabolism?
a. glucose → glucose 6-phosphate
b. phosphoenolpyruvic acid → pyruvic acid
c. glucose → pyruvic acid
d. acetyl CoA → CO_2 + H_2O
e. succinic acid → fumaric acid

3. Which of the following processes does *not* generate ATP?
a. photophosphorylation
b. the Calvin-Benson cycle
c. oxidative phosphorylation
d. substrate-level phosphorylation
e. none of the above

4. Which of the following compounds has the greatest amount of energy for a cell?
a. CO_2
b. ATP
c. glucose
d. O_2
e. lactic acid

5. Which of the following is the best definition of the Krebs cycle?
   a. the oxidation of pyruvic acid
   b. the way cells produce CO_2
   c. a series of chemical reactions in which NADH is produced from the oxidation of pyruvic acid
   d. a method of producing ATP by phosphorylating ADP
   e. a series of chemical reactions in which ATP is produced from the oxidation of pyruvic acid
6. Which of the following is the best definition of *respiration*?
   a. a sequence of carrier molecules with O_2 as the final electron acceptor
   b. a sequence of carrier molecules with an inorganic molecule as the final electron acceptor
   c. a method of generating ATP
   d. the complete oxidation of glucose to CO_2 and H_2O
   e. a series of reactions in which pyruvic acid is oxidized to CO_2 and H_2O

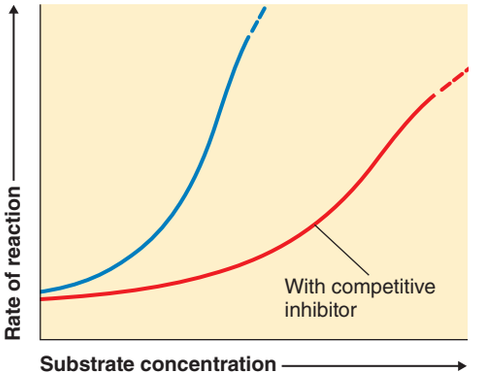
Use the following choices to answer questions 7–10.

   a. *E. coli* growing in glucose broth at 35°C with O_2 for 5 days
   b. *E. coli* growing in glucose broth at 35°C without O_2 for 5 days
   c. both a and b
   d. neither a nor b

7. Which culture produces the most lactic acid?
8. Which culture produces the most ATP?
9. Which culture uses NAD^+?
10. Which culture uses the most glucose?

Critical Thinking

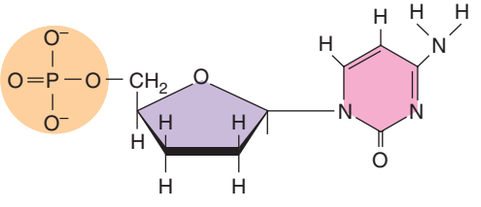
1. Explain why, even under ideal conditions, *Streptococcus* grows slowly.
2. The following graph shows the normal rate of reaction of an enzyme and its substrate (blue) and the rate when an excess of competitive inhibitor is present (red). Explain why the graph appears as it does.



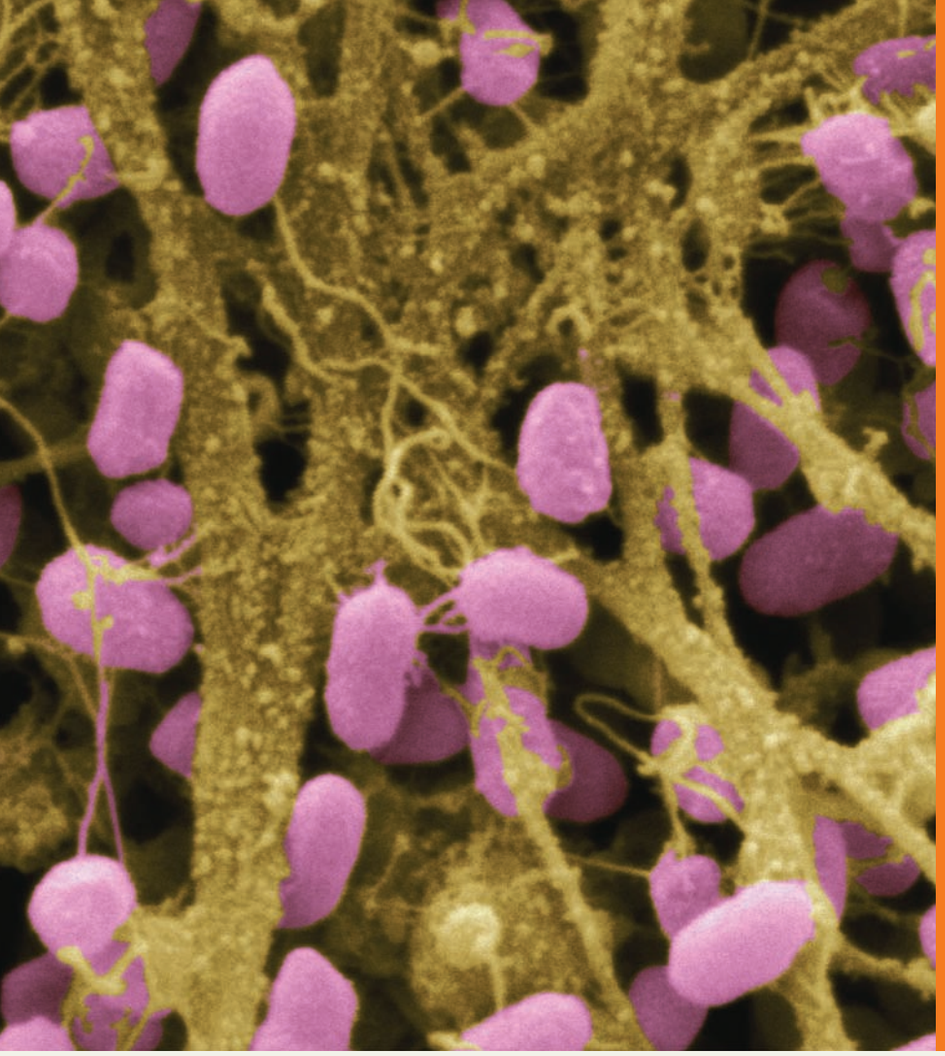
3. Compare and contrast carbohydrate catabolism and energy production in the following bacteria:
   a. *Pseudomonas,* an aerobic chemoheterotroph
   b. *Spirulina,* an oxygenic photoautotroph
   c. *Ectothiorhodospira,* an anoxygenic photoautotroph
4. How much ATP could be obtained from the complete oxidation of one molecule of glucose? From one molecule of butterfat containing one glycerol and three 12-carbon chains?
5. The chemoautotroph *Thiobacillus* can obtain energy from the oxidation of arsenic ($As^{3+} \rightarrow As^{5+}$). How does this reaction provide energy? How can humans put this bacterium to use?

Clinical Applications

1. *Haemophilus influenzae* requires hemin (X factor) to synthesize cytochromes and NAD^+ (V factor) from other cells. For what does it use these two growth factors? What diseases does *H. influenzae* cause?
2. The drug Hivid, also called ddC, inhibits DNA synthesis. It is used to treat HIV infection and AIDS. Compare the following illustration of ddC to Figure 2.16 on page 46. How does this drug work?



3. The bacterial enzyme streptokinase is used to digest fibrin (blood clots) in patients with atherosclerosis. Why doesn't injection of streptokinase cause a streptococcal infection? How do we know the streptokinase will digest fibrin only and not good tissues?



6

Microbial Growth

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When we talk about microbial growth, we are really referring to the *number* of cells, not the *size* of the cells. Microbes that are "growing" are increasing in number, accumulating into *colonies* (groups of cells large enough to be seen without a microscope) of hundreds of thousands of cells or *populations* of billions of cells. Although individual cells approximately double in size during their lifetime, this change is not very significant compared with the size increases observed during the lifetime of plants and animals.

Many bacteria survive and grow slowly in nutrient-poor environments by forming biofilms. The *Serratia marcescens* bacteria in the photo have formed a biofilm on a piece of plastic. Biofilms are frequently sources of health care associated infections such as the one described in the Clinical Case.

Microbial populations can become incredibly large in a very short time. By understanding the conditions necessary for microbial growth, we can determine how to control the growth of microbes that cause diseases and food spoilage. We can also learn how to encourage the growth of helpful microbes and those we wish to study.

In this chapter we will examine the physical and chemical requirements for microbial growth, the various kinds of culture media, bacterial cell division, the phases of microbial growth, and the methods of measuring microbial growth.

The Requirements for Growth

LEARNING OBJECTIVES

6-1 Classify microbes into five groups on the basis of preferred temperature range.

6-2 Identify how and why the pH of culture media is controlled.

6-3 Explain the importance of osmotic pressure to microbial growth.

6-4 Name a use for each of the four elements (carbon, nitrogen, sulfur, and phosphorus) needed in large amounts for microbial growth.

6-5 Explain how microbes are classified on the basis of oxygen requirements.

6-6 Identify ways in which aerobes avoid damage by toxic forms of oxygen.

The requirements for microbial growth can be divided into two main categories: physical and chemical. Physical aspects include temperature, pH, and osmotic pressure. Chemical requirements include sources of carbon, nitrogen, sulfur, phosphorus, oxygen, trace elements, and organic growth factors.

Clinical Case: Glowing in the Dark

Reginald MacGruder, an investigator at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, has a mystery on his hands. Earlier this year, he was involved in the recall of an intravenous heparin solution that was blamed for causing *Pseudomonas fluorescens* bloodstream infections in patients in four different states. It seemed that everything was under control, but now, three months after the recall, 19 patients in two other states develop the same *P. fluorescens* bloodstream infections. It makes no sense to Dr. MacGruder; how could this infection be popping up again so soon after the recall? Could another heparin batch be tainted?

What is *P. fluorescens*? Read on to find out.

154 166 175 177

Physical Requirements

Temperature

Most microorganisms grow well at the temperatures that humans favor. However, certain bacteria are capable of growing at extremes of temperature that would certainly hinder the survival of almost all eukaryotic organisms.

Microorganisms are classified into three primary groups on the basis of their preferred range of temperature: **psychrophiles** (cold-loving microbes), **mesophiles** (moderate-temperature–loving microbes), and **thermophiles** (heat-loving microbes). Most bacteria grow only within a limited range of temperatures, and their maximum and minimum growth temperatures are only about 30°C apart. They grow poorly at the high and low temperature extremes within their range.

Each bacterial species grows at particular minimum, optimum, and maximum temperatures. The **minimum growth temperature** is the lowest temperature at which the species will grow. The **optimum growth temperature** is the temperature at which the species grows best. The **maximum growth temperature** is the highest temperature at which growth is possible. By graphing the growth response over a temperature range, we can see that the optimum growth temperature is usually near the top of the range; above that temperature the rate of growth drops off rapidly (Figure 6.1). This happens presumably because the high temperature has inactivated necessary enzymatic systems of the cell.

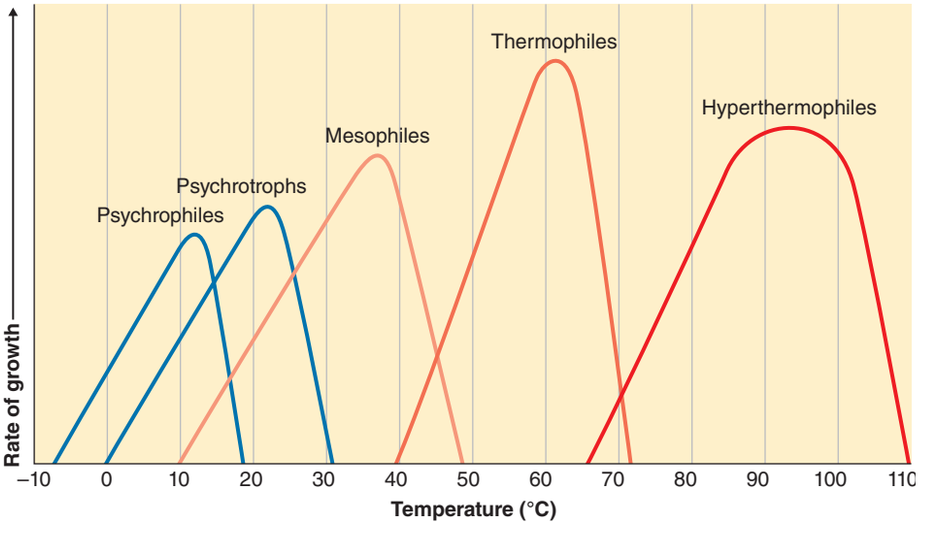


Figure 6.1 Typical growth rates of different types of microorganisms in response to temperature. The peak of the curve represents optimum growth (fastest reproduction). Notice that the reproductive rate drops off very quickly at temperatures only a little above the optimum. At either extreme of the temperature range, the reproductive rate is much lower than the rate at the optimum temperature.

Q Why is it difficult to define *psychrophile, mesophile,* and *thermophile*?

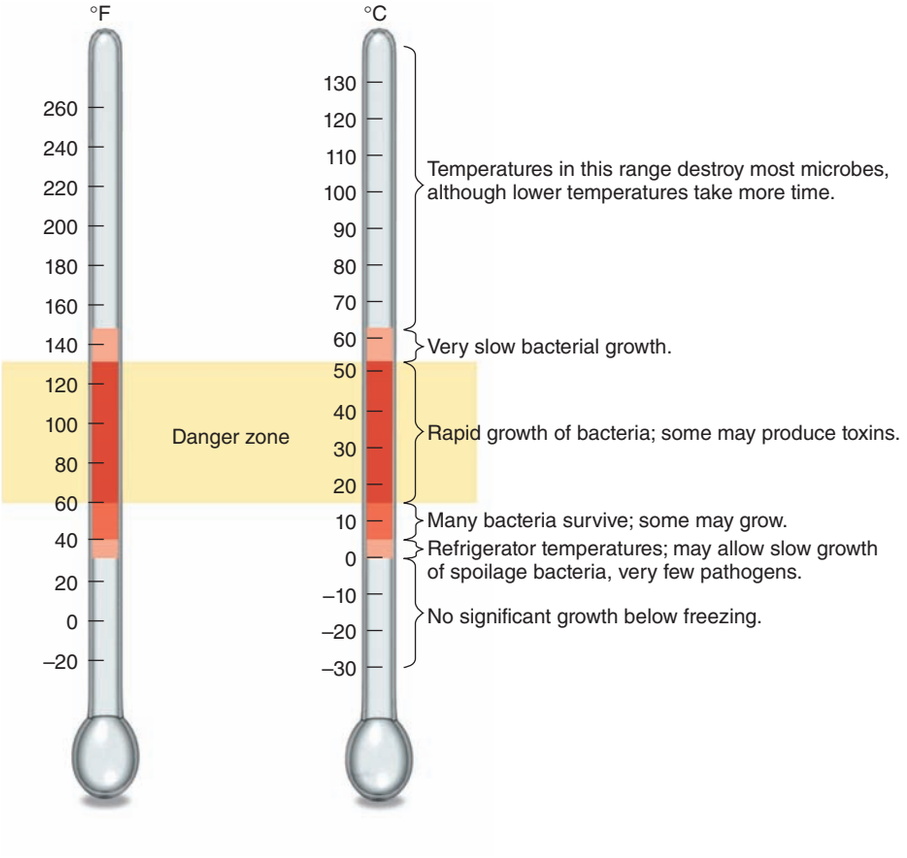


Figure 6.2 Food preservation temperatures. Low temperatures decrease microbial reproduction rates, which is the basic principle of refrigeration. There are always some exceptions to the temperature responses shown here; for example, certain bacteria grow well at temperatures that would kill most bacteria, and a few bacteria can actually grow at temperatures well below freezing.

Q Which bacterium would theoretically be more likely to grow at refrigerator temperatures: a human intestinal pathogen or a soilborne plant pathogen?

The ranges and maximum growth temperatures that define bacteria as psychrophiles, mesophiles, or thermophiles are not rigidly defined. Psychrophiles, for example, were originally considered simply to be organisms capable of growing at 0°C. However, there seem to be two fairly distinct groups capable of growth at that temperature. One group, composed of psychrophiles in the strictest sense, can grow at 0°C but has an optimum growth temperature of about 15°C. Most of these organisms are so sensitive to higher temperatures that they will not even grow in a reasonably warm room (25°C). Found mostly in the oceans' depths or in certain polar regions, such organisms seldom cause problems in food preservation. The other group that can grow at 0°C has higher optimum temperatures, usually 20–30°C and cannot grow above about 40°C. Organisms of this type are much more common than psychrophiles and are the most likely to be encountered in low-temperature food spoilage because they grow fairly well at refrigerator temperatures. We will use the term **psychrotrophs,** which food microbiologists favor, for this group of spoilage microorganisms.

Refrigeration is the most common method of preserving household food supplies. It is based on the principle that microbial reproductive rates decrease at low temperatures. Although microbes usually survive even subfreezing temperatures (they might become entirely dormant), they gradually decline in number. Some species decline faster than others. Psychrotrophs actually do not grow well at low temperatures, except in comparison with other organisms; given time, however, they are able to slowly degrade food. Such spoilage might take the form of mold mycelium, slime on food surfaces, or off-tastes or off-colors in foods. The temperature inside a properly set refrigerator will greatly slow the growth of most spoilage organisms and will entirely prevent the growth of all but a few pathogenic bacteria. **Figure 6.2** illustrates the importance of low temperatures for preventing the growth of spoilage and disease organisms. When large amounts of food must be refrigerated, it is important to keep in mind the slow cooling rate of a large quantity of warm food (**Figure 6.3**).

Mesophiles, with an optimum growth temperature of 25–40°C, are the most common type of microbe. Organisms that have adapted to live in the bodies of animals usually have an optimum temperature close to that of their hosts. The optimum temperature for many pathogenic bacteria is about 37°C, and incubators for clinical cultures are usually set at about this temperature. The mesophiles include most of the common spoilage and disease organisms.

Thermophiles are microorganisms capable of growth at high temperatures. Many of these organisms have an optimum growth temperature of 50–60°C, about the temperature of water from a hot water tap. Such temperatures can also be reached in sunlit soil and in thermal waters such as hot springs. Remarkably, many thermophiles cannot grow at temperatures below

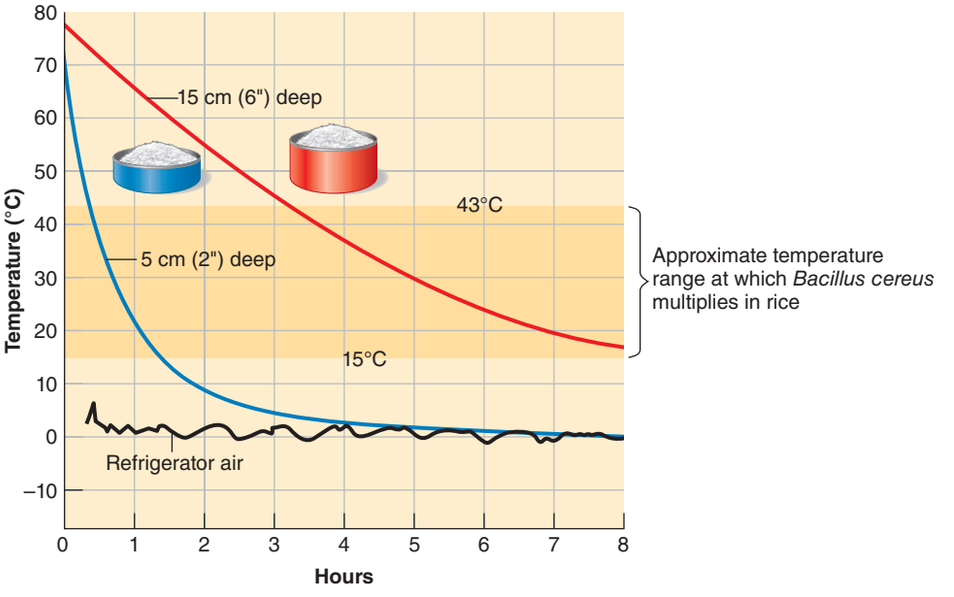


Figure 6.3 The effect of the amount of food on its cooling rate in a refrigerator and its chance of spoilage. Notice that in this example, the pan of rice with a depth of 5 cm (2 in) cooled through the incubation temperature range of the *Bacillus cereus* in about 1 hour, whereas the pan of rice with a depth of 15 cm (6 in) remained in this temperature range for about 5 hours.

Q Given a shallow pan and a deep pot with the same volume, which would cool faster? Why?

about 45°C. Endospores formed by thermophilic bacteria are unusually heat resistant and may survive the usual heat treatment given canned goods. Although elevated storage temperatures may cause surviving endospores to germinate and grow, thereby spoiling the food, these thermophilic bacteria are not considered a public health problem. Thermophiles are important in organic compost piles (see Figure 27.10 on page 782), in which the temperature can rise rapidly to 50–60°C.

Some microbes, members of the Archaea (page 4), have an optimum growth temperature of 80°C or higher. These organisms are called **hyperthermophiles,** or sometimes **extreme thermophiles.** Most of these organisms live in hot springs associated with volcanic activity; sulfur is usually important in their metabolic activity. The known record for bacterial growth and replication at high temperatures is about 121°C near deep-sea hydrothermal vents. See the box on the facing page. The immense pressure in the ocean depths prevents water from boiling even at temperatures well above 100°C.

pH

Recall from Chapter 2 (pages 34–35) that pH refers to the acidity or alkalinity of a solution. Most bacteria grow best in a narrow pH range near neutrality, between pH 6.5 and 7.5. Very few bacteria grow at an acidic pH below about pH 4. This is why a number of foods, such as sauerkraut, pickles, and many cheeses, are preserved from spoilage by acids produced by bacterial fermentation. Nonetheless, some bacteria, called **acidophiles,** are remarkably tolerant of acidity. One type of chemoautotrophic bacteria, which is found in the drainage water from coal mines and oxidizes sulfur to form sulfuric acid, can survive at a pH value of 1. Molds and yeasts will grow over a greater pH range than bacteria will, but the optimum pH of molds and yeasts is generally below that of bacteria, usually about pH 5 to 6. Alkalinity also inhibits microbial growth but is rarely used to preserve foods.

When bacteria are cultured in the laboratory, they often produce acids that eventually interfere with their own growth. To neutralize the acids and maintain the proper pH, chemical buffers are included in the growth medium. The peptones and amino acids in some media act as buffers, and many media also contain phosphate salts. Phosphate salts have the advantage of exhibiting their buffering effect in the pH growth range of most bacteria. They are also nontoxic; in fact, they provide phosphorus, an essential nutrient.

Osmotic Pressure

Microorganisms obtain almost all their nutrients in solution from the surrounding water. Thus, they require water for growth, and their composition is 80–90% water. High osmotic pressures have the effect of removing necessary water from a cell. When a microbial cell is in a solution whose concentration of solutes is higher than in the cell (the environment is *hypertonic* to the cell), the cellular water passes out through the plasma membrane to the high solute concentration. (See the discussion of osmosis in Chapter 4, pages 92–93, and review Figure 4.18 for the three types of solution environments a cell may encounter.) This osmotic loss of water causes **plasmolysis,** or shrinkage of the cell's cytoplasm (Figure 6.4).

The importance of this phenomenon is that the growth of the cell is inhibited as the plasma membrane pulls away from the cell wall. Thus, the addition of salts (or other solutes) to a solution, and the resulting increase in osmotic pressure, can be used to preserve foods. Salted fish, honey, and sweetened condensed milk are preserved largely by this mechanism; the high salt or sugar concentrations draw water out of any microbial cells that are present

Life in the Extreme

Until humans explored the deep-ocean floor, scientists believed that only a few forms of life could survive in that high-pressure, completely dark, oxygen-poor environment. Then, in 1977, *Alvin,* the deep-sea submersible carried two scientists 2600 meters below the surface at the Galápagos Rift (about 350 km northeast of the Galápagos Islands). There, amid the vast expanse of barren basalt rocks, the scientists found unexpectedly rich oases of life. Superheated water from beneath the seafloor rises through fractures in the Earth's crust called vents. Mats of bacteria grow along the sides of the vents, where temperatures exceed 100°C (see the figure).

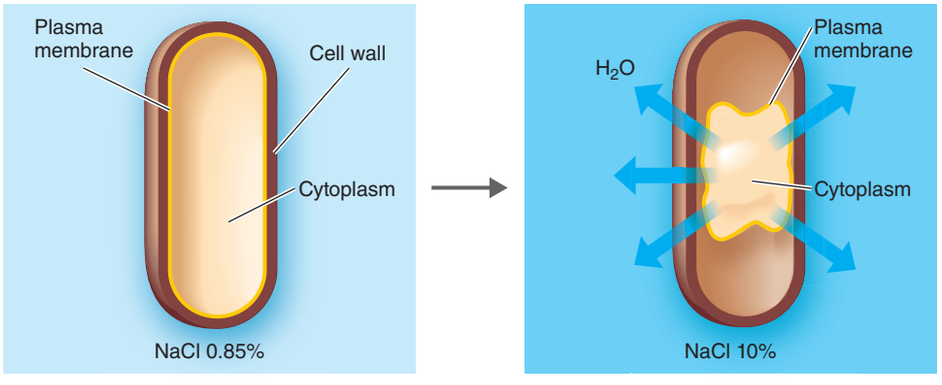
Ecosystem of the Hydrothermal Vents

Life at the surface of the world's oceans depends on photosynthetic organisms, such as plants and algae, which harness the sun's energy to fix carbon dioxide (CO_2) to make carbohydrates. At the deep-ocean floor, where no light penetrates, photosynthesis is not possible. The scientists found that the primary producers at the ocean floor are chemoautotrophic bacteria. Using chemical energy from hydrogen sulfide (H_2S) as a source of energy to fix CO_2, the chemoautotrophs create an environment that supports higher life forms. Hydrothermal vents in the seafloor supply the H_2S and CO_2.

New Products from Hydrothermal Vents

Terrestrial fungi and bacteria have had a major impact on the development of antimicrobial and antitumor compounds since the 1930s. Hydrothermal vents are the next frontier in the hunt for new drugs. In 2010 a peptide produced by *Thermovibrio ammonificans* was shown to induce apoptosis (cell death) and thus potential anticancer activity. Currently, researchers are growing *Pyrococcus furiosus* because it produces alternative fuels, hydrogen gas, and butanol. DNA polymerases (enzymes that synthesize DNA) isolated from two archaea living near deep-sea vents are being used in the polymerase chain reaction (PCR), a technique for making many copies of DNA. In PCR, single-stranded DNA is made by heating a chromosome fragment to 98°C and cooling it so that DNA polymerase can copy each strand. DNA polymerase from *Thermococcus litoralis*, called $Vent_R$, and from *Pyrococcus*, called Deep $Vent_R$, are not denatured at 98°C. These enzymes can be used in automatic thermalcyclers to repeat the heating and cooling cycles, allowing many copies of DNA to be made easily and quickly.

A white microbial biofilm is visible on this deep-sea hydrothermal vent. Water is being emitted through the ocean floor at temperatures above 100°C.



(a) Cell in isotonic solution. Under these conditions, the solute concentration in the cell is equivalent to a solute concentration of 0.85% sodium chloride (NaCl). See Figure 4.18.

(b) Plasmolyzed cell in hypertonic solution. If the concentration of solutes such as NaCl is higher in the surrounding medium than in the cell (the environment is hypertonic), water tends to leave the cell. Growth of the cell is inhibited.

Figure 6.4 **Plasmolysis.**

Q Why is osmotic pressure an important factor in microbial growth?

and thus prevent their growth. These effects of osmotic pressure are roughly related to the *number* of dissolved molecules and ions in a volume of solution.

Some organisms, called **extreme halophiles**, have adapted so well to high salt concentrations that they actually require them for growth. In this case, they may be termed **obligate halophiles.** Organisms from such saline waters as the Dead Sea often require nearly 30% salt, and the inoculating loop (a device for handling bacteria in the laboratory) used to transfer them must first be dipped into a saturated salt solution. More common are **facultative halophiles,** which do not require high salt concentrations but are able to grow at salt concentrations up to 2%, a concentration that inhibits the growth of many other organisms. A few species of facultative halophiles can tolerate even 15% salt.

Most microorganisms, however, must be grown in a medium that is nearly all water. For example, the concentration of agar (a complex polysaccharide isolated from marine algae) used to solidify microbial growth media is usually about 1.5%. If markedly higher concentrations are used, the increased osmotic pressure can inhibit the growth of some bacteria.

If the osmotic pressure is unusually low (the environment is *hypotonic*)—such as in distilled water, for example—water tends to enter the cell rather than leave it. Some microbes that have a relatively weak cell wall may be lysed by such treatment.

CHECK YOUR UNDERSTANDING

- Why are hyperthermophiles that grow at temperatures above 100°C seemingly limited to oceanic depths? **6-1**
- Other than controlling acidity, what is an advantage of using phosphate salts as buffers in growth media? **6-2**
- Why might primitive civilizations have used food preservation techniques that rely on osmotic pressure? **6-3**

Chemical Requirements

Carbon

Besides water, one of the most important requirements for microbial growth is carbon. Carbon is the structural backbone of living matter; it is needed for all the organic compounds that make up a living cell. Half the dry weight of a typical bacterial cell is carbon. Chemoheterotrophs get most of their carbon from the source of their energy—organic materials such as proteins, carbohydrates, and lipids. Chemoautotrophs and photoautotrophs derive their carbon from carbon dioxide.

Nitrogen, Sulfur, and Phosphorus

In addition to carbon, microorganisms need other elements to synthesize cellular material. For example, protein synthesis requires considerable amounts of nitrogen as well as some sulfur. The syntheses of DNA and RNA also require nitrogen and some phosphorus, as does the synthesis of ATP, the molecule so important for the storage and transfer of chemical energy within the cell. Nitrogen makes up about 14% of the dry weight of a bacterial cell, and sulfur and phosphorus together constitute about another 4%.

Organisms use nitrogen primarily to form the amino group of the amino acids of proteins. Many bacteria meet this requirement by decomposing protein-containing material and reincorporating the amino acids into newly synthesized proteins and other nitrogen-containing compounds. Other bacteria use nitrogen from ammonium ions (NH_4^+), which are already in the reduced form and are usually found in organic cellular material. Still other bacteria are able to derive nitrogen from nitrates (compounds that dissociate to give the nitrate ion, NO_3^-, in solution).

Some important bacteria, including many of the photosynthesizing cyanobacteria (page 137), use gaseous nitrogen (N_2) directly from the atmosphere. This process is called **nitrogen fixation.** Some organisms that can use this method are free-living, mostly in the soil, but others live cooperatively in symbiosis with the roots of legumes such as clover, soybeans, alfalfa, beans, and peas. The nitrogen fixed in the symbiosis is used by both the plant and the bacterium (see Chapter 27).

Sulfur is used to synthesize sulfur-containing amino acids and vitamins such as thiamine and biotin. Important natural sources of sulfur include the sulfate ion (SO_4^{2-}), hydrogen sulfide, and the sulfur-containing amino acids.

Phosphorus is essential for the synthesis of nucleic acids and the phospholipids of cell membranes. Among other places, it is also found in the energy bonds of ATP. A source of phosphorus is the phosphate ion (PO_4^{3-}). Potassium, magnesium, and calcium are also elements that microorganisms require, often as cofactors for enzymes (see Chapter 5, pages 114–115).

Trace Elements

Microbes require very small amounts of other mineral elements, such as iron, copper, molybdenum, and zinc; these are referred to as **trace elements.** Most are essential for the functions of certain enzymes, usually as cofactors. Although these elements are sometimes added to a laboratory medium, they are usually assumed to be naturally present in tap water and other components of media. Even most distilled waters contain adequate amounts, but tap water is sometimes specified to ensure that these trace minerals will be present in culture media.

Oxygen

We are accustomed to thinking of molecular oxygen (O_2) as a necessity of life, but it is actually in a sense a poisonous gas. Very little molecular oxygen existed in the atmosphere during most of Earth's history—in fact, it is possible that life could not have arisen had oxygen been present. However, many current forms of life have metabolic systems that require oxygen for aerobic respiration. As we have seen, hydrogen atoms that have been stripped from organic compounds combine with oxygen to form water, as shown in Figure 5.14 (page 127). This process yields a great deal of energy while neutralizing a potentially toxic gas—a very neat solution, all in all.

TABLE 6.1 The Effect of Oxygen on the Growth of Various Types of Bacteria

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Microaerophiles
Effect of Oxygen on Growth	Only aerobic growth; oxygen required.	Both aerobic and anaerobic growth; greater growth in presence of oxygen.	Only anaerobic growth; ceases in presence of oxygen.	Only anaerobic growth; but continues in presence of oxygen.	Only aerobic growth; oxygen required in low concentration.
Bacterial Growth in Tube of Solid Growth Medium					
Explanation of Growth Patterns	Growth occurs only where high concentrations of oxygen have diffused into the medium.	Growth is best where most oxygen is present, but occurs throughout tube.	Growth occurs only where there is no oxygen.	Growth occurs evenly; oxygen has no effect.	Growth occurs only where a low concentration of oxygen has diffused into medium.
Explanation of Oxygen's Effects	Presence of enzymes catalase and superoxide dismutase (SOD) allows toxic forms of oxygen to be neutralized; can use oxygen.	Presence of enzymes catalase and SOD allows toxic forms of oxygen to be neutralized; can use oxygen.	Lacks enzymes to neutralize harmful forms of oxygen; cannot tolerate oxygen.	Presence of one enzyme, SOD, allows harmful forms of oxygen to be partially neutralized; tolerates oxygen.	Produce lethal amounts of toxic forms of oxygen if exposed to normal atmospheric oxygen.

Microbes that use molecular oxygen (aerobes) extract more energy from nutrients than microbes that do not use oxygen (anaerobes). Organisms that require oxygen to live are called **obligate aerobes** (Table 6.1a).

Obligate aerobes are at a disadvantage because oxygen is poorly soluble in the water of their environment. Therefore, many of the aerobic bacteria have developed, or retained, the ability to continue growing in the absence of oxygen. Such organisms are called **facultative anaerobes** (Table 6.1b). In other words, facultative anaerobes can use oxygen when it is present but are able to continue growth by using fermentation or anaerobic respiration when oxygen is not available. However, their efficiency in producing energy decreases in the absence of oxygen. Examples of facultative anaerobes are the familiar *Escherichia coli* that are found in the human intestinal tract. Many yeasts are also facultative anaerobes. Recall from the discussion of anaerobic respiration in Chapter 5 (page 130) that many microbes are able to substitute other electron acceptors, such as nitrate ions, for oxygen, which is something humans are unable to do.

Obligate anaerobes (Table 6.1c) are bacteria that are unable to use molecular oxygen for energy-yielding reactions. In fact, most are harmed by it. The genus *Clostridium* (klôs-tri′dē-um), which contains the species that cause tetanus and botulism, is the most familiar example. These bacteria do use oxygen atoms present in cellular materials; the atoms are usually obtained from water.

Understanding how organisms can be harmed by oxygen requires a brief discussion of the toxic forms of oxygen:

1. **Singlet oxygen** ($^1O_2^-$) is normal molecular oxygen (O_2) that has been boosted into a higher-energy state and is extremely reactive.
2. **Superoxide radicals** ($O_2^{\cdot-}$), or **superoxide anions,** are formed in small amounts during the normal respiration of organisms that use oxygen as a final electron acceptor, forming water. In the presence of oxygen, obligate anaerobes also appear to form some superoxide radicals, which are so toxic to cellular components that all organisms attempting to grow in atmospheric oxygen must produce an enzyme, **superoxide dismutase (SOD),** to neutralize them. Their toxicity is caused by their great instability, which leads them to steal an electron from a neighboring molecule, which in turn becomes a radical and steals an electron, and so on. Aerobic bacteria, facultative anaerobes growing aerobically, and aerotolerant anaerobes (discussed shortly) produce SOD, with which they convert the superoxide radical into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2):

$$O_2^{\cdot-} + O_2^{\cdot-} + 2\,H^+ \longrightarrow H_2O_2 + O_2$$

3. The hydrogen peroxide produced in this reaction contains the **peroxide anion** O_2^{2-} and is also toxic. In Chapter 7 (page 199) we will encounter it as the active principle in the antimicrobial agents hydrogen peroxide and benzoyl peroxide. Because the hydrogen peroxide produced during normal aerobic respiration is toxic, microbes have developed enzymes to neutralize it. The most familiar of these is **catalase,** which converts it into water and oxygen:

$$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$$

Catalase is easily detected by its action on hydrogen peroxide. When a drop of hydrogen peroxide is added to a colony of bacterial cells producing catalase, oxygen bubbles are released. Anyone who has put hydrogen peroxide on a wound will recognize that cells in human tissue also contain catalase. The other enzyme that breaks down hydrogen peroxide is **peroxidase,** which differs from catalase in that its reaction does not produce oxygen:

$$H_2O_2 + 2\ H^+ \longrightarrow 2\ H_2O$$

Another important form of reactive oxygen, **ozone (O_3),** is also discussed on page 199.

4. The **hydroxyl radical** ($OH\cdot$) is another intermediate form of oxygen and probably the most reactive. It is formed in the cellular cytoplasm by ionizing radiation. Most aerobic respiration produces traces of hydroxyl radicals, but they are transient.

These toxic forms of oxygen are an essential component of one of the body's most important defenses against pathogens, phagocytosis (see page 460 and Figure 16.7). In the phagolysosome of the phagocytic cell, ingested pathogens are killed by exposure to singlet oxygen, superoxide radicals, peroxide anions of hydrogen peroxide, and hydroxyl radicals and other oxidative compounds.

Obligate anaerobes usually produce neither superoxide dismutase nor catalase. Because aerobic conditions probably lead to an accumulation of superoxide radicals in their cytoplasm, obligate anaerobes are extremely sensitive to oxygen.

Aerotolerant anaerobes (Table 6.1d) cannot use oxygen for growth, but they tolerate it fairly well. On the surface of a solid medium, they will grow without the use of special techniques (discussed later) required for obligate anaerobes. Many of the aerotolerant bacteria characteristically ferment carbohydrates to lactic acid. As lactic acid accumulates, it inhibits the growth of aerobic competitors and establishes a favorable ecological niche for lactic acid producers. A common example of lactic acid–producing aerotolerant anaerobes is the lactobacilli used in the production of many acidic fermented foods, such as pickles and cheese. In the laboratory, they are handled and grown much like any other bacteria, but they make no use of the oxygen in the air. These bacteria can tolerate oxygen because they possess SOD or an equivalent system that neutralizes the toxic forms of oxygen previously discussed.

A few bacteria are **microaerophiles** (Table 6.1e). They are aerobic; they do require oxygen. However, they grow only in oxygen concentrations lower than those in air. In a test tube of solid nutrient medium, they grow only at a depth where small amounts of oxygen have diffused into the medium; they do not grow near the oxygen-rich surface or below the narrow zone of adequate oxygen. This limited tolerance is probably due to their sensitivity to superoxide radicals and peroxides, which they produce in lethal concentrations under oxygen-rich conditions.

Organic Growth Factors

Essential organic compounds an organism is unable to synthesize are known as **organic growth factors;** they must be directly obtained from the environment. One group of organic growth factors for humans is vitamins. Most vitamins function as coenzymes, the organic cofactors required by certain enzymes in order to function. Many bacteria can synthesize all their own vitamins and do not depend on outside sources. However, some bacteria lack the enzymes needed for the synthesis of certain vitamins, and for them those vitamins are organic growth factors. Other organic growth factors required by some bacteria are amino acids, purines, and pyrimidines.

CHECK YOUR UNDERSTANDING

- If bacterial cells were given a sulfur source containing radioactive sulfur (^{35}S) in their culture media, in what molecules would the ^{35}S be found in the cells? 6-4
- How would one determine whether a microbe is a strict anaerobe? 6-5
- Oxygen is so pervasive in the environment that it would be very difficult for a microbe to always avoid physical contact with it. What, therefore, is the most obvious way for a microbe to avoid damage? 6-6

Biofilms

LEARNING OBJECTIVE

6-7 Describe the formation of biofilms and their potential for causing infection.

In nature, microorganisms seldom live in the isolated single-species colonies that we see on laboratory plates. They more typically live in communities called **biofilms.** This fact was not well appreciated until the development of confocal microscopy (see page 61) made the three-dimensional structure of biofilms more visible. Biofilms reside in a matrix made up primarily of polysaccharides, but also containing DNA and proteins, that is often informally called *slime.* A biofilm also can be considered a *hydrogel,* which is a complex polymer containing many times its dry weight in water. Cell-to-cell chemical communication, or *quorum sensing,* allows bacteria to coordinate their activity and group together into communities that provide benefits not unlike those of multicellular organisms (see the box in Chapter 3, page 56). Therefore, biofilms are not just bacterial

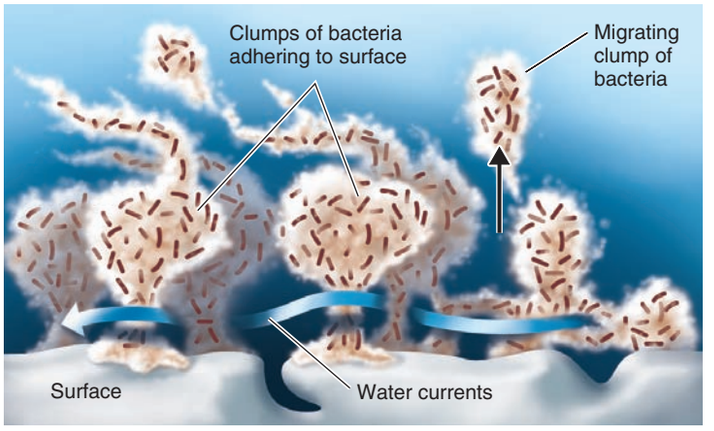
slime layers but biological systems; the bacteria are organized into a coordinated, functional community. Biofilms are usually attached to a surface, such as a rock in a pond, a human tooth (plaque; see Figure 25.3 on page 714), or a mucous membrane. This community might be of a single species or of a diverse group of microorganisms. Biofilms also might take other, more varied forms. The floc that forms in certain types of sewage treatment (see Figure 27.19, page 791) is an example. In fast-flowing streams, the biofilm might be in the form of filamentous streamers. Within a biofilm community, the bacteria are able to share nutrients and are sheltered from harmful factors in the environment, such as desiccation, antibiotics, and the body's immune system. The close proximity of microorganisms within a biofilm might also have the advantage of facilitating the transfer of genetic information by, for example, conjugation.

A biofilm usually begins to form when a free-swimming (*planktonic*) bacterium attaches to a surface. If these bacteria grew in a uniformly thick monolayer, they would become overcrowded, nutrients would not be available in lower depths, and toxic wastes could accumulate. Microorganisms in biofilm communities sometimes avoid these problems by forming pillar-like structures (**Figure 6.5**) with channels between them, through which water can carry incoming nutrients and outgoing wastes. This constitutes a primitive circulatory system. Individual microbes and clumps of slime occasionally leave the established biofilm and move to a new location where the biofilm becomes extended. Such a biofilm is generally composed of a surface layer about 10 µm thick, with pillars that extend up to 200 µm above it.

The microorganisms in biofilms can work cooperatively to carry out complex tasks. For example, the digestive systems of ruminant animals, such as cattle, require many different microbial species to break down cellulose. The microbes in a ruminant's digestive system are located mostly within biofilm communities. Biofilms are also essential elements in the proper functioning of sewage treatment systems, which we will discuss in Chapter 27. They can also, however, be a problem in pipes and tubing, where their accumulations impede circulation.

Biofilms are an important factor in human health. For example, microbes in biofilms are probably 1000 times more resistant to microbicides. Experts at the Centers for Disease Control and Prevention (CDC) estimate that 70% of human bacterial infections involve biofilms. Most nosocomial infections (infections acquired in health care facilities) are probably related to biofilms on medical catheters (see Figure 1.8 on page 18 and Figure 21.3 on page 592). In fact, biofilms form on almost all indwelling medical devices, including mechanical heart valves. Biofilms, which also can include those formed by fungi such as *Candida,* are encountered in many disease conditions, such as infections related to the use of contact lenses, dental caries (see page 713), and infections by pseudomonad bacteria (see page 307).

One approach to preventing biofilm formation is to incorporate antimicrobials into surfaces on which biofilms might form (see page 56). Because the chemical signals that allow quorum sensing are essential to biofilm formation, research is underway to determine the makeup of these chemical signals and perhaps block them. Another approach involves the discovery that lactoferrin (see page 473), which is abundant in many human secretions, can inhibit biofilm formation. Lactoferrin binds iron, especially among the pseudomonads that are responsible for cystic fibrosis biofilms, the cause of the pathology of this hereditary disease. The lack of iron inhibits the surface motility essential for the aggregation of the bacteria into biofilms.



Water currents move, as shown by the blue arrow, among pillars of slime formed by the growth of bacteria attached to solid surfaces. This allows efficient access to nutrients and removal of bacterial waste products. Individual slime-forming bacteria or bacteria in clumps of slime detach and move to new locations. See Figure 1.8.

Figure 6.5 **Biofilms.**

Q Why is the prevention of biofilms important in a health care environment?

Most laboratory methods in microbiology today use organisms being cultured in their planktonic mode. However, microbiologists now predict that there will be an increasing focus on how microorganisms actually live in relation with one another and that this will be considered in industrial and medical research.

CHECK YOUR UNDERSTANDING

✓ Identify a way in which pathogens find it advantageous to form biofilms. **6-7**

Culture Media

LEARNING OBJECTIVES

6-8 Distinguish chemically defined and complex media.

6-9 Justify the use of each of the following: anaerobic techniques, living host cells, candle jars, selective and differential media, enrichment medium.

6-10 Differentiate biosafety levels 1, 2, 3, and 4.

A nutrient material prepared for the growth of microorganisms in a laboratory is called a **culture medium.** Some bacteria can

grow well on just about any culture medium; others require special media, and still others cannot grow on any nonliving medium yet developed. Microbes that are introduced into a culture medium to initiate growth are called an **inoculum.** The microbes that grow and multiply in or on a culture medium are referred to as a **culture.**

Suppose we want to grow a culture of a certain microorganism, perhaps the microbes from a particular clinical specimen. What criteria must the culture medium meet? First, it must contain the right nutrients for the specific microorganism we want to grow. It should also contain sufficient moisture, a properly adjusted pH, and a suitable level of oxygen, perhaps none at all. The medium must initially be **sterile**—that is, it must initially contain no living microorganisms—so that the culture will contain only the microbes (and their offspring) we add to the medium. Finally, the growing culture should be incubated at the proper temperature.

A wide variety of media are available for the growth of microorganisms in the laboratory. Most of these media, which are available from commercial sources, have premixed components and require only the addition of water and then sterilization. Media are constantly being developed or revised for use in the isolation and identification of bacteria that are of interest to researchers in such fields as food, water, and clinical microbiology.

When it is desirable to grow bacteria on a solid medium, a solidifying agent such as agar is added to the medium. A complex polysaccharide derived from a marine alga, **agar** has long been used as a thickener in foods such as jellies and ice cream.

Agar has some very important properties that make it valuable to microbiology, and no satisfactory substitute has ever been found. Few microbes can degrade agar, so it remains solid. Also, agar liquefies at about 100°C (the boiling point of water) and at sea level remains liquid until the temperature drops to about 40°C. For laboratory use, agar is held in water baths at about 50°C. At this temperature, it does not injure most bacteria when it is poured over them (as shown in Figure 6.17a, page 173). Once the agar has solidified, it can be incubated at temperatures approaching 100°C before it again liquefies; this property is particularly useful when thermophilic bacteria are being grown.

Agar media are usually contained in test tubes or *Petri dishes.* The test tubes are called *slants* when their contents are allowed to solidify with the tube held at an angle so that a large surface area for growth is available. When the agar solidifies in a vertical tube, it is called a *deep.* Petri dishes, named for their inventor, are shallow dishes with a lid that nests over the bottom to prevent contamination; when filled, they are called *Petri* (or culture) *plates.*

Chemically Defined Media

To support microbial growth, a medium must provide an energy source, as well as sources of carbon, nitrogen, sulfur, phosphorus, and any organic growth factors the organism is unable to synthesize. A **chemically defined medium** is one whose exact chemical composition is known. For a chemoheterotroph, the chemically defined medium must contain organic growth factors that serve as a source of carbon and energy. For example, as shown in **Table 6.2**, glucose is included in the medium for growing the chemoheterotroph *E. coli.*

TABLE 6.2 A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *Escherichia coli*

Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic ($NH_4H_2PO_4$)	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Potassium phosphate, dibasic (K_2HPO_4)	1.0 g
Water	1 liter

As **Table 6.3** shows, many organic growth factors must be provided in the chemically defined medium used to cultivate a species of *Leuconostoc*. Organisms that require many growth factors are described as *fastidious.* Organisms of this type, such as *Lactobacillus* (page 316), are sometimes used in tests that determine the concentration of a particular vitamin in a substance. To perform such a *microbiological assay,* a growth medium is prepared that contains all the growth requirements of the bacterium except the vitamin being assayed. Then the medium, test substance, and bacterium are combined, and the growth of bacteria is measured. This bacterial growth, which is reflected by the amount of lactic acid produced, will be proportional to the amount of vitamin in the test substance. The more lactic acid, the more the *Lactobacillus* cells have been able to grow, so the more vitamin is present.

Complex Media

Chemically defined media are usually reserved for laboratory experimental work or for the growth of autotrophic bacteria. Most heterotrophic bacteria and fungi, such as you would work with in an introductory lab course, are routinely grown on **complex media** made up of nutrients including extracts from yeasts, meat, or plants, or digests of proteins from these and other sources. The exact chemical composition varies slightly from batch to batch. **Table 6.4** shows one widely used recipe.

In complex media, the energy, carbon, nitrogen, and sulfur requirements of the growing microorganisms are provided primarily by protein. Protein is a large, relatively insoluble molecule that a minority of microorganisms can

TABLE 6.3 Defined Culture Medium for *Leuconostoc mesenteroides*

Carbon and Energy
Glucose, 25 g
Salts
NH_4Cl, 3.0 g K_2HPO_4*, 0.6 g KH_2PO_4*, 0.6 g $MgSO_4$, 0.1 g
Amino Acids, 100–200 µg each
Alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine
Purines and Pyrimidines, 10 mg of each
Adenine, guanine, uracil, xanthine
Vitamins, 0.01–1 mg each
Biotin, folate, nicotinic acid, pyridoxal, pyridoxamine, pyridoxine, riboflavin, thiamine, pantothenate, *p*-aminobenzoic acid
Trace Elements, 2–10 µg each
Fe, Co, Mn, Zn, Cu, Ni, Mo
Buffer, pH 7
Sodium acetate, 25 g
Distilled Water, 1,000 ml
*Also serves as buffer.

TABLE 6.4 Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria

Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter

utilize directly, but a partial digestion by acids or enzymes reduces protein to shorter chains of amino acids called *peptones*. These small, soluble fragments can be digested by most bacteria.

Vitamins and other organic growth factors are provided by meat extracts or yeast extracts. The soluble vitamins and minerals from the meats or yeasts are dissolved in the extracting water, which is then evaporated so that these factors are concentrated. (These extracts also supplement the organic nitrogen and carbon compounds.) Yeast extracts are particularly rich in the B vitamins. If a complex medium is in liquid form, it is called **nutrient broth.** When agar is added, it is called **nutrient agar.** (This terminology can be confusing; just remember that agar itself is not a nutrient.)

Anaerobic Growth Media and Methods

The cultivation of anaerobic bacteria poses a special problem. Because anaerobes might be killed by exposure to oxygen, special media called **reducing media** must be used. These media contain ingredients, such as sodium thioglycolate, that chemically combine with dissolved oxygen and deplete the oxygen in the culture medium. To routinely grow and maintain pure cultures of obligate anaerobes, microbiologists use reducing media stored in ordinary, tightly capped test tubes. These media are heated shortly before use to drive off absorbed oxygen.

When the culture must be grown in Petri plates to observe individual colonies, several methods are available. Laboratories that work with relatively few culture plates at a time can use systems that can incubate the microorganisms in sealed boxes and jars in which the oxygen is chemically removed after the culture plates have been introduced and the container sealed. Some systems require that water be added to an envelope of chemicals before the container is closed, as shown in Figure 6.6, and require a catalyst. The chemicals produce hydrogen and carbon dioxide (about 4–10%) and remove the oxygen in the container by combining it, in the presence of the catalyst, with hydrogen to form water. In another commercially available system, the envelope of chemicals (the active ingredient is ascorbic acid) is simply opened to expose it to oxygen in the container's atmosphere. No water or catalyst is needed. The atmosphere in such containers usually has less than 5% oxygen, about 18% CO_2, and no hydrogen. In a recently introduced system, each individual Petri plate (OxyPlate) becomes an anaerobic chamber. The medium in the plate contains an enzyme, oxyrase, which combines oxygen with hydrogen, removing oxygen as water is formed.

Laboratories that have a large volume of work with anaerobes often use an anaerobic chamber, such as that shown in Figure 6.7. The chamber is filled with inert gases (typically about 85% N_2, 10% H_2, and 5% CO_2) and is equipped with air locks to introduce cultures and materials.

Special Culture Techniques

Many bacteria have never been successfully grown on artificial laboratory media. *Mycobacterium leprae,* the leprosy bacillus, is now usually grown in armadillos, which have a relatively low body temperature that matches the requirements of the microbe.

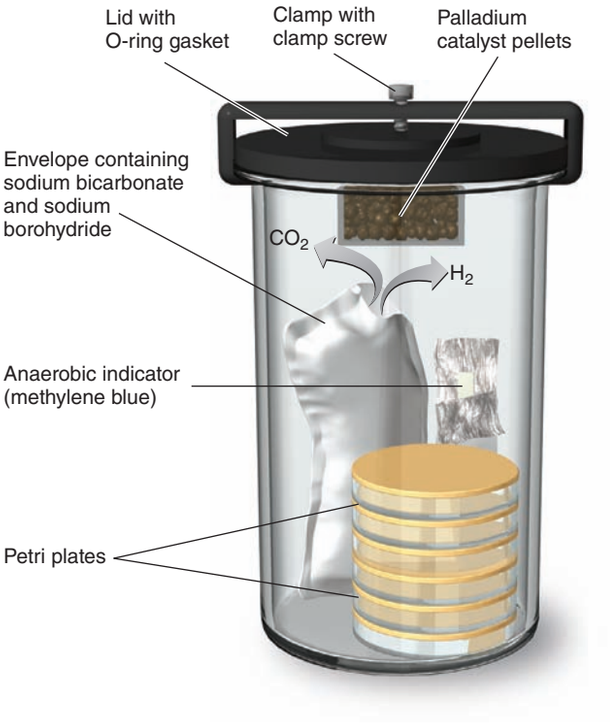


Figure 6.6 A jar for cultivating anaerobic bacteria on Petri plates. When water is mixed with the chemical packet containing sodium bicarbonate and sodium borohydride, hydrogen and carbon dioxide are generated. Reacting on the surface of a palladium catalyst in a screened reaction chamber, which may also be incorporated into the chemical packet, the hydrogen and atmospheric oxygen in the jar combine to form water. The oxygen is thus removed. Also in the jar is an anaerobic indicator containing methylene blue, which is blue when oxidized and turns colorless when the oxygen is removed (as shown here).

Q What is the technical name for bacteria that require a higher-than-atmospheric-concentration of CO_2 for growth?

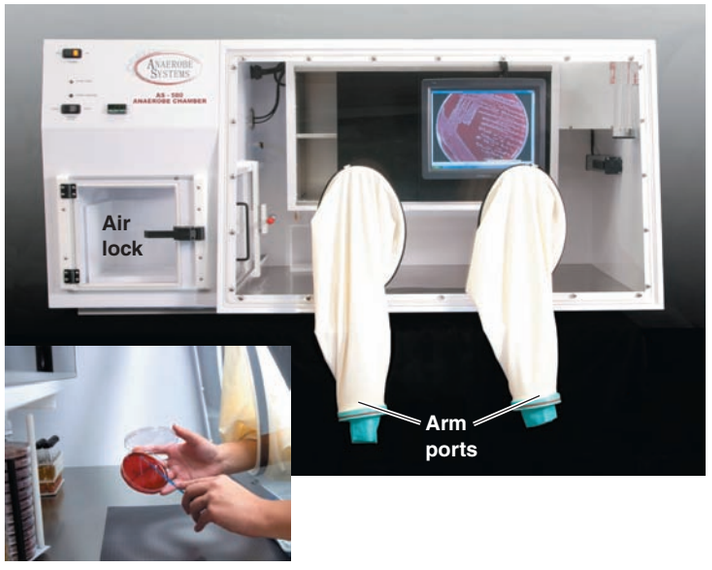


Figure 6.7 An anaerobic chamber. Materials are introduced through the small doors in the air-lock chamber at the left. The operator works through arm ports in airtight sleeves. The airtight sleeves extend into the cabinet when it is in use. This unit also features an internal camera and monitor.

Q In what way would an anaerobic chamber resemble the Space Laboratory orbiting in the vacuum of space?

Another example is the syphilis spirochete, although certain nonpathogenic strains of this microbe have been grown on laboratory media. With few exceptions, the obligate intracellular bacteria, such as the rickettsias and the chlamydias, do not grow on artificial media. Like viruses, they can reproduce only in a living host cell. See the discussion of cell culture, page 379.

Many clinical laboratories have special *carbon dioxide incubators* in which to grow aerobic bacteria that require concentrations of CO_2 higher or lower than that found in the atmosphere. Desired CO_2 levels are maintained by electronic controls. High CO_2 levels are also obtained with simple *candle jars.* Cultures are placed in a large sealed jar containing a lighted candle, which consumes oxygen. The candle stops burning when the air in the jar has a lowered concentration of oxygen (at about 17% O_2, still adequate for the growth of aerobic bacteria). An elevated concentration of CO_2 (about 3%) is also present. Microbes that grow better at high CO_2 concentrations are called **capnophiles.** The low-oxygen, high-CO_2 conditions resemble those found in the intestinal tract, respiratory tract, and other body tissues where pathogenic bacteria grow.

Candle jars are still used occasionally, but more often commercially available chemical packets are used to generate carbon dioxide atmospheres in containers. When only one or two Petri plates of cultures are to be incubated, clinical laboratory investigators often use small plastic bags with self-contained chemical gas generators that are activated by crushing the packet or moistening it with a few milliliters of water. These packets are sometimes specially designed to provide precise concentrations of carbon dioxide (usually higher than can be obtained in candle jars) and oxygen for culturing organisms such as the microaerophilic *Campylobacter* bacteria (page 313).

Some microorganisms are so dangerous that they can be handled only under extraordinary systems of containment called *biosafety level 4 (BSL-4).* Level 4 labs are popularly known as "the hot zone." Only a handful of such labs exists in the United States. The lab is a sealed environment within a larger building and has an atmosphere under negative pressure, so that aerosols containing pathogens will not escape. Both intake and exhaust air is filtered through high-efficiency particulate air filters (see HEPA filters, page 188); the exhaust air is filtered twice. All waste materials leaving the lab are rendered noninfectious. The personnel wear "space suits" that are connected to an air supply (**Figure 6.8**).

Figure 6.8 Technicians in a biosafety level 4 (BSL-4) laboratory. Personnel working in a BSL-4 facility wear a "space suit" that is connected to an outside air supply.

Q If a technician were working with pathogenic prions, how would material leaving the lab be rendered noninfectious? (*Hint*: See Chapter 7.)

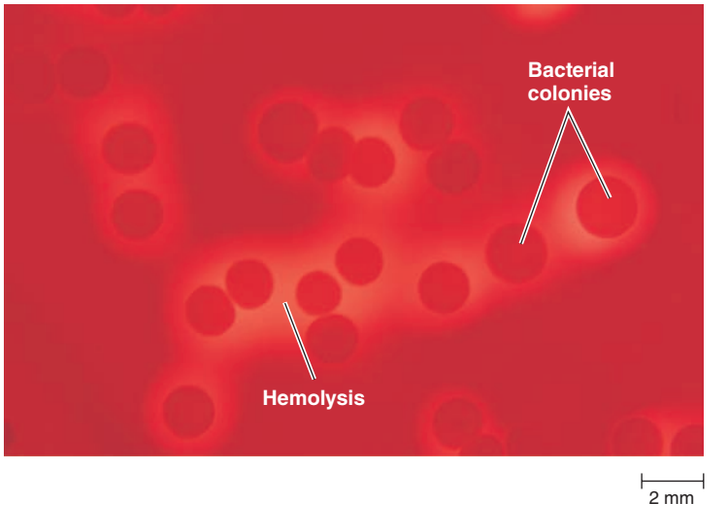


Figure 6.9 Blood agar, a differential medium containing red blood cells. The bacteria have lysed the red blood cells (beta-hemolysis), causing the clear areas around the colonies.

Q Of what value are hemolysins to pathogens?

Less dangerous organisms are handled at lower levels of biosafety. For example, a basic microbiology teaching laboratory would be BSL-1. Organisms that present a moderate risk of infection can be handled at BSL-2 levels, that is, on open laboratory benchtops with appropriate gloves, lab coats, or possibly face and eye protection. BSL-3 labs are intended for highly infectious airborne pathogens such as the tuberculosis agent. Biological safety cabinets similar in appearance to the anaerobic chamber shown in Figure 6.7 are used. The laboratory itself should be negatively pressurized and equipped with air filters to prevent release of the pathogen from the laboratory.

Selective and Differential Media

In clinical and public health microbiology, it is frequently necessary to detect the presence of specific microorganisms associated with disease or poor sanitation. For this task, selective and differential media are used. **Selective media** are designed to suppress the growth of unwanted bacteria and encourage the growth of the desired microbes. For example, bismuth sulfite agar is one medium used to isolate the typhoid bacterium, the gram-negative *Salmonella typhi* (tī′fē), from feces. Bismuth sulfite inhibits gram-positive bacteria and most gram-negative intestinal bacteria (other than *S. typhi*), as well. Sabouraud's dextrose agar, which has a pH of 5.6, is used to isolate fungi that outgrow most bacteria at this pH.

Differential media make it easier to distinguish colonies of the desired organism from other colonies growing on the same plate. Similarly, pure cultures of microorganisms have identifiable reactions with differential media in tubes or plates. Blood agar (which contains red blood cells) is a medium that microbiologists often use to identify bacterial species that destroy red blood cells. These species, such as *Streptococcus pyogenes* (pī-äj′en-ēz), the bacterium that causes strep throat, show a clear ring around their colonies (beta-hemolysis, page 317) where they have lysed the surrounding blood cells (**Figure 6.9**).

Sometimes, selective and differential characteristics are combined in a single medium. Suppose we want to isolate the common bacterium *Staphylococcus aureus,* found in the nasal passages. This organism has a tolerance for high concentrations of sodium chloride; it can also ferment the carbohydrate mannitol to form acid. Mannitol salt agar contains 7.5% sodium chloride, which will discourage the growth of competing organisms and thus *select for* (favor the growth of) *S. aureus.* This salty medium also contains a pH indicator that changes color if the mannitol in the medium is fermented to acid; the mannitol-fermenting colonies of *S. aureus* are thus *differentiated from* colonies of bacteria that do not ferment mannitol. Bacteria that grow at the high salt concentration *and* ferment mannitol to acid can be readily identified by the color change (**Figure 6.10**). These are probably colonies of *S. aureus,* and their identification can be confirmed by additional tests. The use of differential media to identify toxin-producing *E. coli* is discussed in Chapter 5, page 136.

Enrichment Culture

Because bacteria present in small numbers can be missed, especially if other bacteria are present in much larger numbers, it is sometimes necessary to use an **enrichment culture.**

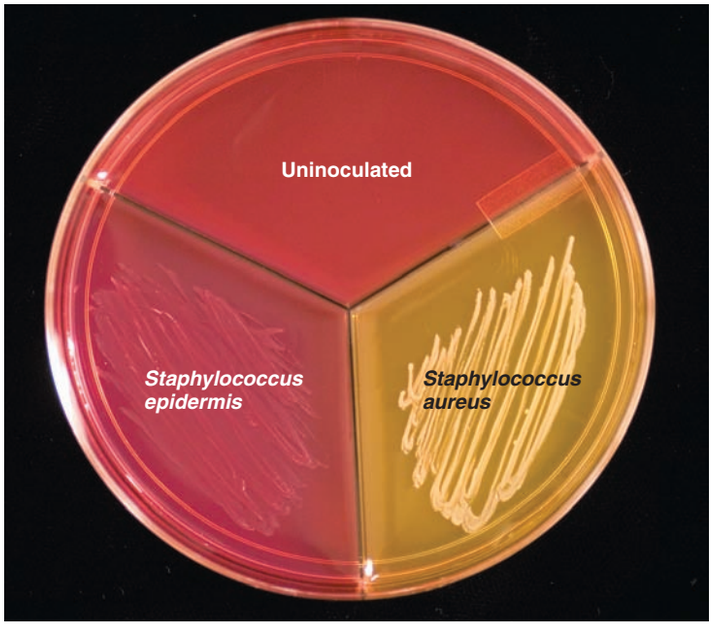


Figure 6.10 Differential medium. This medium is mannitol salt agar, and bacteria capable of fermenting the mannitol in the medium to acid (*Staphylococcus aureus*) cause the medium to change color to yellow. This **differentiates** between bacteria that can ferment mannitol and those that cannot. Actually, this medium is also *selective* because the high salt concentration prevents the growth of most bacteria but not *Staphlylococcus* spp.

Q Are bacteria capable of growing at a high osmotic pressure likely to be capable of growing in the mucus found in nostrils?

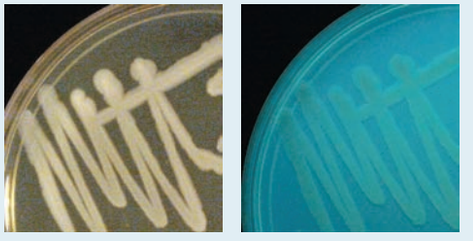
This is often the case for soil or fecal samples. The medium (enrichment medium) for an enrichment culture is usually liquid and provides nutrients and environmental conditions that favor the growth of a particular microbe but not others. In this sense, it is also a selective medium, but it is designed to increase very small numbers of the desired type of organism to detectable levels.

Suppose we want to isolate from a soil sample a microbe that can grow on phenol and is present in much smaller numbers than other species. If the soil sample is placed in a liquid enrichment medium in which phenol is the only source of carbon and energy, microbes unable to metabolize phenol will not grow. The culture medium is allowed to incubate for a few days, and then a small amount of it is transferred into another flask of the same medium. After a series of such transfers, the surviving population will consist of bacteria capable of metabolizing phenol. The bacteria are given time to grow in the medium between transfers; this is the enrichment stage. (See the box in Chapter 28 page 808.) Any nutrients in the original inoculum are rapidly diluted out with the successive transfers. When the last dilution is streaked onto a solid medium of the same composition, only those colonies of organisms capable of using phenol should grow. A remarkable aspect of this particular technique is that phenol is normally lethal to most bacteria.

Table 6.5 summarizes the purposes of the main types of culture media.

Clinical Case

P. fluorescens is an aerobic, gram-negative rod that grows best between 25°C and 30°C and grows poorly at the standard hospital microbiology incubation temperatures (35°C to 37°C). The bacteria are so named because they produce a pigment that fluoresces under ultraviolet light. While reviewing the facts of the latest outbreak, Dr. MacGruder learns that the most recent patients were last exposed to the contaminated heparin 84 to 421 days before onset of their infections. On-site investigations confirmed that the patients' clinics are no longer using the recalled heparin and had, in fact, returned all unused inventory. Concluding that these patients did not develop infections during the previous outbreak, Dr. MacGruder must look for a new source of infection. The patients all have indwelling venous catheters: tubes that are inserted into a vein for long-term delivery of concentrated solutions, such as anticancer drugs. Dr. MacGruder orders cultures of the new heparin being used, but the results do not recover any organisms. He then orders blood and catheter cultures from each of the patients.



Illuminated with white light | Illuminated with ultraviolet light

The organism cultured from both the patients' blood and their catheters is shown in the figure. What organism is it?

154 **166** 175 177

CHECK YOUR UNDERSTANDING

- Could humans exist on chemically defined media, at least under laboratory conditions? **6-8**
- Could Louis Pasteur, in the 1800s, have grown rabies viruses in cell culture instead of in living animals? **6-9**
- What BSL is your laboratory? **6-10**

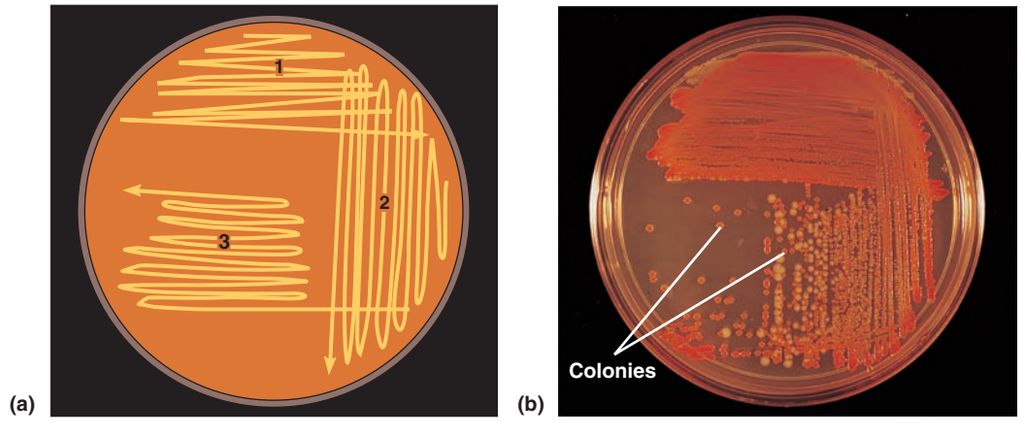


Figure 6.11 The streak plate method for isolating pure bacterial cultures. (**a**) Arrows indicate the direction of streaking. Streak series 1 is made from the original bacterial culture. The incoculating loop is sterilized following each streak series. In series 2 and 3, the loop picks up bacteria from the previous series, diluting the number of cells each time. There are numerous variants of such patterns. (**b**) In series 3 of this example, notice that well-isolated colonies of bacteria of two different types, red and yellow, have been obtained.

Q Is a colony formed as a result of streaking a plate always derived from a single bacterium? Why or why not?

Obtaining Pure Cultures

LEARNING OBJECTIVES

6-11 Define *colony*.

6-12 Describe how pure cultures can be isolated by using the streak plate method.

Most infectious materials, such as pus, sputum, and urine, contain several different kinds of bacteria; so do samples of soil, water, or food. If these materials are plated out onto the surface of a solid medium, colonies will form that are exact copies of the original organism. A visible **colony** theoretically arises from a single spore or vegetative cell or from a group of the same microorganisms attached to one another in clumps or chains. Estimates are that only about 1% of bacteria in ecosystems produce colonies by conventional culture methods. Microbial colonies often have a distinctive appearance that distinguishes one microbe from another (see Figure 6.10). The bacteria must be distributed widely enough so that the colonies are visibly separated from each other.

Most bacteriological work requires pure cultures, or clones, of bacteria. The isolation method most commonly used to get pure cultures is the **streak plate method** (Figure 6.11). A sterile inoculating loop is dipped into a mixed culture that contains more than one type of microbe and is streaked in a pattern over the surface of the nutrient medium. As the pattern is traced, bacteria are rubbed off the loop onto the medium. The last cells to be rubbed off the loop are far enough apart to grow into isolated colonies. These colonies can be picked up with an inoculating loop and transferred to a test tube of nutrient medium to form a pure culture containing only one type of bacterium.

The streak plate method works well when the organism to be isolated is present in large numbers relative to the total population. However, when the microbe to be isolated is present only in very small numbers, its numbers must be greatly increased by selective enrichment before it can be isolated with the streak plate method.

TABLE 6.5 Culture Media

Type	Purpose
Chemically Defined	Growth of chemoautotrophs and photoautotrophs; microbiological assays
Complex	Growth of most chemoheterotrophic organisms
Reducing	Growth of obligate anaerobes
Selective	Suppression of unwanted microbes; encouraging desired microbes
Differential	Differentiation of colonies of desired microbes from others
Enrichment	Similar to selective media but designed to increase numbers of desired microbes to detectable levels

CHECK YOUR UNDERSTANDING

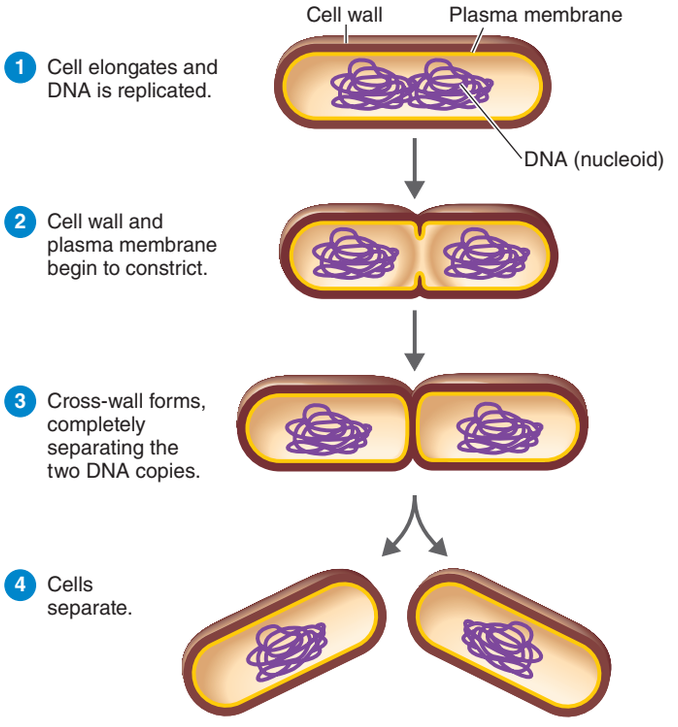
- Can you think of any reason why a colony does not grow to an infinite size, or at least fill the confines of the Petri plate? 6-11
- Could a pure culture of bacteria be obtained by the streak plate method if there were only one desired microbe in a bacterial suspension of billions? 6-12

Preserving Bacterial Cultures

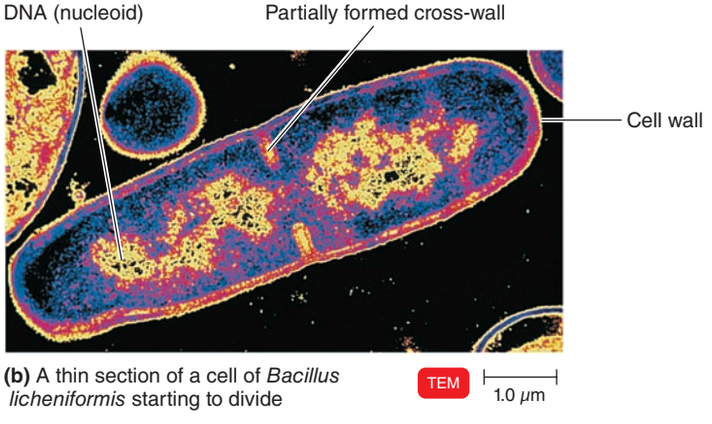
LEARNING OBJECTIVE

6-13 Explain how microorganisms are preserved by deep-freezing and lyophilization (freeze-drying).

Refrigeration can be used for the short-term storage of bacterial cultures. Two common methods of preserving microbial cultures



(a) A diagram of the sequence of cell division



(b) A thin section of a cell of *Bacillus licheniformis* starting to divide

Figure 6.12 **Binary fission in bacteria.**

Q In what way is budding different from binary fission?

for long periods are deep-freezing and lyophilization. **Deep-freezing** is a process in which a pure culture of microbes is placed in a suspending liquid and quick-frozen at temperatures ranging from –50°C to –95°C. The culture can usually be thawed and cultured even several years later. During **lyophilization (freeze-drying),** a suspension of microbes is quickly frozen at temperatures ranging from –54°C to –72°C, and the water is removed by a high vacuum (sublimation). While under vacuum, the container is sealed by melting the glass with a high-temperature torch. The remaining powderlike residue that contains the surviving microbes can be stored for years. The organisms can be revived at any time by hydration with a suitable liquid nutrient medium.

CHECK YOUR UNDERSTANDING

If the Space Station in Earth orbit suddenly ruptured, the humans on board would die instantly from cold and the vacuum of space. Would all the bacteria in the capsule also be killed? 6-13

The Growth of Bacterial Cultures

LEARNING OBJECTIVES

6-14 Define *bacterial growth,* including *binary fission.*

6-15 Compare the phases of microbial growth, and describe their relation to generation time.

6-16 Explain four direct methods of measuring cell growth.

6-17 Differentiate direct and indirect methods of measuring cell growth.

6-18 Explain three indirect methods of measuring cell growth.

Being able to represent graphically the enormous populations resulting from the growth of bacterial cultures is an essential part of microbiology. It is also necessary to be able to determine microbial numbers, either directly, by counting, or indirectly, by measuring their metabolic activity.

Bacterial Division

As we mentioned at the beginning of the chapter, bacterial growth refers to an increase in bacterial numbers, not an increase in the size of the individual cells. Bacteria normally reproduce by **binary fission** (Figure 6.12).

A few bacterial species reproduce by **budding;** they form a small initial outgrowth (a bud) that enlarges until its size approaches that of the parent cell, and then it separates. Some filamentous bacteria (certain actinomycetes) reproduce by producing chains of conidiospores carried externally at the tips of the filaments. A few filamentous species simply fragment, and the fragments initiate the growth of new cells. MM **Animations** Binary Fission; Bacterial Growth: Overview

Generation Time

For purposes of calculating the generation time of bacteria, we will consider only reproduction by binary fission, which is by far the most common method. As you can see in Figure 6.13, one cell's division produces two cells, two cells' divisions produce four cells, and so on. When the number of cells in each generation is expressed as a power of 2, the exponent tells the number of doublings (generations) that have occurred.

The time required for a cell to divide (and its population to double) is called the **generation time.** It varies considerably among organisms and with environmental conditions, such as temperature. Most bacteria have a generation time of 1 to 3 hours; others require more than 24 hours per generation. (The math required to calculate generation times is

Numbers of Cells	Numbers Expressed as a Power of 2	Visual Representation of Numbers
1	2^0	●
2	2^1	●●
4	2^2	●●●●
8	2^3	●●●●●●●●
16	2^4	●●●●●●●●●●●●●●●●
32	2^5	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●

(a) Visual representation of increase in bacterial number over five generations. The number of bacteria doubles in each generation. The superscript indicates the generation; that is, $2^5 = 5$ generations.

Generation Number	Number of Cells	Log_{10} of Number of Cells
0	$2^0 = 1$	0
5	$2^5 = 32$	1.51
10	$2^{10} = 1{,}024$	3.01
15	$2^{15} = 32{,}768$	4.52
16	$2^{16} = 65{,}536$	4.82
17	$2^{17} = 131{,}072$	5.12
18	$2^{18} = 262{,}144$	5.42
19	$2^{19} = 524{,}288$	5.72
20	$2^{20} = 1{,}048{,}576$	6.02

(b) Conversion of the number of cells in a population into the logarithmic expression of this number. To arrive at the numbers in the center column, use the y^x key on your calculator. Enter 2 on the calculator; press y^x; enter 5; then press the = sign. The calculator will show the number 32. Thus, the fifth-generation population of bacteria will total 32 cells. To arrive at the numbers in the right-hand column, use the log key on your calculator. Enter the number 32; then press the log key. The calculator will show, rounded off, that the $\log_{10}$ of 32 is 1.51.

Figure 6.13 **Cell division.**

Q If a single bacterium reproduced every 30 minutes, how many would there be in 2 hours?

presented in Appendix B.) If binary fission continues unchecked, an enormous number of cells will be produced. If a doubling occurred every 20 minutes—which is the case for *E. coli* under favorable conditions—after 20 generations a single initial cell would increase to over 1 million cells. This would require a little less than 7 hours. In 30 generations, or 10 hours, the population would be 1 billion, and in 24 hours it would be a number trailed by 21 zeros. It is difficult to graph population changes of such enormous magnitude by using arithmetic numbers. This is why logarithmic scales are generally used to graph bacterial growth. Understanding logarithmic representations of bacterial populations requires some use of mathematics and is necessary for anyone studying microbiology. (See Appendix B.)

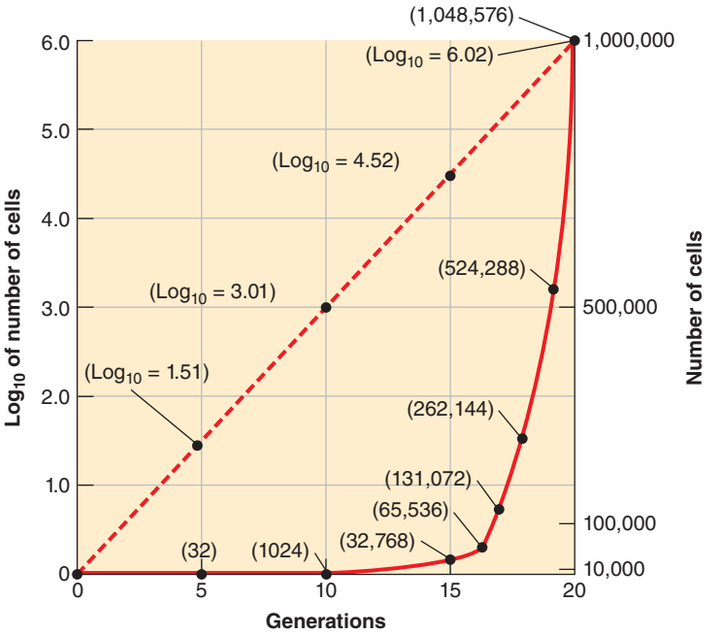


Figure 6.14 **A growth curve for an exponentially increasing population, plotted logarithmically (dashed line) and arithmetically (solid line).** For demonstration purposes, this graph has been drawn so that the arithmetic and logarithmic curves intersect at 1 million cells. This figure demonstrates why it is necessary to graph changes in the immense numbers of bacterial populations by logarithmic plots rather than by arithmetic numbers. For example, note that at ten generations the line representing arithmetic numbers has not even perceptibly left the baseline, whereas the logarithmic plot point for the tenth generation (3.01) is halfway up the graph.

Q If the arithmetic numbers (solid line) were plotted for two more generations, would the line still be on the page?

Logarithmic Representation of Bacterial Populations

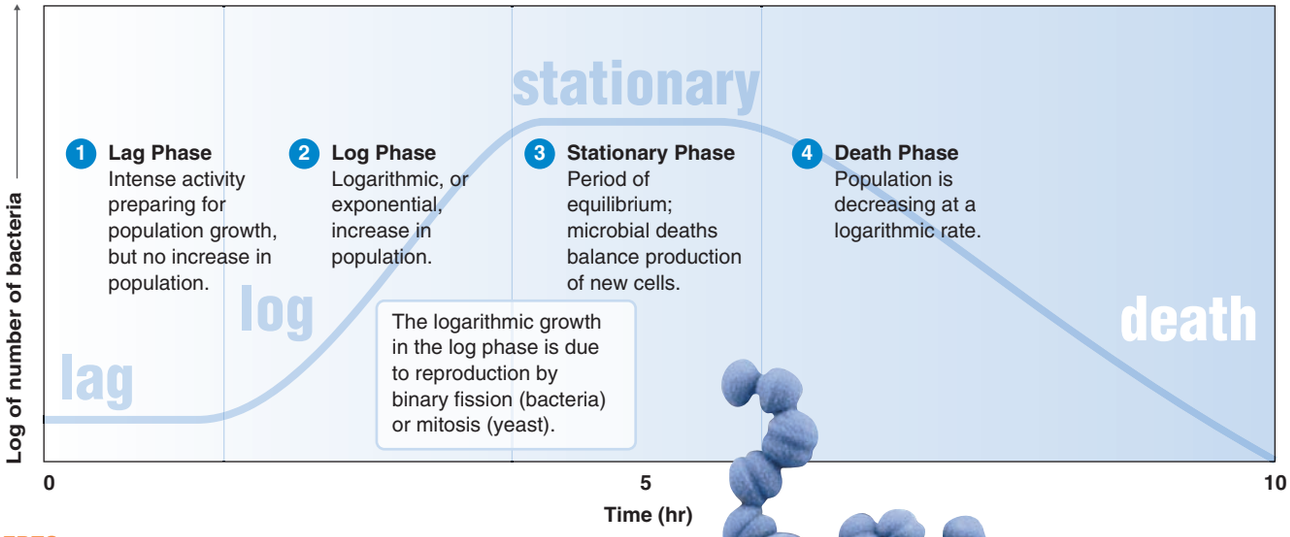
To illustrate the difference between logarithmic and arithmetic graphing of bacterial populations, let's express 20 bacterial generations both logarithmically and arithmetically. In five generations (2^5), there would be 32 cells; in ten generations (2^{10}), there would be 1024 cells, and so on. (If your calculator has a y^x key and a log key, you can duplicate the numbers in the third column of Figure 6.13.)

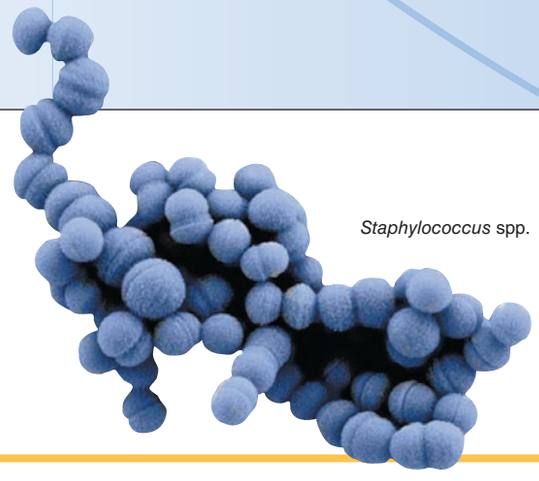
In **Figure 6.14**, notice that the arithmetically plotted line (solid) does not clearly show the population changes in the early stages of the growth curve at this scale. In fact, the first ten generations do not even appear to leave the baseline. Furthermore, another one or two arithmetic generations graphed to the same scale would greatly increase the height of the graph and take the line off the page.

The dashed line in Figure 6.14 shows how these plotting problems can be avoided by graphing the $\log_{10}$ of the population numbers. The $\log_{10}$ of the population is plotted at 5, 10, 15, and 20 generations. Notice that a straight line is formed and that a

FOUNDATION FIGURE **6.15**

Understanding the Bacterial Growth Curve





Staphylococcus spp.

KEY CONCEPTS

- Bacterial populations follow a sequential series of growth phases: the lag, log, stationary, and death phases.
- Knowledge of the bacterial growth curve is critical to understanding population dynamics and population control in the course of infectious diseases, in food preservation and spoilage, as well as in industrial microbiology processes, such as ethanol production.

thousand times this population (1,000,000,000, or $\log_{10}$ 9.0) could be accommodated in relatively little extra space. However, this advantage is obtained at the cost of distorting our "common sense" perception of the actual situation. We are not accustomed to thinking in logarithmic relationships, but it is necessary for a proper understanding of graphs of microbial populations.

CHECK YOUR UNDERSTANDING

Can a complex organism, such as a beetle, divide by binary fission? **6-14**

Phases of Growth

When a few bacteria are inoculated into a liquid growth medium and the population is counted at intervals, it is possible to plot a **bacterial growth curve** that shows the growth of cells over time (**Figure 6.15**). There are four basic phases of growth: the lag, log, stationary, and death phases. **Animation** Bacterial Growth Curve

The Lag Phase

For a while, the number of cells changes very little because the cells do not immediately reproduce in a new medium. This period of little or no cell division is called the **lag phase,** and it can last for 1 hour or several days. During this time, however, the cells are not dormant. The microbial population is undergoing a period of intense metabolic activity involving, in particular, synthesis of enzymes and various molecules. (The situation is analogous to a factory being equipped to produce automobiles; there is considerable tooling-up activity but no immediate increase in the automobile population.)

The Log Phase

Eventually, the cells begin to divide and enter a period of growth, or logarithmic increase, called the **log phase,** or **exponential growth phase.** Cellular reproduction is most active during this period, and generation time reaches a constant minimum. Because the generation time is constant, a logarithmic plot of growth during the log phase is a straight line. The log phase is the time when cells are most active metabolically and is preferred for industrial purposes where, for example, a product needs to be produced efficiently.

The Stationary Phase

If exponential growth continued unchecked, startlingly large numbers of cells could arise. For example, a single bacterium

(at a weight of 9.5×10^{-13} g per cell) dividing every 20 minutes for only 25.5 hours can theoretically produce a population equivalent in weight to that of an 80,000-ton aircraft carrier. In reality, this does not happen. Eventually, the growth rate slows, the number of microbial deaths balances the number of new cells, and the population stabilizes. This period of equilibrium is called the **stationary phase.**

What causes exponential growth to stop is not always clear. The exhaustion of nutrients, accumulation of waste products, and harmful changes in pH may all play a role.

The Death Phase

The number of deaths eventually exceeds the number of new cells formed, and the population enters the **death phase,** or **logarithmic decline phase.** This phase continues until the population is diminished to a tiny fraction of the number of cells in the previous phase or until the population dies out entirely. Some species pass through the entire series of phases in only a few days; others retain some surviving cells almost indefinitely. Microbial death will be discussed further in Chapter 7.

CHECK YOUR UNDERSTANDING

- If two mice started a family within a fixed enclosure, with a fixed food supply, would the population curve be the same as a bacterial growth curve? **6-15**

Direct Measurement of Microbial Growth

The growth of microbial populations can be measured in a number of ways. Some methods measure cell numbers; other methods measure the population's total mass, which is often directly proportional to cell numbers. Population numbers are usually recorded as the number of cells in a milliliter of liquid or in a gram of solid material. Because bacterial populations are usually very large, most methods of counting them are based on direct or indirect counts of very small samples; calculations then determine the size of the total population. Assume, for example, that a millionth of a milliliter (10^{-6} ml) of sour milk is found to contain 70 bacterial cells. Then there must be 70 times 1 million, or 70 million, cells per milliliter.

However, it is not practical to measure out a millionth of a milliliter of liquid or a millionth of a gram of food. Therefore, the procedure is done indirectly, in a series of dilutions. For example, if we add 1 ml of milk to 99 ml of water, each milliliter of this dilution now has one-hundredth as many bacteria as each milliliter of the original sample had. By making a series of such dilutions, we can readily estimate the number of bacteria in our original sample. To count microbial populations in solid foods (such as hamburger), an homogenate of one part food to nine parts water is finely ground in a food blender. Samples of this initial one-tenth dilution can then be transferred with a pipette for further dilutions or cell counts.

Plate Counts

The most frequently used method of measuring bacterial populations is the **plate count.** An important advantage of this method is that it measures the number of viable cells. One disadvantage may be that it takes some time, usually 24 hours or more, for visible colonies to form. This can be a serious problem in some applications, such as quality control of milk, when it is not possible to hold a particular lot for this length of time.

Plate counts assume that each live bacterium grows and divides to produce a single colony. This is not always true, because bacteria frequently grow linked in chains or as clumps (see Figure 4.1, page 77). Therefore, a colony often results, not from a single bacterium, but from short segments of a chain or from a bacterial clump. To reflect this reality, plate counts are often reported as **colony-forming units (CFU).**

When a plate count is performed, it is important that only a limited number of colonies develop in the plate. When too many colonies are present, some cells are overcrowded and do not develop; these conditions cause inaccuracies in the count. The U.S. Food and Drug Administration convention is to count only plates with 25 to 250 colonies, but many microbiologists prefer plates with 30 to 300 colonies. To ensure that some colony counts will be within this range, the original inoculum is diluted several times in a process called **serial dilution** (**Figure 6.16**).

Serial Dilutions Let's say, for example, that a milk sample has 10,000 bacteria per milliliter. If 1 ml of this sample were plated out, there would theoretically be 10,000 colonies formed in the Petri plate of medium. Obviously, this would not produce a countable plate. If 1 ml of this sample were transferred to a tube containing 9 ml of sterile water, each milliliter of fluid in this tube would now contain 1000 bacteria. If 1 ml of this sample were inoculated into a Petri plate, there would still be too many potential colonies to count on a plate. Therefore, another serial dilution could be made. One milliliter containing 1000 bacteria would be transferred to a second tube of 9 ml of water. Each milliliter of this tube would now contain only 100 bacteria, and if 1 ml of the contents of this tube were plated out, potentially 100 colonies would be formed—an easily countable number.

Pour Plates and Spread Plates A plate count is done by either the pour plate method or the spread plate method. The **pour plate method** follows the procedure shown in **Figure 6.17a**. Either 1.0 ml or 0.1 ml of dilutions of the bacterial suspension is introduced into a Petri dish. The nutrient medium, in which the agar is kept liquid by holding it in a water bath at about 50°C, is poured over the sample, which is then mixed into the medium by gentle agitation of the plate. When the agar solidifies, the plate is incubated. With the pour plate technique, colonies will grow within the nutrient agar (from cells suspended in the nutrient medium as the agar solidifies) as well as on the surface of the agar plate.

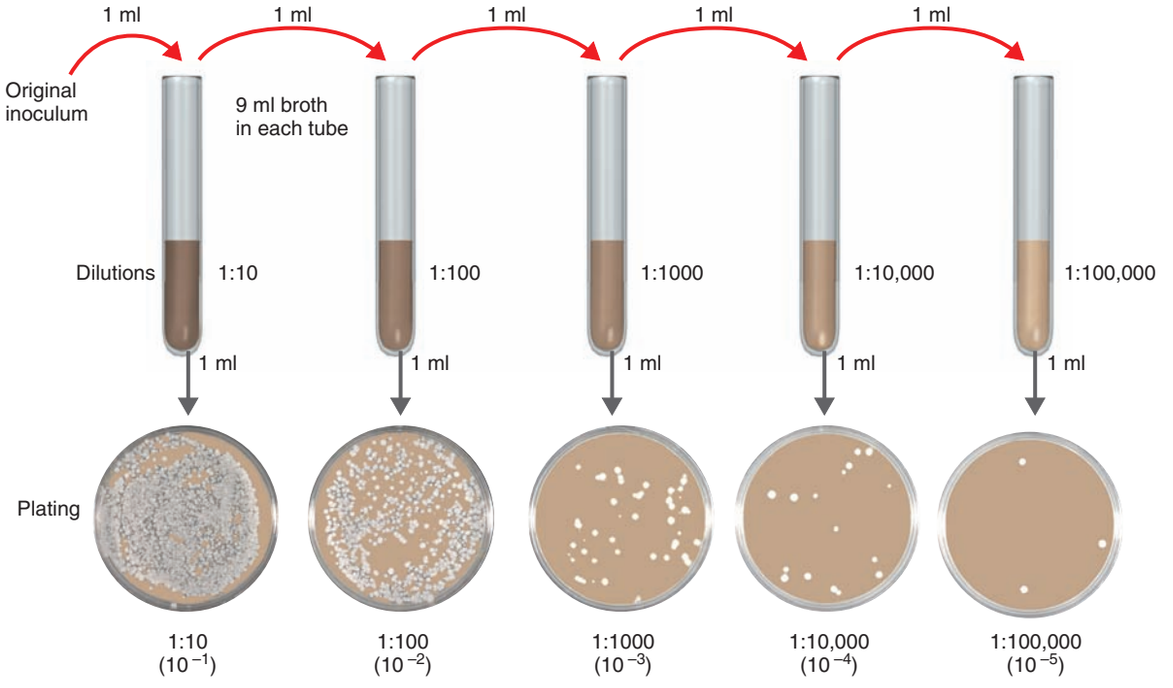


Figure 6.16 Serial dilutions and plate counts. In serial dilutions, the original inoculum is diluted in a series of dilution tubes. In our example, each succeeding dilution tube will have only one-tenth the number of microbial cells as the preceding tube. Then, samples of the dilution are used to inoculate Petri plates, on which colonies grow and can be counted. This count is then used to estimate the number of bacteria in the original sample.

Q Why were the dilutions of 1:10,000 and 1:100,000 not counted? Theoretically, how many colonies should appear on the 1:100 plate?

This technique has some drawbacks because some relatively heat-sensitive microorganisms may be damaged by the melted agar and will therefore be unable to form colonies. Also, when certain differential media are used, the distinctive appearance of the colony on the surface is essential for diagnostic purposes. Colonies that form beneath the surface of a pour plate are not satisfactory for such tests. To avoid these problems, the **spread plate method** is frequently used instead (Figure 6.17b). A 0.1-ml inoculum is added to the surface of a prepoured, solidified agar medium. The inoculum is then spread uniformly over the surface of the medium with a specially shaped, sterilized glass or metal rod. This method positions all the colonies on the surface and avoids contact between the cells and melted agar.

Filtration

When the quantity of bacteria is very small, as in lakes or relatively pure streams, bacteria can be counted by **filtration** methods (Figure 6.18). In this technique, at least 100 ml of water are passed through a thin membrane filter whose pores are too small to allow bacteria to pass. Thus, the bacteria are filtered out and retained on the surface of the filter. This filter is then transferred to a Petri dish containing a pad soaked in liquid nutrient medium, where colonies arise from the bacteria on the filter's surface. This method is applied frequently to detection and enumeration of coliform bacteria, which are indicators of fecal contamination of food or water (see Chapter 27). The colonies formed by these bacteria are distinctive when a differential nutrient medium is used. (The colonies shown in Figure 6.18b are examples of coliforms.)

The Most Probable Number (MPN) Method

Another method for determining the number of bacteria in a sample is the **most probable number (MPN) method,** illustrated in Figure 6.19. This statistical estimating technique is based on the fact that the greater the number of bacteria in a sample, the more dilution is needed to reduce the density to the point at which no bacteria are left to grow in the tubes in a dilution series. The MPN method is most useful when the

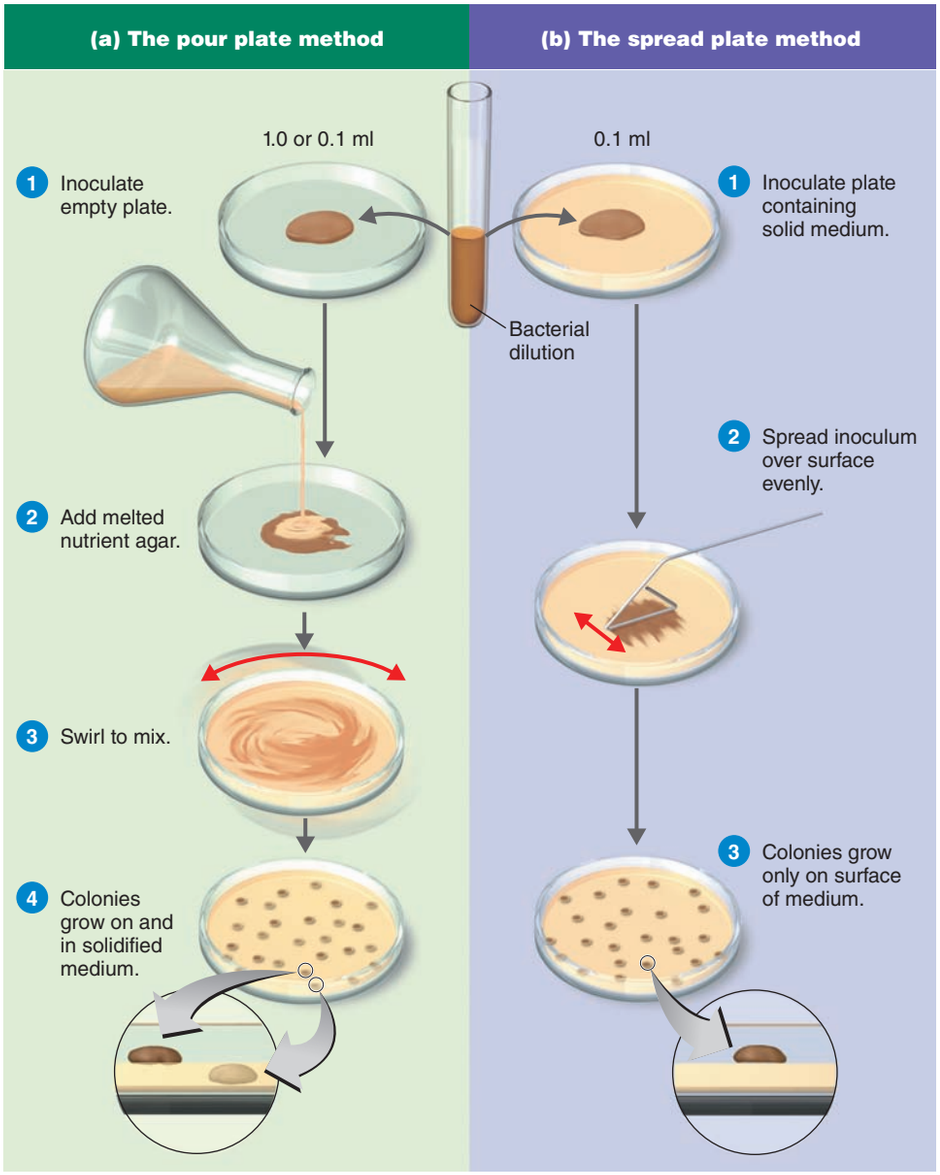


Figure 6.17 Methods of preparing plates for plate counts. (**a**) The pour plate method. (**b**) The spread plate method.

In what instances would the pour plate method be more appropriate than the spread plate method?

microbes being counted will not grow on solid media (such as the chemoautotrophic nitrifying bacteria). It is also useful when the growth of bacteria in a liquid differential medium is used to identify the microbes (such as coliform bacteria, which selectively ferment lactose to acid, in water testing). The MPN is only a statement that there is a 95% chance that the bacterial population falls within a certain range and that the MPN is statistically the most probable number.

Direct Microscopic Count

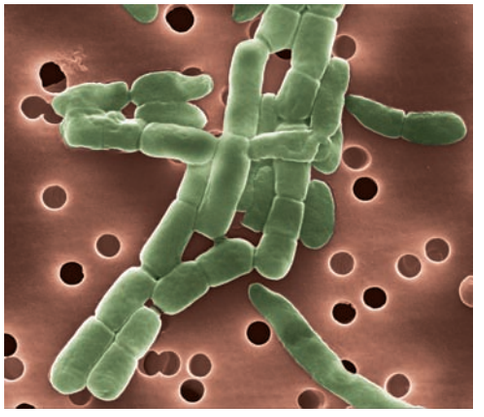
In the method known as the **direct microscopic count,** a measured volume of a bacterial suspension is placed within a defined area on a microscope slide. Because of time considerations, this method is often used to count the number of bacteria in milk. A 0.01-ml sample is spread over a marked square centimeter of slide, stain is added so that the bacteria can be seen, and the sample is viewed under the oil immersion objective lens. The area of the viewing field of this objective can be determined. Once the number of bacteria has been counted in several different fields, the average number of bacteria per viewing field can be calculated. From these data, the number of bacteria in the square centimeter over which the sample was spread can also be calculated. Because this area on the slide contained 0.01 ml of sample, the number of bacteria in each milliliter of the suspension is the number of bacteria in the sample times 100.

A specially designed slide called a *Petroff-Hausser cell counter* is also used in direct microscopic counts (Figure 6.20).

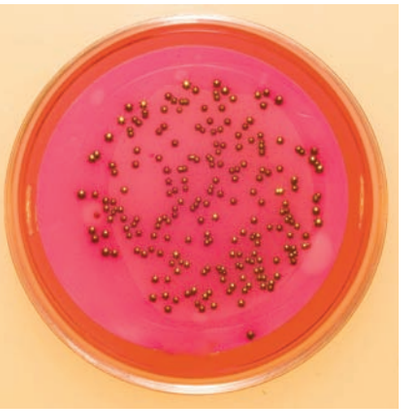
Motile bacteria are difficult to count by this method, and, as happens with other microscopic methods, dead cells are about

Figure 6.18 **Counting bacteria by filtration.**

Q Could you make a pour plate in the usual Petri dish with a 10-ml inoculum? Why or why not?



(a) The bacterial populations in bodies of water can be determined by passing a sample through a membrane filter. Here, the bacteria in a 100 ml water sample have been sieved out onto the surface of a membrane filter. These bacteria form visible colonies when placed on the surface of a suitable medium.



(b) A membrane filter with bacteria on its surface, as described in (a), has been placed on Endo agar. This medium is selective for gram-negative bacteria; lactose fermenters, such as the coliforms, form distinctive colonies. There are 214 colonies visible, so we would record 214 bacteria per 100 ml in the water sample.

Volume of Inoculum for Each Set of Five Tubes	Tubes of Nutrient Medium (Sets of Five Tubes)	Number of Positive Tubes in Set
10 ml		5
1 ml		3
0.1 ml		1

(a) Most probable number (MPN) dilution series. In this example, there are three sets of tubes and five tubes in each set. Each tube in the first set of five tubes receives 10 ml of the inoculum, such as a sample of water. Each tube in the second set of five tubes receives 1 ml of the sample, and the third set, 0.1 ml each. There were enough bacteria in the sample so that all five tubes in the first set showed bacterial growth and were recorded as positive. In the second set, which received only one-tenth as much inoculum, only three tubes were positive. In the third set, which received one-hundredth as much inoculum, only one tube was positive.

Combination of Positives	MPN Index/ 100 ml	95% Confidence Limits	
		Lower	Upper
4-2-0	22	6.8	50
4-2-1	26	9.8	70
4-3-0	27	9.9	70
4-3-1	33	10	70
4-4-0	34	14	100
5-0-0	23	6.8	70
5-0-1	31	10	70
5-0-2	43	14	100
5-1-0	33	10	100
5-1-1	46	14	120
5-1-2	63	22	150
5-2-0	49	15	150
5-2-1	70	22	170
5-2-2	94	34	230
5-3-0	79	22	220
5-3-1	110	34	250
5-3-2	140	52	400

(b) MPN table. MPN tables enable us to calculate for a sample the microbial numbers that are statistically likely to lead to such a result. The number of positive tubes is recorded for each set: in the shaded example, 5, 3, and 1. If we look up this combination in an MPN table, we find that the MPN index per 100 ml is 110. Statistically, this means that 95% of the water samples that give this result contain 34–250 bacteria, with 110 being the most probable number.

Figure 6.19 **The most probable number (MPN) method.**

Q Under what circumstances is the MPN method used to determine the number of bacteria in a sample?

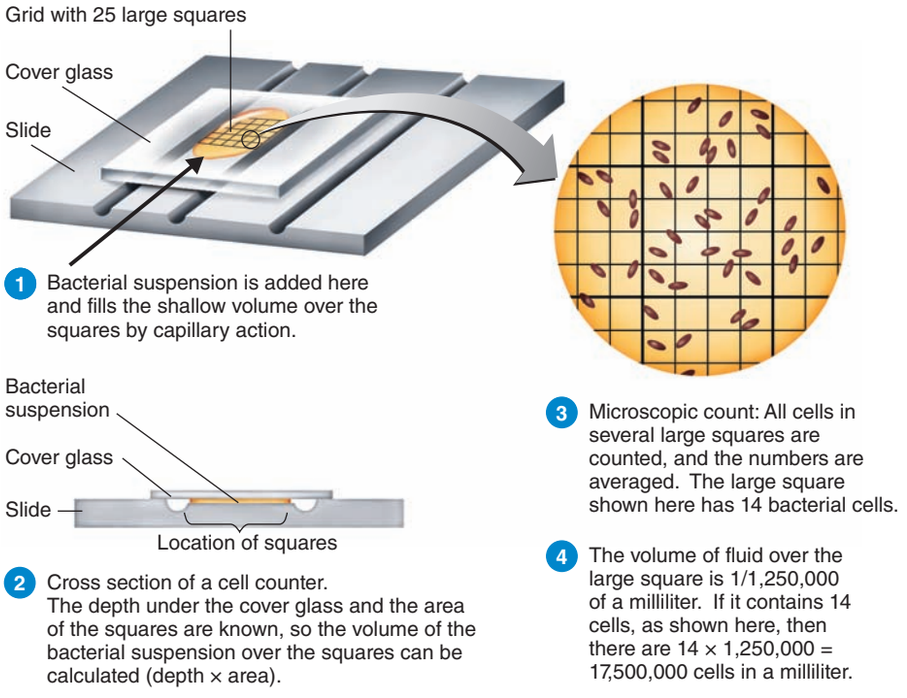


Figure 6.20 Direct microscopic count of bacteria with a Petroff-Hausser cell counter. The average number of cells within a large square multiplied by a factor of 1,250,000 gives the number of bacteria per milliliter.

Q This type of counting, despite its obvious disadvantages, is often used in estimating the bacterial population in dairy products. Why?

as likely to be counted as live ones. In addition to these disadvantages, a rather high concentration of cells is required to be countable—about 10 million bacteria per milliliter. The chief advantage of microscopic counts is that no incubation time is required, and they are usually reserved for applications in which time is the primary consideration. This advantage also holds for *electronic cell counters,* sometimes known as *Coulter counters,* which automatically count the number of cells in a measured volume of liquid. These instruments are used in some research laboratories and hospitals.

Clinical Case

The bacteria in the blood and catheter cultures fluoresce under ultraviolet light. The results from the culture show that *P. fluorescens* is present in the blood of 15 patients, in 17 catheters, and in the blood and catheters of four patients. The bacteria survived even after the heparin recall. Dr. MacGruder would like to have some idea how many bacteria are colonizing a patient's catheter. Because the amount of nutrients in a patient's catheter is minimal, he concludes that the bacteria grow slowly. He does some calculations based on the assumption that five *Pseudomonas* cells, with a generation time of 35 hours, may have been originally introduced into the catheters.

Approximately how many cells would there be after a month?

154 166 **175** 177

CHECK YOUR UNDERSTANDING

Why is it difficult to measure realistically the growth of a filamentous mold isolate by the plate count method? 6-16

Estimating Bacterial Numbers by Indirect Methods

It is not always necessary to count microbial cells to estimate their numbers. In science and industry, microbial numbers and activity are determined by some of the following indirect means as well.

Turbidity

For some types of experimental work, estimating **turbidity** is a practical way of monitoring bacterial growth. As bacteria multiply in a liquid medium, the medium becomes turbid, or cloudy with cells.

The instrument used to measure turbidity is a *spectrophotometer* (or colorimeter). In the spectrophotometer, a beam of light is transmitted through a bacterial suspension to a light-sensitive detector (Figure 6.21). As bacterial numbers increase, less light will reach the detector. This change of light will register on the instrument's scale as the *percentage of transmission.* Also printed on the instrument's scale is a logarithmic expression called the *absorbance* (sometimes called *optical density,* or *OD,* which is calculated as Abs = 2 − log of % transmittance). The absorbance is used to plot bacterial growth. When the bacteria are in logarithmic growth or decline, a graph of absorbance versus time will form an approximately straight line. If absorbance readings are matched with plate counts of the same culture, this correlation can be used in future estimations of bacterial numbers obtained by measuring turbidity.

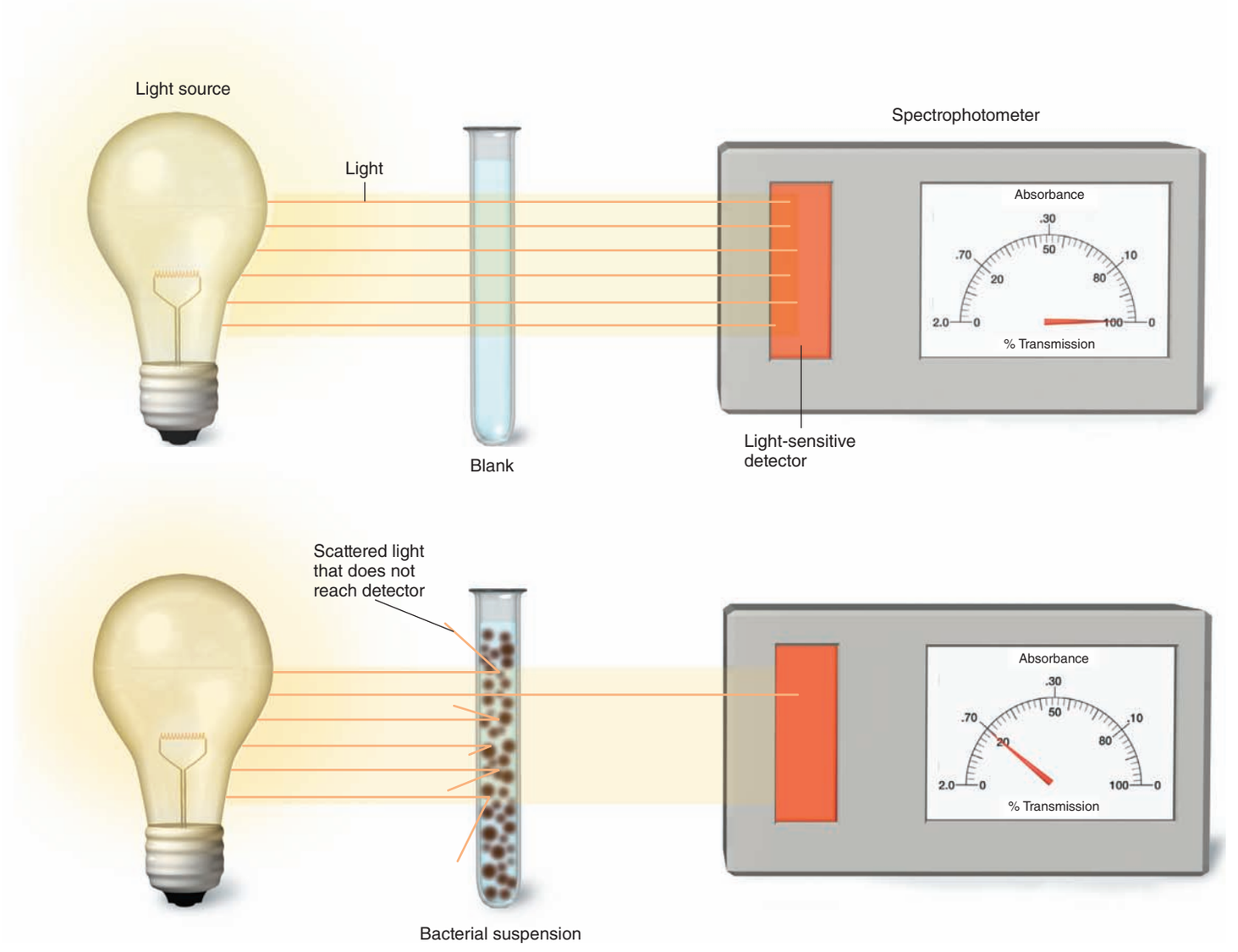


Figure 6.21 Turbidity estimation of bacterial numbers. The amount of light striking the light-sensitive detector on the spectrophotometer is inversely proportional to the number of bacteria under standardized conditions. The less light transmitted, the more bacteria in the sample. The turbidity of the sample could be reported as either 20% transmittance or 0.7 absorbance. Readings in absorbance are a logarithmic function and are sometimes useful in plotting data.

Q Why is turbidity more useful in measuring contamination of liquids by large numbers, rather than small numbers, of bacteria?

More than a million cells per milliliter must be present for the first traces of turbidity to be visible. About 10 million to 100 million cells per milliliter are needed to make a suspension turbid enough to be read on a spectrophotometer. Therefore, turbidity is not a useful measure of contamination of liquids by relatively small numbers of bacteria.

Metabolic Activity

Another indirect way to estimate bacterial numbers is to measure a population's *metabolic activity*. This method assumes that the amount of a certain metabolic product, such as acid or CO_2, is in direct proportion to the number of bacteria present. An example of a practical application of a metabolic test is the microbiological assay in which acid production is used to determine amounts of vitamins.

Dry Weight

For filamentous bacteria and molds, the usual measuring methods are less satisfactory. A plate count would not measure this increase in filamentous mass. In plate counts of actinomycetes (see Figure 11.20, page 320) and molds, it is mostly the number of asexual spores that is counted instead. This is not a good measure of growth. One of the better ways to measure

the growth of filamentous organisms is by *dry weight*. In this procedure, the fungus is removed from the growth medium, filtered to remove extraneous material, and dried in a desiccator. It is then weighed. For bacteria, the same basic procedure is followed.

CHECK YOUR UNDERSTANDING

- Direct methods usually require an incubation time for a colony. Why is this not always feasible for analyzing foods? **6-17**
- If there is no good method for analyzing a product for its vitamin content, what is a feasible method of determining the vitamin content? **6-18**

* * *

You now have a basic understanding of the requirements for, and measurements of, microbial growth. In Chapter 7, we will look at how this growth is controlled in laboratories, hospitals, industry, and our homes.

Clinical Case Resolved

Biofilms are dense accumulations of cells. Five cells might go through 20 generations in a month, producing 7.79×10^6 cells. Now Dr. MacGruder knows that the *P. fluorescens* bacteria are present in the patients' indwelling catheters. He orders the catheters to be replaced and has the CDC examine the used catheters with scanning electron microscopy. They discover that the *P. fluorescens* colonized the inside of the catheters by forming biofilms. In his report to the CDC, Dr. MacGruder explains that the *P. fluorescens* bacteria may have entered the bloodstreams of these patients at the same time as the first outbreak, but not in sufficient quantities to cause symptoms at that time. Biofilm formation enabled the bacteria to persist in the patients' catheters. He notes that previous electron microscopy studies indicate that nearly all indwelling vascular catheters become colonized by microorganisms that are embedded in a biofilm layer and that heparin has been reported to stimulate biofilm formation. Dr. MacGruder concludes that the bacteria in the biofilm were dislodged by subsequent uncontaminated intravenous solutions and released into the bloodstream, finally causing infections months after initial colonization.

154 166 175 177

Study Outline

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Test your understanding with quizzes, microbe review, and a chapter post-test at www.masteringmicrobiology.com.

The Requirements for Growth (pp. 154–160)

1. The growth of a population is an increase in the number of cells.
2. The requirements for microbial growth are both physical and chemical.

Physical Requirements (pp. 154–158)

3. On the basis of preferred temperature ranges, microbes are classified as psychrophiles (cold-loving), mesophiles (moderate-temperature–loving), and thermophiles (heat-loving).
4. The minimum growth temperature is the lowest temperature at which a species will grow, the optimum growth temperature is the temperature at which it grows best, and the maximum growth temperature is the highest temperature at which growth is possible.
5. Most bacteria grow best at a pH value between 6.5 and 7.5.
6. In a hypertonic solution, most microbes undergo plasmolysis; halophiles can tolerate high salt concentrations.

Chemical Requirements (pp. 158–160)

7. All organisms require a carbon source; chemoheterotrophs use an organic molecule, and autotrophs typically use carbon dioxide.
8. Nitrogen is needed for protein and nucleic acid synthesis. Nitrogen can be obtained from the decomposition of proteins or from NH_4^+ or NO_3^-; a few bacteria are capable of nitrogen (N_2) fixation.
9. On the basis of oxygen requirements, organisms are classified as obligate aerobes, facultative anaerobes, obligate anaerobes, aerotolerant anaerobes, and microaerophiles.
10. Aerobes, facultative anaerobes, and aerotolerant anaerobes must have the enzymes superoxide dismutase ($2\ O_2^{\cdot-} + 2\ H^+ \longrightarrow O_2 + H_2O_2$) and either catalase ($2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$) or peroxidase ($H_2O_2 + 2\ H^+ \longrightarrow 2\ H_2O$).
11. Other chemicals required for microbial growth include sulfur, phosphorus, trace elements, and, for some microorganisms, organic growth factors.

Biofilms (pp. 160–161)

1. Microbes adhere to surfaces and accumulate as biofilms on solid surfaces in contact with water.
2. Biofilms form on teeth, contact lenses, and catheters.
3. Microbes in biofilms are more resistant to antibiotics than are free-swimming microbes.

Culture Media (pp. 161–166)

1. A culture medium is any material prepared for the growth of bacteria in a laboratory.
2. Microbes that grow and multiply in or on a culture medium are known as a culture.
3. Agar is a common solidifying agent for a culture medium.

Chemically Defined Media (p. 162)

4. A chemically defined medium is one in which the exact chemical composition is known.

Complex Media (pp. 162–163)

5. A complex medium is one in which the exact chemical composition varies slightly from batch to batch.

Anaerobic Growth Media and Methods (p. 163)

6. Reducing media chemically remove molecular oxygen (O_2) that might interfere with the growth of anaerobes.
7. Petri plates can be incubated in an anaerobic jar, anaerobic chamber, or OxyPlate.

Special Culture Techniques (pp. 163–165)

8. Some parasitic and fastidious bacteria must be cultured in living animals or in cell cultures.
9. CO_2 incubators or candle jars are used to grow bacteria that require an increased CO_2 concentration.
10. Procedures and equipment to minimize exposure to pathogenic microorganisms are designated as biosafety levels 1 through 4.

Selective and Differential Media (p. 165)

11. By inhibiting unwanted organisms with salts, dyes, or other chemicals, selective media allow growth of only the desired microbes.
12. Differential media are used to distinguish different organisms.

Enrichment Culture (pp. 165–166)

13. An enrichment culture is used to encourage the growth of a particular microorganism in a mixed culture.

Obtaining Pure Cultures (p. 167)

1. A colony is a visible mass of microbial cells that theoretically arose from one cell.
2. Pure cultures are usually obtained by the streak plate method.

Preserving Bacterial Cultures (pp. 167–168)

1. Microbes can be preserved for long periods of time by deep-freezing or lyophilization (freeze-drying).

The Growth of Bacterial Cultures (pp. 168–177)

Bacterial Division (p. 168)

1. The normal reproductive method of bacteria is binary fission, in which a single cell divides into two identical cells.
2. Some bacteria reproduce by budding, aerial spore formation, or fragmentation.

Generation Time (pp. 168–169)

3. The time required for a cell to divide or a population to double is known as the generation time.

Logarithmic Representation of Bacterial Populations (pp. 169–170)

4. Bacterial division occurs according to a logarithmic progression (two cells, four cells, eight cells, and so on).

Phases of Growth (pp. 170–171)

5. During the lag phase, there is little or no change in the number of cells, but metabolic activity is high.
6. During the log phase, the bacteria multiply at the fastest rate possible under the conditions provided.
7. During the stationary phase, there is an equilibrium between cell division and death.
8. During the death phase, the number of deaths exceeds the number of new cells formed.

Direct Measurement of Microbial Growth (pp. 171–175)

9. A heterotrophic plate count reflects the number of viable microbes and assumes that each bacterium grows into a single colony; plate counts are reported as number of colony-forming units (CFU).
10. A plate count may be done by either the pour plate method or the spread plate method.
11. In filtration, bacteria are retained on the surface of a membrane filter and then transferred to a culture medium to grow and subsequently be counted.
12. The most probable number (MPN) method can be used for microbes that will grow in a liquid medium; it is a statistical estimation.
13. In a direct microscopic count, the microbes in a measured volume of a bacterial suspension are counted with the use of a specially designed slide.

Estimating Bacterial Numbers by Indirect Methods (pp. 175–177)

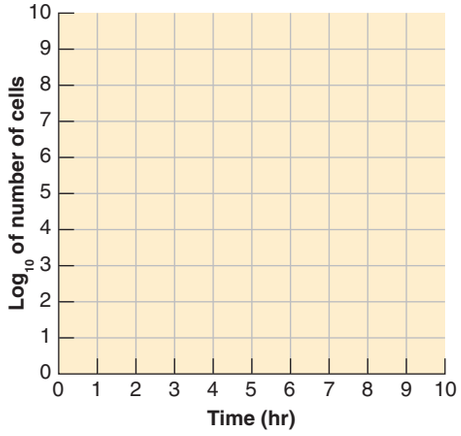
14. A spectrophotometer is used to determine turbidity by measuring the amount of light that passes through a suspension of cells.
15. An indirect way of estimating bacterial numbers is measuring the metabolic activity of the population (for example, acid production or oxygen consumption).
16. For filamentous organisms such as fungi, measuring dry weight is a convenient method of growth measurement.

Study Questions

Answers to the Review and Multiple Choice questions can be found by turning to the Answers tab at the back of the textbook.

Review

1. Describe binary fission.
2. Macronutrients (needed in relatively large amounts) are often listed as CHONPS. What does each of these letters indicate, and why are they needed by the cell?
3. Define and explain the importance of each of the following:
   - **a.** catalase
   - **b.** hydrogen peroxide
   - **c.** peroxidase
   - **d.** superoxide radical
   - **e.** superoxide dismutase
4. Seven methods of measuring microbial growth were explained in this chapter. Categorize each as either a direct or an indirect method.
5. By deep-freezing, bacteria can be stored without harm for extended periods. Why do refrigeration and freezing preserve foods?
6. A pastry chef accidentally inoculated a cream pie with six *S. aureus* cells. If *S. aureus* has a generation time of 60 minutes, how many cells would be in the cream pie after 7 hours?
7. Nitrogen and phosphorus added to beaches following an oil spill encourage the growth of natural oil-degrading bacteria. Explain why the bacteria do not grow if nitrogen and phosphorus are not added.
8. Differentiate complex and chemically defined media.
9. DRAW IT Draw the following growth curves for *E. coli,* starting with 100 cells with a generation time of 30 minutes at 35°C, 60 minutes at 20°C, and 3 hours at 5°C.
   - **a.** The cells are incubated for 5 hours at 35°C.
   - **b.** After 5 hours, the temperature is changed to 20°C for 2 hours.
   - **c.** After 5 hours at 35°C, the temperature is changed to 5°C for 2 hours followed by 35°C for 5 hours.



10. NAME IT A prokaryotic cell hitched a ride to Earth on a space shuttle from some unknown planet. The organism is a psychrophile, an obligate halophile, and an obligate aerobe. Based on the characteristics of the microbe, describe the planet.

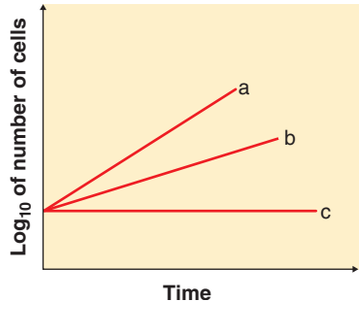
Multiple Choice

Use the following information to answer questions 1 and 2. Two culture media were inoculated with four different bacteria. After incubation, the following results were obtained:

Organism	Medium 1	Medium 2
Escherichia coli	Red colonies	No growth
Staphylococcus aureus	No growth	Growth
Staphylococcus epidermidis	No growth	Growth
Salmonella enterica	Colorless colonies	No growth

1. Medium 1 is
   - **a.** selective.
   - **b.** differential.
   - **c.** both selective and differential.
2. Medium 2 is
   - **a.** selective.
   - **b.** differential.
   - **c.** both selective and differential.

Use the following graph to answer questions 3 and 4.

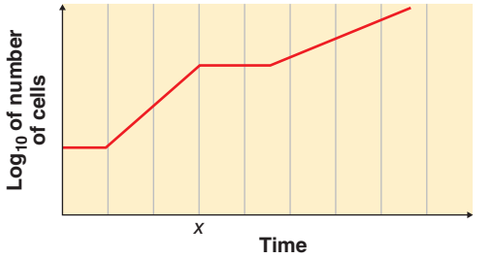


3. Which of the lines best depicts the log phase of a thermophile incubated at room temperature?
4. Which of the lines best depicts the log phase of *Listeria monocytogenes* growing in a human?
5. Assume you inoculated 100 facultatively anaerobic cells onto nutrient agar and incubated the plate aerobically. You then inoculated 100 cells of the same species onto nutrient agar and incubated the second plate anaerobically. After incubation for 24 hours, you should have
   - **a.** more colonies on the aerobic plate.
   - **b.** more colonies on the anaerobic plate.
   - **c.** the same number of colonies on both plates.
6. The term *trace elements* refers to
   - **a.** the elements CHONPS.
   - **b.** vitamins.
   - **c.** nitrogen, phosphorus, and sulfur.
   - **d.** small mineral requirements.
   - **e.** toxic substances.

7. Which one of the following temperatures would most likely kill a mesophile?
   a. −50°C
   b. 0°C
   c. 9°C
   d. 37°C
   e. 60°C
8. Which of the following is *not* a characteristic of biofilms?
   a. antibiotic resistance
   b. hydrogel
   c. iron deficiency
   d. quorum sensing
9. Which of the following types of media would *not* be used to culture aerobes?
   a. selective media
   b. reducing media
   c. enrichment media
   d. differential media
   e. complex media
10. An organism that has peroxidase and superoxide dismutase but lacks catalase is most likely an
    a. aerobe.
    b. aerotolerant anaerobe.
    c. obligate anaerobe.

Critical Thinking

1. *E. coli* was incubated with aeration in a nutrient medium containing two carbon sources, and the following growth curve was made from this culture.
   a. Explain what happened at the time marked *x*.
   b. Which substrate provided "better" growth conditions for the bacteria? How can you tell?



2. *Clostridium* and *Streptococcus* are both catalase-negative. *Streptococcus* grows by fermentation. Why is *Clostridium* killed by oxygen, whereas *Streptococcus* is not?
3. Most laboratory media contain a fermentable carbohydrate and peptone because the majority of bacteria require carbon, nitrogen, and energy sources in these forms. How are these three needs met by glucose–minimal salts medium? (*Hint:* See Table 6.2.)
4. Flask A contains yeast cells in glucose–minimal salts broth incubated at 30°C with aeration. Flask B contains yeast cells in glucose–minimal salts broth incubated at 30°C in an anaerobic jar. The yeasts are facultative anaerobes.
   a. Which culture produced more ATP?
   b. Which culture produced more alcohol?
   c. Which culture had the shorter generation time?
   d. Which culture had the greater cell mass?
   e. Which culture had the higher absorbance?

Clinical Applications

1. Assume that after washing your hands, you leave ten bacterial cells on a new bar of soap. You then decide to do a plate count of the soap after it was left in the soap dish for 24 hours. You dilute 1 g of the soap 1:10^6 and plate it on heterotrophic plate count agar. After 24 hours of incubation, there are 168 colonies. How many bacteria were on the soap? How did they get there?
2. Heat lamps are commonly used to maintain foods at about 50°C for as long as 12 hours in cafeteria serving lines. The following experiment was conducted to determine whether this practice poses a potential health hazard.

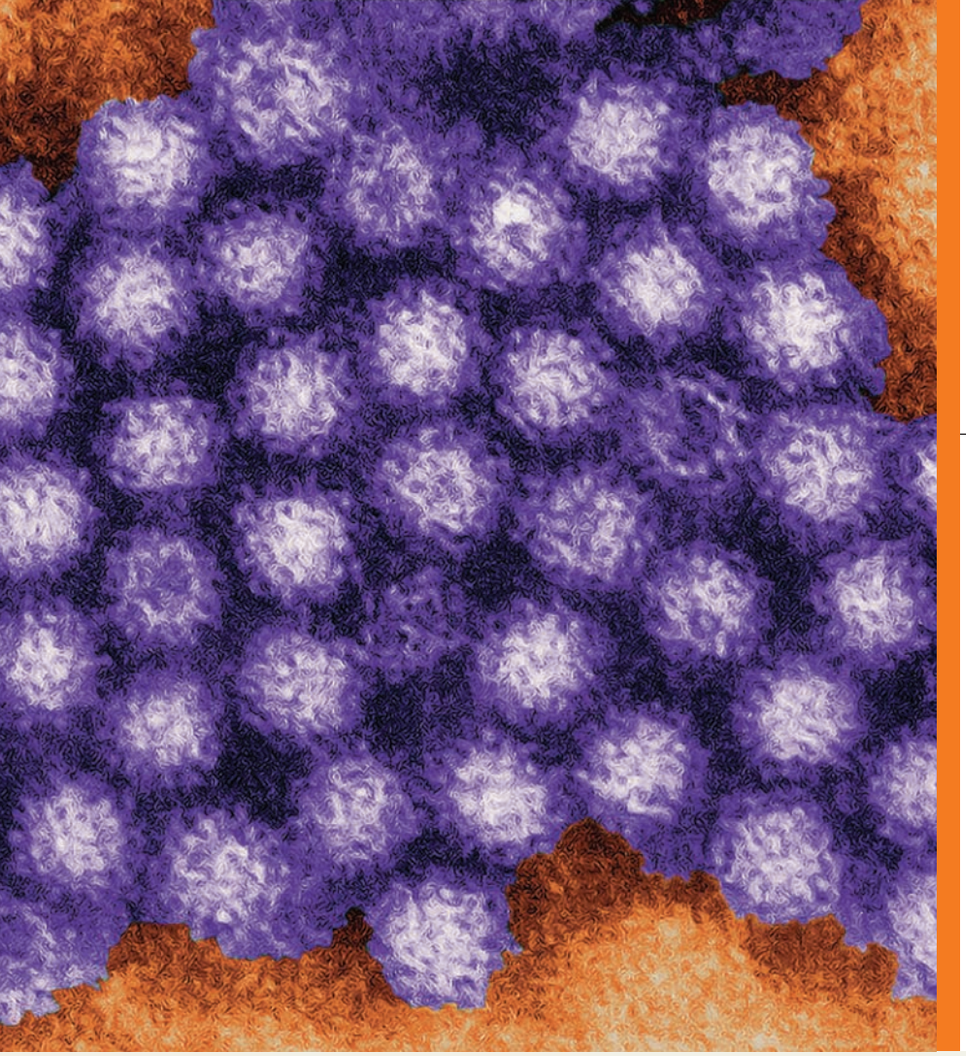
   Beef cubes were surface-inoculated with 500,000 bacterial cells and incubated at 43–53°C to establish temperature limits for bacterial growth. The following results were obtained from heterotrophic plate counts performed on beef cubes at 6 and 12 hours after inoculation:

		Bacteria per Gram of Beef After	
	Temp. (°C)	6 hr	12 hr
Staphylococcus aureus	43	140,000,000	740,000,000
	51	810,000	59,000
	53	650	300
Salmonella typhimurium	43	3,200,000	10,000,000
	51	950,000	83,000
	53	1,200	300
Clostridium perfringens	43	1,200,000	3,600,000
	51	120,000	3,800
	53	300	300

   Draw the growth curves for each organism. What holding temperature would you recommend? Assuming that cooking kills bacteria in foods, how could these bacteria contaminate the cooked foods? What disease does each organism cause? (*Hint:* See Chapter 25.)
3. The number of bacteria in saliva samples was determined by collecting the saliva, making serial dilutions, and inoculating nutrient agar by the pour plate method. The plates were incubated aerobically for 48 hours at 37°C.

	Bacteria per ml Saliva	
	Before Using Mouthwash	After Using Mouthwash
Mouthwash 1	13.1×10^6	10.9×10^6
Mouthwash 2	11.7×10^6	14.2×10^5
Mouthwash 3	9.3×10^5	7.7×10^5

   What can you conclude from these data? Did all the bacteria present in each saliva sample grow?



7

The Control of Microbial Growth

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The scientific control of microbial growth began only about 100 years ago. Recall from Chapter 1 that Pasteur's work on microorganisms led scientists to believe that microbes were a possible cause of disease. In the mid-1800s, the Hungarian physician Ignaz Semmelweis and English physician Joseph Lister used this thinking to develop some of the first microbial control practices for medical procedures. These practices included washing hands with microbe-killing chloride of lime and using the techniques of **aseptic surgery** to prevent microbial contamination of surgical wounds. Until that time, hospital-acquired infections, or *nosocomial infections,* were the cause of death in at least 10% of surgical cases, and as high as 25% in delivering mothers. Ignorance of microbes was such that during the American Civil War, a surgeon might have cleaned his scalpel on his boot sole between incisions. We now know that handwashing is the best way to prevent transmission of pathogens such as the norovirus in the photo. Controlling noroviruses on environmental surfaces is the topic of the Clinical Case.

Over the last century, scientists have continued to develop a variety of physical methods and chemical agents to control microbial growth. In Chapter 20 we will discuss methods for controlling microbes once infection has occurred, mainly antibiotic chemotherapy.

The Terminology of Microbial Control

LEARNING OBJECTIVE

7-1 Define the following key terms related to microbial control: *sterilization, disinfection, antisepsis, degerming, sanitization, biocide, germicide, bacteriostasis,* and *asepsis*.

A word frequently used, and misused, in discussing the control of microbial growth is *sterilization*. **Sterilization** is the removal or destruction of *all* living microorganisms. Heating is the most common method used for killing microbes, including the most resistant forms, such as endospores. A sterilizing agent is called a **sterilant.** Liquids or gases can be sterilized by filtration.

One would think that canned food in the supermarket is completely sterile. In reality, the heat treatment required to ensure absolute sterility would unnecessarily degrade the quality of the food. Instead, food is subjected only to enough heat to destroy the endospores of *Clostridium botulinum* (bo-tū-lī′ num), which can produce a deadly toxin. This limited heat treatment is termed **commercial sterilization.** The endospores of a number of thermophilic bacteria, capable of causing food spoilage but not human disease, are considerably more resistant to heat than *C. botulinum*. If present, they will survive, but their survival is usually of no practical consequence; they will not grow at normal food storage temperatures. If canned foods in a supermarket were incubated at temperatures in the growth range of these thermophiles (above about 45°C), significant food spoilage would occur.

Complete sterilization is often not required in other settings. For example, the body's normal defenses can cope with a few microbes entering a surgical wound. A drinking glass or a fork in a restaurant requires only enough microbial control to prevent the transmission of possibly pathogenic microbes from one person to another.

Control directed at destroying harmful microorganisms is called **disinfection.** It usually refers to the destruction of vegetative (non–endospore-forming) pathogens, which is not the same thing as complete sterility. Disinfection might make use of chemicals, ultraviolet radiation, boiling water, or steam. In practice, the term is most commonly applied to the use of a chemical (a *disinfectant*) to treat an inert surface or substance. When this treatment is directed at living tissue, it is called **antisepsis,** and the chemical is then called an *antiseptic*. Therefore, in practice the same chemical might be called a disinfectant for one use and an antiseptic for another. Of course, many chemicals suitable for wiping a tabletop would be too harsh to use on living tissue.

There are modifications of disinfection and antisepsis. For example, when someone is about to receive an injection, the skin is swabbed with alcohol—the process of **degerming** (or *degermation*), which mostly results in the mechanical removal, rather than the killing, of most of the microbes in a limited area. Restaurant glassware, china, and tableware are subjected to **sanitization,** which is intended to lower microbial counts to safe public health levels and minimize the chances of disease transmission from one user to another. This is usually accomplished by high-temperature washing or, in the case of glassware in a bar, washing in a sink followed by a dip in a chemical disinfectant.

Table 7.1 summarizes the terminology relating to the control of microbial growth.

Names of treatments that cause the outright death of microbes have the suffix *-cide,* meaning kill. A **biocide,** or **germicide,** kills microorganisms (usually with certain exceptions, such as endospores); a *fungicide* kills fungi; a *virucide* inactivates viruses; and so on. Other treatments only inhibit the growth and multiplication of bacteria; their names have the suffix *-stat* or *-stasis,* meaning to stop or to steady, as in **bacteriostasis.** Once a bacteriostatic agent is removed, growth might resume.

Sepsis, from the Greek for decay or putrid, indicates bacterial contamination, as in septic tanks for sewage treatment. (The term is also used to describe a disease condition; see Chapter 23, page 646.) *Aseptic* means that an object or area is free of pathogens. Recall from Chapter 1 that **asepsis** is the absence of significant contamination. Aseptic techniques are important in surgery to minimize contamination from the instruments, operating personnel, and the patient.

CHECK YOUR UNDERSTANDING

The usual definition of *sterilization* is the removal or destruction of all forms of microbial life; how could there be practical exceptions to this simple definition? **7-1**

Clinical Case: A School Epidemic

It is 9:00 A.M. on a Wednesday morning, and Amy Garza, the school nurse at Westview Elementary School in Rockville, Maryland, has been on the phone since she came in to work at 7:00 A.M. So far this morning, she has received reports of students unable to attend school today because of some sort of gastrointestinal ailment. They all have the same symptoms: nausea and vomiting, diarrhea, and a low-grade fever. As Amy picks up the phone to call the principal to give her an update, she receives her eighth call of the day. Keith Jackson, a first-grade teacher who has been out sick since Monday, calls to tell Amy that his physician sent his stool sample to the laboratory for testing. The results came back positive for norovirus.

What is norovirus? Read on to find out.

182 197 199 201

The Rate of Microbial Death

LEARNING OBJECTIVE

7-2 Describe the patterns of microbial death caused by treatments with microbial control agents.

TABLE 7.1 Terminology Relating to the Control of Microbial Growth

	Definition	Comments
Sterilization	Destruction or removal of all forms of microbial life, including endospores but with the possible exception of prions.	Usually done by steam under pressure or a sterilizing gas, such as ethylene oxide.
Commercial Sterilization	Sufficient heat treatment to kill endospores of *Clostridium botulinum* in canned food.	More-resistant endospores of thermophilic bacteria may survive, but they will not germinate and grow under normal storage conditions.
Disinfection	Destruction of vegetative pathogens.	May make use of physical or chemical methods.
Antisepsis	Destruction of vegetative pathogens on living tissue.	Treatment is almost always by chemical antimicrobials.
Degerming	Removal of microbes from a limited area, such as the skin around an injection site.	Mostly a mechanical removal by an alcohol-soaked swab.
Sanitization	Treatment is intended to lower microbial counts on eating and drinking utensils to safe public health levels.	May be done with high-temperature washing or by dipping into a chemical disinfectant.

When bacterial populations are heated or treated with antimicrobial chemicals, they usually die at a constant rate. For example, suppose a population of 1 million microbes has been treated for 1 minute, and 90% of the population has died. We are now left with 100,000 microbes. If the population is treated for another minute, 90% of *those* microbes die, and we are left with 10,000 survivors. In other words, for each minute the treatment is applied, 90% of the remaining population is killed (**Table 7.2**). If the death curve is plotted logarithmically, the death rate is constant, as shown by the straight line in **Figure 7.1a**.

Several factors influence the effectiveness of antimicrobial treatments:

- *The number of microbes.* The more microbes there are to begin with, the longer it takes to eliminate the entire population (**Figure 7.1b**).
- *Environmental influences.* The presence of organic matter often inhibits the action of chemical antimicrobials. In hospitals, the presence of organic matter in blood, vomitus, or feces influences the selection of disinfectants. Microbes in surface biofilms (see page 160) are difficult for biocides to reach effectively. Because their activity is due to temperature-dependent chemical reactions, disinfectants work somewhat better under warm conditions.

  The nature of the suspending medium is also a factor in heat treatment. Fats and proteins are especially protective, and a medium rich in these substances protects microbes, which will then have a higher survival rate. Heat is also measurably more effective under acidic conditions.
- *Time of exposure.* Chemical antimicrobials often require extended exposure to affect more-resistant microbes or endospores. See the discussion of equivalent treatments on page 188.
- *Microbial characteristics.* The concluding section of this chapter discusses how microbial characteristics affect the choice of chemical and physical control methods.

TABLE 7.2 Microbial Exponential Death Rate: An Example

Time (min)	Deaths per Minute	Number of Survivors
0	0	1,000,000
1	900,000	100,000
2	90,000	10,000
3	9000	1000
4	900	100
5	90	10
6	9	1

CHECK YOUR UNDERSTANDING

How is it possible that a solution containing a million bacteria would take longer to sterilize than one containing a half-million bacteria? **7-2**

Actions of Microbial Control Agents

LEARNING OBJECTIVE

7-3 Describe the effects of microbial control agents on cellular structures.

In this section, we examine the ways various agents actually kill or inhibit microbes.

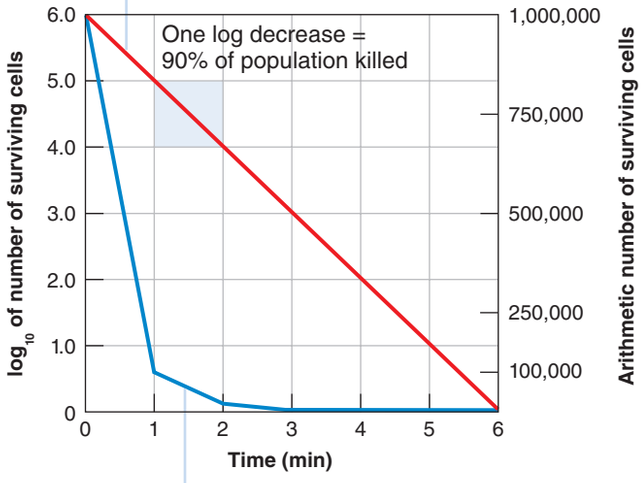
Alteration of Membrane Permeability

A microorganism's plasma membrane (see Figure 4.14, page 89), located just inside the cell wall, is the target of many microbial control agents. This membrane actively regulates the passage of

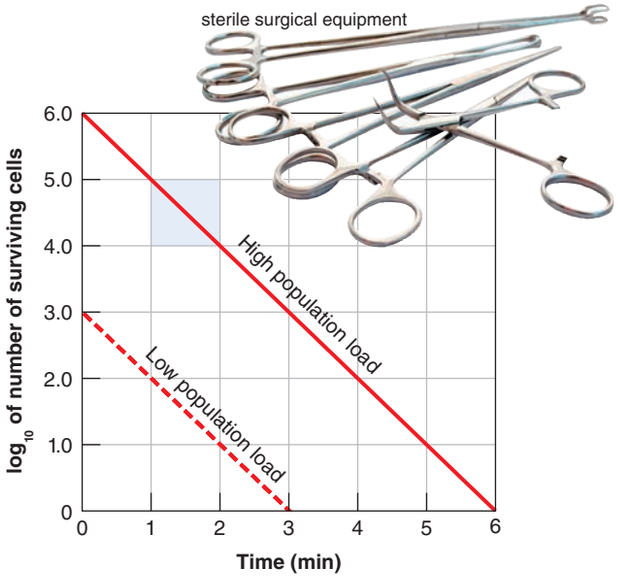
FOUNDATION FIGURE **7.1**

Understanding the Microbial Death Curve

Plotting the typical microbial death curve **logarithmically** (**red line**) results in a straight line.



(a) Plotting the typical microbial death curve **arithmetically** (**blue line**) is impractical: at 3 minutes the population of 1000 cells would only be a hundredth of the graphed distance between 100,000 and the baseline.



(b) Logarithmic plotting (**red**) reveals that if the rate of killing is the same, it will take longer to kill all members of a larger population than a smaller one, whether using heat or chemical treatments.

KEY CONCEPTS

- Bacterial populations usually die at a constant rate when heated or when treated with antimicrobial chemicals.
- It is necessary to use logarithmic numbers to graph bacterial populations effectively.
- Understanding logarithmic death curves for microbial populations, including the elements of time and the size of the initial population, is especially useful in food preservation and in the sterilization of media or medical supplies.

preserved food

nutrients into the cell and the elimination of wastes from the cell. Damage to the lipids or proteins of the plasma membrane by antimicrobial agents causes cellular contents to leak into the surrounding medium and interferes with the growth of the cell.

Damage to Proteins and Nucleic Acids

Bacteria are sometimes thought of as "little bags of enzymes." Enzymes, which are primarily protein, are vital to all cellular activities. Recall that the functional properties of proteins are the result of their three-dimensional shape (see Figure 2.15, page 45). This shape is maintained by chemical bonds that link adjoining portions of the amino acid chain as it folds back and forth upon itself. Some of those bonds are hydrogen bonds, which are susceptible to breakage by heat or certain chemicals; breakage results in denaturation of the protein. Covalent bonds are stronger but are also subject to attack. For example, disulfide bridges, which play an important role in protein structure by joining amino acids with exposed sulfhydryl (—SH) groups, can be broken by certain chemicals or sufficient heat.

The nucleic acids DNA and RNA are the carriers of the cell's genetic information. Damage to these nucleic acids by heat, radiation, or chemicals is frequently lethal to the cell; the cell can no longer replicate, nor can it carry out normal metabolic functions such as the synthesis of enzymes.

CHECK YOUR UNDERSTANDING

- Would a chemical microbial control agent that affects plasma membranes affect humans? **7-3**

Physical Methods of Microbial Control

LEARNING OBJECTIVES

7-4 Compare the effectiveness of moist heat (boiling, autoclaving, pasteurization) and dry heat.

7-5 Describe how filtration, low temperatures, high pressure, desiccation, and osmotic pressure suppress microbial growth.

7-6 Explain how radiation kills cells.

As early as the Stone Age, humans likely were already using some physical methods of microbial control to preserve foods. Drying (desiccation) and salting (osmotic pressure) were probably among the earliest techniques.

When selecting methods of microbial control, one must consider what else, besides the microbes, a particular method will affect. For example, heat might inactivate certain vitamins or antibiotics in a solution. Repeated heating damages many laboratory and hospital materials, such as rubber and latex tubing. There are also economic considerations; for example, it may be less expensive to use presterilized, disposable plasticware than to repeatedly wash and resterilize glassware.

Heat

A visit to any supermarket will demonstrate that heat-preserved canned goods represent one of the most common methods of food preservation. Heat is also usually used to sterilize laboratory media and glassware and hospital instruments. Heat appears to kill microorganisms by denaturing their enzymes; the resultant changes to the three-dimensional shapes of these proteins inactivate them (see Figure 5.6, page 117).

Heat resistance varies among different microbes; these differences can be expressed through the concept of thermal death point. **Thermal death point (TDP)** is the lowest temperature at which all the microorganisms in a particular liquid suspension will be killed in 10 minutes.

Another factor to be considered in sterilization is the length of time required. This is expressed as **thermal death time (TDT),** the minimal length of time for all bacteria in a particular liquid culture to be killed at a given temperature. Both TDP and TDT are useful guidelines that indicate the severity of treatment required to kill a given population of bacteria.

Decimal reduction time (**DRT,** or *D value*) is a third concept related to bacterial heat resistance. DRT is the time, in minutes, in which 90% of a population of bacteria at a given temperature will be killed (in Table 7.2 and Figure 7.1a, DRT is 1 minute). In Chapter 28 you can find an important application of DRT in the canning industry, as well as a discussion of the 12D treatment of canned goods.

Moist Heat Sterilization

Moist heat kills microorganisms primarily by coagulating proteins (denaturation), which is caused by breakage of the hydrogen bonds that hold the proteins in their three-dimensional structure. This coagulation process is familiar to anyone who has watched an egg white frying.

One type of moist heat "sterilization" is boiling, which kills vegetative forms of bacterial pathogens, almost all viruses, and fungi and their spores within about 10 minutes, usually much faster. Free-flowing (unpressurized) steam is essentially the same temperature as boiling water. Endospores and some viruses, however, are not destroyed this quickly. Some hepatitis viruses, for example, can survive up to 30 minutes of boiling, and some bacterial endospores can resist boiling for more than 20 hours. Boiling is therefore not always a reliable sterilization procedure. However, brief boiling, even at high altitudes, will kill most pathogens. The use of boiling to sanitize glass baby bottles is a familiar example.

Reliable sterilization with moist heat requires temperatures above that of boiling water. These high temperatures are most commonly achieved by steam under pressure in an **autoclave** (**Figure 7.2**). Autoclaving is the preferred method of sterilization, unless the material to be sterilized can be damaged by heat or moisture.

The higher the pressure in the autoclave, the higher the temperature. For example, when free-flowing steam at a temperature of 100°C is placed under a pressure of 1 atmosphere above sea level pressure—that is, about 15 pounds of pressure per square inch (psi)—the temperature rises to 121°C. Increasing the pressure to 20 psi raises the temperature to 126°C. The relationship between temperature and pressure is shown in **Table 7.3**.

Sterilization in an autoclave is most effective when the organisms either are contacted by the steam directly or are contained in a small volume of aqueous (primarily water) liquid. Under these conditions, steam at a pressure of about 15 psi (121°C) will kill *all* organisms (but not prions, see page 395) and their endospores in about 15 minutes.

Autoclaving is used to sterilize culture media, instruments, dressings, intravenous equipment, applicators, solutions, syringes, transfusion equipment, and numerous other items that can withstand high temperatures and pressures. Large industrial autoclaves are called *retorts* (see Figure 28.2 on page 801), but the same principle applies for the common household pressure cooker used in the home canning of foods.

Heat requires extra time to reach the center of solid materials, such as canned meats, because such materials do not develop the efficient heat-distributing convection currents that occur in liquids. Heating large containers also requires extra time. **Table 7.4** shows the different time requirements for sterilizing liquids in various container sizes.

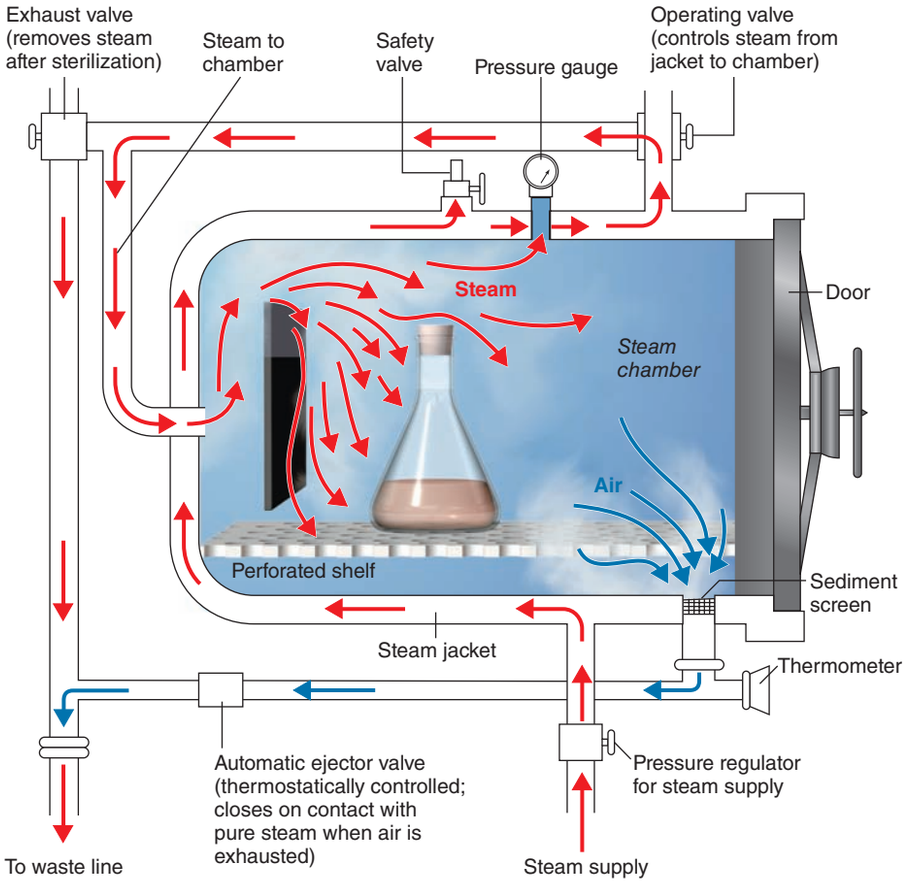


Figure 7.2 An autoclave. The entering steam forces the air out of the bottom (blue arrows). The automatic ejector valve remains open as long as an air-steam mixture is passing out of the waste line. When all the air has been ejected, the higher temperature of the pure steam closes the valve, and the pressure in the chamber increases.

Q How would an empty, uncapped flask be positioned for sterilization in an autoclave?

Unlike sterilizing aqueous solutions, sterilizing the surface of a solid requires that steam actually contact it. To sterilize dry glassware, bandages, and the like, care must be taken to ensure that steam contacts all surfaces. For example, aluminum foil is impervious to steam and should not be used to wrap dry materials that are to be sterilized; paper should be used instead. Care should also be taken to avoid trapping air in the bottom of a dry container: trapped air will not be replaced by steam, because steam is

TABLE 7.3 The Relationship between the Pressure and Temperature of Steam at Sea Level*

Pressure (psi in Excess of Atmospheric Pressure)	Temperature (°C)
0	100
5	110
10	116
15	121
20	126
30	135

*At higher altitudes, the atmospheric pressure is less, a phenomenon that must be taken into account in operating an autoclave. For example, to reach sterilizing temperatures (121°C) in Denver, Colorado, whose altitude is 5280 feet (1600 meters), the pressure shown on the autoclave gauge would need to be higher than the 15 psi shown in the table.

TABLE 7.4 The Effect of Container Size on Autoclave Sterilization Times for Liquid Solutions*

Container Size	Liquid Volume	Sterilization Time (min)
Test tube: 18 × 150 mm	10 ml	15
Erlenmeyer flask: 125 ml	95 ml	15
Erlenmeyer flask: 2000 ml	1500 ml	30
Fermentation bottle: 9000 ml	6750 ml	70

*Sterilization times in the autoclave include the time for the contents of the containers to reach sterilization temperatures. For smaller containers, this is only 5 min or less, but for a 9000-ml bottle it might be as much as 70 min. A container is usually not filled past 75% of its capacity.

lighter than air. The trapped air is the equivalent of a small hot-air oven, which, as we will see shortly, requires a higher temperature and longer time to sterilize materials. Containers that can trap air should be placed in a tipped position so that the steam will force out the air. Products that do not permit penetration by moisture, such as mineral oil or petroleum jelly, are not sterilized by the same methods used to sterilize aqueous solutions.

Several commercially available methods can indicate whether heat treatment has achieved sterilization. Some of these are chemical reactions in which an indicator changes color when the proper times and temperatures have been reached (**Figure 7.3**). In some designs, the word *sterile* or *autoclaved* appears on wrappings or tapes. A widely used test consists of preparations of specified species of bacterial endospores impregnated into paper strips. After the strips are autoclaved, they can then be aseptically inoculated into culture media. Growth in the culture media indicates survival of the endospores and therefore inadequate processing. Other designs use endospore suspensions that can be released, after heating, into a surrounding culture medium within the same vial.

Steam under pressure fails to sterilize when the air is not completely exhausted. This can happen with the premature closing of the autoclave's automatic ejector valve (see Figure 7.2). The principles of heat sterilization have a direct bearing on home canning. As anyone familiar with home canning knows, the steam must flow vigorously out of the valve in the lid for several minutes to carry with it all the air before the pressure cooker is sealed. If the air is not completely exhausted, the container will not reach the temperature expected for a given pressure. Because of the possibility of botulism, a kind of food poisoning resulting from improper canning methods (see Chapter 22, page 622), anyone doing home canning should obtain reliable directions and follow them exactly.

Figure 7.3 Examples of sterilization indicators. The strips indicate whether the item has been properly sterilized. The word *NOT* appears if heating has been inadequate. In the illustration, the indicator that was wrapped with aluminum foil was not sterilized because steam couldn't penetrate the foil.

Q What should have been used instead of aluminum foil to wrap the items?

Pasteurization

Recall from Chapter 1 that in the early days of microbiology, Louis Pasteur found a practical method of preventing the spoilage of beer and wine. Pasteur used mild heating, which was sufficient to kill the organisms that caused the particular spoilage problem without seriously damaging the taste of the product. The same principle was later applied to milk to produce what we now call pasteurized milk. The intent of **pasteurization** of milk was to eliminate pathogenic microbes. It also lowers microbial numbers, which prolongs milk's good quality under refrigeration. Many relatively heat-resistant (**thermoduric**) bacteria survive pasteurization, but these are unlikely to cause disease or cause refrigerated milk to spoil.

Products other than milk, such as ice cream, yogurt, and beer, all have their own pasteurization times and temperatures, which often differ considerably. There are several reasons for these variations. For example, heating is less efficient in foods that are more viscous, and fats in food can have a protective effect on microorganisms. The dairy industry routinely uses a test to determine whether products have been pasteurized: the *phosphatase test* (phosphatase is an enzyme naturally present in milk). If the product has been pasteurized, phosphatase will have been inactivated.

Most milk pasteurization today uses temperatures of at least 72°C, but for only 15 seconds. This treatment, known as **high-temperature short-time (HTST) pasteurization,** is applied as the milk flows continuously past a heat exchanger. In addition to killing pathogens, HTST pasteurization lowers total bacterial counts, so the milk keeps well under refrigeration.

Milk can also be sterilized—something quite different from pasteurization—by **ultra-high-temperature (UHT) treatments.** It can then be stored for several months without refrigeration (also see *commercial sterilization,* page 800). UHT-treated milk is widely sold in Europe and is especially necessary in less developed parts of the world where refrigeration facilities are not always available. In the United States, UHT is sometimes used on the small containers of coffee creamers found in restaurants. To avoid giving the milk a cooked taste, the process avoids having the milk touch a surface hotter than the milk itself. Usually, the liquid milk (or juice) is sprayed through a nozzle into a chamber filled with high-temperature steam under pressure. A small volume of fluid sprayed into an atmosphere of high-temperature steam exposes a relatively large surface area on the fluid droplets to heating by

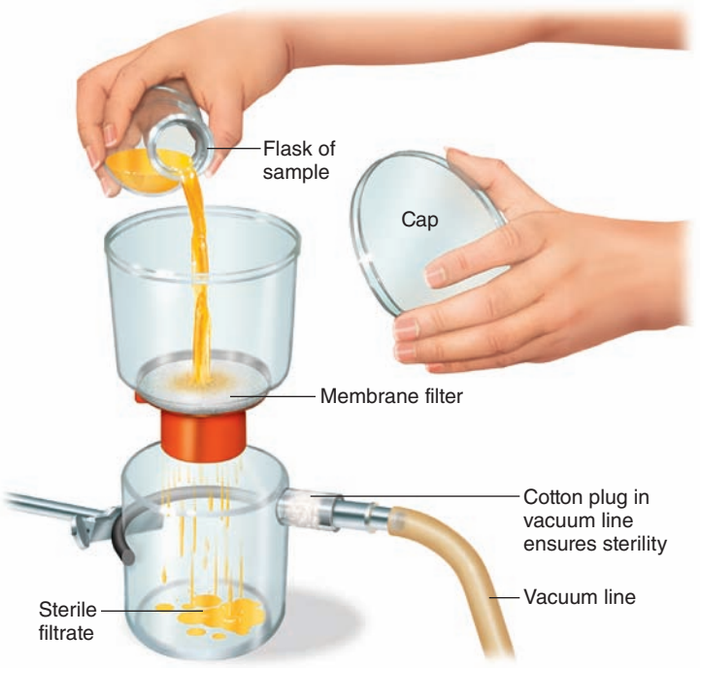


Figure 7.4 Filter sterilization with a disposable, presterilized plastic unit. The sample is placed into the upper chamber and forced through the membrane filter by a vacuum in the lower chamber. Pores in the membrane filter are smaller than the bacteria, so bacteria are retained on the filter. The sterilized sample can then be decanted from the lower chamber. Similar equipment with removable filter disks is used to count bacteria in samples (see Figure 6.18).

Q How is a plastic filtration apparatus presterilized? (Assume the plastic cannot be heat sterilized.)

the steam; sterilizing temperatures are reached almost instantaneously. After reaching a temperature of 140°C for 4 seconds, the fluid is rapidly cooled in a vacuum chamber. The milk or juice is then packaged in a presterilized, airtight container.

The heat treatments we have just discussed illustrate the concept of **equivalent treatments:** as the temperature is increased, much less time is needed to kill the same number of microbes. For example, the destruction of highly resistant endospores might take 70 minutes at 115°C, whereas only 7 minutes might be needed at 125°C. Both treatments yield the same result.

Dry Heat Sterilization

Dry heat kills by oxidation effects. A simple analogy is the slow charring of paper in a heated oven, even when the temperature remains below the ignition point of paper. One of the simplest methods of dry heat sterilization is direct **flaming.** You will use this procedure many times in the microbiology laboratory when you sterilize inoculating loops. To effectively sterilize the inoculating loop, you heat the wire to a red glow. A similar principle is used in *incineration,* an effective way to sterilize and dispose of contaminated paper cups, bags, and dressings.

Another form of dry heat sterilization is **hot-air sterilization.** Items to be sterilized by this procedure are placed in an oven. Generally, a temperature of about 170°C maintained for nearly 2 hours ensures sterilization. The longer period and higher temperature (relative to moist heat) are required because the heat in water is more readily transferred to a cool body than is the heat in air. For example, imagine the different effects of immersing your hand in boiling water at 100°C (212°F) and of holding it in a hot-air oven at the same temperature for the same amount of time.

Filtration

Recall from Chapter 6 that *filtration* is the passage of a liquid or gas through a screenlike material with pores small enough to retain microorganisms (often the same apparatus used for counting; see Figure 6.18, page 174). A vacuum is created in the receiving flask; air pressure then forces the liquid through the filter. Filtration is used to sterilize heat-sensitive materials, such as some culture media, enzymes, vaccines, and antibiotic solutions.

Some operating theaters and rooms occupied by burn patients receive filtered air to lower the numbers of airborne microbes. **High-efficiency particulate air (HEPA) filters** remove almost all microorganisms larger than about 0.3 µm in diameter.

In the early days of microbiology, hollow candle-shaped filters of unglazed porcelain were used to filter liquids. The long and indirect passageways through the walls of the filter adsorbed the bacteria. Unseen pathogens that passed through the filters (causing such diseases as rabies) were called *filterable viruses.* See the discussion of filtration in modern water treatment on page 788.

In recent years, **membrane filters,** composed of such substances as cellulose esters or plastic polymers, have become popular for industrial and laboratory use (Figure 7.4). These filters are only 0.1 mm thick. The pores of membrane filters include, for example, 0.22-µm and 0.45-µm sizes, which are intended for bacteria. Some very flexible bacteria, such as spirochetes, or the wall-less mycoplasma, will sometimes pass through such filters, however. Filters are available with pores as small as 0.01 µm, a size that will retain viruses and even some large protein molecules.

Low Temperatures

The effect of low temperatures on microorganisms depends on the particular microbe and the intensity of the application. For example, at temperatures of ordinary refrigerators (0–7°C), the metabolic rate of most microbes is so reduced that they cannot reproduce or synthesize toxins. In other words, ordinary refrigeration has a bacteriostatic effect. Yet psychrotrophs do grow slowly at refrigerator temperatures and will alter the appearance and taste of foods after a time. For example, a single microbe reproducing only three times a day would reach a population of more than 2 million within a week. Pathogenic bacteria generally will not grow at refrigerator temperatures, but for at

least one important exception, see the discussion of listeriosis in Chapter 22 (page 619).

Surprisingly, some bacteria can grow at temperatures several degrees below freezing. Most foods remain unfrozen until −2°C or lower. Rapidly attained subfreezing temperatures tend to render microbes dormant but do not necessarily kill them. Slow freezing is more harmful to bacteria; the ice crystals that form and grow disrupt the cellular and molecular structure of the bacteria. Thawing, being inherently slower, is actually the more damaging part of a freeze-thaw cycle. Once frozen, one-third of the population of some vegetative bacteria might survive a year, whereas other species might have very few survivors after this time. Many eukaryotic parasites, such as the roundworms that cause human trichinellosis, are killed by several days of freezing temperatures. Some important temperatures associated with microorganisms and food spoilage are shown in Figure 6.2 (page 155).

High Pressure

High pressure applied to liquid suspensions is transferred instantly and evenly throughout the sample. If the pressure is high enough, it alters the molecular structures of proteins and carbohydrates, resulting in the rapid inactivation of vegetative bacterial cells. Endospores are relatively resistant to high pressure. They can, however, be killed by other techniques, such as combining high pressure with elevated temperatures or by alternating pressure cycles that cause spore germination, followed by pressure-caused death of the resulting vegetative cells. Fruit juices preserved by high-pressure treatments have been marketed in Japan and the United States. An advantage is that these treatments preserve the flavors, colors, and nutrient values of the products.

Desiccation

In the absence of water, known as **desiccation,** microorganisms cannot grow or reproduce but can remain viable for years. Then, when water is made available to them, they can resume their growth and division. This is the principle that underlies lyophilization, or freeze-drying, a laboratory process for preserving microbes described in Chapter 6 (page 168). Certain foods are also freeze-dried (for example, coffee and some fruit additives for dry cereals).

The resistance of vegetative cells to desiccation varies with the species and the organism's environment. For example, the gonorrhea bacterium can withstand desiccation for only about an hour, but the tuberculosis bacterium can remain viable for months. Viruses are generally resistant to desiccation, but they are not as resistant as bacterial endospores, some of which have survived for centuries. This ability of certain dried microbes and endospores to remain viable is important in a hospital setting. Dust, clothing, bedding, and dressings might contain infectious microbes in dried mucus, urine, pus, and feces.

Osmotic Pressure

The use of high concentrations of salts and sugars to preserve food is based on the effects of *osmotic pressure.* High concentrations of these substances create a hypertonic environment that causes water to leave the microbial cell (see Figure 6.4, page 157). This process resembles preservation by desiccation, in that both methods deny the cell the moisture it needs for growth. The principle of osmotic pressure is used in the preservation of foods. For example, concentrated salt solutions are used to cure meats, and thick sugar solutions are used to preserve fruits.

As a general rule, molds and yeasts are much more capable than bacteria of growing in materials with low moisture or high osmotic pressures. This property of molds, sometimes combined with their ability to grow under acidic conditions, is the reason that molds, rather than bacteria, cause spoilage of fruits and grains. It is also part of the reason molds are able to form mildew on a damp wall or a shower curtain.

Radiation

Radiation has various effects on cells, depending on its wavelength, intensity, and duration. Radiation that kills microorganisms (sterilizing radiation) is of two types: ionizing and nonionizing.

Ionizing radiation—gamma rays, X rays, or high-energy electron beams—has a wavelength shorter than that of nonionizing radiation, less than about 1 nm. Therefore, it carries much more energy (**Figure 7.5**). *Gamma rays* are emitted by certain radioactive elements such as cobalt, and electron beams are produced by accelerating electrons to high energies in special machines. *X rays,* which are produced by machines in a manner similar to the production of electron beams, are similar to gamma rays. Gamma rays penetrate deeply but may require hours to sterilize large masses; *high-energy electron beams* have much lower penetrating power but usually require only a few seconds of exposure. The principal effect of ionizing radiation is the ionization of water, which forms highly reactive hydroxyl radicals (see the discussion of toxic forms of oxygen in Chapter 6, pages 159–160). These radicals react with organic cellular components, especially DNA.

The so-called target theory of damage by radiation supposes that ionizing particles, or packets of energy, pass through or close to vital portions of the cell; these constitute "hits." One, or a few, hits may only cause nonlethal mutations, some of them conceivably useful. More hits are likely to cause sufficient mutations to kill the microbe.

The food industry has recently renewed its interest in the use of radiation for food preservation (discussed more fully in Chapter 28). Low-level ionizing radiation, used for years in many countries, has been approved in the United States for processing spices and certain meats and vegetables. Ionizing radiation, especially high-energy electron beams, is used to sterilize pharmaceuticals and disposable dental and medical supplies, such as plastic syringes, surgical gloves, suturing

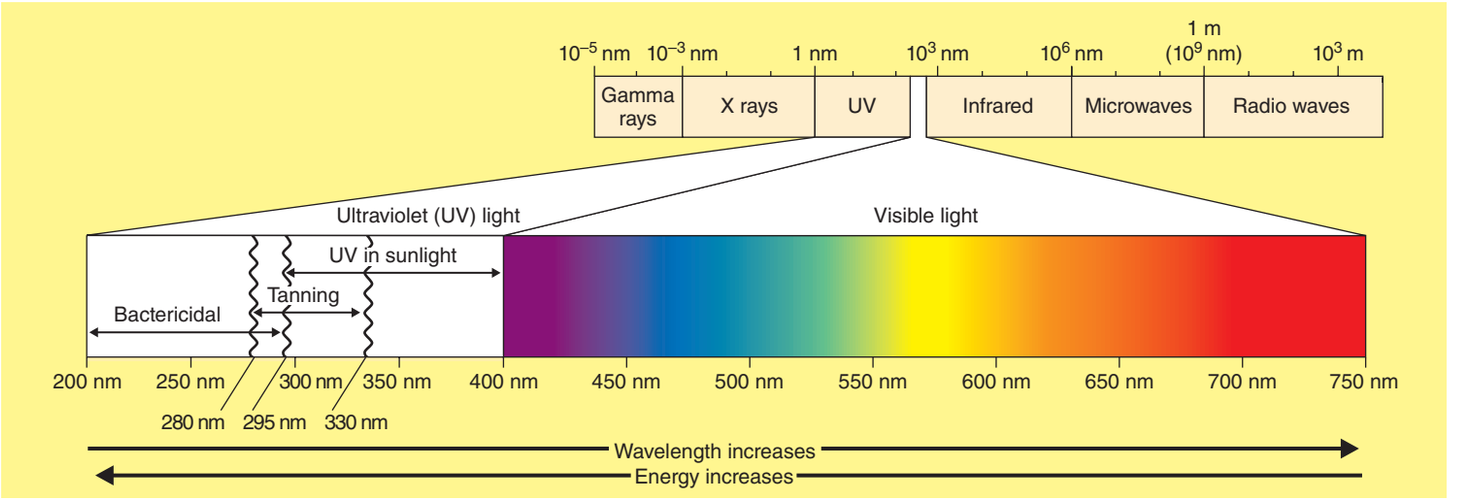


Figure 7.5 The radiant energy spectrum. Visible light and other forms of radiant energy radiate through space as waves of various lengths. Ionizing radiation, such as gamma rays and X rays, has a wavelength shorter than 1 nm. Nonionizing radiation, such as ultraviolet (UV) light, has a wavelength between 1 nm and about 380 nm, where the visible spectrum begins.

Q How might increased UV radiation (due to decrease in the ozone layer) affect the Earth's ecosystems?

materials, and catheters. As a protection against bioterrorism, the postal service often uses electron beam radiation to sterilize certain classes of mail.

Nonionizing radiation has a wavelength longer than that of ionizing radiation, usually greater than about 1 nm. The best example of nonionizing radiation is ultraviolet (UV) light. UV light damages the DNA of exposed cells by causing bonds to form between adjacent pyrimidine bases, usually thymines, in DNA chains (see Figure 8.21, page 228). These *thymine dimers* inhibit correct replication of the DNA during reproduction of the cell. The UV wavelengths most effective for killing microorganisms are about 260 nm; these wavelengths are specifically absorbed by cellular DNA. UV radiation is also used to control microbes in the air. A UV, or "germicidal," lamp is commonly found in hospital rooms, nurseries, operating rooms, and cafeterias. UV light is also used to disinfect vaccines and other medical products. A major disadvantage of UV light as a disinfectant is that the radiation is not very penetrating, so the organisms to be killed must be directly exposed to the rays. Organisms protected by solids and such coverings as paper, glass, and textiles are not affected. Another potential problem is that UV light can damage human eyes, and prolonged exposure can cause burns and skin cancer in humans.

Sunlight contains some UV radiation, but the shorter wavelengths—those most effective against bacteria—are screened out by the ozone layer of the atmosphere. The antimicrobial effect of sunlight is due almost entirely to the formation of singlet oxygen in the cytoplasm (see Chapter 6, page 159). Many pigments produced by bacteria provide protection from sunlight.

Microwaves do not have much direct effect on microorganisms, and bacteria can readily be isolated from the interior of recently operated microwave ovens. Moisture-containing foods are heated by microwave action, and the heat will kill most vegetative pathogens. Solid foods heat unevenly because of the uneven distribution of moisture. For this reason, pork cooked in a microwave oven has been responsible for outbreaks of trichinellosis.

Table 7.5 summarizes the physical methods of microbial control.

CHECK YOUR UNDERSTANDING

- How is microbial growth in canned foods prevented? **7-4**
- Why would a can of pork take longer to sterilize at a given temperature than a can of soup that also contained pieces of pork? **7-5**
- What is the connection between the killing effect of radiation and hydroxyl radical forms of oxygen? **7-6**

Chemical Methods of Microbial Control

LEARNING OBJECTIVES

7-7 List the factors related to effective disinfection.

7-8 Interpret the results of use-dilution tests and the disk-diffusion method.

7-9 Identify the methods of action and preferred uses of chemical disinfectants.

7-10 Differentiate halogens used as antiseptics from halogens used as disinfectants.

TABLE 7.5 Physical Methods Used to Control Microbial Growth

Methods	Mechanism of Action	Comment	Preferred Use
Heat			
1. Moist heat			
a. Boiling or flowing steam	Protein denaturation	Kills vegetative bacterial and fungal pathogens and almost all viruses within 10 min; less effective on endospores.	Dishes, basins, pitchers, various equipment
b. Autoclaving	Protein denaturation	Very effective method of sterilization; at about 15 psi of pressure (121°C), all vegetative cells and their endospores are killed in about 15 min.	Microbiological media, solutions, linens, utensils, dressings, equipment, and other items that can withstand temperature and pressure
2. Pasteurization	Protein denaturation	Heat treatment for milk (72°C for about 15 sec) that kills all pathogens and most nonpathogens.	Milk, cream, and certain alcoholic beverages (beer and wine)
3. Dry heat			
a. Direct flaming	Burning contaminants to ashes	Very effective method of sterilization.	Inoculating loops
b. Incineration	Burning to ashes	Very effective method of sterilization.	Paper cups, contaminated dressings, animal carcasses, bags, and wipes
c. Hot-air sterilization	Oxidation	Very effective method of sterilization but requires temperature of 170°C for about 2 hr.	Empty glassware, instruments, needles, and glass syringes
Filtration	Separation of bacteria from suspending liquid	Removes microbes by passage of a liquid or gas through a screenlike material; most filters in use consist of cellulose acetate or nitrocellulose.	Useful for sterilizing liquids (enzymes, vaccines) that are destroyed by heat
Cold			
1. Refrigeration	Decreased chemical reactions and possible changes in proteins	Has a bacteriostatic effect.	Food, drug, and culture preservation
2. Deep-freezing (see Chapter 6, page 168)	Decreased chemical reactions and possible changes in proteins	An effective method for preserving microbial cultures, in which cultures are quick-frozen between − 50° and − 95°C.	Food, drug, and culture preservation
3. Lyophilization (see Chapter 6, page 168)	Decreased chemical reactions and possible changes in proteins	Most effective method for long-term preservation of microbial cultures; water removed by high vacuum at low temperature.	Food, drug, and culture preservation
High Pressure	Alteration of molecular structure of proteins and carbohydrates	Preserves of colors, flavors, nutrient values.	Fruit juices
Desiccation	Disruption of metabolism	Involves removing water from microbes; primarily bacteriostatic.	Food preservation
Osmotic Pressure	Plasmolysis	Results in loss of water from microbial cells.	Food preservation
Radiation			
1. Ionizing	Destruction of DNA	Not widespread in routine sterilization.	Sterilizing pharmaceuticals and medical and dental supplies
2. Nonionizing	Damage to DNA	Radiation is not very penetrating.	Control of closed environment with UV (germicidal) lamp

7-11 Identify the appropriate uses for surface-active agents.

7-12 List the advantages of glutaraldehyde over other chemical disinfectants.

7-13 Identify chemical sterilizers.

Chemical agents are used to control the growth of microbes on both living tissue and inanimate objects. Unfortunately, few chemical agents achieve sterility; most of them merely reduce microbial populations to safe levels or remove vegetative forms of pathogens from objects. A common problem in disinfection

is the selection of an agent. No single disinfectant is appropriate for all circumstances.

Principles of Effective Disinfection

By reading the label, we can learn a great deal about a disinfectant's properties. Usually the label indicates what groups of organisms the disinfectant is effective against. Remember that the concentration of a disinfectant affects its action, so it should always be diluted exactly as specified by the manufacturer.

Also consider the nature of the material being disinfected. For example, are organic materials present that might interfere with the action of the disinfectant? Similarly, the pH of the medium often has a great effect on a disinfectant's activity.

Another very important consideration is whether the disinfectant will easily make contact with the microbes. An area might need to be scrubbed and rinsed before the disinfectant is applied. In general, disinfection is a gradual process. Thus, to be effective, a disinfectant might need to be left on a surface for several hours.

Evaluating a Disinfectant

Use-Dilution Tests

There is a need to evaluate the effectiveness of disinfectants and antiseptics. The current standard is the American Official Analytical Chemist's **use-dilution test.** Metal or glass cylinders (8 mm × 10 mm) are dipped into standardized cultures of the test bacteria grown in liquid media, removed, and dried at 37°C for a short time. The dried cultures are then placed into a solution of the disinfectant at the concentration recommended by the manufacturer and left there for 10 minutes at 20°C. Following this exposure, the cylinders are transferred to a medium that permits the growth of any surviving bacteria. The effectiveness of the disinfectant can then be determined by the number of cultures that grow.

Variations of this method are used for testing the effectiveness of antimicrobial agents against endospores, mycobacteria that cause tuberculosis, viruses, and fungi, because they are difficult to control with chemicals. Also, tests of antimicrobials intended for special purposes, such as dairy utensil disinfection, may substitute other test bacteria.

The Disk-Diffusion Method

The **disk-diffusion method** is used in teaching laboratories to evaluate the efficacy of a chemical agent. A disk of filter paper is soaked with a chemical and placed on an agar plate that has been previously inoculated and incubated with the test organism. After incubation, if the chemical is effective, a clear zone representing inhibition of growth can be seen around the disk (**Figure 7.6**).

Disks containing antibiotics are commercially available and used to determine microbial susceptibility to antibiotics (see Figure 20.17, page 578).

Types of Disinfectants

Phenol and Phenolics

Lister was the first to use **phenol** (carbolic acid) to control surgical infections in the operating room. Its use had been suggested by its effectiveness in controlling odor in sewage. It is now rarely used as an antiseptic or disinfectant because it irritates the skin and has a disagreeable odor. It is often used in throat lozenges for its local anesthetic effect but has little antimicrobial effect at the low concentrations used. At concentrations above 1% (such as in some throat sprays), however, phenol has a significant antibacterial effect. The structure of a phenol molecule is shown in **Figure 7.7a**.

Derivatives of phenol, called **phenolics,** contain a molecule of phenol that has been chemically altered to reduce its irritating qualities or increase its antibacterial activity in combination with a soap or detergent. Phenolics exert antimicrobial activity by injuring lipid-containing plasma membranes, which results in leakage of cellular contents. The cell wall of mycobacteria, the causes of tuberculosis and leprosy, are rich in lipids, which make them susceptible to phenol derivatives. A useful property of phenolics as disinfectants is that they remain active in the presence of organic compounds, are stable, and persist for long periods after application. For these reasons, phenolics are suitable agents for disinfecting pus, saliva, and feces.

One of the most frequently used phenolics is derived from coal tar, a group of chemicals called *cresols*. A very important cresol is *O-phenylphenol* (see Figure 7.6 and **Figure 7.7b**), the main ingredient in most formulations of Lysol. Cresols are very good surface disinfectants.

Bisphenols

Bisphenols are derivatives of phenol that contain two phenolic groups connected by a bridge (*bis* indicates *two*). One bisphenol, *hexachlorophene* (Figure 7.6 and **Figure 7.7c**), is an ingredient of a prescription lotion, pHisoHex, used for surgical and hospital microbial control procedures. Gram-positive staphylococci and streptococci, which can cause skin infections in newborns, are particularly susceptible to hexachlorophene, so it is often used to control such infections in nurseries. However, excessive use of this bisphenol, such as bathing infants with it several times a day, can lead to neurological damage.

Another widely used bisphenol is *triclosan* (**Figure 7.7d**), an ingredient in antibacterial soaps and at least one toothpaste. Triclosan has even been incorporated into kitchen cutting boards and the handles of knives and other plastic kitchenware. Its use is now so widespread that resistant bacteria have been reported, and concerns about its effect on microbes' resistance to certain antibiotics have been raised. Triclosan inhibits an enzyme needed for the biosynthesis of fatty acids (lipids), which mainly affects the integrity of the plasma membrane. It is especially effective against gram-positive bacteria but also

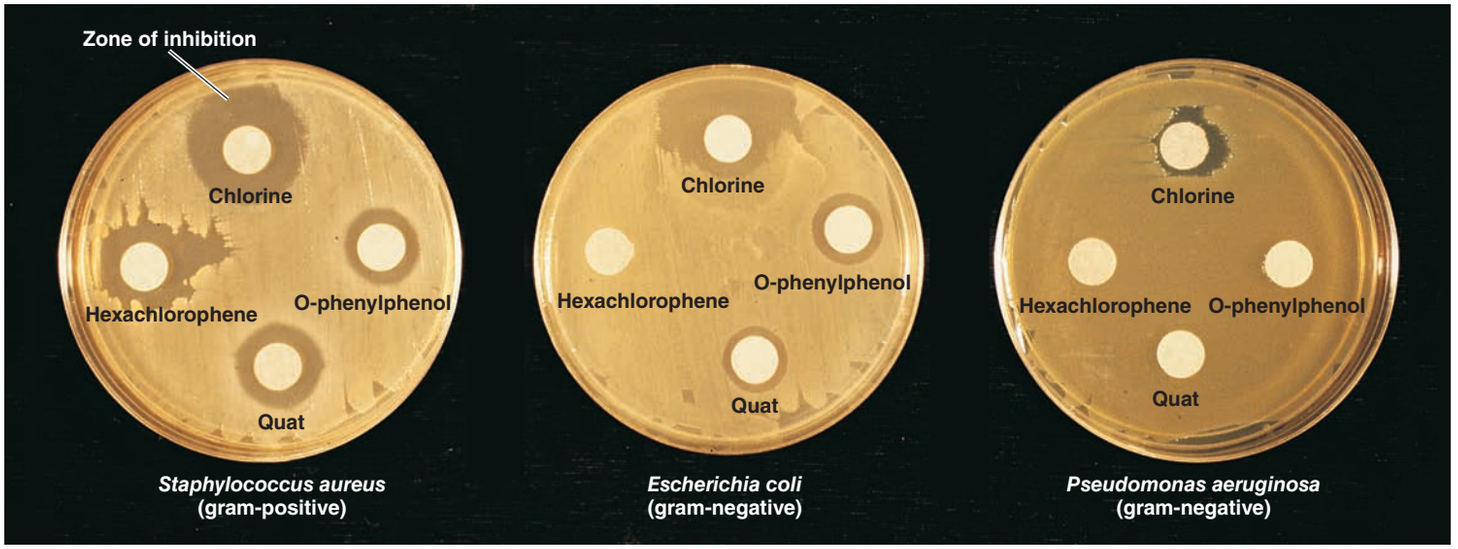


Figure 7.6 Evaluation of disinfectants by the disk-diffusion method. In this experiment, paper disks are soaked in a solution of disinfectant and placed on the surface of a nutrient medium on which a culture of test bacteria has been spread to produce uniform growth.

At the top of each plate, the tests show that chlorine (as sodium hypochlorite) was effective against all the test bacteria but was more effective against gram-positive bacteria.

At the bottom row of each plate, the tests show that the quaternary ammonium compound ("quat") was also more effective against the gram-positive bacteria, but it did not affect the pseudomonads at all.

At the left side of each plate, the tests show that hexachlorophene was effective against gram-positive bacteria only.

At the right sides, O-phenylphenol was ineffective against pseudomonads but was almost equally effective against the gram-positive bacteria and the gram-negative bacteria.

All four chemicals worked against the gram-positive test bacteria, but only one of the four chemicals affected pseudomonads.

Q Why are the pseudomonads less affected by the four chemicals shown in the figure?

works well against yeasts and gram-negative bacteria. There are certain exceptions, such as *Pseudomonas aeruginosa,* a gram-negative bacterium that is very resistant to triclosan, as well as to many other antibiotics and disinfectants (see the discussions on pages 307, 415, and 596).

Biguanides

Biguanides have a broad spectrum of activity, with a mode of action primarily affecting bacterial cell membranes. They are especially effective against gram-positive bacteria. Biguanides are also effective against gram-negative bacteria, with the significant exception of most pseudomonads. Biguanides are not sporicidal but have some activity against enveloped viruses. The best known biguanide is *chlorhexidine,* which is frequently used for microbial control on skin and mucous membranes. Combined with a detergent or alcohol, chlorhexidine is very often used for surgical hand scrubs and preoperative skin preparation in patients. *Alexidine* is a similar biguanide and is more rapid in its action than chlorhexidine. Eventually, alexidine is expected to replace Betadine in many applications see below.

Halogens

The **halogens,** particularly iodine and chlorine, are effective antimicrobial agents, both alone and as constituents of inorganic or organic compounds. *Iodine* (I_2) is one of the oldest and most effective antiseptics. It is active against all kinds of bacteria, many endospores, various fungi, and some viruses. Iodine impairs protein synthesis and alters cell membranes, apparently by forming complexes with amino acids and unsaturated fatty acids.

Iodine is available as a **tincture**—that is, in solution in aqueous alcohol—and as an iodophor. An **iodophor** is a combination of iodine and an organic molecule, from which the iodine is released slowly. Iodophors have the antimicrobial activity of iodine, but they do not stain and are less irritating. The most common

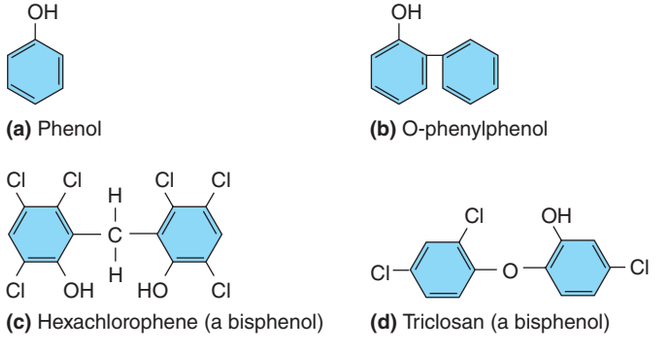


Figure 7.7 The structure of phenolics and bisphenols.

Q Some lozenges intended to alleviate the symptoms of a sore throat contain phenol. Why include this ingredient?

TABLE 7.6 **Biocidal Action of Various Concentrations of Ethanol in Aqueous Solution against *Streptococcus pyogenes***

Concentration of Ethanol (%)	Time of Exposure (sec)				
	10	20	30	40	50
100	G	G	G	G	G
95	NG	NG	NG	NG	NG
90	NG	NG	NG	NG	NG
80	NG	NG	NG	NG	NG
70	NG	NG	NG	NG	NG
60	NG	NG	NG	NG	NG
50	G	G	NG	NG	NG
40	G	G	G	G	G

Note:
G = growth
NG = no growth

commercial preparation is Betadine, which is a *povidone-iodine.* Povidone is a surface-active iodophor that improves the wetting action and serves as a reservoir of free iodine. Iodines are used mainly for skin disinfection and wound treatment. Many campers are familiar with using iodine for water treatment.

Chlorine (Cl_2), as a gas or in combination with other chemicals, is another widely used disinfectant. Its germicidal action is caused by the hypochlorous acid (HOCl) that forms when chlorine is added to water:

(1)

$$\underset{\text{Chlorine}}{Cl_2} + \underset{\text{Water}}{H_2O} \rightleftharpoons \underset{\text{Hydrogen ion}}{H^+} + \underset{\text{Chloride ion}}{Cl^-} + \underset{\text{Hypochlorous acid}}{HOCl}$$

(2)

$$\underset{\text{Hypochlorous acid}}{HOCl} \rightleftharpoons \underset{\text{Hydrogen ion}}{H^+} + \underset{\text{Hypochlorite ion}}{OCl^-}$$

Hypochlorous acid is a strong oxidizing agent that prevents much of the cellular enzyme system from functioning. Hypochlorous acid is the most effective form of chlorine because it is neutral in electrical charge and diffuses as rapidly as water through the cell wall. Because of its negative charge, the hypochlorite ion (OCl^-) cannot enter the cell freely.

A liquid form of compressed chlorine gas is used extensively for disinfecting municipal drinking water, water in swimming pools, and sewage. Several compounds of chlorine are also effective disinfectants. For example, solutions of *calcium hypochlorite* [$Ca(OCl)_2$] are used to disinfect dairy equipment and restaurant eating utensils. This compound, once called chloride of lime, was used as early as 1825, long before the concept of a germ theory for disease, to soak hospital dressings in Paris hospitals. It was also the disinfectant used in the 1840s by Semmelweis to control hospital infections during childbirth, as mentioned in Chapter 1, page 11. Another chlorine compound, *sodium hypochlorite* (NaOCl; see Figure 7.6), is used as a household disinfectant and bleach (Clorox) and as a disinfectant in dairies, food-processing establishments, and hemodialysis systems. When the quality of drinking water is in question, household bleach can provide a rough equivalent of municipal chlorination. After two drops of bleach are added to a liter of water (four drops if the water is cloudy) and the mixture has sat for 30 minutes, the water is considered safe for drinking under emergency conditions.

The food-processing industry makes wide use of chlorine dioxide solution as a surface disinfectant because it does not leave residual tastes or odors. As a disinfectant, it has a broad spectrum of activity against bacteria and viruses and at high concentrations is even effective against cysts and endospores. At low concentrations, chlorine dioxide can be used as an antiseptic. (Also, see page 198 for the use of chlorine dioxide as a sterilant and disinfectant.)

An important group of chlorine compounds are the *chloramines,* combinations of chlorine and ammonia. Most municipal water-treatment systems mix ammonia with chlorine to form chloramines. (Chloramines are toxic to aquarium fish, but pet shops sell chemicals to neutralize them.) U.S. military forces in the field are issued tablets (Chlor-Floc) that contain *sodium dichloroisocyanurate,* a chloramine combined with an agent that flocculates (coagulates) suspended materials in a water sample, causing them to settle out, clarifying the water. Chloramines are also used to sanitize glassware and eating utensils and to treat dairy and food-manufacturing equipment. They are relatively stable compounds that release chlorine over long periods. Chloramines are relatively effective in organic matter but have the disadvantages of acting more slowly and being less effective than hypochlorite.

Alcohols

Alcohols effectively kill bacteria and fungi but not endospores and nonenveloped viruses. The mechanism of action of alcohol is usually protein denaturation, but alcohol can also disrupt membranes and dissolve many lipids, including the lipid component of enveloped viruses. Alcohols have the advantage of acting and then evaporating rapidly and leaving no residue. When the skin is swabbed (degermed) before an injection, most of the microbial control activity comes from simply wiping away dirt and microorganisms, along with skin oils. However, alcohols are unsatisfactory antiseptics when applied to wounds. They cause coagulation of a layer of protein under which bacteria continue to grow.

Two of the most commonly used alcohols are ethanol and isopropanol. The recommended optimum concentration of *ethanol*

is 70%, but concentrations between 60% and 95% seem to kill as well (**Table 7.6**). Pure ethanol is less effective than aqueous solutions (ethanol mixed with water) because denaturation requires water. *Isopropanol,* often sold as rubbing alcohol, is slightly superior to ethanol as an antiseptic and disinfectant. Moreover, it is less volatile, less expensive, and more easily obtained than ethanol.

Washing hands with soap and water is an effective sanitation method. Use soap and *warm* water (if possible), and rub hands together for 20 seconds (imagine singing "Happy Birthday" twice through). Then rinse, dry with a paper towel or air dryer, and try to use a paper towel to turn off the faucet. Alcohol-based (about 62% alcohol) hand sanitizers such as Purell and Germ-X are very popular for use when hands are not visibly soiled. Rub the product over the surfaces of the hands and fingers until they are dry. Claims that products will kill 99.9% of germs should be viewed with caution; such effectiveness is seldom reached under typical user's conditions. Also, certain pathogens, such as the spore-forming *Clostridium difficile* and viruses that lack a lipid envelope, are comparatively resistant to alcohol-based hand sanitizers.

Ethanol and isopropanol are often used to enhance the effectiveness of other chemical agents. For example, an aqueous solution of Zephiran (described on page 196) kills about 40% of the population of a test organism in 2 minutes, whereas a tincture of Zephiran kills about 85% in the same period. To compare the effectiveness of tinctures and aqueous solutions, see Figure 7.10 on page 196.

Heavy Metals and Their Compounds

Several heavy metals can be biocidal or antiseptic, including silver, mercury, and copper. The ability of very small amounts of heavy metals, especially silver and copper, to exert antimicrobial activity is referred to as **oligodynamic action** (*oligo* means few). Centuries ago, Egyptians found that putting silver coins in water barrels served to keep the water clean of unwanted organic growths. This action can be seen when we place a coin or other clean piece of metal containing silver or copper on a culture on an inoculated Petri plate. Extremely small amounts of metal diffuse from the coin and inhibit the growth of bacteria for some distance around the coin (**Figure 7.8**). This effect is produced by the action of heavy metal ions on microbes. When the metal ions combine with the sulfhydryl groups on cellular proteins, denaturation results.

Silver is used as an antiseptic in a 1% *silver nitrate* solution. At one time, many states required that the eyes of newborns be treated with a few drops of silver nitrate to guard against an infection of the eyes called ophthalmia neonatorum, which the infants might have contracted as they passed through the birth canal. In recent years, antibiotics have replaced silver nitrate for this purpose.

Recently, there has been renewed interest in the use of silver as an antimicrobial agent. Silver-impregnated dressings that slowly release silver ions have proven especially useful against antibiotic-resistant bacteria. The enthusiasm for incorporating silver in all manner of consumer products is increasing. Among the newer products being sold are plastic food containers

Figure 7.8 Oligodynamic action of heavy metals. Clear zones where bacterial growth has been inhibited are seen around the sombrero charm (pushed aside), the dime, and the penny. The charm and the dime contain silver; the penny contains copper.

Q The coins used in this demonstration were minted many years ago; why were more contemporary coins not used?

infused with silver nanoparticles, which are intended to keep food fresher, and silver-infused athletic shirts and socks, which are claimed to minimize odors.

A combination of silver and the drug sulfadiazine, *silver-sulfadiazine,* is the most common formulation. It is available as a topical cream for use on burns. Silver can also be incorporated into indwelling catheters, which are a common source of hospital infections, and in wound dressings. *Surfacine* is a relatively new antimicrobial for application to surfaces, either animate or inanimate. It contains water-insoluble silver iodide in a polymer carrier and is very persistent, lasting at least 13 days. When a bacterium contacts the surface, the cell's outer membrane is recognized, and a lethal amount of silver ions is released.

Inorganic mercury compounds, such as *mercuric chloride,* have a long history of use as disinfectants. They have a very broad spectrum of activity; their effect is primarily bacteriostatic. However, their use is now limited because of their toxicity, corrosiveness, and ineffectiveness in organic matter. At present, the primary use of mercurials is to control mildew in paints.

Copper in the form of *copper sulfate* or other copper-containing additives is used chiefly to destroy green algae (algicide) that grow in reservoirs, stock ponds, swimming pools, and fish tanks. If the water does not contain excessive organic matter, copper compounds are effective in concentrations of one part per million of water. To prevent mildew, copper compounds such as *copper 8-hydroxyquinoline* are sometimes included in paint. In the nineteenth century, the wine regions of Europe were plagued by fungal diseases that affected the grapevines.

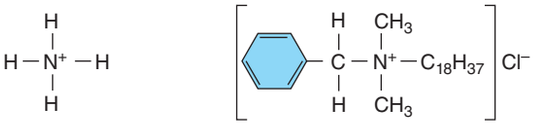


Figure 7.9 The ammonium ion and a quaternary ammonium compound, benzalkonium chloride (Zephiran). Notice how other groups replace the hydrogens of the ammonium ion.

Q Are quats most effective against gram-positive or gram-negative bacteria?

It was observed that vines near the road were less affected than those further afield. The reason was that these roadside vines had been sprayed with a mixture of copper sulfate and lime (both visible and bitter to the taste) to deter passers-by on the road from eating the grapes. Because of this chance observation, mixtures based on copper ions (known as Bordeaux mixture) have long been used to control fungal diseases of plants.

Long term use of alcohol-based hand sanitizers often causes problems with skin dryness. A relatively new hand sanitizer, Xgel, does not contain alcohol but uses copper contained in a skin lotion formulation. Xgel may be more effective as an antimicrobial than alcohol-based hand sanitizers.

Another metal used as an antimicrobial is zinc. The effect of trace amounts of zinc can be seen on weathered roofs of buildings down-slope from galvanized (zinc-coated) fittings. The roof is lighter-colored where biological growth, mostly algae, is impeded. Copper- and zinc-treated shingles are available. *Zinc chloride* is a common ingredient in mouthwashes, and *zinc pyrithione* is an ingredient in antidandruff shampoos.

Surface-Active Agents

Surface-active agents, or **surfactants,** can decrease surface tension among molecules of a liquid. Such agents include soaps and detergents.

Soaps and Detergents Soap has little value as an antiseptic, but it does have an important function in the mechanical removal of microbes through scrubbing. The skin normally contains dead cells, dust, dried sweat, microbes, and oily secretions from oil glands. Soap breaks the oily film into tiny droplets, a process called *emulsification,* and the water and soap together lift up the emulsified oil and debris and float them away as the lather is washed off. In this sense, soaps are good degerming agents.

Acid-Anionic Sanitizers *Acid-anionic* surface-active sanitizers are very important in cleaning dairy utensils and equipment. Their sanitizing ability is related to the negatively charged portion (anion) of the molecule, which reacts with the plasma membrane. These sanitizers, which act on a wide spectrum of microbes, including troublesome thermoduric bacteria, are nontoxic, noncorrosive, and fast acting.

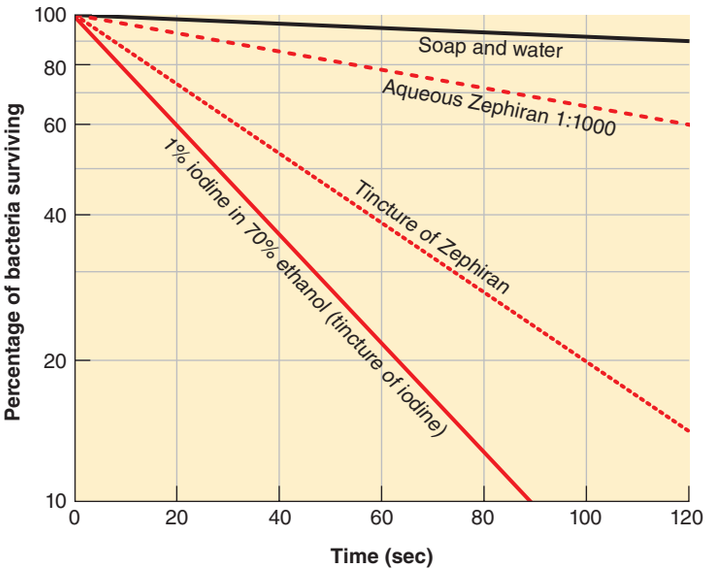


Figure 7.10 A comparison of the effectiveness of various antiseptics. The steeper the downward slope of the killing curve, the more effective the antiseptic is. A 1% iodine in 70% ethanol solution is the most effective; soap and water are the least effective. Notice that a tincture of Zephiran is more effective than an aqueous solution of the same antiseptic.

Q Why is the tincture of Zephiran more effective than the aqueous solution?

Quaternary Ammonium Compounds (Quats) The most widely used surface-active agents are the cationic detergents, especially the **quaternary ammonium compounds (quats).** Their cleansing ability is related to the positively charged portion—the cation—of the molecule. Their name is derived from the fact that they are modifications of the four-valence ammonium ion, NH_4^+ (**Figure 7.9**). Quaternary ammonium compounds are strongly bactericidal against gram-positive bacteria and less active against gram-negative bacteria (see Figure 7.6).

Quats are also fungicidal, amebicidal, and virucidal against enveloped viruses. They do not kill endospores or mycobacteria. (See the box on page 198.) Their chemical mode of action is unknown, but they probably affect the plasma membrane. They change the cell's permeability and cause the loss of essential cytoplasmic constituents, such as potassium.

Two popular quats are Zephiran, a brand name of *benzalkonium chloride* (see Figure 7.9), and Cepacol, a brand name of *cetylpyridinium chloride.* They are strongly antimicrobial, colorless, odorless, tasteless, stable, easily diluted, and nontoxic, except at high concentrations. If your mouthwash bottle fills with foam when shaken, the mouthwash probably contains a quat. However, organic matter interferes with their activity, and they are rapidly neutralized by soaps and anionic detergents.

Anyone involved in medical applications of quats should remember that certain bacteria, such as some species of *Pseudomonas,* not only survive in quaternary ammonium compounds but

actively grow in them. These microbes are resistant not only to the disinfectant solution but also to gauze and bandages moistened with it, because the fibers tend to neutralize the quats.

Before we move on to the next group of chemical agents, refer to **Figure 7.10**, which compares the effectiveness of some of the antiseptics we have discussed so far.

Chemical Food Preservatives

Chemical preservatives are frequently added to foods to retard spoilage. *Sulfur dioxide* (SO_2) has long been used as a disinfectant, especially in wine-making. Homer's *Odyssey,* written nearly 2800 years ago, mentions its use. Among the more common additives are sodium benzoate, sorbic acid, and calcium propionate. These chemicals are simple organic acids, or salts of organic acids, which the body readily metabolizes and which are generally judged to be safe in foods. *Sorbic acid,* or its more soluble salt *potassium sorbate,* and *sodium benzoate* prevent molds from growing in certain acidic foods, such as cheese and soft drinks. Such foods, usually with a pH of 5.5 or lower, are most susceptible to spoilage by molds. *Calcium propionate,* an effective fungistat used in bread, prevents the growth of surface molds and the *Bacillus* bacterium that causes ropy bread. These organic acids inhibit mold growth, not by affecting the pH but by interfering with the mold's metabolism or the integrity of the plasma membrane.

Sodium nitrate and *sodium nitrite* are added to many meat products, such as ham, bacon, hot dogs, and sausage. The active ingredient is sodium nitrite, which certain bacteria in the meats can also produce from sodium nitrate. These bacteria use nitrate as a substitute for oxygen under anaerobic conditions. The nitrite has two main functions: to preserve the pleasing red color of the meat by reacting with blood components in the meat, and to prevent the germination and growth of any botulism endospores that might be present. Nitrite selectively inhibits certain iron-containing enzymes of *Clostridium botulinum.* There has been some concern that the reaction of nitrites with amino acids can form certain carcinogenic products known as **nitrosamines,** and the amount of nitrites added to foods has generally been reduced recently for this reason. However, the use of nitrites continues because of their established value in preventing botulism. Because nitrosamines are formed in the body from other sources, the added risk posed by a limited use of nitrates and nitrites in meats is lower than was once thought.

Antibiotics

The antimicrobials discussed in this chapter are not useful for ingestion or injection to treat disease. Antibiotics are used for this purpose. The use of antibiotics is highly restricted; however, at least two have considerable use in food preservation. Neither is of value for clinical purposes. *Nisin* is often added to cheese to inhibit the growth of certain endospore-forming spoilage bacteria. It is an example of a bacteriocin, a protein that is produced by one bacterium and inhibits another (see Chapter 8, page 235). Nisin is present naturally in small amounts in many dairy products. It is tasteless, readily digested, and nontoxic. *Natamycin* (pimaricin) is an antifungal antibiotic approved for use in foods, mostly cheese.

Aldehydes

Aldehydes are among the most effective antimicrobials. Two examples are formaldehyde and glutaraldehyde. They inactivate proteins by forming covalent cross-links with several organic functional groups on proteins (—NH_2, —OH, —COOH, and —SH). *Formaldehyde gas* is an excellent disinfectant. However, it is more commonly available as *formalin,* a 37% aqueous solution of formaldehyde gas. Formalin was once used extensively to preserve biological specimens and inactivate bacteria and viruses in vaccines.

Glutaraldehyde is a chemical relative of formaldehyde that is less irritating and more effective than formaldehyde. Glutaraldehyde is used to disinfect hospital instruments, including endoscopes and respiratory therapy equipment, but they must be carefully cleaned first. When used in a 2% solution (Cidex), it is bactericidal, tuberculocidal, and virucidal in 10 minutes and sporicidal in 3 to 10 hours. Glutaraldehyde is one of the few liquid chemical disinfectants that can be considered a sterilizing agent. However, 30 minutes is often considered the maximum time allowed for a sporicide to act, which is a criterion glutaraldehyde cannot meet. Both glutaraldehyde and formalin are used by morticians for embalming.

A possible replacement for glutaraldehyde for many uses is *ortho-phthalaldehyde* (OPA), which is more effective against many microbes and has fewer irritating properties.

Clinical Case

Norovirus, a nonenveloped virus, is one cause of acute gastroenteritis. It can be spread by consuming fecally contaminated food or water, coming in direct contact with an infected person, or touching a contaminated surface. Amy is able to rule out foodborne transmission immediately. The small private school does not have a school lunch program; all students and staff bring their lunches from home. After meeting with the principal, Amy speaks to the custodial staff and directs them to use a quat to clean the school. She asks them to pay special attention to areas with high potential for fecal contamination, especially toilet seats, flush handles, toilet stall inner door handles, and restroom door inner handles. Amy is sure she has avoided a major outbreak, but by Friday, 42 students and six more staff members call in to report similar symptoms.

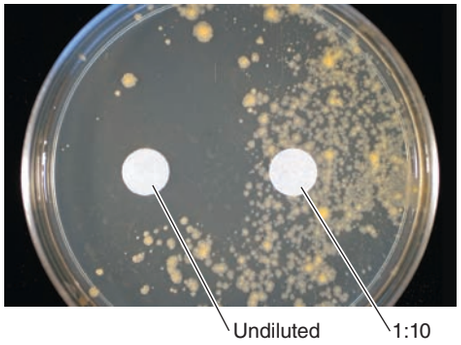
Why didn't the quat work to kill the virus?

182 **197** 199 201

Infection Following Steroid Injection

As you read through this box, you will encounter a series of questions that infection control officers ask themselves as they track the source of infection. Try to answer each question before going on to the next one.

1. Dr. Priya Agarwal, an infectious disease physician, called the department of health to report that in the last 3 months, she had seen 12 patients with *Mycobacterium abscessus* joint and soft-tissue infections. Slow-growing mycobacteria, including *M. tuberculosis* and *M. leprae,* are common human pathogens, but Dr. Agarwal was concerned because these infections were caused by rapidly growing mycobacteria (RGM).
   Where are these RGM normally found? (Hint: Read page 319.)
2. RGM are usually found in soil and water. In Dr. Agarwal's report, she noted that all 12 patients received injections for arthritis from the same physician. The injection procedure consisted of cleaning the skin with cotton balls soaked in diluted (1:10) Zephiran, painting the skin with commercially prepared iodine swabs, anesthetizing the area with 0.5 ml of 1% lidocaine in a sterile 22-gauge needle and syringe, and injecting 0.5–1.0 ml of betamethasone, a cortisone-like steroid, into the joint with a sterile 20-gauge needle and syringe.
   What did Dr. Agarwal need to do to determine the source of the infection?
3. Dr. Agarwal ordered cultures from the inside surface of an open metal container of forceps and the inside surface of a metal container of cotton balls. She also requested cultures of the iodine prep swabs, Zephiran-soaked cotton balls, solutions of lidocaine and betamethasone, diluted and undiluted Zephiran, and a sealed bottle of distilled water used to dilute the Zephiran.
   What type of disinfectant is Zephiran?
4. Zephiran is a quat. Results of cultures from the laboratory showed that only the cotton balls soaked in Zephiran had grown *M. abscessus*. Dr. Agarwal then performed a disk-diffusion assay (see the figure above) of both diluted and undiluted Zephiran.
   What did the assay reveal? What should Dr. Agarwal tell the physician about preventing future infections?



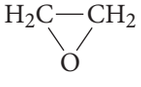
Disk-diffusion test of Zephiran against *M. abscessus*.

5. Recent research has shown that soaking cotton balls in disinfectant selects for resistant bacteria. Additionally, the disinfecting ability of Zephiran and other quats is reduced by the presence of organic material, such as cotton balls. Dr. Agarwal informed the physician that cotton balls should not be stored in the disinfectant; doing so can cause patients to be inoculated with bacteria.

Source: Adapted from *Clinical Infectious Diseases* 43:823–830 (2006).

Chemical Sterilization

Sterilization with liquid chemicals is possible, but even sporicidal chemicals such as glutaraldehyde are usually not considered to be practical sterilants. However, the gaseous chemosterilants are frequently used as substitutes for physical sterilization processes. Their application requires a closed chamber similar to a steam autoclave. Probably the most familiar example is *ethylene oxide:*



Its activity depends on *alkylation,* that is, replacing the proteins' labile hydrogen atoms in a chemical group (such as —SH, —COOH, or —CH_2CH_2OH) with a chemical radical. This leads to cross-linking of nucleic acids and proteins and inhibits its vital cellular functions. Ethylene oxide kills all microbes and endospores but requires a lengthy exposure period of several hours. It is toxic and explosive in its pure form, so it is usually mixed with a nonflammable gas, such as carbon dioxide. Among its advantages is that it carries out sterilization at ambient temperatures and it is highly penetrating. Larger hospitals often are able to sterilize even mattresses in special ethylene oxide sterilizers.

Chlorine dioxide is a short-lived gas that is usually manufactured at the place of use. Notably, it has been used to fumigate enclosed building areas contaminated with endospores of anthrax. It is much more stable in aqueous solution. Its most common use is in water treatment prior to chlorination, where its purpose is to remove, or reduce the formation of, certain carcinogenic compounds sometimes formed in the chlorination of water.

Plasmas

In addition to the traditional three states of matter—liquid, gas, and solid—there might be considered to exist a fourth state of matter, plasma. **Plasma** is a state of matter in which a gas is excited, in this case by an electromagnetic field, to make a mixture of nuclei with assorted electrical charges and free electrons. Health care facilities are increasingly facing the challenge of sterilizing metal or plastic surgical instruments used for many newer procedures in arthroscopic or laparoscopic surgery. Such devices have long, hollow tubes, many with an interior diameter of only a few millimeters, and are difficult to sterilize. *Plasma sterilization* is a

reliable method for this. The instruments are placed in a container in which a combination of a vacuum, electromagnetic field, and chemicals such as hydrogen peroxide (sometimes with peracetic acid, as well) form the plasma. Such plasmas have many free radicals that quickly destroy even endospore-forming microbes. The advantage of plasma sterilization, which has elements of both physical and chemical sterilization, is that it requires only low temperatures, but it is relatively expensive.

Supercritical Fluids

The use of supercritical fluids in sterilization combines chemical and physical methods. When carbon dioxide is compressed into a "supercritical" state, it has properties of both a liquid (with increased solubility) and a gas (with a lowered surface tension). Organisms exposed to *supercritical carbon dioxide* are inactivated, including most vegetative organisms that cause spoilage and foodborne pathogens. Even endospore inactivation requires a temperature of only about 45°C. Used for a number of years in treating certain foods, supercritical carbon dioxide has more recently been used to decontaminate medical implants, such as bone, tendons, or ligaments taken from donor patients.

Peroxygens and Other Forms of Oxygen

Peroxygens are a group of oxidizing agents that includes hydrogen peroxide and peracetic acid.

Hydrogen peroxide is an antiseptic found in many household medicine cabinets and in hospital supply rooms. It is not a good antiseptic for open wounds. It is quickly broken down to water and gaseous oxygen by the action of the enzyme catalase, which is present in human cells (see Chapter 6, page 160). However, hydrogen peroxide does effectively disinfect inanimate objects; in such applications, it is even sporicidal at high concentrations. On a nonliving surface, the normally protective enzymes of aerobic bacteria and facultative anaerobes are overwhelmed by high concentrations of peroxide. Because of these factors, and its rapid degradation into harmless water and oxygen, the food industry is increasing its use of hydrogen peroxide for aseptic packaging (see Figure 28.4 on page 802). The packaging material passes through a hot solution of the chemical before being assembled into a container. In addition, many wearers of contact lenses are familiar with hydrogen peroxide's use as a disinfectant. After the lens is disinfected, a platinum catalyst in the lens-disinfecting kit destroys residual hydrogen peroxide so that it does not persist on the lens, where it might cause eye irritation.

Heated, gaseous hydrogen peroxide can be used as a sterilant of atmosphere and surfaces. Hospital rooms, for example, can be decontaminated quickly and routinely with equipment available under the brand name Bioquell. The room is sealed with the generating apparatus inside and the controls on the outside. Once the sealed room has undergone a decontamination cycle, the hydrogen peroxide vapor is catalytically converted into water vapor and oxygen.

Peracetic acid (*peroxyactic acid,* or *PAA*) is one of the most effective liquid chemical sporicides available and can be used as a sterilant. Its mode of action is similar to that of hydrogen peroxide. It is generally effective on endospores and viruses within 30 minutes and kills vegetative bacteria and fungi in less than 5 minutes. PAA has many applications in the disinfection of food-processing and medical equipment, especially endoscopes, because it leaves no toxic residues (only water and small amounts of acetic acid) and is minimally affected by the presence of organic matter. The FDA has approved use of PAA for the washing of fruits and vegetables.

Other oxidizing agents include *benzoyl peroxide*, which is probably most familiar as the main ingredient in over-the-counter medications for acne. *Ozone (O_3)* is a highly reactive form of oxygen that is generated by passing oxygen through high-voltage electrical discharges (see Figure 27.16, page 789). It is responsible for the air's rather fresh odor after a lightning storm, in the vicinity of electrical sparking, or around an ultraviolet light. Ozone is often used to supplement chlorine in the disinfection of water because it helps neutralize tastes and odors. Although ozone is a more effective killing agent than chlorine, its residual activity is difficult to maintain in water.

Clinical Case

Quats are virucidal against enveloped viruses. By, Monday, a total of 103 out of 266 staff and students call in sick with vomiting and diarrhea. With almost half the school either out sick or returning to school after being sick, Amy decides to call the Maryland State Health Department. After going over her records with a health department statistician, she finds out that the most significant risk factors for infection are contact with an ill person or being in the first grade. All but five first-graders have reported sick with diarrheal illness. Because the school is so small, the first-grade classroom also houses the computer lab for the school. Both students and staff share these computers. The health department sends someone to swab the first-grade classroom, and norovirus is cultured from a computer mouse.

How did the virus get from a computer mouse in the first grade classroom to all of the other grades and staff?

182 197 **199** 201

CHECK YOUR UNDERSTANDING

- If you wanted to disinfect a surface contaminated by vomit and a surface contaminated by a sneeze, why would your choice of disinfectant make a difference? **7-7**
- Which is more likely to be used in a medical clinic laboratory, a use-dilution test or a disk-diffusion test? **7-8**
- Why is alcohol effective against some viruses and not others? **7-9**
- Is Betadine an antiseptic or a disinfectant when it is used on skin? **7-10**
- What characteristics make surface-active agents attractive to the dairy industry? **7-11**
- What chemical disinfectants can be considered sporicides? **7-12**
- What chemicals are used to sterilize? **7-13**

Microbial Characteristics and Microbial Control

LEARNING OBJECTIVE

7-14 Explain how the type of microbe affects the control of microbial growth.

Many biocides tend to be more effective against gram-positive bacteria, as a group, than against gram-negative bacteria. This principle is illustrated in **Figure 7.11**, which presents a simplified hierarchy of relative resistance of major microbial groups to biocides. A principal factor in this relative resistance to biocides is the external lipopolysaccharide layer of gram-negative bacteria. Within gram-negative bacteria, members of the genera *Pseudomonas* and *Burkholderia* are of special interest. These closely related bacteria are unusually resistant to biocides (see Figure 7.6) and will even grow actively in some disinfectants and antiseptics, most notably the quaternary ammonium compounds. In Chapter 20, you will see that these bacteria are also resistant to many antibiotics. This resistance to chemical antimicrobials is related mostly to the characteristics of their *porins* (structural openings in the wall of gram-negative bacteria; see Figure 4.13c, page 85). Porins are highly selective of molecules that they permit to enter the cell.

The mycobacteria are another group of non–endospore-forming bacteria that exhibit greater than normal resistance to chemical biocides. (See the box on page 198.) This group includes *Mycobacterium tuberculosis,* the pathogen that causes tuberculosis. The cell wall of this organism and other members of this genus have a waxy, lipid-rich component. Instruction labels on disinfectants often state whether they are tuberculocidal, indicating that they are effective against mycobacteria. Special tuberculocidal tests have been developed to evaluate the effectiveness of biocides against this bacterial group.

Bacterial endospores are affected by relatively few biocides. (The activity of the major chemical antimicrobial groups against mycobacteria and endospores is summarized in **Table 7.7**.) The cysts and oocysts of protozoa are also relatively resistant to chemical disinfection.

The resistance of viruses to biocides largely depends on the presence or absence of an envelope. Antimicrobials that are lipid-soluble

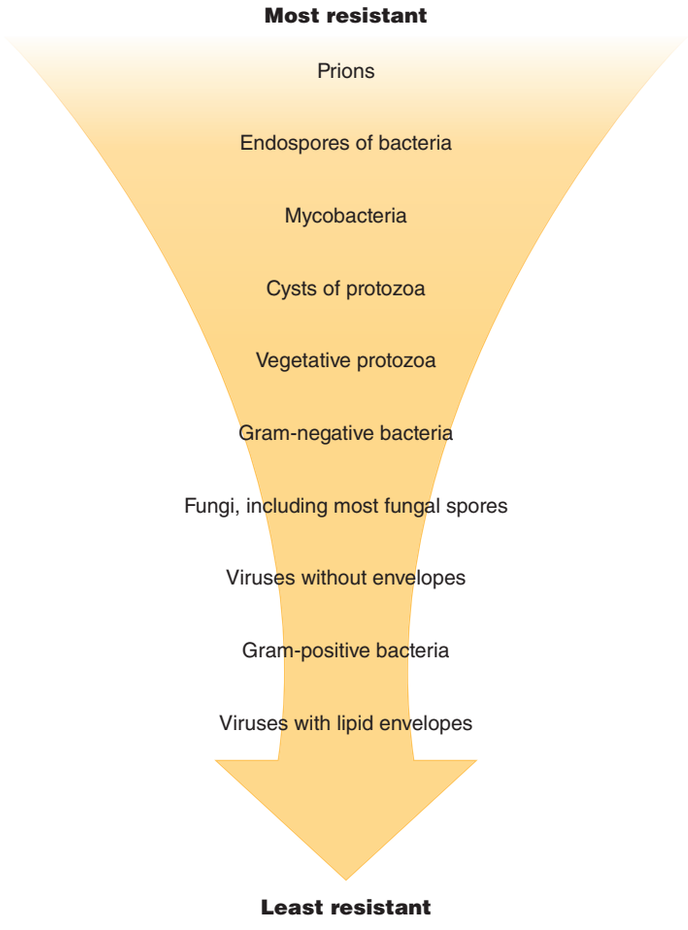


Figure 7.11 **Decreasing order of resistance of microorganisms to chemical biocides**.

Q Why are viruses with lipid-containing envelopes relatively susceptible to certain biocides?

are more likely to be effective against enveloped viruses. The label of such an agent will indicate that it is effective against lipophilic viruses. Nonenveloped viruses, which have only a protein coat, are more resistant—fewer biocides are active against them.

A special problem, not yet completely solved, is the reliable killing of prions. Prions are infectious proteins that are the cause of neurological diseases known as spongiform encephalopathies, such as the popularly named mad cow disease (see Chapter 22, page 637). To destroy prions, infected animal carcasses are incinerated. A major problem is the disinfection of surgical instruments exposed to prion contamination. Normal autoclaving has proven to be inadequate. The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) have recommended the combined use of a solution of sodium hydroxide and autoclaving at 134°C. Recent reports indicate that surgical instruments have been successfully treated to inactivate prions, which are proteins, by addition of protease enzymes to the cleaning solution. Surgeons sometimes resort to using disposable instruments.

TABLE 7.7 The Effectiveness of Chemical Antimicrobials against Endospores and Mycobacteria

Chemical Agent	Endospores	Mycobacteria
Mercury	No activity	No activity
Phenolics	Poor	Good
Bisphenols	No activity	No activity
Quats	No activity	No activity
Chlorines	Fair	Fair
Iodine	Poor	Good
Alcohols	Poor	Good
Glutaraldehyde	Fair	Good
Chlorhexidine	No activity	Fair

In summary, it is important to remember that microbial control methods, especially biocides, are not uniformly effective against all microbes.

Table 7.8 summarizes chemical agents used to control microbial growth.

CHECK YOUR UNDERSTANDING

- The presence or absence of endospores has an obvious effect on microbial control, but why are gram-negative bacteria more resistant to chemical biocides than gram-positive bacteria? **7-14**

Clinical Case Resolved

Norovirus is an extremely contagious virus and can spread quickly from person to person. It is also nonenveloped, so it cannot be easily destroyed by a biocide. Amy asks the principal if, once the school is back to full capacity, she can hold an assembly to discuss the importance of handwashing with the students and staff. Proper washing with soap and water can eliminate the transmission of norovirus to other people or surfaces. Amy also meets again with the custodial staff to discuss the health department's recommendations. According to the health department, when cleaning environmental surfaces that are visibly soiled with feces or vomitus, the staff should wear masks and gloves, use a disposable towel that has been soaked in dilute detergent to wipe the surface for at least 10 seconds, and then apply a 1:10 household bleach solution for at least 1 minute. Although Amy knows this won't be the last time her school is affected by a virus, she is certain she has taken a positive step toward protecting her students and staff from this particular virus.

182 197 199 **201**

The compounds discussed in this chapter are not generally useful in the treatment of diseases. Antibiotics and the pathogens against which they are active will be discussed in Chapter 20.

TABLE 7.8 Chemical Agents Used to Control Microbial Growth

Chemical Agent	Mechanism of Action	Preferred Use	Comment
Phenol and Phenolics			
Phenol	Disruption of plasma membrane, denaturation of enzymes.	Rarely used, except as a standard of comparison.	Seldom used as a disinfectant or antiseptic because of its irritating qualities and disagreeable odor.
Phenolics	Disruption of plasma membrane, denaturation of enzymes.	Environmental surfaces, instruments, skin surfaces, and mucous membranes.	Derivatives of phenol that are reactive even in the presence of organic material; O-phenylphenol is an example.
Bisphenols	Probably disruption of plasma membrane.	Disinfectant hand soaps and skin lotions.	Triclosan is an especially common example of a bisphenol. Broad spectrum, but most effective against gram-positives.
Biguanides (Chlorhexidine)	Disruption of plasma membrane.	Skin disinfection, especially for surgical scrubs.	Bactericidal to gram-positives and gram-negatives; nontoxic, persistent.

(continued)

TABLE 7.8 Chemical Agents Used to Control Microbial Growth *(continued)*

Chemical Agent	Mechanism of Action	Preferred Use	Comment
Halogens	Iodine inhibits protein function and is a strong oxidizing agent; chlorine forms the strong oxidizing agent hypochlorous acid, which alters cellular components.	Iodine is an effective antiseptic available as a tincture and an iodophor; chlorine gas is used to disinfect water; chlorine compounds are used to disinfect dairy equipment, eating utensils, household items, and glassware.	Iodine and chlorine may act alone or as components of inorganic and organic compounds.
Alcohols	Protein denaturation and lipid dissolution.	Thermometers and other instruments. When the skin is swabbed with alcohol before an injection, most of the disinfecting action probably comes from a simple wiping away (degerming) of dirt and some microbes.	Bactericidal and fungicidal, but not effective against endospores or nonenveloped viruses; commonly used alcohols are ethanol and isopropanol.
Heavy Metals and Their Compounds	Denaturation of enzymes and other essential proteins.	Silver nitrate may be used to prevent ophthalmia neonatorum; silver-sulfadiazine is used as a topical cream on burns; copper sulfate is an algicide.	Heavy metals such as silver and mercury are biocidal.
Surface-Active Agents			
Soaps and detergents	Mechanical removal of microbes through scrubbing.	Skin degerming and removal of debris.	Many antibacterial soaps contain antimicrobials.
Acid-anionic sanitizers	Not certain; may involve enzyme inactivation or disruption.	Sanitizers in dairy and food-processing industries.	Wide spectrum of activity; nontoxic, noncorrosive, fast-acting.
Quaternary ammonium compounds (cationic detergents)	Enzyme inhibition, protein denaturation, and disruption of plasma membranes.	Antiseptic for skin, instruments, utensils, rubber goods.	Bactericidal, bacteriostatic, fungicidal, and virucidal against enveloped viruses. Examples of quats are Zephiran and Cepacol.
Chemical Food Preservatives			
Organic acids	Metabolic inhibition, mostly affecting molds; action not related to their acidity.	Sorbic acid and benzoic acid effective at low pH; parabens much used in cosmetics, shampoos; calcium propionate used in bread.	Widely used to control mold and some bacteria in foods and cosmetics.
Nitrates/nitrites	Active ingredient is nitrite, which is produced by bacterial action on nitrate. Nitrite inhibits certain iron-containing enzymes of anaerobes.	Meat products such as ham, bacon, hot dogs, sausage.	Prevents growth of *Clostridium botulinum* in food; also imparts a red color.
Aldehydes	Protein denaturation.	Glutaraldehyde (Cidex) is less irritating than formaldehyde and is used for disinfecting medical equipment.	Very effective antimicrobials.
Chemical Sterilization			
Ethylene oxide and other gaseous sterilants	Inhibits vital cellular functions.	Mainly for sterilization of materials that would be damaged by heat.	Ethylene oxide is the most commonly used. Heated hydrogen peroxide and chlorine dioxide have special uses.
Plasma sterilization	Inhibits vital cellular functions.	Especially useful for tubular medical instruments.	Usually hydrogen peroxide excited in a vacuum by an electromagnetic field.
Supercritical fluids	Inhibits vital cellular functions.	Especially useful for sterilizing organic medical implants.	Carbon dioxide compressed to a supercritical state.
Peroxygens and Other Forms of Oxygen	Oxidation.	Contaminated surfaces; some deep wounds, in which they are very effective against oxygen-sensitive anaerobes.	Ozone is widely used as a supplement for chlorination; hydrogen peroxide is a poor antiseptic but a good disinfectant. Peracetic acid is especially effective.

Study Outline

MasteringMICROBIOLOGY™

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The Terminology of Microbial Control (pp. 182–183)

1. The control of microbial growth can prevent infections and food spoilage.
2. Sterilization is the process of removing or destroying all microbial life on an object.
3. Commercial sterilization is heat treatment of canned foods to destroy *C. botulinum* endospores.
4. Disinfection is the process of reducing or inhibiting microbial growth on a nonliving surface.
5. Antisepsis is the process of reducing or inhibiting microorganisms on living tissue.
6. The suffix *-cide* means to kill; the suffix *-stat* means to inhibit.
7. Sepsis is bacterial contamination.

The Rate of Microbial Death (p. 183)

1. Bacterial populations subjected to heat or antimicrobial chemicals usually die at a constant rate.
2. Such a death curve, when plotted logarithmically, shows this constant death rate as a straight line.
3. The time it takes to kill a microbial population is proportional to the number of microbes.
4. Microbial species and life cycle phases (e.g., endospores) have different susceptibilities to physical and chemical controls.
5. Organic matter may interfere with heat treatments and chemical control agents.
6. Longer exposure to lower heat can produce the same effect as shorter time at higher heat.

Actions of Microbial Control Agents (pp. 183–185)

Alteration of Membrane Permeability (pp. 183–184)

1. The susceptibility of the plasma membrane is due to its lipid and protein components.
2. Certain chemical control agents damage the plasma membrane by altering its permeability.

Damage to Proteins and Nucleic Acids (pp. 184–185)

3. Some microbial control agents damage cellular proteins by breaking hydrogen bonds and covalent bonds.
4. Other agents interfere with DNA and RNA and protein synthesis.

Physical Methods of Microbial Control (pp. 185–190)

Heat (pp. 185–188)

1. Heat is frequently used to kill microorganisms.
2. Moist heat kills microbes by denaturing enzymes.
3. Thermal death point (TDP) is the lowest temperature at which all the microbes in a liquid culture will be killed in 10 minutes.
4. Thermal death time (TDT) is the length of time required to kill all bacteria in a liquid culture at a given temperature.
5. Decimal reduction time (DRT) is the length of time in which 90% of a bacterial population will be killed at a given temperature.
6. Boiling (100°C) kills many vegetative cells and viruses within 10 minutes.
7. Autoclaving (steam under pressure) is the most effective method of moist heat sterilization. The steam must directly contact the material to be sterilized.
8. In HTST pasteurization, a high temperature is used for a short time (72°C for 15 seconds) to destroy pathogens without altering the flavor of the food. Ultra-high-temperature (UHT) treatment (140°C for 4 seconds) is used to sterilize dairy products.
9. Methods of dry heat sterilization include direct flaming, incineration, and hot-air sterilization. Dry heat kills by oxidation.
10. Different methods that produce the same effect (reduction in microbial growth) are called equivalent treatments.

Filtration (p. 188)

11. Filtration is the passage of a liquid or gas through a filter with pores small enough to retain microbes.
12. Microbes can be removed from air by high-efficiency particulate air (HEPA) filters.
13. Membrane filters composed of cellulose esters are commonly used to filter out bacteria, viruses, and even large proteins.

Low Temperatures (pp. 188–189)

14. The effectiveness of low temperatures depends on the particular microorganism and the intensity of the application.
15. Most microorganisms do not reproduce at ordinary refrigerator temperatures (0–7°C).
16. Many microbes survive (but do not grow) at the subzero temperatures used to store foods.

High Pressure (p. 189)

17. High pressure denatures proteins in vegetative cells.

Desiccation (p. 189)

18. In the absence of water, microorganisms cannot grow but can remain viable.
19. Viruses and endospores can resist desiccation.

Osmotic Pressure (p. 189)

20. Microorganisms in high concentrations of salts and sugars undergo plasmolysis.
21. Molds and yeasts are more capable than bacteria of growing in materials with low moisture or high osmotic pressure.

Radiation (pp. 189–190)

22. The effects of radiation depend on its wavelength, intensity, and duration.
23. Ionizing radiation (gamma rays, X rays, and high-energy electron beams) has a high degree of penetration and exerts its effect primarily by ionizing water and forming highly reactive hydroxyl radicals.

24. Ultraviolet (UV) radiation, a form of nonionizing radiation, has a low degree of penetration and causes cell damage by making thymine dimers in DNA that interfere with DNA replication; the most effective germicidal wavelength is 260 nm.
25. Microwaves can kill microbes indirectly as materials get hot.

Chemical Methods of Microbial Control (pp. 190–200)

1. Chemical agents are used on living tissue (as antiseptics) and on inanimate objects (as disinfectants).
2. Few chemical agents achieve sterility.

Principles of Effective Disinfection (pp. 191–192)

3. Careful attention should be paid to the properties and concentration of the disinfectant to be used.
4. The presence of organic matter, degree of contact with microorganisms, and temperature should also be considered.

Evaluating a Disinfectant (p. 192)

5. In the use-dilution test, bacterial survival in the manufacturer's recommended dilution of a disinfectant is determined.
6. Viruses, endospore-forming bacteria, mycobacteria, and fungi can also be used in the use-dilution test.
7. In the disk-diffusion method, a disk of filter paper is soaked with a chemical and placed on an inoculated agar plate; a zone of inhibition indicates effectiveness.

Types of Disinfectants (pp. 192–200)

8. Phenolics exert their action by injuring plasma membranes.
9. Bisphenols such as triclosan (over the counter) and hexachlorophene (prescription) are widely used in household products.
10. Biguanides damage plasma membranes of vegetative cells.
11. Some halogens (iodine and chlorine) are used alone or as components of inorganic or organic solutions.
12. Iodine may combine with certain amino acids to inactivate enzymes and other cellular proteins.
13. Iodine is available as a tincture (in solution with alcohol) or as an iodophor (combined with an organic molecule).
14. The germicidal action of chlorine is based on the formation of hypochlorous acid when chlorine is added to water.
15. Alcohols exert their action by denaturing proteins and dissolving lipids.
16. In tinctures, they enhance the effectiveness of other antimicrobial chemicals.
17. Aqueous ethanol (60–95%) and isopropanol are used as disinfectants.
18. Silver, mercury, copper, and zinc are used as germicides.
19. They exert their antimicrobial action through oligodynamic action. When heavy metal ions combine with sulfhydryl (—SH) groups, proteins are denatured.
20. Surface-active agents decrease the surface tension among molecules of a liquid; soaps and detergents are examples.
21. Soaps have limited germicidal action but assist in removing microorganisms.
22. Acid-anionic detergents are used to clean dairy equipment.
23. Quats are cationic detergents attached to NH_4^+.
24. By disrupting plasma membranes, quats allow cytoplasmic constituents to leak out of the cell.
25. Quats are most effective against gram-positive bacteria.
26. SO_2, sorbic acid, benzoic acid, and propionic acid inhibit fungal metabolism and are used as food preservatives.
27. Nitrate and nitrite salts prevent germination of *C. botulinum* endospores in meats.
28. Nisin and natamycin are anibiotics used to preserve foods, especially cheese.
29. Aldehydes such as formaldehyde and glutaraldehyde exert their antimicrobial effect by inactivating proteins.
30. They are among the most effective chemical disinfectants.
31. Ethylene oxide is the gas most frequently used for sterilization.
32. It penetrates most materials and kills all microorganisms by protein denaturation.
33. Free radicals in plasma gases are used to sterilize plastic instruments.
34. Supercritical fluids, which have properties of liquid and gas, can sterilize at low temperatures.
35. Hydrogen peroxide, peracetic acid, benzoyl peroxide, and ozone exert their antimicrobial effect by oxidizing molecules inside cells.

Microbial Characteristics and Microbial Control (pp. 200–202)

1. Gram-negative bacteria are generally more resistant than gram-positive bacteria to disinfectants and antiseptics.
2. Mycobacteria, endospores, and protozoan cysts and oocysts are very resistant to disinfectants and antiseptics.
3. Nonenveloped viruses are generally more resistant than enveloped viruses to disinfectants and antiseptics.
4. Prions are resistant to disinfection and autoclaving.

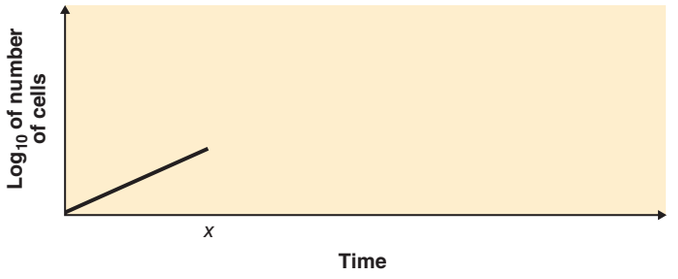
Study Questions

Answers to the Review and Multiple Choice questions can be found by turning to the Answers tab at the back of the textbook.

Review

1. The thermal death time for a suspension of *Bacillus subtilis* endospores is 30 minutes in dry heat and less than 10 minutes in an autoclave. Which type of heat is more effective? Why?
2. If pasteurization does not achieve sterilization, why is pasteurization used to treat food?
3. Thermal death point is not considered an accurate measure of the effectiveness of heat sterilization. List three factors that can alter thermal death point.
4. The antimicrobial effect of gamma radiation is due to (a) _____. The antimicrobial effect of ultraviolet radiation is due to (b) _____.

5. DRAW IT A bacterial culture was in log phase in the following figure. At time *x,* an antibacterial compound was added to the culture. Draw the lines indicating addition of a bactericidal compound and a bacteriostatic compound. Explain why the viable count does not immediately drop to zero at *x.*



6. How do autoclaving, hot air, and pasteurization illustrate the concept of equivalent treatments?
7. How do salts and sugars preserve foods? Why are these considered physical rather than chemical methods of microbial control? Name one food that is preserved with sugar and one preserved with salt. How do you account for the occasional growth of *Penicillium* mold in jelly, which is 50% sucrose?
8. The use-dilution values for two disinfectants tested under the same conditions are as follows: Disinfectant A—1:2; Disinfectant B—1:10,000. If both disinfectants are designed for the same purpose, which would you select?
9. A large hospital washes burn patients in a stainless steel tub. After each patient, the tub is cleaned with a quat. It was noticed that 14 of 20 burn patients acquired *Pseudomonas* infections after being bathed. Provide an explanation for this high rate of infection.
10. NAME IT What bacterium has porins, is resistant to triclosan, and survives and may grow in quats?

Multiple Choice

1. Which of the following does *not* kill endospores?
   a. autoclaving
   b. incineration
   c. hot-air sterilization
   d. pasteurization
   e. All of the above kill endospores.
2. Which of the following is most effective for sterilizing mattresses and plastic Petri dishes?
   a. chlorine
   b. ethylene oxide
   c. glutaraldehyde
   d. autoclaving
   e. nonionizing radiation
3. Which of these disinfectants does *not* act by disrupting the plasma membrane?
   a. phenolics
   b. phenol
   c. quaternary ammonium compounds
   d. halogens
   e. biguanides
4. Which of the following *cannot* be used to sterilize a heat-labile solution stored in a plastic container?
   a. gamma radiation
   b. ethylene oxide
   c. supercritical fluids
   d. autoclaving
   e. short-wavelength radiation
5. Which of the following is *not* a characteristic of quaternary ammonium compounds?
   a. bactericidal against gram-positive bacteria
   b. sporicidal
   c. amoebicidal
   d. fungicidal
   e. kills enveloped viruses
6. A classmate is trying to determine how a disinfectant might kill cells. You observed that when he spilled the disinfectant in your reduced litmus milk, the litmus turned blue again. You suggest to your classmate that
   a. the disinfectant might inhibit cell wall synthesis.
   b. the disinfectant might oxidize molecules.
   c. the disinfectant might inhibit protein synthesis.
   d. the disinfectant might denature proteins.
   e. he take his work away from yours.
7. Which of the following is most likely to be bactericidal?
   a. membrane filtration
   b. ionizing radiation
   c. lyophilization (freeze-drying)
   d. deep-freezing
   e. all of the above
8. Which of the following is used to control microbial growth in foods?
   a. organic acids
   b. alcohols
   c. aldehydes
   d. heavy metals
   e. all of the above

Use the following information to answer questions 9 and 10. The data were obtained from a use-dilution test comparing four disinfectants against *Salmonella choleraesuis. G* = growth, *NG* = no growth

	Bacterial Growth after Exposure to			
Dilution	**Disinfectant A**	**Disinfectant B**	**Disinfectant C**	**Disinfectant D**
1:2	NG	G	NG	NG
1:4	NG	G	NG	G
1:8	NG	G	G	G
1:16	G	G	G	G

9. Which disinfectant is the most effective?
10. Which disinfectant(s) is (are) bactericidal?
    a. A, B, C, and D
    b. A, C, and D
    c. A only
    d. B only
    e. none of the above

Critical Thinking

1. The disk-diffusion method was used to evaluate three disinfectants. The results were as follows:

Disinfectant	Zone of Inhibition
X	0 mm
Y	5 mm
Z	10 mm

   a. Which disinfectant was the most effective against the organism?
   b. Can you determine whether compound Y was bactericidal or bacteriostatic?

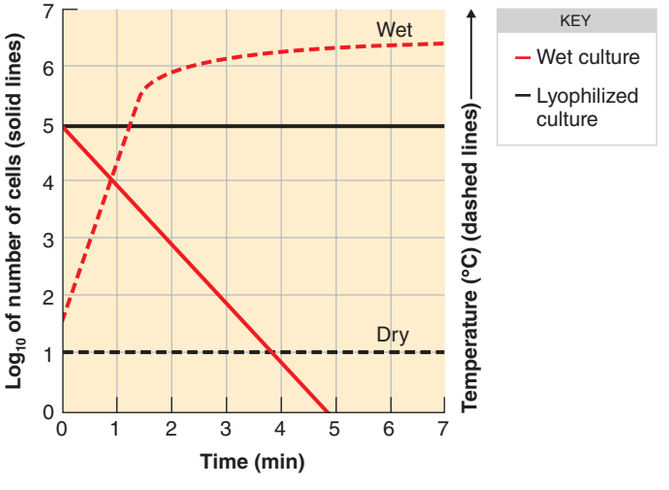
2. For each of the following bacteria, explain why it is often resistant to disinfectants.
   a. *Mycobacterium*
   b. *Pseudomonas*
   c. *Bacillus*

3. A use-dilution test was used to evaluate two disinfectants against *Salmonella choleraesuis.* The results were as follows:

	Bacterial Growth after Exposures		
Time of Exposure (min)	Disinfectant A	Disinfectant B Diluted with Distilled Water	Disinfectant B Diluted with Tap Water
10	G	NG	G
20	G	NG	NG
30	NG	NG	NG

   a. Which disinfectant was the most effective?
   b. Which disinfectant should be used against *Staphylococcus*?

4. To determine the lethal action of microwave radiation, two 10^5 suspensions of *E. coli* were prepared. One cell suspension was exposed to microwave radiation while wet, whereas the other was lyophilized (freeze-dried) and then exposed to radiation. The results are shown in the following figure. Dashed lines indicate the temperature of the samples. What is the most likely method of lethal action of microwave radiation? How do you suppose these data might differ for *Clostridium*?

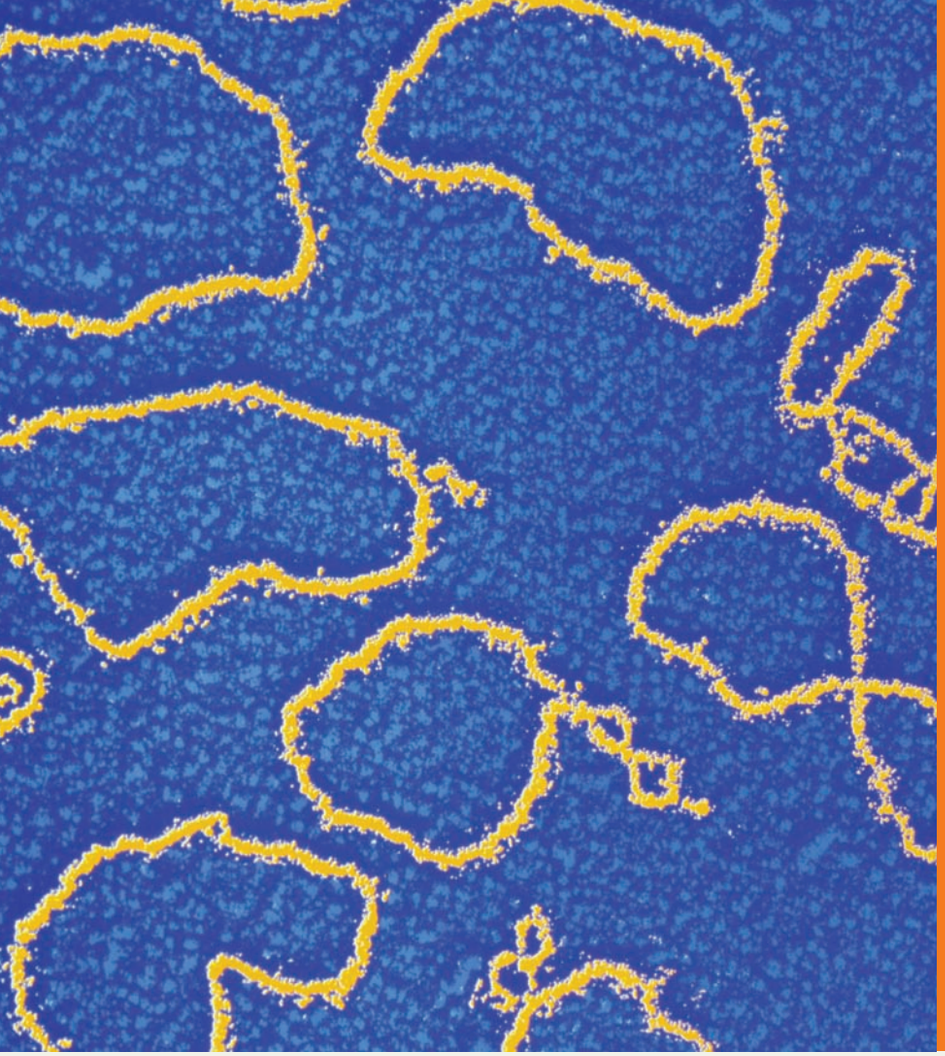


Clinical Applications

1. *Entamoeba histolytica* and *Giardia lamblia* were isolated from the stool sample of a 45-year-old man, and *Shigella sonnei* was isolated from the stool sample of an 18-year-old woman. Both patients experienced diarrhea and severe abdominal cramps, and prior to onset of digestive symptoms both had been treated by the same chiropractor. The chiropractor had administered colonic irrigations (enemas) to these patients. The device used for this treatment was a gravity-dependent apparatus using 12 liters of tap water. There were no check valves to prevent backflow, so all parts of the apparatus could have become contaminated with feces during each colonic treatment. The chiropractor provided colonic treatment to four or five patients per day. Between patients, the adaptor piece that is inserted into the rectum was placed in a "hot-water sterilizer."
   What two errors were made by the chiropractor?

2. Between March 9 and April 12, five chronic peritoneal dialysis patients at one hospital became infected with *Pseudomonas aeruginosa.* Four patients developed peritonitis (inflammation of the abdominal cavity), and one developed a skin infection at the catheter insertion site. All patients with peritonitis had low-grade fever, cloudy peritoneal fluid, and abdominal pain. All patients had permanent indwelling peritoneal catheters, which the nurse wiped with gauze that had been soaked with an iodophor solution each time the catheter was connected to or disconnected from the machine tubing. Aliquots of the iodophor were transferred from stock bottles to small in-use bottles. Cultures from the dialysate concentrate and the internal areas of the dialysis machines were negative; iodophor from a small in-use plastic container yielded a pure culture of *P. aeruginosa.*
   What improper technique led to this infection?

3. You are investigating a national outbreak of *Ralstonia mannitolilytica* associated with use of a contaminated oxygen-delivery device among pediatric patients. The device adds moisture to and warms the oxygen. Each hospital followed the manufacturer's recommendation to use a detergent to clean the reusable components of the device between patients. Tap water is permitted in the device because the device uses a reusable 0.01-μm filter as a biological barrier between the air and water compartments. *Ralstonia* is a gram-negative rod commonly found in water.
   Why did disinfection fail?
   What do you recommend for disinfecting? The device cannot be autoclaved.



8

Microbial Genetics

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Virtually all the microbial traits you have read about in earlier chapters are controlled or influenced by heredity. The inherited traits of microbes include their shape and structural features, their metabolism, their ability to move or behave in various ways, and their ability to interact with other organisms—perhaps causing disease. Individual organisms transmit these characteristics to their offspring through genes.

The development of antibiotic resistance in microorganisms depends on genetics. Antibiotic resistance is often carried on plasmids such as those in the figure. Plasmids are readily transferred between bacterial cells. They are responsible for the emergence of methicillin-resistant *Staphylococcus aureus* and the recent emergence of carbapenem-resistant *Klebsiella pneumoniae*. The emergence of vancomycin-resistant *S. aureus* (VRSA) poses a serious threat to patient care. In this chapter you will see how VRSA acquired this trait.

Emerging diseases provide another reason why it is important to understand genetics. New diseases are the results of genetic changes in some existing organism; for example, *E. coli* O157:H7 acquired the genes for Shiga toxin from *Shigella*.

Currently, microbiologists are using genetics to discover relatedness among organisms, to explore the origins of organisms such as HIV and H1N1 influenza virus, and to study how genes are expressed.

Structure and Function of the Genetic Material

LEARNING OBJECTIVES

8-1 Define *genetics, genome, chromosome, gene, genetic code, genotype, phenotype,* and *genomics.*

8-2 Describe how DNA serves as genetic information.

8-3 Describe the process of DNA replication.

8-4 Describe protein synthesis, including transcription, RNA processing, and translation.

8-5 Compare protein synthesis in prokaryotes and eukaryotes.

Genetics is the science of heredity; it includes the study of what genes are, how they carry information, how they are replicated and passed to subsequent generations of cells or passed between organisms, and how the expression of their information within an organism determines the particular characteristics of that organism. The genetic information in a cell is called the **genome.** A cell's genome includes its chromosomes and plasmids. **Chromosomes** are structures containing DNA that physically carry hereditary information; the chromosomes contain the genes. **Genes** are segments of DNA (except in some viruses, in which they are made of RNA) that code for functional products.

Clinical Case: Where There's Smoke

Marcel DuBois, a 70-year-old grandfather of 12, quietly hangs up the phone. His doctor has just called him with the results of his stool DNA test that he undertook at the Mayo Clinic last week. Marcel's doctor suggested this experimental, noninvasive screening tool for colorectal cancer because Marcel is not comfortable with the colonoscopy procedure and usually tries to postpone getting one. The stool DNA test, however, uses stool samples, which contain cells that have been shed from the colon lining. The DNA from these cells is tested for DNA markers that may indicate the presence of precancerous polyps or cancerous tumors. Marcel makes an appointment to come in to see his doctor the next afternoon.

Once in the office, the doctor explains to Marcel and his wife, Janice, that the stool DNA test detected the presence of serrated colorectal polyps. This type of polyp is usually difficult to see with a colonoscopy because it is not raised and can be the same color as the colon wall.

How can DNA show whether a person has cancer? Read on to find out.

208 226 231 232

We saw in Chapter 2 that DNA is a macromolecule composed of repeating units called *nucleotides.* Recall that each nucleotide consists of a nucleobase (adenine, thymine, cytosine, or guanine), deoxyribose (a pentose sugar), and a phosphate group (see Figure 2.16, page 46). The DNA within a cell exists as long strands of nucleotides twisted together in pairs to form a double helix. Each strand has a string of alternating sugar and phosphate groups (its *sugar-phosphate backbone*), and a nitrogenous base is attached to each sugar in the backbone. The two strands are held together by hydrogen bonds between their nitrogenous bases. The **base pairs** always occur in a specific way: adenine always pairs with thymine, and cytosine always pairs with guanine. Because of this specific base pairing, the base sequence of one DNA strand determines the base sequence of the other strand. The two strands of DNA are thus *complementary.*

The structure of DNA helps explain two primary features of biological information storage. First, the linear sequence of bases provides the actual information. Genetic information is encoded by the sequence of bases along a strand of DNA, in much the same way as our written language uses a linear sequence of letters to form words and sentences. The genetic language, however, uses an alphabet with only four letters—the four kinds of nucleobases in DNA (or RNA). But 1000 of these four bases, the number contained in an average-sized gene, can be arranged in 4^{1000} different ways. This astronomically large number explains how genes can be varied enough to provide all the information a cell needs to grow and perform its functions. The **genetic code,** the set of rules that determines how a nucleotide sequence is converted into the amino acid sequence of a protein, is discussed in more detail later in the chapter.

Second, the complementary structure allows for the precise duplication of DNA during cell division. Each offspring cell receives one of the original strands from the parent; thus ensuring one strand that functions correctly.

Much of cellular metabolism is concerned with translating the genetic message of genes into specific proteins. A gene usually codes for a messenger RNA (mRNA) molecule, which ultimately results in the formation of a protein. Alternatively, the gene product can be a ribosomal RNA (rRNA), transfer RNA (tRNA), or microRNA (miRNA). As we will see, all of these types of RNA are involved in the process of protein synthesis. When the ultimate molecule for which a gene codes (a protein, for example) has been produced, we say that the gene has been *expressed.*

Genotype and Phenotype

The **genotype** of an organism is its genetic makeup, the information that codes for all the particular characteristics of the organism. The genotype represents *potential* properties, but not the properties themselves. **Phenotype** refers to *actual, expressed* properties, such as the organism's ability to perform a particular chemical reaction. Phenotype, then, is the manifestation of genotype.

In molecular terms, an organism's genotype is its collection of genes, its entire DNA. What constitutes the organism's phenotype in molecular terms? In a sense, an organism's phenotype is its collection of proteins. Most of a cell's properties derive from the structures and functions of its proteins. In microbes, most proteins are either *enzymatic* (catalyze particular reactions) or *structural* (participate in large functional complexes such as membranes or flagella). Even phenotypes that depend on structural macromolecules other than proteins (such as lipids or polysaccharides) rely indirectly on proteins. For instance, the structure of a complex lipid or polysaccharide molecule results from the catalytic activities of enzymes that synthesize, process, and degrade those molecules. Thus, although it is not completely accurate to say that phenotypes are due only to proteins, it is a useful simplification.

DNA and Chromosomes

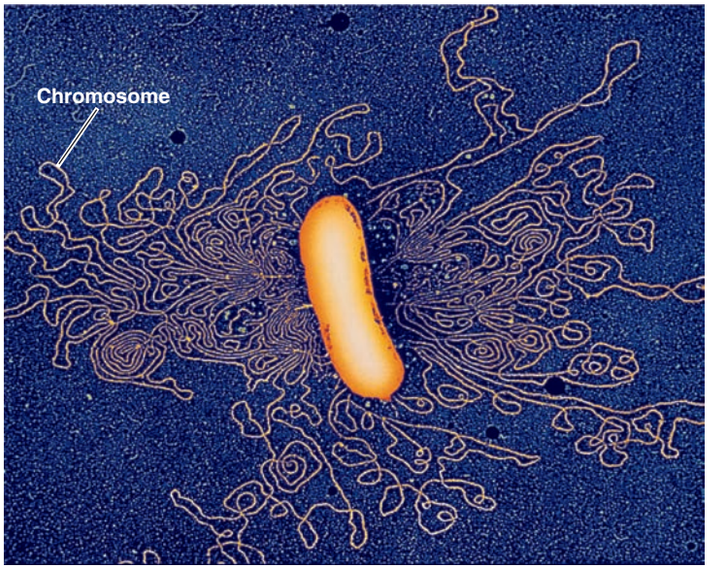
Bacteria typically have a single circular chromosome consisting of a single circular molecule of DNA with associated proteins. The chromosome is looped and folded and attached at one or several points to the plasma membrane. The DNA of *E. coli* has about 4.6 million base pairs and is about 1 mm long—1000 times longer than the entire cell (Figure 8.1a). However, the chromosome takes up only about 10% of the cell's volume because the DNA is twisted, or *supercoiled*.

Originally, the location of genes on a bacterial chromosome was determined by experiments on the transfer of genes from one cell to another. These processes will be discussed later in this chapter. Therefore, the bacterial chromosome map is marked in minutes corresponding to when the genes are transferred from a donor cell to a recipient cell (Figure 8.1b). The entire genome does not consist of back-to-back genes. Noncoding regions called **short tandem repeats (STRs)** occur in most genomes, including that of *E. coli.* STRs are repeating sequences of two- to five-base sequences. These are used in DNA fingerprinting (discussed on page 261).

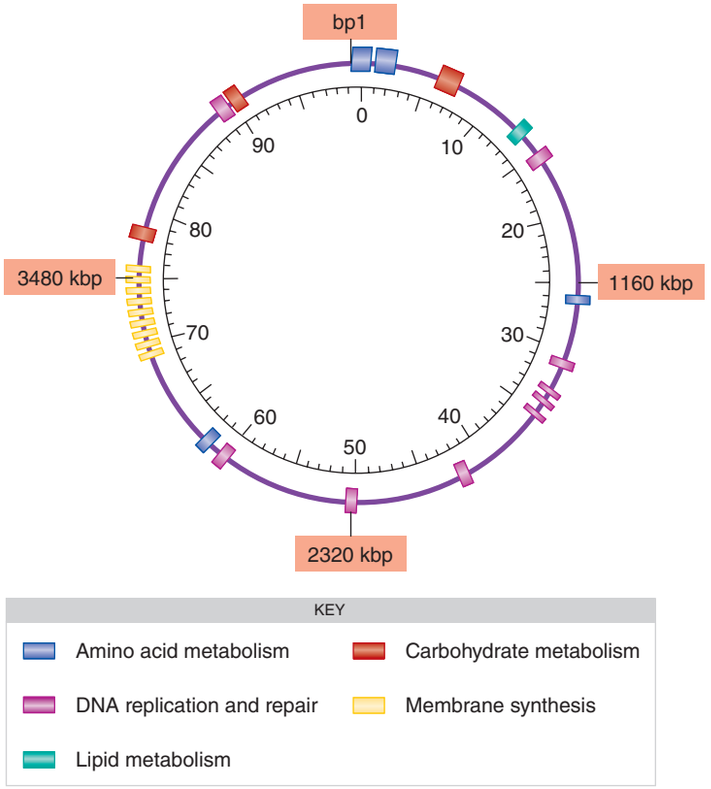
Now, the complete base sequences of chromosomes can be determined. Computers are used to search for *open-reading frames,* that is, regions of DNA that are likely to encode a protein. As you will see later, these are base sequences between start and stop codons. The sequencing and molecular characterization of genomes is called **genomics.** The use of genomics to track West Nile virus is described in the box on page 220.

The Flow of Genetic Information

DNA replication makes possible the flow of genetic information from one generation to the next. As shown in Figure 8.2, the DNA of a cell replicates before cell division so that each offspring cell receives a chromosome identical to the parent's. Within each metabolizing cell, the genetic information contained in DNA also flows in another way: it is transcribed into mRNA and then translated into protein. We describe the processes of transcription and translation later in this chapter.



(a) The tangled mass and looping strands of DNA emerging from this disrupted *E. coli* cell are part of its single chromosome.



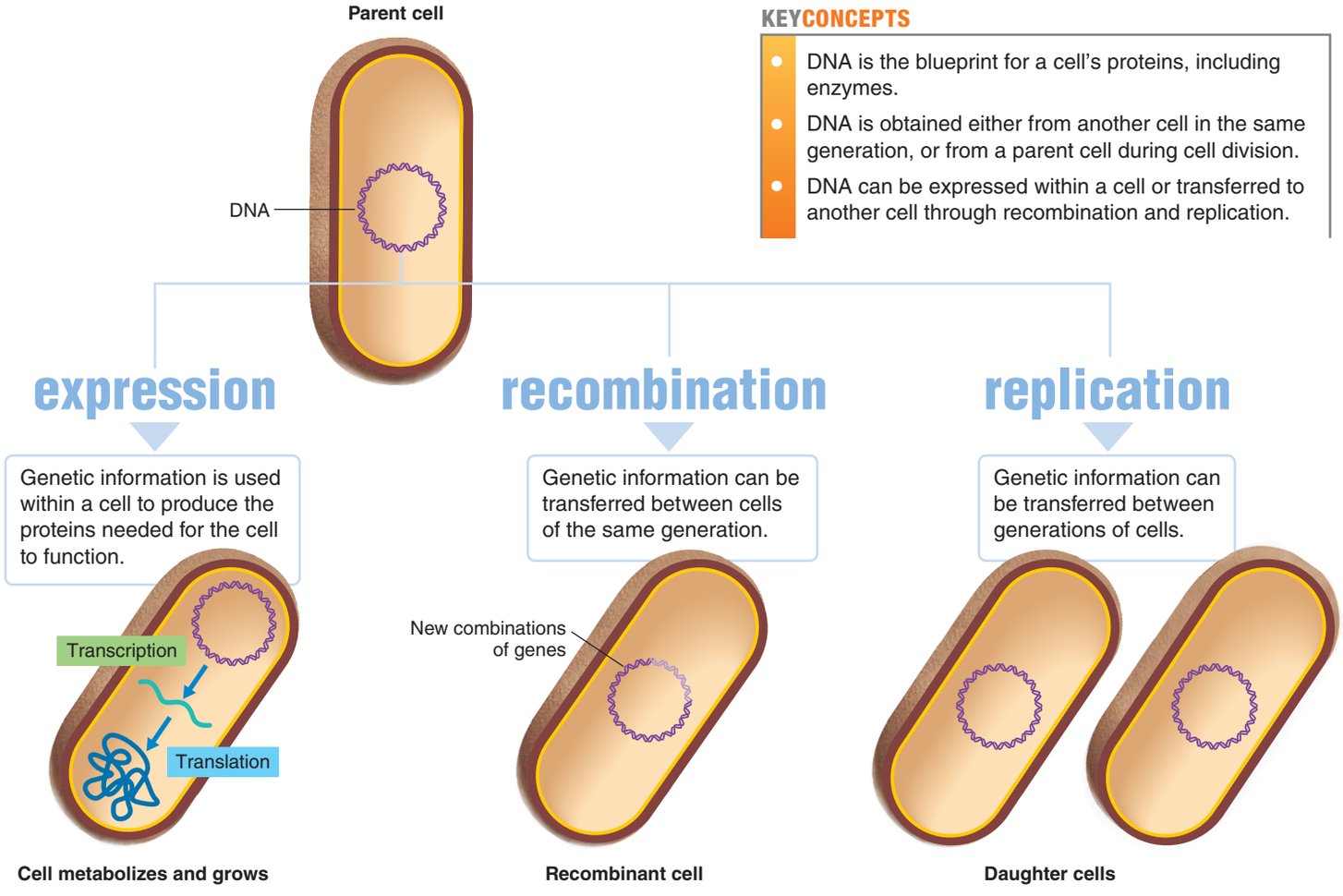
(b) A genetic map of the chromosome of *E. coli.* The numbers inside the circle indicate the number of minutes it takes to transfer the genes during mating between two cells; the numbers in colored boxes indicate the number of base pairs. 1 kbp = 1000 base pairs.

Figure 8.1 A prokaryotic chromosome.

Q What is a gene? What is an open-reading frame?

FOUNDATION FIGURE **8.2**

The Flow of Genetic Information



CHECK YOUR UNDERSTANDING

- Give a clinical application of genomics. **8-1**
- Why is the base pairing in DNA important? **8-2**

DNA Replication

In DNA replication, one "parental" double-stranded DNA molecule is converted to two identical "daughter" molecules. The complementary structure of the nitrogenous base sequences in the DNA molecule is the key to understanding DNA replication. Because the bases along the two strands of double-helical DNA are complementary, one strand can act as a template for the production of the other strand (**Figure 8.3a**).

DNA replication requires the presence of several cellular proteins that direct a particular sequence of events. Enzymes involved in DNA replication and other processes are listed in **Table 8.1.** When replication begins, the supercoiling is relaxed by *topoisomerase* or *gyrase,* and the two strands of parental DNA are unwound by *helicase* and separated from each other in one small DNA segment after another. Free nucleotides present in the cytoplasm of the cell are matched up to the exposed bases of the single-stranded parental DNA. Where thymine is present on the original strand, only adenine can fit into place on the new strand; where guanine is present on the original strand, only cytosine can fit into place, and so on. Any bases that are improperly base-paired are removed and replaced by replication enzymes. Once aligned, the newly added nucleotide is joined to the growing DNA strand by an enzyme called **DNA polymerase.** Then the parental DNA is unwound a bit further to allow the addition of the next nucleotides. The point at which replication occurs is called the *replication fork.*

As the replication fork moves along the parental DNA, each of the unwound single strands combines with new nucleotides. The original strand and this newly synthesized daughter strand then rewind. Because each new double-stranded DNA molecule contains

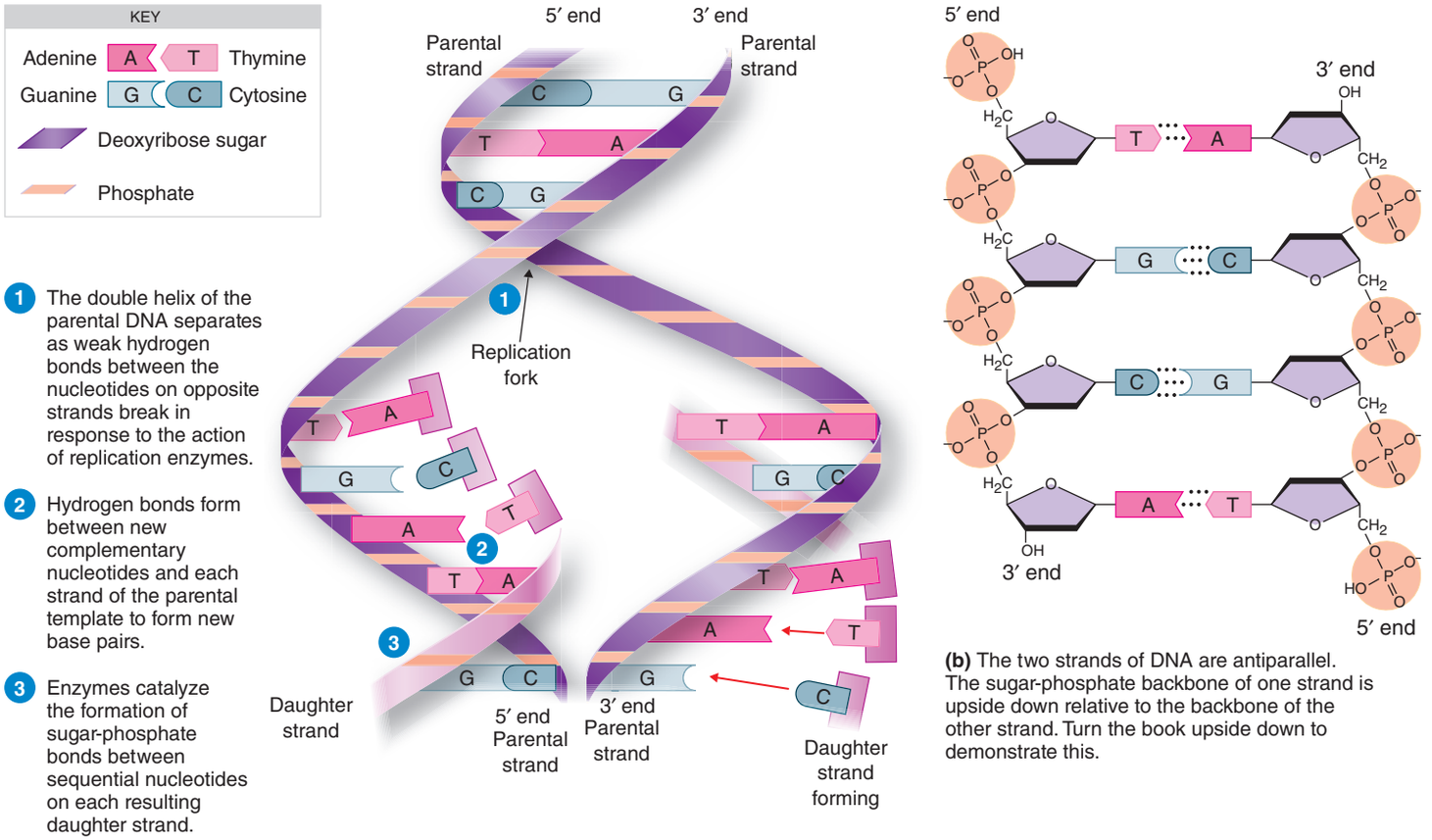


Figure 8.3 **DNA replication.**

Q What is the advantage of semiconservative replication?

TABLE 8.1 Important Enzymes in DNA Replication, Expression, and Repair

DNA Gyrase	Relaxes supercoiling ahead of the replication fork
DNA Ligase	Makes covalent bonds to join DNA strands; joins Okazaki fragments and new segments in excision repair
DNA Polymerase	Synthesizes DNA; proofreads and repairs DNA
Endonucleases	Cut DNA backbone in a strand of DNA; facilitate repair and insertions
Exonucleases	Cut DNA from an exposed end of DNA; facilitate repair
Helicase	Unwinds double-stranded DNA
Methylase	Adds methyl group to selected bases in newly made DNA
Photolyase	Uses visible light energy to separate UV-induced pyrimidine dimers
Ribozyme	RNA enzyme that removes introns and splices exons together
RNA Polymerase	Copies RNA from a DNA template
RNA Primase	An RNA polymerase that makes RNA primers from a DNA template
snRNP	RNA-protein complex that removes introns and splices exons together
Topoisomerase	Relaxes supercoiling ahead of the replication fork; separates DNA circles at the end of DNA replication
Transposase	Cuts DNA backbone, leaving single-stranded "sticky ends"

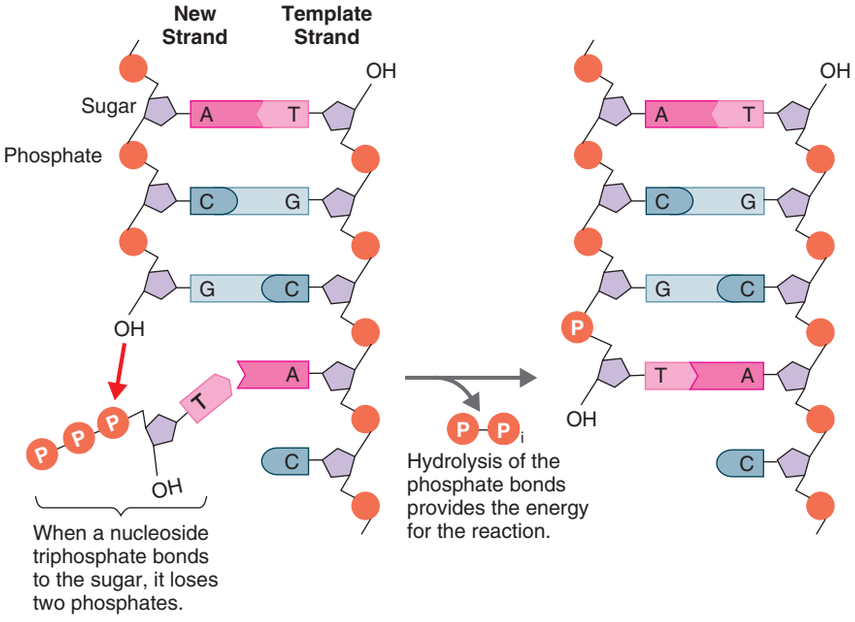


Figure 8.4 **Adding a nucleotide to DNA.**

Q Why is one strand "upside down" relative to the other strand? Why can't both strands "face" the same way?

one original (conserved) strand and one new strand, the process of replication is referred to as **semiconservative replication.**

Before looking at DNA replication in more detail, let's take a closer look at the structure of DNA (see Figure 2.16, on page 46). It is important to understand the concept that the paired DNA strands are oriented in opposite directions relative to each other. Notice in Figure 2.16 that the carbon atoms of the sugar component of each nucleotide are numbered 1′ (pronounced "one prime") to 5′. For the paired bases to be next to each other, the sugar components in one strand are upside down relative to the other. The end with

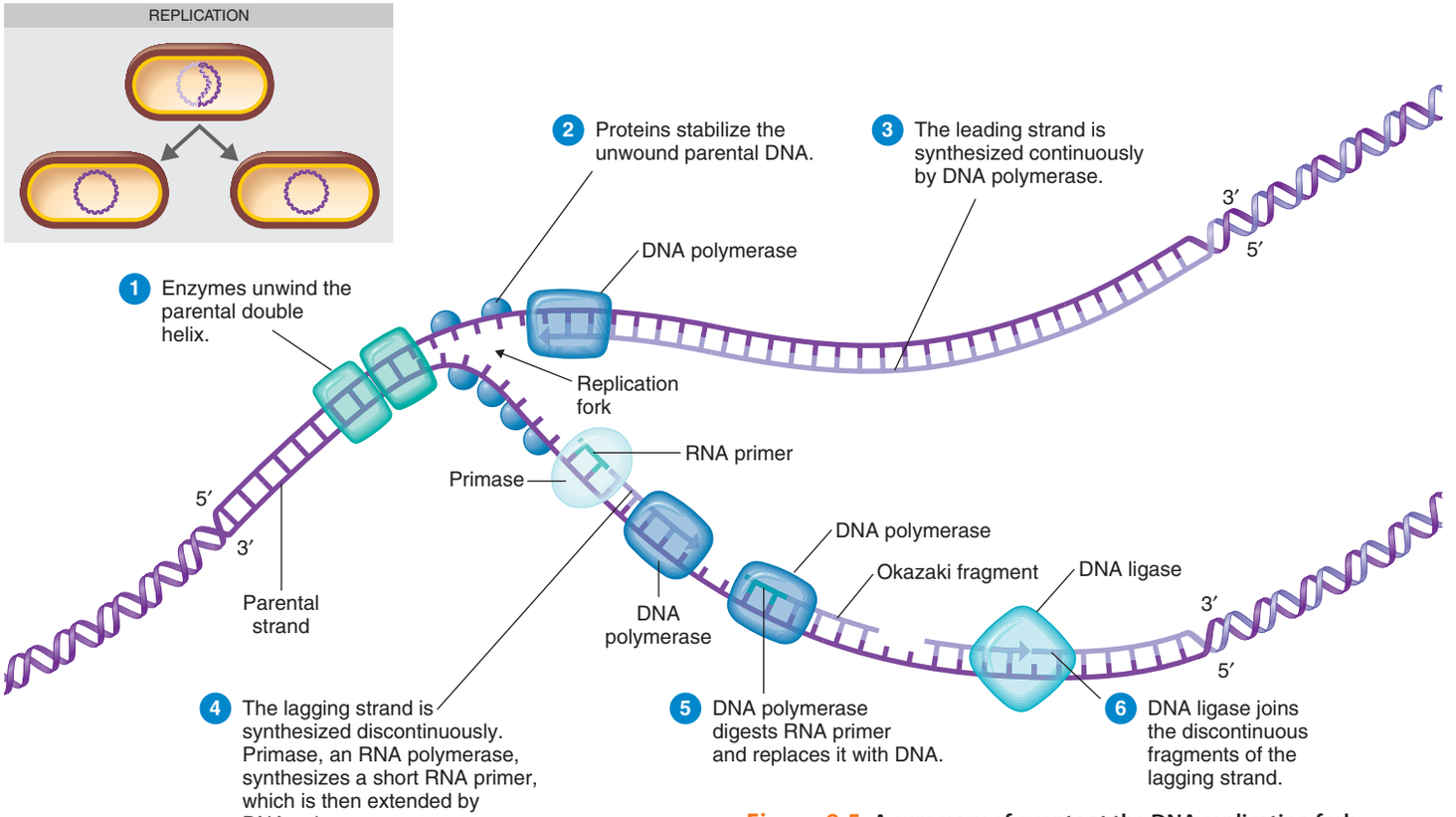
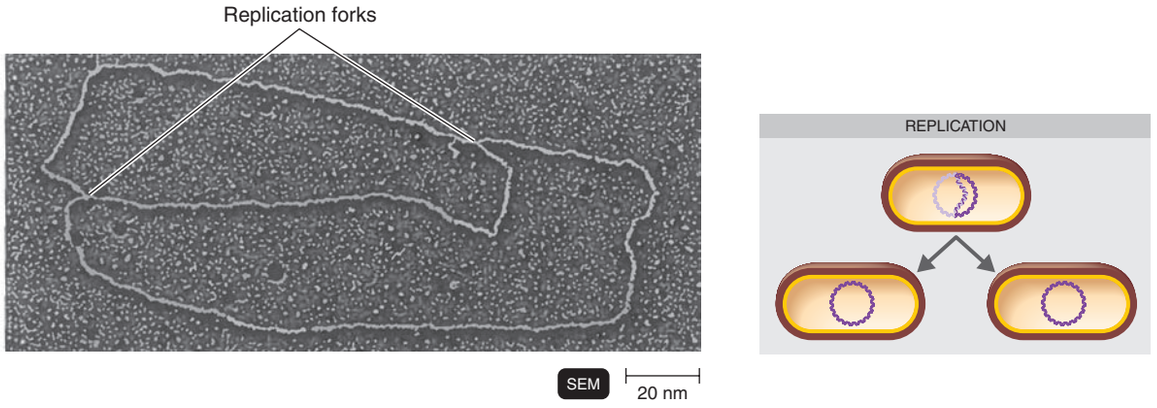
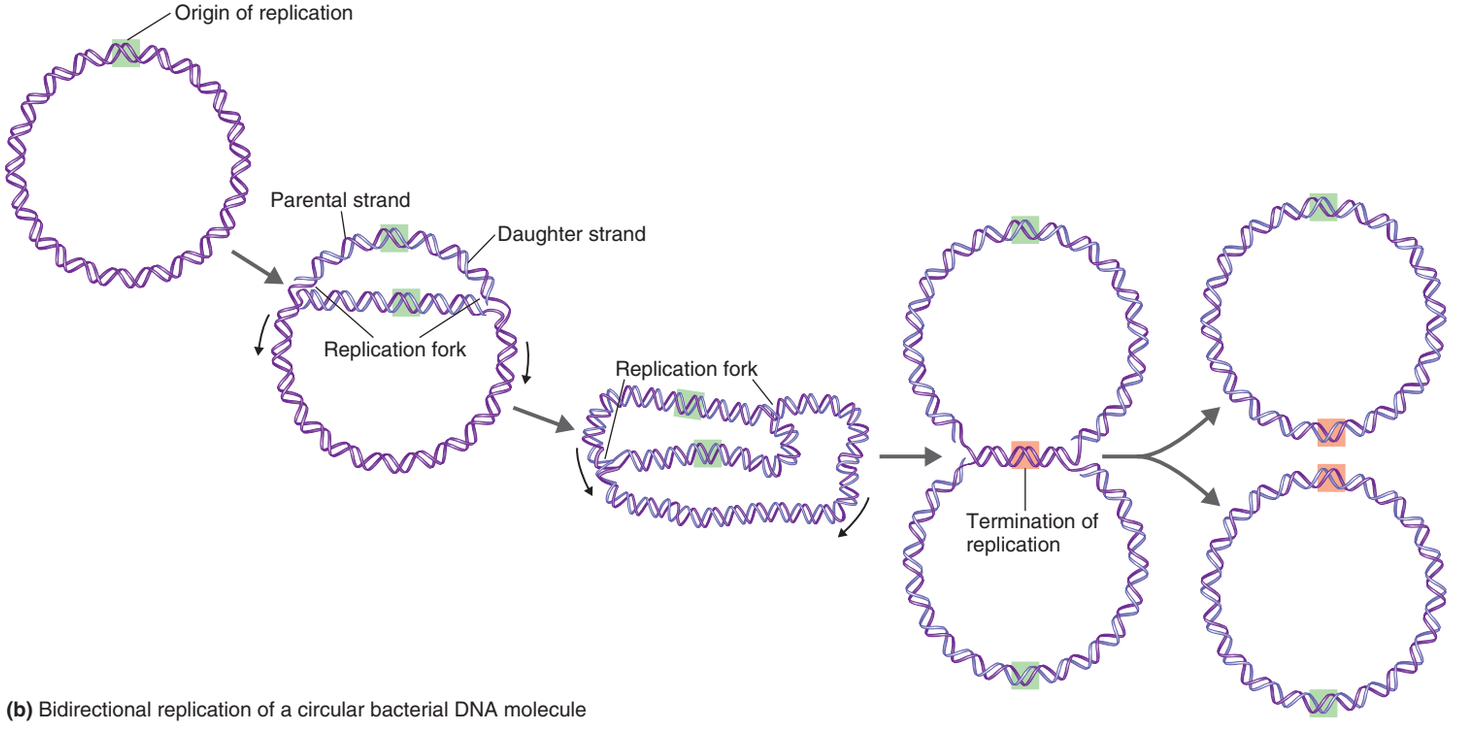


Figure 8.5 **A summary of events at the DNA replication fork.**

Q Why is one strand of DNA synthesized discontinuously?



(a) An *E. coli* chromosome in the process of replicating



(b) Bidirectional replication of a circular bacterial DNA molecule

Figure 8.6 **Replication of bacterial DNA.**

Q What is the origin of replication?

the hydroxyl attached to the 3′ carbon is called the 3′ end of the DNA strand; the end having a phosphate attached to the 5′ carbon is called the 5′ end. The way in which the two strands fit together dictates that the 5′ ⟶ 3′ direction of one strand runs counter to the 5′ ⟶ 3′ direction of the other strand (**Figure 8.3b**). This structure of DNA affects the replication process because DNA polymerases can add new nucleotides to the 3′ end only. Therefore, as the replication fork moves along the parental DNA, the two new strands must grow in different directions.

DNA replication requires a great deal of energy. The energy is supplied from the nucleotides, which are actually nucleoside triphosphates. You already know about ATP; the only difference between ATP and the adenine nucleotide in DNA is the sugar component. Deoxyribose is the sugar in the nucleosides used to synthesize DNA, and nucleoside triphosphates with ribose are used to synthesize RNA. Two phosphate groups are removed to add the nucleotide to a growing strand of DNA; hydrolysis of the nucleoside is exergonic and provides energy to make the new bonds in the DNA strand (**Figure 8.4**).

Figure 8.5 provides more detail about the many steps that go into this complex process.

DNA replication by some bacteria, such as *E. coli,* goes *bidirectionally* around the chromosome (**Figure 8.6**). Two replication forks move in opposite directions away from the origin of replication. Because the bacterial chromosome is a closed loop, the replication forks eventually meet when replication is completed. The two

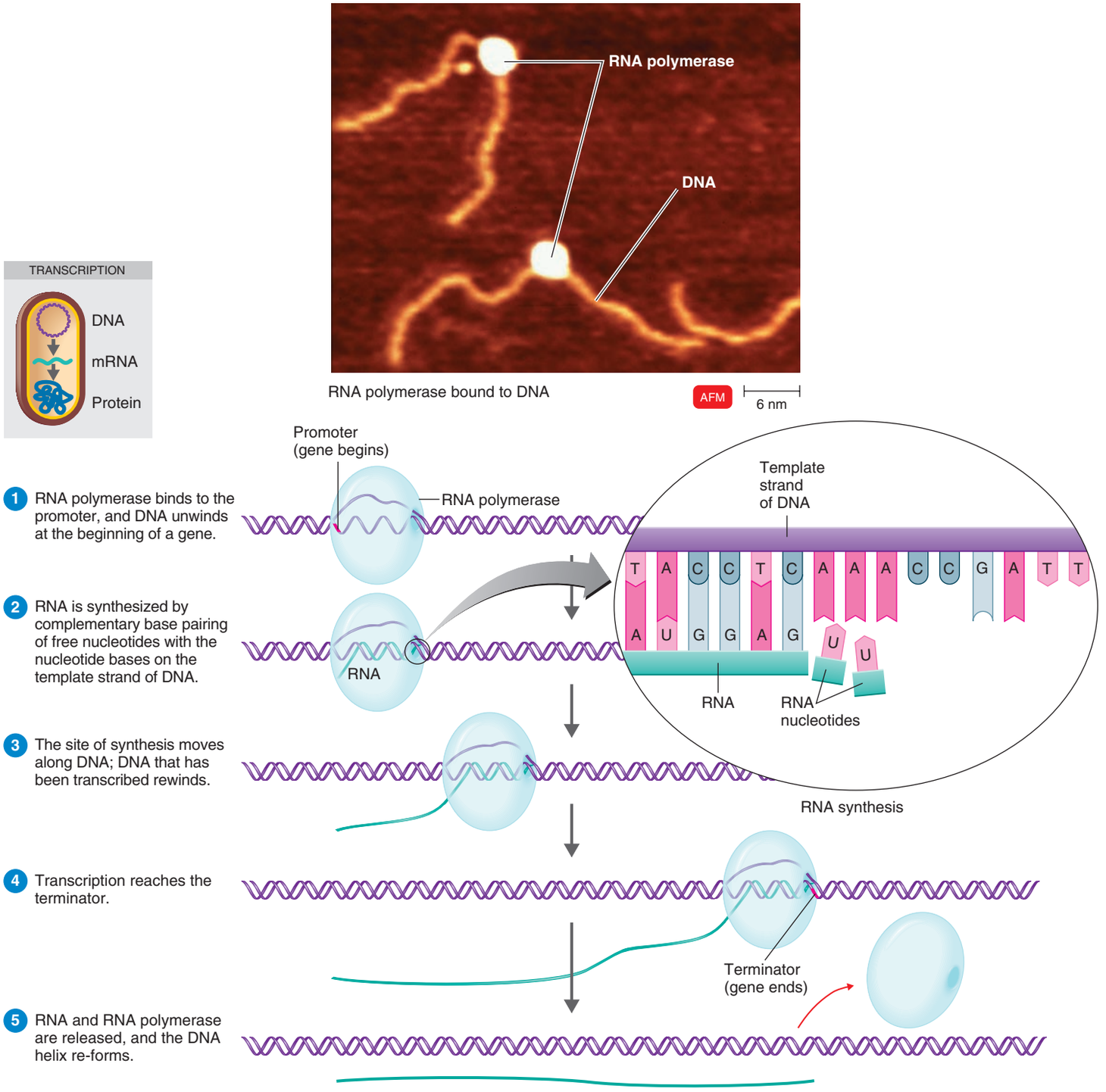


Figure 8.7 The process of transcription. The orienting diagram indicates the relationship of transcription to the overall flow of genetic information within a cell.

Q When does transcription stop?

loops must be separated by a topoisomerase. Much evidence shows an association between the bacterial plasma membrane and the origin of replication. After duplication, if each copy of the origin binds to the membrane at opposite poles, then each daughter cell receives one copy of the DNA molecule—that is, one complete chromosome.

DNA replication is an amazingly accurate process. Typically, mistakes are made at a rate of only 1 in every 10^{10} bases incorporated. Such accuracy is largely due to the *proofreading* capability of DNA polymerase. As each new base is added, the enzyme evaluates whether it forms the proper complementary

base-pairing structure. If not, the enzyme excises the improper base and replaces it with the correct one. In this way, DNA can be replicated very accurately, allowing each daughter chromosome to be virtually identical to the parental DNA. **Animations** DNA Replication: Overview, Forming the Replication Fork, Replication Proteins, Synthesis

CHECK YOUR UNDERSTANDING

- Describe DNA replication, including the functions of DNA gyrase, DNA ligase, and DNA polymerase. **8-3**

RNA and Protein Synthesis

How is the information in DNA used to make the proteins that control cell activities? In the process of *transcription,* genetic information in DNA is copied, or transcribed, into a complementary base sequence of RNA. The cell then uses the information encoded in this RNA to synthesize specific proteins through the process of *translation*. We now take a closer look at these two processes as they occur in a bacterial cell.

Transcription

Transcription is the synthesis of a complementary strand of RNA from a DNA template. We will discuss transcription in prokaryotic cells here. Transcription in eukaryotes is discussed on page 218. **Ribosomal RNA (rRNA)** forms an integral part of ribosomes, the cellular machinery for protein synthesis. Transfer RNA is also involved in protein synthesis, as we will see. **Messenger RNA (mRNA)** carries the coded information for making specific proteins from DNA to ribosomes, where proteins are synthesized.

During transcription, a strand of mRNA is synthesized using a specific portion of the cell's DNA as a template. In other words, the genetic information stored in the sequence of nitrogenous bases of DNA is rewritten so that the same information appears in the base sequence of mRNA. As in DNA replication, a G in the DNA template dictates a C in the mRNA being made, a C in the DNA template dictates a G in the mRNA, and a T in the DNA template dictates an A in the mRNA. However, an A in the DNA template dictates a uracil (U) in the mRNA, because RNA contains U instead of T. (U has a chemical structure slightly different from T, but it base-pairs in the same way.) If, for example, the template portion of DNA has the base sequence 3′-ATGCAT, the newly synthesized mRNA strand will have the complementary base sequence 5′-UACGUA.

The process of transcription requires both an enzyme called *RNA polymerase* and a supply of RNA nucleotides (**Figure 8.7**). Transcription begins when RNA polymerase binds to the DNA at a site called the **promoter.** Only one of the two DNA strands serves as the template for RNA synthesis for a given gene. Like DNA, RNA is synthesized in the 5′ → 3′ direction. RNA synthesis continues until RNA polymerase reaches a site on the DNA called the **terminator.**

The process of transcription allows the cell to produce short-term copies of genes that can be used as the direct source of information for protein synthesis. Messenger RNA acts as an intermediate between the permanent storage form, DNA, and the process that uses the information, translation. **Animations** Transcription: Overview, Process

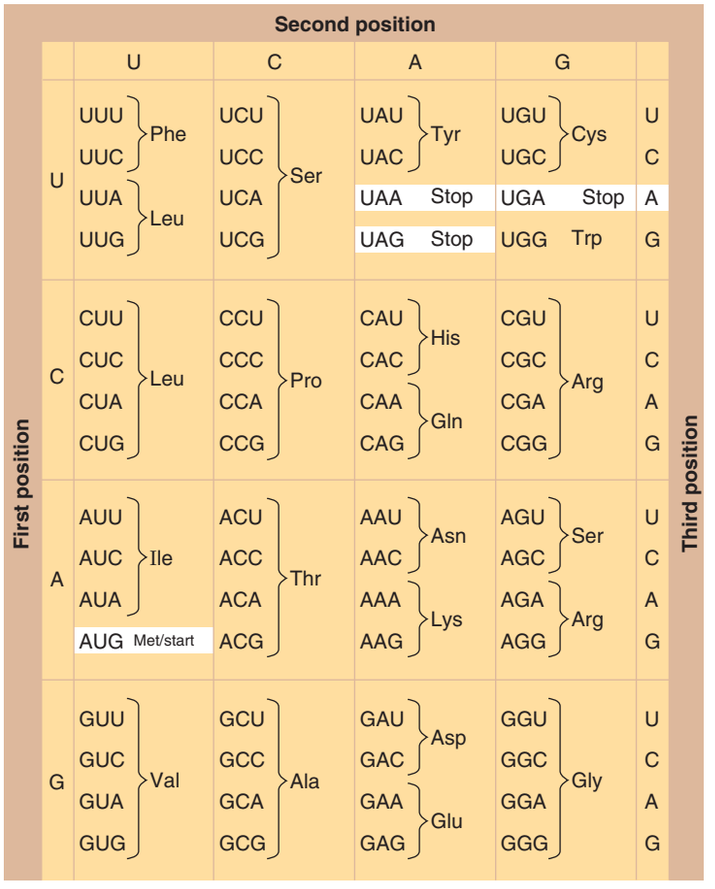


Figure 8.8 The genetic code. The three nucleotides in an mRNA codon are designated, respectively, as the first position, second position, and third position of the codon on the mRNA. Each set of three nucleotides specifies a particular amino acid, represented by a three-letter abbreviation (see Table 2.5, page 42). The codon AUG, which specifies the amino acid methionine, is also the start of protein synthesis. The word *Stop* identifies the nonsense codons that signal the termination of protein synthesis.

Q What is the advantage of the degeneracy of the genetic code?

Translation

We have seen how the genetic information in DNA is transferred to mRNA during transcription. Now we will see how mRNA serves as the source of information for the synthesis of proteins. Protein synthesis is called **translation** because it involves decoding the "language" of nucleic acids and converting that information into the "language" of proteins.

The language of mRNA is in the form of **codons,** groups of three nucleotides, such as AUG, GGC, or AAA. The sequence of codons on an mRNA molecule determines the sequence of amino acids that will be in the protein being synthesized. Each codon "codes" for a particular amino acid. This is the genetic code (**Figure 8.8**).

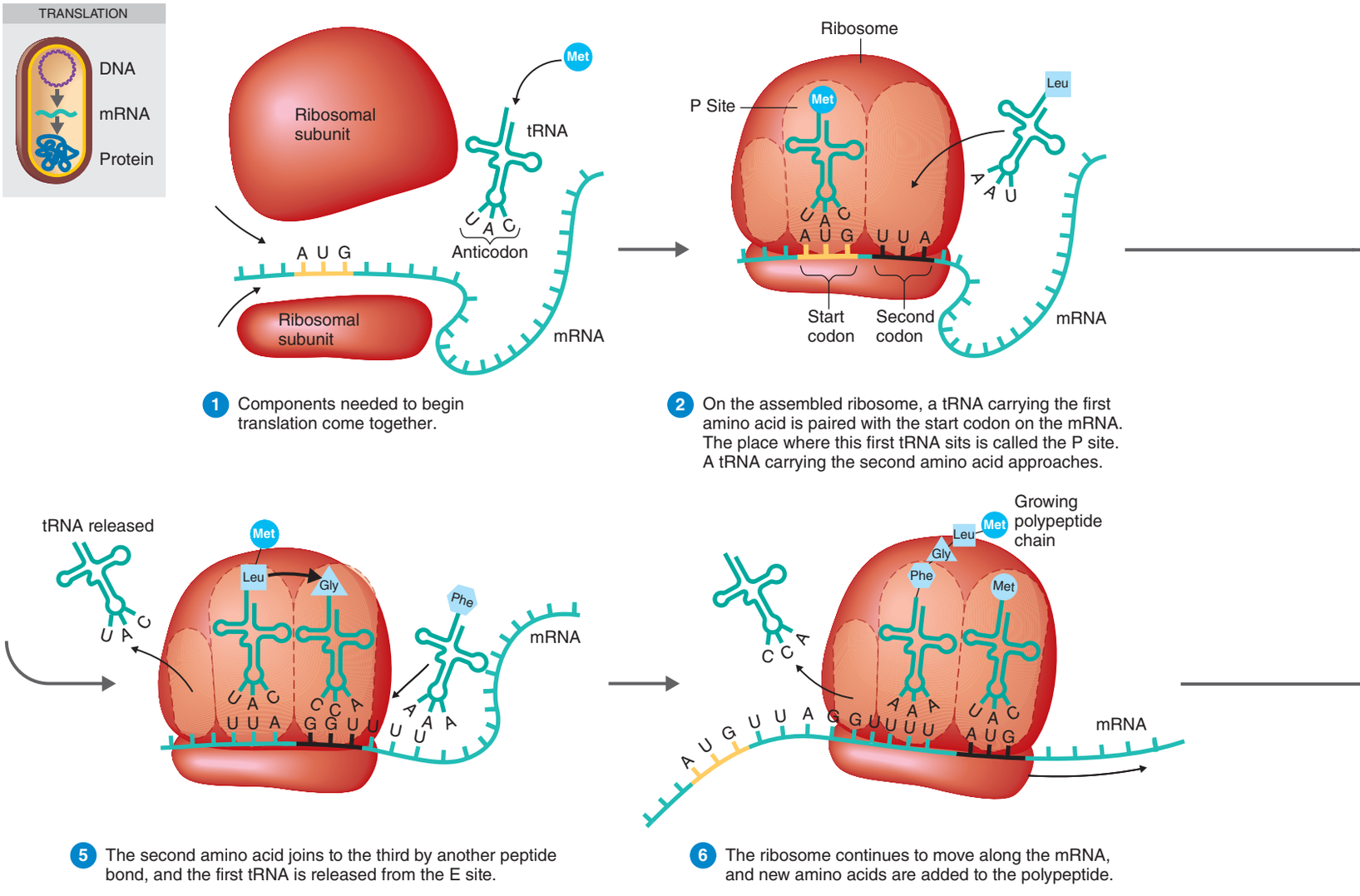


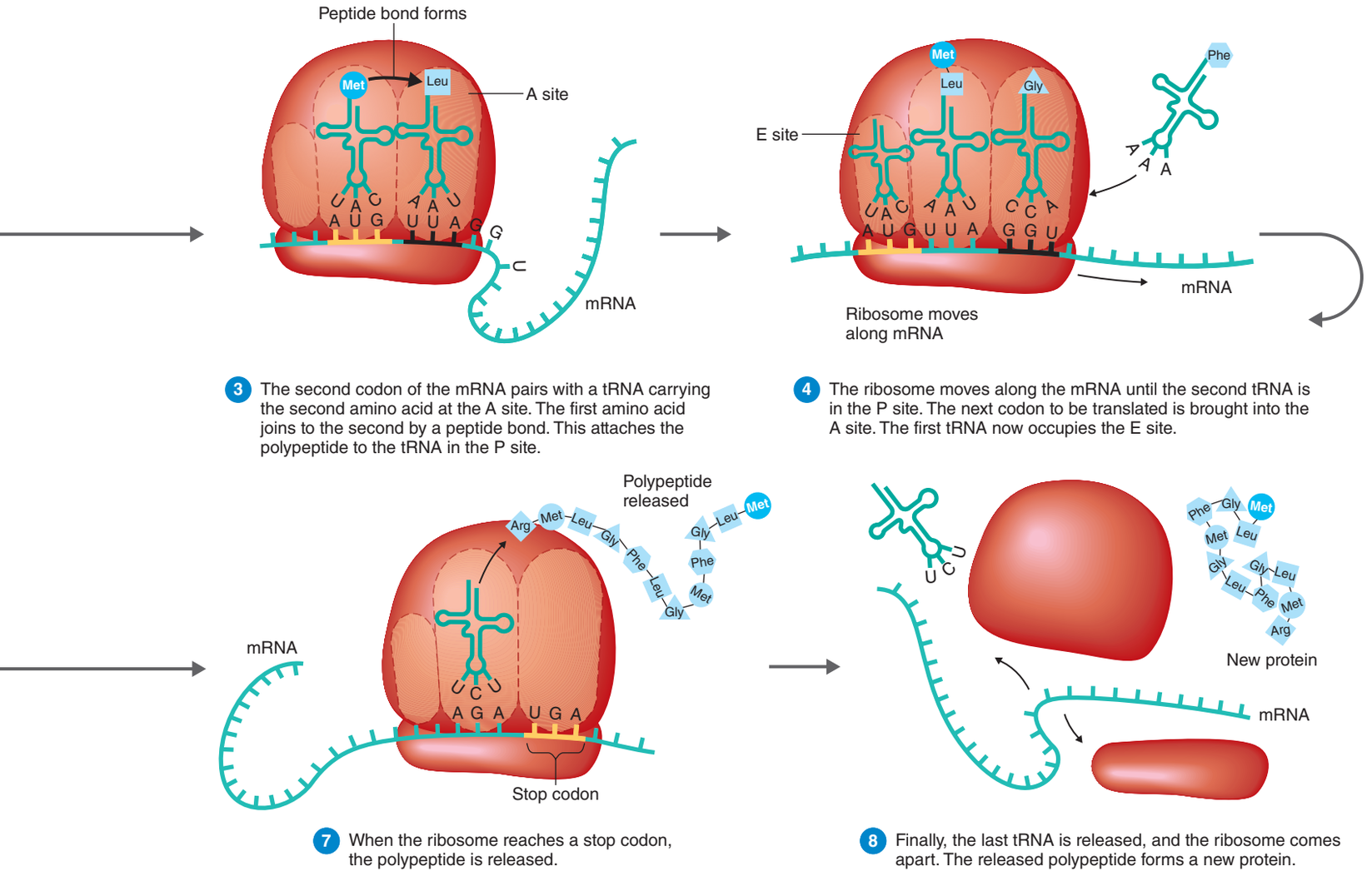
Figure 8.9 The process of translation. The overall goal of translation is to produce proteins using mRNAs as the source of biological information. The complex cycle of events illustrated here shows the primary role of tRNA and ribosomes in the decoding of this information. The ribosome acts as the site where the mRNA-encoded information is decoded, as well as the site where individual amino acids are connected into polypeptide chains. The tRNA molecules act as the actual "translators"—one end of each tRNA recognizes a specific mRNA codon, while the other end carries the amino acid coded for by that codon.

Q When does translation stop?

Codons are written in terms of their base sequence in mRNA. Notice that there are 64 possible codons but only 20 amino acids. This means that most amino acids are signaled by several alternative codons, a situation referred to as the **degeneracy** of the code. For example, leucine has six codons, and alanine has four codons. Degeneracy allows for a certain amount of change, or mutation, in the DNA without affecting the protein ultimately produced.

Of the 64 codons, 61 are sense codons, and 3 are nonsense codons. **Sense codons** code for amino acids, and **nonsense codons** (also called *stop codons*) do not. Rather, the nonsense codons—UAA, UAG, and UGA—signal the end of the protein molecule's synthesis. The start codon that initiates the synthesis of the protein molecule is AUG, which is also the codon for methionine. In bacteria, the start AUG codes for formylmethionine rather than the methionine found in other parts of the protein. The initiating methionine is often removed later, so not all proteins contain methionine.

The codons of mRNA are converted into protein through the process of translation. The codons of an mRNA are "read" sequentially; and, in response to each codon, the appropriate amino acid is assembled into a growing chain. The site of translation is the ribosome, and **transfer RNA (tRNA)** molecules both recognize the specific codons and transport the required amino acids.



3 The second codon of the mRNA pairs with a tRNA carrying the second amino acid at the A site. The first amino acid joins to the second by a peptide bond. This attaches the polypeptide to the tRNA in the P site.

4 The ribosome moves along the mRNA until the second tRNA is in the P site. The next codon to be translated is brought into the A site. The first tRNA now occupies the E site.

7 When the ribosome reaches a stop codon, the polypeptide is released.

8 Finally, the last tRNA is released, and the ribosome comes apart. The released polypeptide forms a new protein.

Figure 8.9 **The process of translation.** ***(continued)***

Each tRNA molecule has an **anticodon,** a sequence of three bases that is complementary to a codon. In this way, a tRNA molecule can base-pair with its associated codon. Each tRNA can also carry on its other end the amino acid encoded by the codon that the tRNA recognizes. The functions of the ribosome are to direct the orderly binding of tRNAs to codons and to assemble the amino acids brought there into a chain, ultimately producing a protein.

Figure 8.9 shows the details of translation. The necessary components assemble: the two ribosomal subunits, a tRNA with the anticodon UAC, and the mRNA molecule to be translated, along with several additional protein factors. This sets up the start codon (AUG) in the proper position to allow translation to begin. After the ribosome joins the first two amino acids with a peptide bond, the first tRNA molecule leaves the ribosome. The ribosome then moves along the mRNA to the next codon. As the proper amino acids are brought into line one by one, peptide bonds are formed between them, and a polypeptide chain results. (See Figure 2.14, page 44.) Translation ends when one of the three nonsense codons in the mRNA is reached. The ribosome then comes apart into its two subunits, and the mRNA and newly synthesized polypeptide chain are released. The ribosome, the mRNA, and the tRNAs are then available to be used again.

The ribosome moves along the mRNA in the 5′ → 3′ direction. As a ribosome moves along the mRNA, it will soon allow the start codon to be exposed. Additional ribosomes can then assemble and begin synthesizing protein. In this way, there are usually a number of ribosomes attached to a single mRNA, all at various stages of protein synthesis. In prokaryotic cells, the translation of mRNA into protein can begin even before

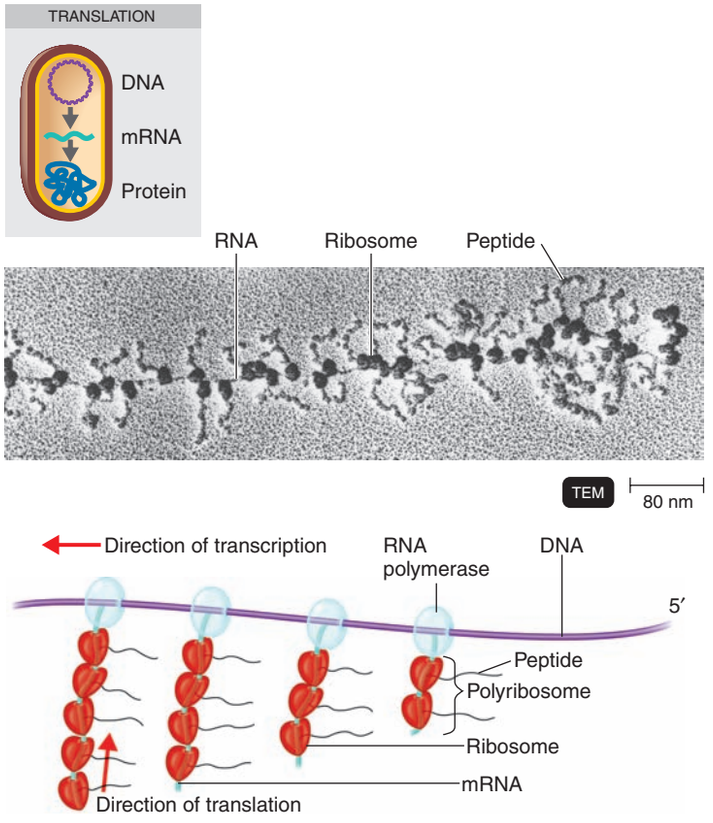


Figure 8.10 Simultaneous transcription and translation in bacteria. Many molecules of mRNA are being synthesized simultaneously. The longest mRNA molecules were the first to be transcribed at the promoter. Note the ribosomes attached to the newly forming mRNA. The micrograph shows a polyribosome (many ribosomes) in a single bacterial gene.

Q Why can translation begin before transcription is complete in prokaryotes but not in eukaryotes?

transcription is complete (Figure 8.10). Because mRNA is produced in the cytoplasm, the start codons of an mRNA being transcribed are available to ribosomes before the entire mRNA molecule is even made.

In eukaryotic cells, transcription takes place in the nucleus. The mRNA must be completely synthesized and moved through the nuclear membrane to the cytoplasm before translation can begin. In addition, the RNA undergoes processing before it leaves the nucleus. In eukaryotic cells, the regions of genes that code for proteins are often interrupted by noncoding DNA. Thus, eukaryotic genes are composed of **exons,** the regions of DNA *expressed,* and **introns,** the *intervening* regions of DNA that do not encode protein. In the nucleus, RNA polymerase synthesizes a molecule called an RNA transcript that contains copies of the introns. Particles called **small nuclear ribonucleoproteins,** abbreviated **snRNPs** and pronounced "snurps," remove the introns and splice the exons together. In some organisms, the introns act as ribozymes to catalyze their own removal (Figure 8.11).

* * *

To summarize, genes are the units of biological information encoded by the sequence of nucleotide bases in DNA. A gene is expressed, or turned into a product within the cell, through the processes of transcription and translation. The genetic information carried in DNA is transferred to a temporary mRNA molecule by transcription. Then, during translation, the mRNA directs the assembly of amino acids into a polypeptide chain: a ribosome attaches to mRNA, tRNAs deliver the amino acids to the ribosome as directed by the mRNA codon sequence, and the ribosome assembles the amino acids into the chain that will be the newly synthesized protein. MM **Animations** Translation: Overview, Genetic Code, Process

CHECK YOUR UNDERSTANDING

- What is the role of the promoter, terminator, and mRNA in transcription? 8-4
- How does mRNA production in eukaryotes differ from the process in prokaryotes? 8-5

The Regulation of Bacterial Gene Expression

LEARNING OBJECTIVES

8-6 Define *operon.*

8-7 Explain pre-transcriptional regulation of gene expression in bacteria.

8-8 Explain post-transcriptional regulation of gene expression.

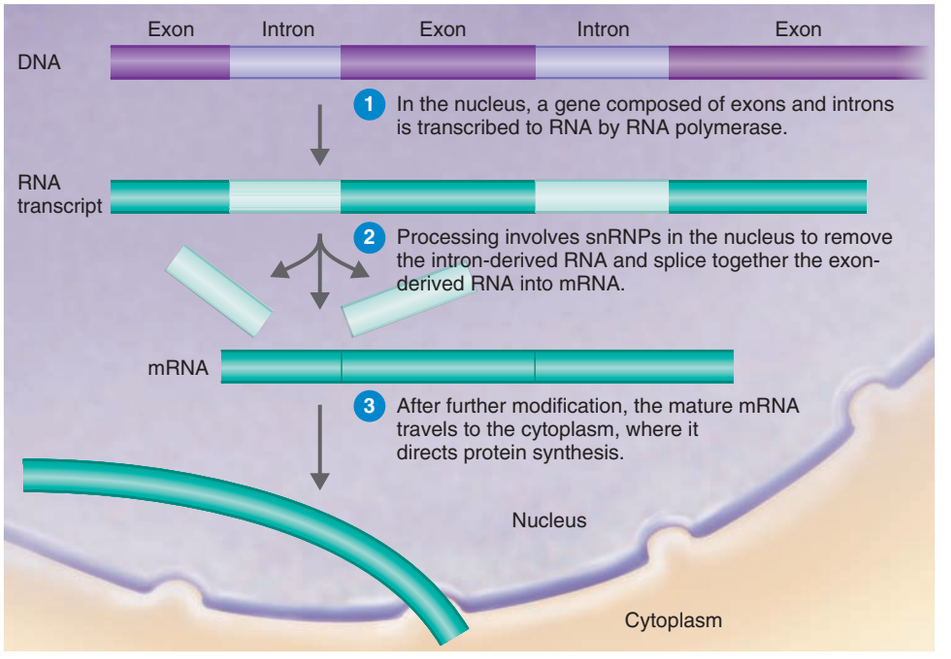
A cell's genetic machinery and its metabolic machinery are integrated and interdependent. Recall from Chapter 5 that the bacterial cell carries out an enormous number of metabolic reactions. The common feature of all metabolic reactions is that they are catalyzed by enzymes. Also recall from Chapter 5 (page 118) that feedback inhibition stops a cell from performing unneeded chemical reactions. Feedback inhibition stops enzymes that have already been synthesized. We will now look at mechanisms to prevent synthesis of enzymes that are not needed.

We have seen that genes, through transcription and translation, direct the synthesis of proteins, many of which serve as enzymes—the very enzymes used for cellular metabolism. Because protein synthesis requires a huge amount of energy, regulation of protein synthesis is important to the cell's energy economy. Cells save energy by making only those proteins needed at a particular time. Next we look at how chemical reactions are regulated by controlling the synthesis of the enzymes.

Many genes, perhaps 60–80%, are not regulated but are instead *constitutive,* meaning that their products are constantly

Figure 8.11 **RNA processing in eukaryotic cells.**

Q Why can't the RNA transcript be used for translation?



produced at a fixed rate. Usually these genes, which are effectively turned on all the time, code for enzymes that the cell needs in fairly large amounts for its major life processes; the enzymes of glycolysis are examples. The production of other enzymes is regulated so that they are present only when needed. *Trypanosoma,* the protozoan parasite that causes African sleeping sickness, has hundreds of genes coding for surface glycoproteins. Each protozoan cell turns on only one glycoprotein gene at a time. As the host's immune system kills parasites with one type of surface molecule, parasites expressing a different surface glycoprotein can continue to grow.

Pre-transcriptional Control

Two genetic control mechanisms known as repression and induction regulate the transcription of mRNA and consequently the synthesis of enzymes from them. These mechanisms control the formation and amounts of enzymes in the cell, not the activities of the enzymes.

Repression

The regulatory mechanism that inhibits gene expression and decreases the synthesis of enzymes is called **repression.** Repression is usually a response to the overabundance of an end-product of a metabolic pathway; it causes a decrease in the rate of synthesis of the enzymes leading to the formation of that product. Repression is mediated by regulatory proteins called **repressors,** which block the ability of RNA polymerase to initiate transcription from the repressed genes. The default position of a repressible gene is *on*.

Induction

The process that turns on the transcription of a gene or genes is **induction.** A substance that acts to induce transcription of a gene is called an **inducer,** and enzymes that are synthesized in the presence of inducers are *inducible enzymes.* The genes required for lactose metabolism in *E. coli* are a well-known example of an inducible system. One of these genes codes for the enzyme β-galactosidase, which splits the substrate lactose into two simple sugars, glucose and galactose. (β refers to the type of linkage that joins the glucose and galactose.) If *E. coli* is placed into a medium in which no lactose is present, the organisms contain almost no β-galactosidase; however, when lactose is added to the medium, the bacterial cells produce a large quantity of the enzyme. Lactose is converted in the cell to the related compound allolactose, which is the inducer for these genes; the presence of lactose thus indirectly induces the cells to synthesize more enzyme. The default position of an inducible gene is *off.* MM **Animations** Operons: Induction, Repression

The Operon Model of Gene Expression

Details of the control of gene expression by induction and repression are described by the operon model. François Jacob and Jacques Monod formulated this general model in 1961 to account for the regulation of protein synthesis. They based their model on studies of the induction of the enzymes of lactose catabolism in *E. coli.* In addition to β-galactosidase, these enzymes include lac permease, which is involved in the transport of lactose into the cell, and transacetylase, which metabolizes certain disaccharides other than lactose.

Tracking West Nile Virus

On August 23, 1999, an infectious disease physician from a hospital in northern Queens contacted the New York City Department of Health (NYCDOH) to report two patients with encephalitis. On investigation, the NYCDOH initially identified a cluster of six patients with encephalitis. At the same time, local health officials observed increased fatalities among New York City birds. No bacteria were cultured from the patients' blood or cerebrospinal fluid. Viruses transmitted by mosquitoes are a likely cause of aseptic encephalitis during the summer months. These viruses are called arboviruses. Arboviruses, *ar*thropod-*bo*rne, are viruses that are maintained in nature through biological transmission between susceptible vertebrate hosts by blood-feeding arthropods, such as mosquitoes.

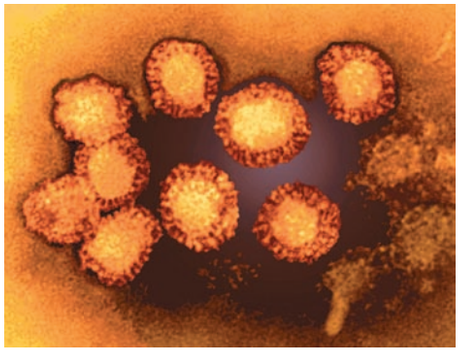
Nucleic acid sequencing of isolates from birds was performed at the CDC on September 23. Comparison of the nucleic acid sequences to databases indicated that the viruses were closely related to West Nile virus (WNV, see the photo), which had never been isolated in the Western Hemisphere.

By 2007, WNV had been found in birds in all states except Alaska and Hawaii. By 2009, the CDC considered West Nile virus endemic in the United States. The recognition of WNV in the Western Hemisphere in the summer of 1999 marked the first introduction in recent history of an Old World flavivirus into the New World.

West Nile virus was first isolated in 1937 in the West Nile district of Uganda. In the early 1950s, scientists recognized WNV encephalitis outbreaks in humans in Egypt and Israel. Initially considered a minor arbovirus, WNV has emerged as a major public health and veterinary concern in southern Europe, the Mediterranean basin, and North America.

Researchers looked at the virus's genome for clues about its path around the world. The flavivirus genome consists of a positive, single-stranded RNA 11,000 to 12,000 nucleotides long. (Positive RNA can act as mRNA and be translated.) The virus has acquired several mutations, and researchers are looking for clues in these mutations to determine the virus's journey.

1. Using the portions of the genomes (shown below) that encode viral proteins, can you determine how similar are these viruses? Can you figure out its movement around the world?
   Determine the amino acids encoded, and group the viruses based on percentage of similarity to the Uganda strain.



West Nile virus TEM 50 nm

2. Based on amino acids, there are two groups called clades.
   Which group is older?
3. The North American and Australian strains have accumulated more mutations, so these should be more recent.
   Calculate the percentage of difference between nucleotides to see how the viruses are related within their clade.
4. Although genetically related groups or clades can be seen, the actual journey of the virus remains elusive.

Source: Adapted from CDC data.

Australia	A	C	C	C	C	G	T	C	C	A	C	C	C	T	T	T	C	A	A	T	T
Egypt	A	A	T	C	G	A	T	C	A	T	C	T	T	C	G	T	C	G	A	T	C
France	A	A	T	C	G	A	T	C	A	T	C	G	T	C	G	T	C	G	A	T	C
Israel	A	T	C	C	A	T	T	C	A	T	C	C	T	C	A	T	C	G	A	T	T
Italy	A	T	C	C	A	C	T	C	A	T	C	C	T	C	G	T	C	G	A	T	T
Kenya	A	T	C	C	A	C	T	C	A	T	C	C	T	C	G	T	C	G	A	T	T
Mexico	A	A	C	C	C	T	T	C	C	T	C	C	C	C	T	T	C	G	A	T	T
United States	A	A	C	C	C	C	T	C	C	T	C	C	C	C	T	T	C	G	A	T	T
Uganda	A	T	A	C	G	A	T	C	A	T	G	C	T	C	G	T	C	C	A	T	C

The genes for the three enzymes involved in lactose uptake and utilization are next to each other on the bacterial chromosome and are regulated together (**Figure 8.12**). These genes, which determine the structures of proteins, are called *structural genes* to distinguish them from an adjoining control region on the DNA. When lactose is introduced into the culture medium, the *lac* structural genes are all transcribed and translated rapidly and simultaneously. We will now see how this regulation occurs.

In the control region of the *lac* operon are two relatively short segments of DNA. One, the *promoter,* is the region of DNA

where RNA polymerase initiates transcription. The other is the **operator,** which is like a traffic light that acts as a go or stop signal for transcription of the structural genes. A set of operator and promoter sites and the structural genes they control define an **operon;** thus, the combination of the three *lac* structural genes and the adjoining control regions is called the *lac* operon.

A regulatory gene called the *I gene* encodes a **repressor** protein that switches inducible and repressible operons on or off. The *lac* operon is an **inducible operon** (see Figure 8.12). In the absence of lactose, the repressor binds to the operator site, thus preventing transcription. If lactose is present, the repressor binds to a metabolite of lactose instead of to the operator, and lactose-digesting enzymes are transcribed.

In **repressible operons,** the structural genes are transcribed until they are turned off, or *repressed* (Figure 8.13). The genes for the enzymes involved in the synthesis of tryptophan are regulated in this manner. The structural genes are transcribed and translated, leading to tryptophan synthesis. When excess tryptophan is present, the tryptophan acts as a **corepressor** binding to the repressor protein. The repressor protein can now bind to the operator, stopping further tryptophan synthesis.

Animation Operons: Overview

CHECK YOUR UNDERSTANDING

Use the following metabolic pathway to answer the questions that follow it. **8-6**

$$\text{Substrate } A \xrightarrow{\text{enzyme } a} \text{Intermediate } B \xrightarrow{\text{enzyme } b} \text{End-product } C$$

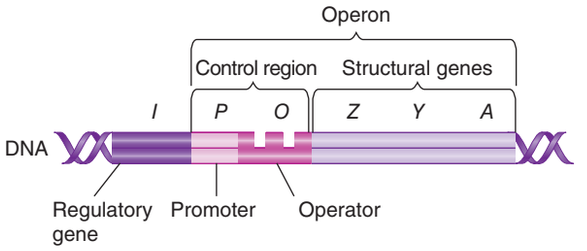
a. If enzyme *a* is inducible and is not being synthesized at present, a (1) _____ protein must be bound tightly to the (2) _____ site. When the inducer is present, it will bind to the (3) _____ so that (4) _____ can occur.

b. If enzyme *a* is repressible, end-product *C*, called a (1) _____, causes the (2) _____ to bind to the (3) _____. What causes derepression?

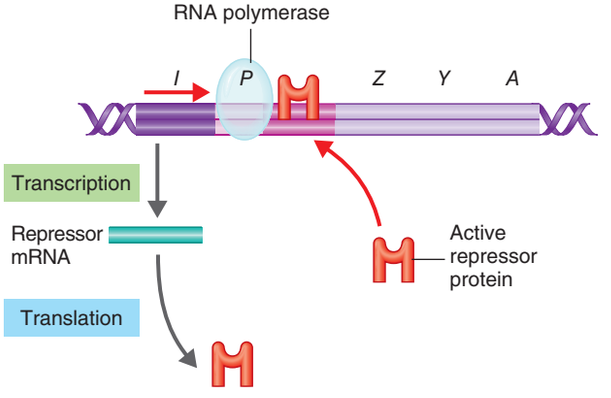
Positive Regulation

Regulation of the lactose operon also depends on the level of glucose in the medium, which in turn controls the intracellular level of the small molecule **cyclic AMP (cAMP)**, a substance derived from ATP that serves as a cellular alarm signal. Enzymes that metabolize glucose are constitutive, and cells grow at their maximal rate with glucose as their carbon source because they can use it most efficiently (Figure 8.14). When glucose is no longer available, cAMP accumulates in the cell. The cAMP binds to the allosteric site of *catabolic activator protein (CAP).* CAP then binds to the *lac* promoter, which initiates transcription by making it easier for RNA polymerase to bind to the promoter. Thus transcription of the *lac* operon requires both the presence of lactose and the absence of glucose (Figure 8.15).

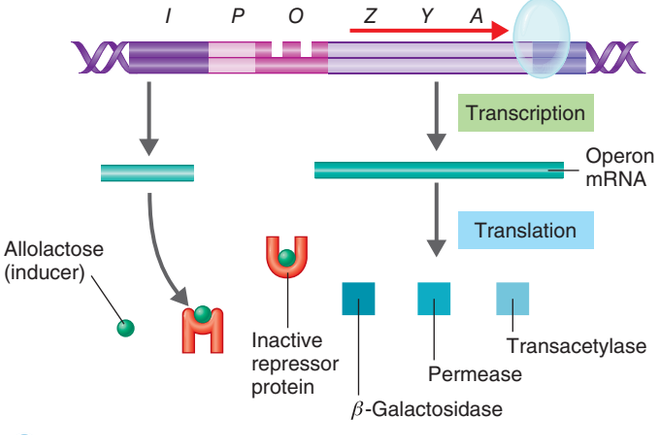
Cyclic AMP is an example of an *alarmone,* a chemical alarm signal that promotes a cell's response to environmental or nutritional stress. (In this case, the stress is the lack of glucose.)



1 Structure of the operon. The operon consists of the promoter (*P*) and operator (*O*) sites and structural genes that code for the protein. The operon is regulated by the product of the regulatory gene (*I*).



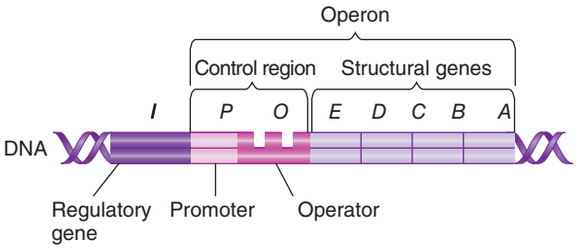
2 Repressor active, operon off. The repressor protein binds with the operator, preventing transcription from the operon.



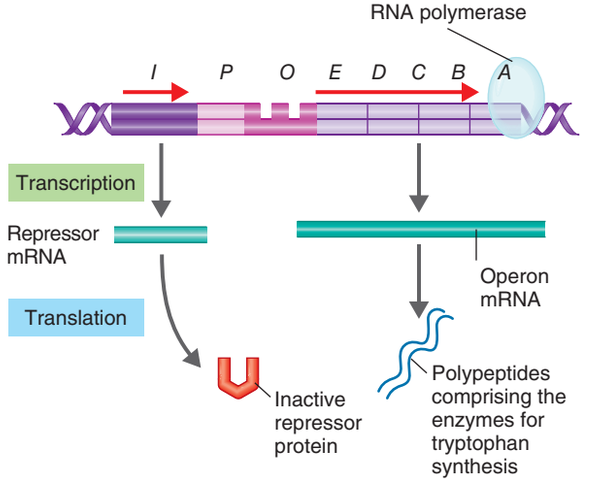
3 Repressor inactive, operon on. When the inducer allolactose binds to the repressor protein, the inactivated repressor can no longer block transcription. The structural genes are transcribed, ultimately resulting in the production of the enzymes needed for lactose catabolism.

Figure 8.12 An inducible operon. Lactose-digesting enzymes are produced in the presence of lactose. In *E. coli*, the genes for the three enzymes are in the *lac* operon. β-galactosidase is encoded by *lacZ*. The *lacY* gene encodes the lac permease, and *lacA* encodes transacetylase, whose function in lactose metabolism is still unclear.

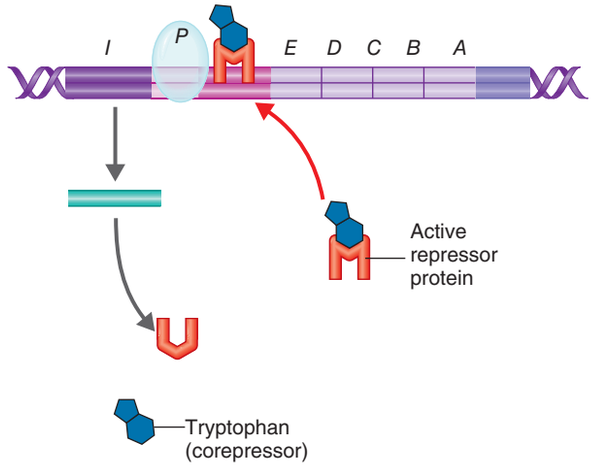
Q What causes transcription of an inducible enzyme?



1 Structure of the operon. The operon consists of the promoter (*P*) and operator (*O*) sites and structural genes that code for the protein. The operon is regulated by the product of the regulatory gene (*I*).



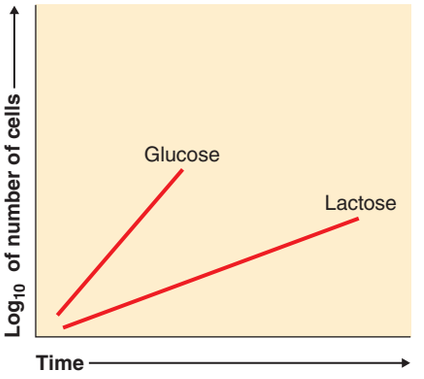
2 Repressor inactive, operon on. The repressor is inactive, and transcription and translation proceed, leading to the synthesis of tryptophan.



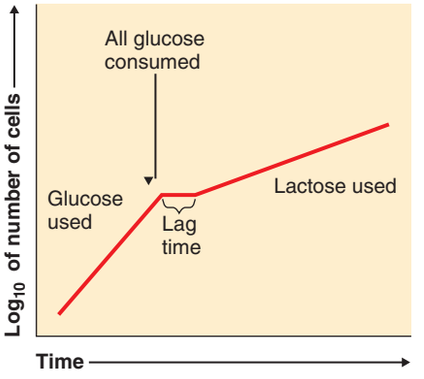
3 Repressor active, operon off. When the corepressor tryptophan binds to the repressor protein, the activated repressor binds with the operator, preventing transcription from the operon.

Figure 8.13 A repressible operon. Tryptophan, an amino acid, is produced by anabolic enzymes encoded by five structural genes. Accumulation of tryptophan represses transcription of these genes, preventing further synthesis of tryptophan. The *E. coli trp* operon is shown here.

Q What causes transcription of a repressible enzyme?



(a) Bacteria growing on glucose as the sole carbon source grow faster than on lactose.



(b) Bacteria growing in a medium containing glucose and lactose first consume the glucose and then, after a short lag time, the lactose. During the lag time, intracellular cAMP increases, the *lac* operon is transcribed, more lactose is transported into the cell, and β-galactosidase is synthesized to break down lactose.

Figure 8.14 The growth rate of *E. coli* on glucose and lactose.

Q When both glucose and lactose are present, why will cells use glucose first?

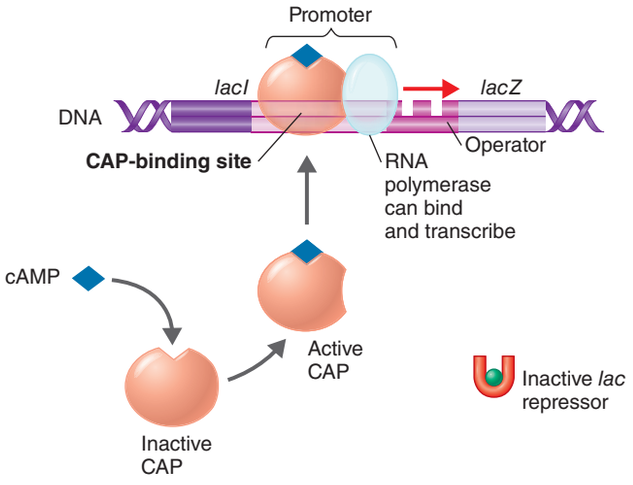
The same mechanism involving cAMP allows the cell to grow on other sugars. Inhibition of the metabolism of alternative carbon sources by glucose is termed **catabolite repression** (or the *glucose effect*). When glucose is available, the level of cAMP in the cell is low, and consequently CAP is not bound.

Epigenetic Control

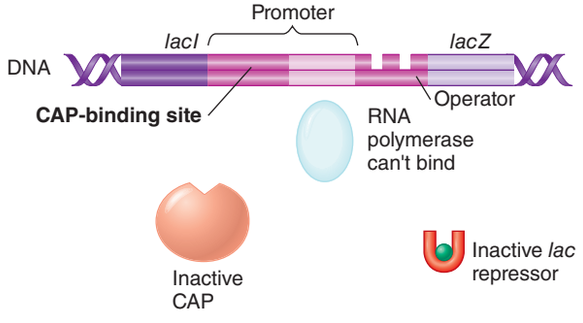
Eukaryotic and bacterial cells can turn genes off by methylating certain nucleotides. The methylated (off) genes are passed to offspring cells. Unlike mutations, this isn't permanent, and the genes can be turned on in a later generation. This is called *epigenetic inheritance* (*epigenetic* = on genes). Epigenetics may explain why bacteria behave differently in a biofilm (see the box on page 56).

Post-transcriptional Control

Some regulatory mechanisms stop protein synthesis after transcription has occurred. Single-stranded RNA molecules of approximately 22 nucleotides, called **microRNAs (miRNAs),** inhibit protein production in eukaryotic cells. In humans, miRNAs produced during development allow different cells to



(a) Lactose present, glucose scarce (cAMP level high). If glucose is scarce, the high level of cAMP activates CAP, and the *lac* operon produces large amounts of mRNA for lactose digestion.



(b) Lactose present, glucose present (cAMP level low). When glucose is present, cAMP is scarce, and CAP is unable to stimulate transcription.

Figure 8.15 **Positive regulation of the *lac* operon.**

Q Will transcription of the *lac* operon occur in the presence of lactose and glucose? In the presence of lactose and the absence of glucose? In the presence of glucose and the absence of lactose?

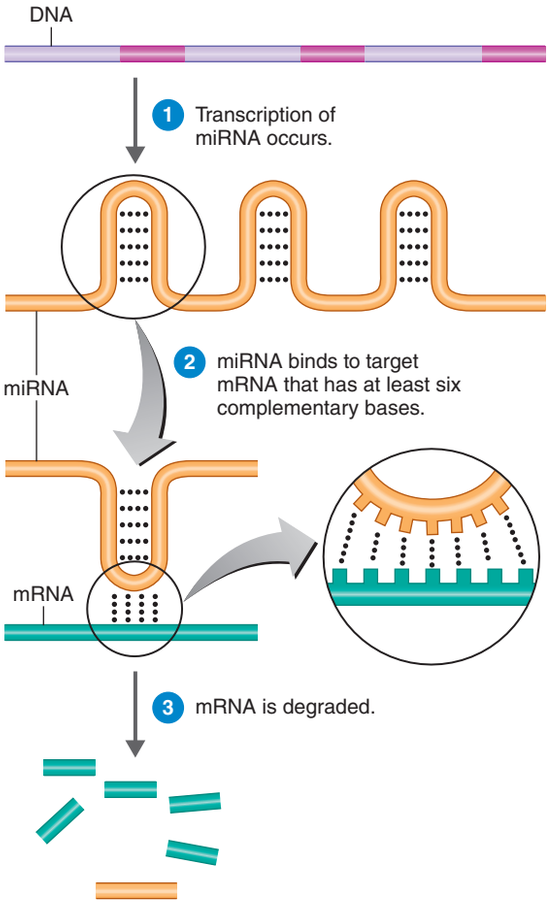


Figure 8.16 **MicroRNAs control a wide range of activities in cells.**

Q In mammals, some miRNAs hybridize with viral RNA. What would happen if a mutation occurred in the miRNA gene?

produce different proteins. Heart cells and skin cells have the same genes, but the cells in each organ produce different proteins because of miRNAs produced in each cell type during development. Similar short RNAs in bacteria enable the cell to cope with environmental stresses, such as low temperature or oxidative damage. An miRNA base-pairs with a complementary mRNA, forming a double-standard RNA. This double-standard RNA is enzymatically destroyed so that the mRNA-encoded protein is not made (**Figure 8.16**). The action of another type of RNA, siRNA, is similar and is discussed on page 258.

CHECK YOUR UNDERSTANDING

- What is the role of cAMP in regulating gene expression? **8-7**
- How does miRNA stop protein synthesis? **8-8**

Mutation: Change in the Genetic Material

LEARNING OBJECTIVES

8-9 Classify mutations by type.

8-10 Describe two ways mutations can be repaired.

8-11 Describe the effect of mutagens on the mutation rate.

8-12 Outline the methods of direct and indirect selection of mutants.

8-13 Identify the purpose of and outline the procedure for the Ames test.

A **mutation** is a permanent change in the base sequence of DNA. Such a change in the base sequence of a gene will sometimes cause a change in the product encoded by that gene. For example, when the gene for an enzyme mutates, the enzyme encoded by the gene may become inactive or less active because its amino acid sequence has changed. Such a change in genotype may be disadvantageous, or even lethal, if the cell loses a

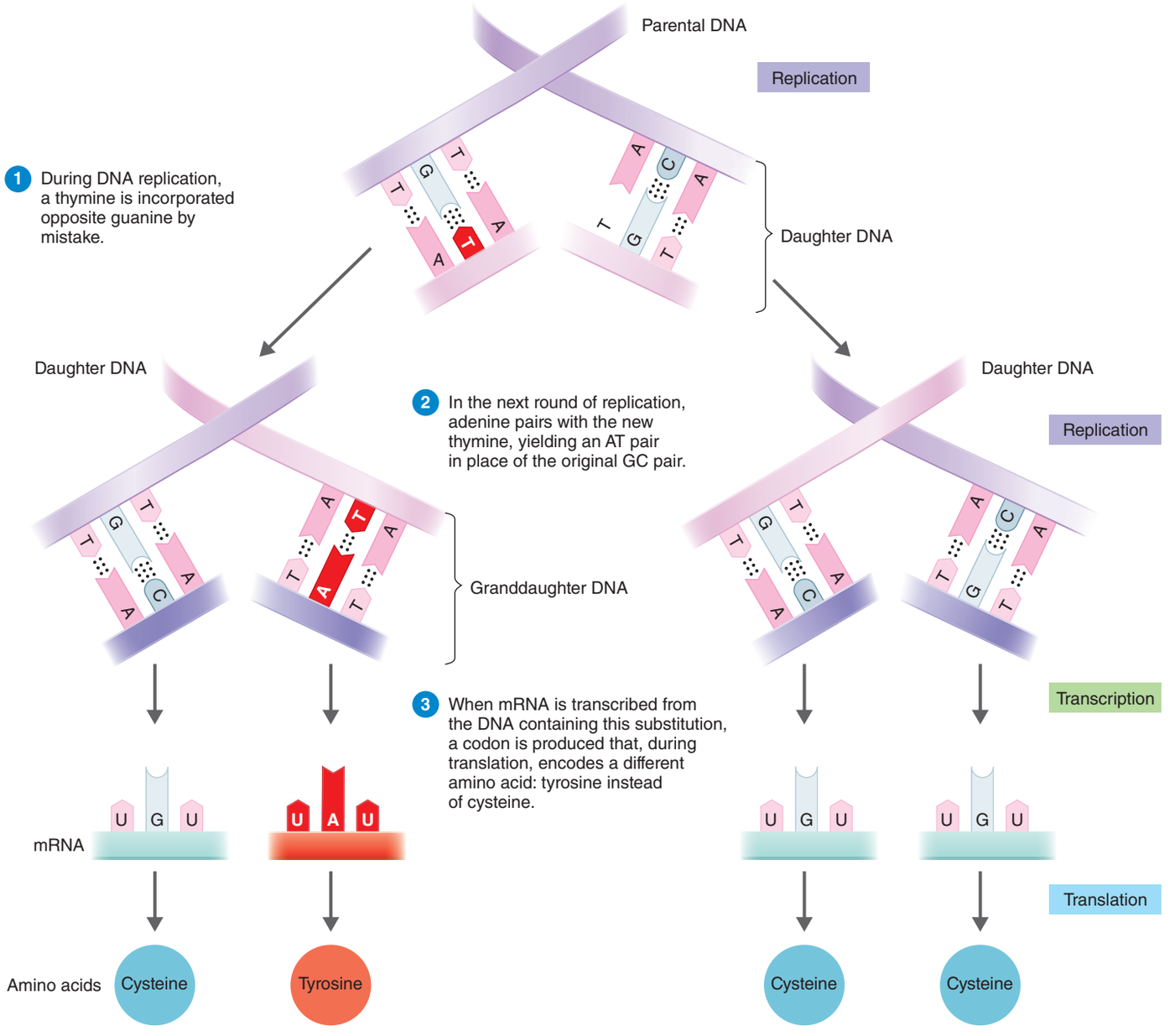


Figure 8.17 Base substitutions. This mutation leads to an altered protein in a granddaughter cell.

Q Does a base substitution always result in a different amino acid?

phenotypic trait it needs. However, a mutation can be beneficial if, for instance, the altered enzyme encoded by the mutant gene has a new or enhanced activity that benefits the cell.

Many simple mutations are silent (neutral); the change in DNA base sequence causes no change in the activity of the product encoded by the gene. Silent mutations commonly occur when one nucleotide is substituted for another in the DNA, especially at a location corresponding to the third position of the mRNA codon. Because of the degeneracy of the genetic code, the resulting new codon might still code for the same amino acid. Even if the amino acid is changed, the function of the protein may not change if the amino acid is in a nonvital portion of the protein, or is chemically very similar to the original amino acid.

Types of Mutations

The most common type of mutation involving single base pairs is **base substitution** (or *point mutation*), in which a single base at one point in the DNA sequence is replaced with a different base. When the DNA replicates, the result is a substituted base