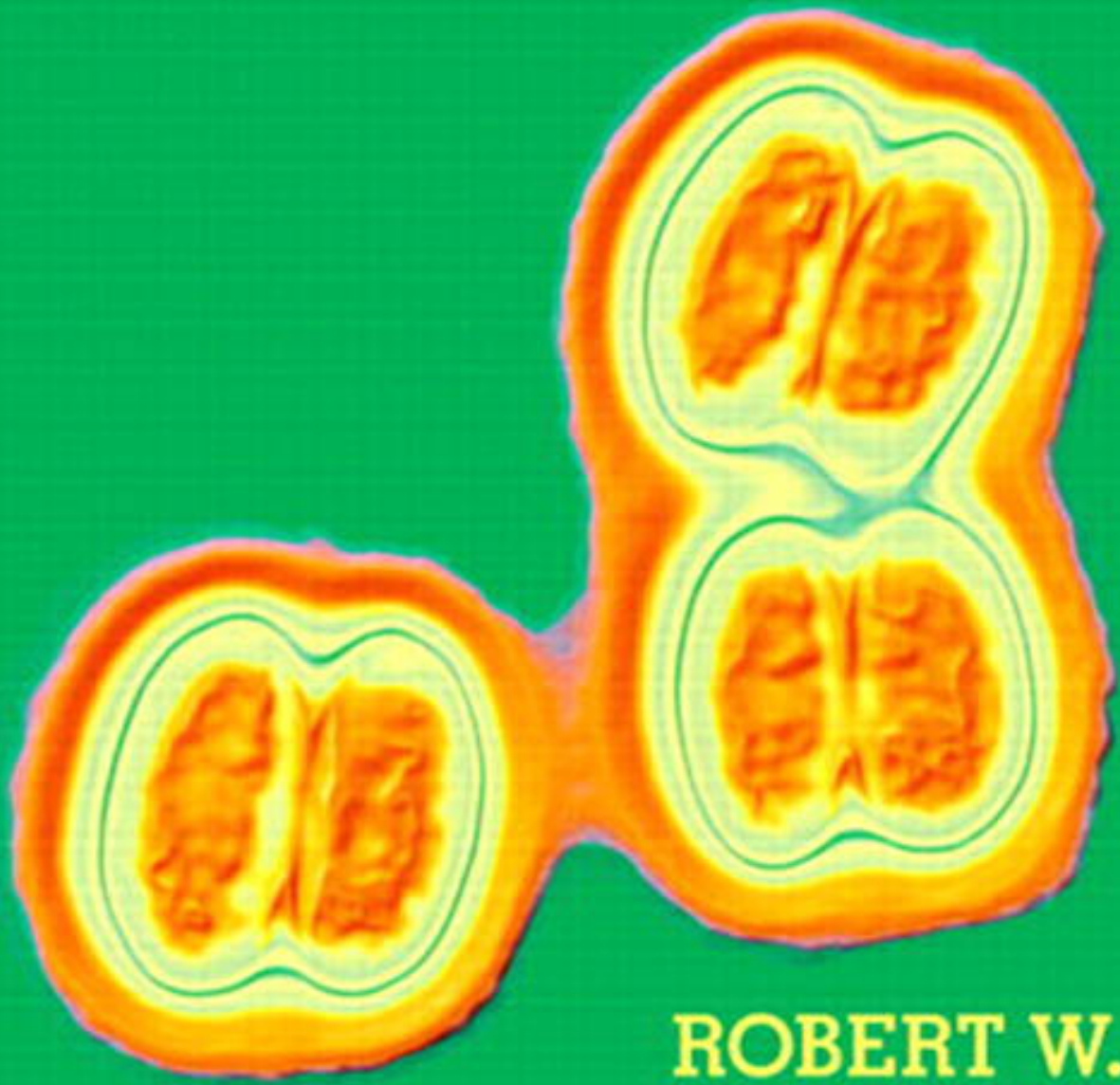
FOURTH EDITION

MICROBIOLOGY

WITH DISEASES BY TAXONOMY



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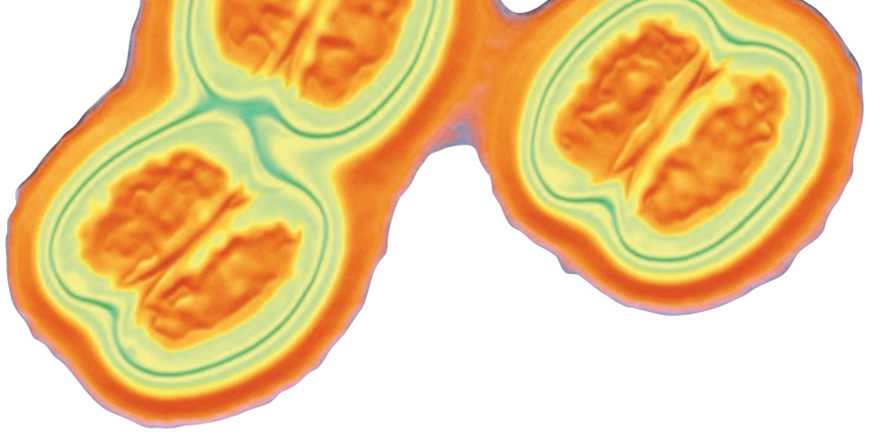

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MICROBIOLOGY

WITH DISEASES BY TAXONOMY

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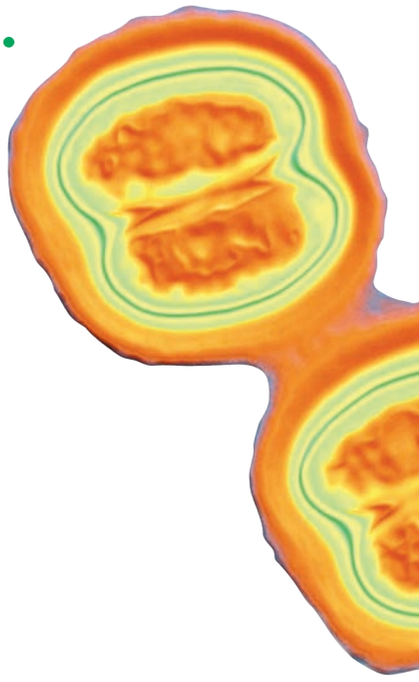

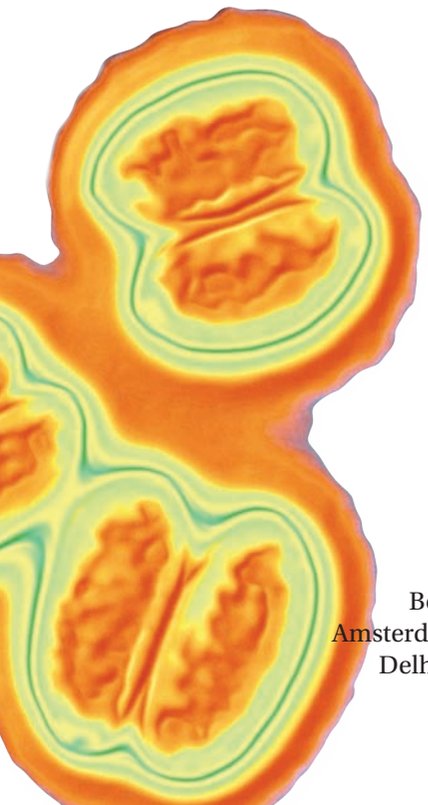

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To Michelle:
My best friend, my closest confidant, my cheerleader,
my partner, my love. Thirty years! I love you more now than then.

—Robert

About the Author

ROBERT W. BAUMAN is a professor of biology and past chairman of the Department of Biological Sciences at Amarillo College in Amarillo, Texas. He teaches microbiology, human anatomy and physiology, and botany. In 2004, the students of Amarillo College selected Dr. Bauman as the recipient of the John F. Mead Faculty Excellence Award. He received an M.A. degree in botany from the University of Texas at Austin and a Ph.D. in biology from Stanford University. His research interests have included the morphology and ecology of freshwater algae, the cell biology of marine algae (particularly the deposition of cell walls and intercellular communication), and environmentally triggered chromogenesis in butterflies. He is a member of the American Society of Microbiology (ASM) where he has held national offices, the Texas Community College Teacher's Association (TCCTA), the American Association for the Advancement of Science (AAAS), the Human Anatomy and Physiology Society (HAPS), and the Lepidopterists Society. When he is not writing books, he enjoys spending time with his family: gardening, hiking, camping, rock climbing, backpacking, cycling, snowshoeing, skiing, and reading by a crackling fire or in a gently swaying hammock.

About the Clinical Consultants

CECILY D. COSBY is nationally certified as both a family nurse practitioner and physician assistant. She is a professor of nursing, currently teaching at Samuel Merritt College in Oakland, California, and has been in clinical practice since 1980, most recently at the University of California, San Francisco, in a preoperative practice. She received her Ph.D. and MS from the University of California, San Francisco; her BSN from California State University, Long Beach; and her PA certificate from the Stanford Primary Care program. She was awarded the Paul C. Samson Clinical Nursing Professional Chair for 2007–2010.

JEAN E. MONTGOMERY is a registered nurse formerly teaching in the associate degree nursing program at Austin Community College in Texas. She received her MSN from the University of Texas Health Science Center at San Antonio, Texas.

Preface

The spread of whooping cough, snail fever, spotted fever rickettsiosis, and other emerging diseases; the cases of strep throat, MRSA, and tuberculosis; the progress of cutting-edge research into microbial genetics; the challenge of increasingly drug-resistant pathogens; the continual discovery of microorganisms previously unknown—these are just a few examples of why exploring microbiology has never been more exciting, or more important. Welcome!

I have taught microbiology to undergraduates for over 25 years and witnessed firsthand how students struggle with the same topics and concepts year after year. To address these challenging topics, I have developed and narrated Video Tutors that walk students through key concepts in microbiology, bringing the art of the textbook to life and important concepts into view. In creating this textbook, my goal was to allow students to see complex topics of microbiology—especially metabolism, genetics, and immunology—in a way that they can understand while at the same time presenting a thorough and accurate overview of microbiology. I also wished to highlight the many positive effects of microorganisms on our lives, along with the medically important microorganisms that cause disease.

NEW TO THIS EDITION

In approaching the fourth edition, my goal was to build upon the strengths and success of the previous editions by updating it with the latest scientific and educational research and data available and by incorporating the many terrific suggestions I have received from colleagues and students alike. The feedback from instructors who adopted previous editions has been immensely gratifying and is much appreciated. Another goal for this edition was to provide additional instruction on important concepts and processes. To that end, I developed and narrated the Video Tutors accessible via QR codes in the textbook and in MasteringMicrobiology. The result is, once again, a collaborative effort of educators, students, editors, and top scientific illustrators: a textbook that, I hope, continues to improve upon conventional explanations and illustrations in substantive and effective ways. In this new edition:

- **New Video Tutors** developed and narrated by the author walk students through key concepts in microbiology, bringing the textbook art to life and helping students visualize and understand tough topics and important processes. These video tutorials are accessible via QR codes in the textbook and accompanied by multiple-choice questions that are assignable in MasteringMicrobiology®.
- **New Clinical Case Study** and **Emerging Disease Case Study boxes** reflect the fourth edition's emphasis on clinical topics and emerging diseases. Focused on the signs, symptoms, diagnosis, and treatment of each disease, these boxes do not assume a student has a medical background or complete understanding of all aspects of health care. They are presented in an engaging style that encourages the student to think critically. (See pp. xxii-xxiii for a full list.)
- **New MicroCareers Coaching Activities and Clinical Case Study Activities**, assignable in MasteringMicrobiology, allow students to explore careers in microbiology by examining diseases and epidemiology.
- **New Numbered Learning Outcomes** in the textbook are used to tag Test Bank questions and all Mastering assets. In addition to being tagged to Learning Outcomes, all Mastering assessments are tagged to the Global Science Learning Outcomes and Bloom's Taxonomy. The complete Mastering Test Bank is also tagged to ASMCUE-recommended outcomes.

- **New Visualize It!** features appear at the end of each chapter. These are short-answer or fill-in-the-blank questions built around illustrations or photos. These are also assignable art labeling activities in MasteringMicrobiology.
- **Over 100 New micrographs and photos** enhance student understanding of the text and boxed features.
- **Improved Lab equipment illustrations** feature increased dimensionality and realism to help students arrive prepared for their lab course.
- **Chapter 3 (Cell Structure and Function)** deemphasizes the term "prokaryote" (a term that is based on an outdated perception of taxonomy and is thus misleading to students) and instead emphasizes the three domains of living organisms, matching the latest taxonomic research. This state-of-the-science organization sets this book apart from all other allied health microbiology books.
- **The immunology chapters (Chapters 15–18),** which have been and continue to be reviewed in-depth by immunology specialists, reflect the most current understanding of this rapidly changing field of any microbiology book available.
- **New Microbe-at-a-Glance art labeling activities**, assignable in MasteringMicrobiology, help students understand form and function relationships with respect to taxonomy.
- **MasteringMicrobiology®** includes not only the new Video Tutors with assessments, the MicroCareers Coaching Activities and Clinical Case Study Activities, and the Visualize It! and Microbe-at-a-Glance art labeling activities but also Microbiology Lab Technique videos with assessment and MicroLab Tutor coaching activties. MicroLab Tutors use lab technique videos, 3D molecular animations, and stepped-out tutorials to actively engage students in making the connection between microbiology lecture, lab, and the real world. Additionally, MasteringMicrobiology and the Study Area include new MicroLab Practical quizzes, which ask students to analyze and interpret important lab tests, techniques, and results.

The following section provides a detailed outline of this edition's chapter-by-chapter revisions.

Chapter-by-Chapter Revisions

Every chapter in this edition has been thoroughly revised, and data in the text, tables, and figures have been updated. The main changes for each chapter are summarized below.

THROUGHOUT THE PATHOGEN CHAPTERS (19–25)

- Updated disease diagnoses, treatments, and incidence and prevalence data
- Updated immunization recommendations and suggested treatments for all diseases
- Added Clinical Case Study boxes as noted below
- Added answers to Clinical Case Study boxes to Instructor's Manual

CHAPTER 1 A BRIEF HISTORY OF MICROBIOLOGY

- Two new figures for increased general interest
- Eight new photos
- Updated map showing countries with acquisition of variant Creutzfeldt-Jakob disease (vJCD)
- New Clinical Case Study boxes on a yellow fever epidemic in the 18th century and stomach ulcers
- New Visualize It! question on Pasteur's experiment
- New Video Tutor: The Scientific Method

CHAPTER 2 THE CHEMISTRY OF MICROBIOLOGY

- Four revised figures for better pedagogy
- Two new photos
- Updated evidence for liquid water and necessary chemicals for life occurring on the moon of Saturn, Enceladus
- Expanded coverage of the nucleosides, which are used as nucleotide analogs in treating a number of diseases
- New figure legend question for enhanced pedagogy
- New Visualize It! question on molecular structure
- New Video Tutor: The Structure of Nucleotides
- New fill-in-the-blank Concept Map on nucleic acids

CHAPTER 3 CELL STRUCTURE AND FUNCTION

- Six revised figures for enhanced pedagogy and accuracy
- Sixteen new photos
- Enhanced discussion of bacterial cytoskeletons
- Enhanced discussion of the roles of glycocalyces in biofilms
- New Clinical Case Study box on streptococcal pharyngitis (strep throat).
- New Visualize It! question on bacterial flagellar arrangements
- New Video Tutor: Bacterial Cell Walls

CHAPTER 4 MICROSCOPY, STAINING, AND CLASSIFICATION

- Three new photos
- Two tables with revised artwork and photos
- Additional coverage of histological stains: Gomori methenamine silver (GMS) stain and hematoxylin and eosin (HE) stain
- Updated coverage of taxonomy; for example, expanded discussion of definitions of microbial species
- New Visualize It! question on parts of the optical microscope
- New Video Tutor: The Light Microscope
- New fill-in-the-blank Concept Map on Gram stain and cell wall structure

CHAPTER 5 MICROBIAL METABOLISM

- One new figure and two revised figures for greater clarity and better pedagogy
- Expanded coverage of vitamins as enzymatic cofactors
- Revisions in text and figure legends to more clearly explain energy transfer in glycolysis, Krebs cycle, and electron transport
- Revisions in the text to clarify that glycolysis, the pentose phosphate pathway, and the Krebs cycle supply many precursor metabolites for anabolism
- Additional discussion of bacterial quorum sensing and biofilms
- New Visualize It! question on glycolysis, the Krebs cycle, and electron transport chains
- New Video Tutor: Electron Transport Chains
- New fill-in-the-blank Concept Map on aerobic respiration

CHAPTER 6 MICROBIAL NUTRITION AND GROWTH

- Two new figures and four revised figures for greater clarity, ease of reading, and better pedagogy
- Four new photos
- Significantly expanded coverage of biofilms and quorum sensing
- Updated section on radiation-tolerant microbes, covering fungi that use radioactivity as an energy source
- New Clinical Case Study box on MRSA infection in a high school
- New Visualize It! question on hemolysis
- New Video Tutor: Bacterial Growth Media

CHAPTER 7 MICROBIAL GENETICS

- Two new figures and thirteen revised figures for greater clarity, accuracy, and pedagogy
- Three new photos
- Revisions in the text to better explain differences between archaeal, bacterial, and eukaryotic genetics
- Extended coverage of differences between nucleoside and nucleotide (many antimicrobial drugs are analogs of the former, not the latter)
- Extended coverage of codons and tRNAs for 21st and 22nd amino acids
- Modified artwork reflecting changes in our understanding of molecular biology. For example, where possible, enzyme shapes are based upon actual 3D profiles as revealed by X-ray crystallography (e.g., Figure 7.28) and eukaryotic histone shape is more accurately represented to conform to new discoveries (Figure 7.3)
- Clearer section on operons, introduction of the term *polycistronic*, and new discussion of quorum-sensing as a trigger for inducible and repressible operons
- Revised section on regulatory RNA molecules for clarity and to conform to newly discovered information
- New Clinical Case Study box on nosocomial, enterococcal infection involving horizontal gene transfer

- New Emerging Disease Case Study box on *Vibrio vulnificus* infection
- New Visualize It! question on a phage DNA molecule
- New Video Tutor: Initiation of Translation
- New fill-in-the-blank Concept Map on point mutations

CHAPTER 8 RECOMBINANT DNA TECHNOLOGY

- Six revised figures for enhanced pedagogy and accuracy
- Five new photos
- Additional coverage of new recombinant agricultural crops, including ringspot-virus-resistant papayas
- Increased coverage of the debate concerning genetic modification of agricultural products
- New Highlight boxes on the use of recombinant DNA techniques to address Dengue fever and the progress of developing edible vaccines
- New Visualize It! question on a "DNA fingerprint"
- New Video Tutor: Action of Restriction Enzymes
- New fill-in-the-blank Concept Map on recombinant DNA technology

CHAPTER 9 CONTROLLING MICROBIAL GROWTH IN THE ENVIRONMENT

- One revised figure more clearly illustrating the role of HEPA filters in biological settings
- Six new photos
- Reorganization of the topics "Methods for Evaluating Disinfectants and Antiseptics" and "Biosafety Levels" for better flow and pedagogy
- New Visualize It! question on brass and water safety
- New Video Tutor: Principles of Autoclaving
- New fill-in-the-blank Concept Map on moist heat applications to control microorganisms

CHAPTER 10 CONTROLLING MICROBIAL GROWTH IN THE BODY: ANTIMICROBIAL DRUGS

- One revised figure more accurately illustrating the inhibitory effects of beta-lactams on bacterial cell walls
- Five new photos
- Expanded discussion of use of RNA interference (RNAi) and antisense nucleic acids as antimicrobial therapy
- Increased discussion of biofilms as they relate to drug resistance
- Updated and revised tables of antimicrobials to include all new antimicrobials mentioned in disease chapters, including new antiprotozoan drugs (lumefantrine, nitazoxanide, paromomycin, piperaquine, and tinidazol) and antiviral protease inhibitors (boceprevir, darunavir, and telaprevir)
- Additional coverage of *therapeutic index* and *therapeutic window* as applied to antimicrobials.
- Additional material on transfer of resistance genes between and among bacteria and on research to discover novel antimicrobials
- Nine new Learning Outcomes
- New Clinical Case Study on treating a wound infection
- Three new figure legend and critical thinking questions, including questions on determining minimum inhibitory concentration and on antimicrobial analog function
- New Visualize It! question on a test for antimicrobial efficacy
- New Video Tutor: Action of Some Drugs That Inhibit Prokaryotic Protein Synthesis
- New fill-in-the-blank Concept Map on antimicrobial resistance

CHAPTER 11 CHARACTERIZING AND CLASSIFYING PROKARYOTES

- Three revised figures for better pedagogy and accuracy
- Fifteen new photos
- Updated taxonomy which corresponds more completely with *Bergey's Manual*
- Six new Learning Outcomes in section on proteobacteria
- New Highlight box exploring a possible connection between cyanobacteria and neurological disorders, such as amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease
- New Highlight box concerning the link between oral microbiota and obesity
- New Visualize It! question on endospores
- New Video Tutor: Arrangements of Prokaryotic Cells
- New fill-in-the-blank Concept Map on the domain archaea

CHAPTER 12 CHARACTERIZING AND CLASSIFYING EUKARYOTES

- Revised two figures for greater clarity and accuracy
- Fifteen new photos
- Additional discussion of the use by fungi of radiation as an energy source
- New Visualize It! question on a general fungal life cycle
- New Video Tutor: Principles of Sexual Reproduction in Fungi
- New fill-in-the-blank Concept Map on eukaryotic microorganisms

CHAPTER 13 CHARACTERIZING AND CLASSIFYING VIRUSES, VIROIDS, AND PRIONS

- Revised three figures for better pedagogy and accuracy
- Six new photos
- New coverage of discovery of *Megavirus*—the largest virus
- Expanded coverage of prions
- Updated Emerging Disease box on chikungunya, including map of affected areas
- New Visualize It! question on recognizing viral shapes in transmission electron micrographs
- New Video Tutor: The Lytic Cycle of Viral Replication
- New fill-in-the-blank Concept Map on the replication of animal viruses

CHAPTER 14 INFECTION, INFECTIOUS DISEASES, AND EPIDEMIOLOGY

- Two revised figures for better pedagogy and accuracy, including more recent disease data
- Eight new photos
- Updated graph on the incidence and prevalence of AIDS among U.S. adults
- New information and graphs on the emerging disease human West Nile virus
- Updated table of nationally notifiable infectious diseases
- New example of "epidemic," using hemolytic uremic syndrome (caused by *E. coli*) instead of *Hantavirus* pulmonary syndrome

- New Video Tutor: Some Virulence Factors
- New fill-in-the-blank Concept Map on disease transmission

CHAPTER 15 INNATE IMMUNITY

- Three revised figures for enhanced clarity and better pedagogy, including new rendition to reflect more accurately the sequence of complement cascade
- One new photo
- Expanded coverage of NOD receptor proteins and their role in protecting against hepatitis C, AIDS, and mononucleosis
- New Clinical Case Study box on mycoplasmal pneumonia in a college student
- New Visualize It! question on identification of white blood cells
- New Video Tutor: Inflammation
- New fill-in-the-blank Concept Map on phagocytosis

CHAPTER 16 SPECIFIC DEFENSE: ADAPTIVE IMMUNITY

- Five revised figures for better pedagogy and clarity
- Two new photos
- Updated information on adaptive T cell cancer therapy
- Newly added step numbering in figures of antigen processing to tie text to figures more closely
- New Visualize It! question on a dendritic cell
- New Video Tutor: Clonal Deletion
- New fill-in-the-blank Concept Map on antibodies

CHAPTER 17 IMMUNIZATION AND IMMUNE TESTING

- Two revised figures for better accuracy, specifically revised virus and antigen structure
- Two new photos
- Updated charts showing the effects of immunization in reducing polio and measles rates in the U.S.
- Updated table of vaccine preventable diseases in the U.S.
- Newly revised CDC 2012 vaccination schedule for children, adolescents, and adults
- Additional coverage of quantifying immunoassays—turbidimetry and nephelometry
- New Visualize It! question on a Western blot
- New Video Tutor: ELISA
- New fill-in-the-blank Concept Map on vaccines

CHAPTER 18 HYPERSENSITIVITIES, AUTOIMMUNE DISEASES, AND IMMUNE DEFICIENCIES

- Five new photos for better pedagogy
- Updated, simplified, and corrected material on Graves' disease, tissue transplants, and multiple sclerosis
- New Visualize It! question on recognizing type I, III, and IV hypersensitivities
- New Video Tutor: Hemolytic Disease of the Newborn
- New fill-in-the-blank Concept Map on immediate (type I) hypersensitivity

CHAPTER 19 PATHOGENIC GRAM-POSITIVE BACTERIA

- One revised figure for better accuracy, specifically revised motor neuron shape
- Eleven new photos
- Updated chart on the incidence of toxic-shock syndrome in the U.S.
- Reduced coverage of diphtheria (only fifteen cases in the U.S. since 1994)
- Updated map showing regions affected by *Mycobacterium ulcerans*
- New Clinical Case Study box on tuberculosis
- New Visualize It! question on the action of the botulism toxin
- New fill-in-the-blank Concept Map on tuberculosis

CHAPTER 20 PATHOGENIC GRAM-NEGATIVE COCCI AND BACILLI

- One new figure and one revised figure for better pedagogy and accuracy
- Nine new photos
- Updated disease charts and graphs for gonorrhea, brucellosis, pertussis, and infections of *Salmonella*
- Updated map showing regions affected by melioidiosis
- Updated treatment recommendations for gonorrhea; meningococcal meningitis; cat-scratch disease; pertussis; infections of *Pseudomonas, Moraxella, Acinetobacter,* and *Burkholderia*; Legionnaires' disease; and Q fever
- New Clinical Case Study box on *Francisella tularensis*
- New Visualize It! question on differential/selective medium (MacConkey's)
- New fill-in-the-blank Concept Map on meningitis

CHAPTER 21 RICKETTSIAS, CHLAMYDIAS, SPIROCHETES, AND VIBRIOS

- Eleven new photos
- Updated disease graphs for Rocky Mountain spotted fever, syphilis, Lyme disease, and cholera
- Revised designation of spotted fever rickettsiosis (from Rocky Mountain spotted fever) to match the CDC reportable disease table and to show that rickettsias other than *Rickettsia rickettsii* can cause the condition
- Updated treatment recommendations for spotted fever rickettsioses, such as Rocky Mountain spotted fever, epidemic (louse-borne) typhus, scrub typhus, lymphogranuloma venereum, trachoma, *Chlamydophila (Chlamydia)* pneumonia, psittacosis, Lyme disease, *Campylobacter* infection, and gastric ulcers
- New Emerging Disease Case Study box on the new cause of spotted fever rickettsiosis—*Rickettsia parkeri*
- Two new critical thinking questions: one concerning the use of tetracyclines in children and pregnant women, and one covering the reason for prescribing chloramphenicol instead of doxycycline
- New Visualize It! question on *Chlamydia*

CHAPTER 22 PATHOGENIC FUNGI

- Thirteen new photos for enhanced pedagogy
- Enhanced discussion of the action of antifungal agents griseofulvin and echinocandins
- Updated diagnoses and treatment of fungal diseases and development of vaccines against fungi
- New Clinical Case Study box on *Histoplasmosis*
- New Visualize It! question on fungal genera

CHAPTER 23 PARASITIC PROTOZOA, HELMINTHS, AND ARTHROPOD VECTORS

- Five new photos
- Revised map of global malaria distribution
- Updated treatment recommendations for leishmaniasis, giardiasis, *Trichomonas* vaginosis, malaria, toxoplasmosis, *Echinococcus, Fasciola, Ascaris,* and *Wuchereria* infections
- Introduction of fifth species of *Plasmodium* that causes malaria in humans—an emerging disease
- New Emerging Disease Case Study box on schistosomiasis, including a map of distribution
- New Visualize It! question on identification of parasites in clinical specimens

CHAPTER 24 PATHOGENIC DNA VIRUSES

- Nineteen new photos
- Updated graph for prevalence of acute hepatitis B showing reduction due to vaccination efforts
- Updated map of regions affected by monkeypox
- New Clinical Case Study box on hepatitis B
- New Visualize It! question on identifying diseases
- New fill-in-the-blank Concept Map on herpes virus

CHAPTER 25 PATHOGENIC RNA VIRUSES

- One new figure and two figures revised for better pedagogy and accuracy
- Twelve new photos
- Updated disease graphs for polio, human West Nile virus, Dengue fever, rubella, HIV/AIDS, measles, mumps, and Ebola
- Revised section on AIDS and HIV reflecting new discoveries about the way HIV enters and exits cells
- New Clinical Case Study box on rabies and influenza
- New Beneficial Microbe box on the use of *Wolbachia* to "vaccinate" mosquitoes against the dengue virus
- New critical thinking question on retroviruses
- New Visualize It! question on flu epidemics
- New fill-in-the-blank Concept Map on viral hepatitis

CHAPTER 26 INDUSTRIAL AND ENVIRONMENTAL MICROBIOLOGY

- Seven new photos
- Clarification of the terms *unripened* and *ripened cheeses* and expanded coverage of the processes of making them
- Additional coverage of biomining—the use of microbes to extract insoluble forms of metals from ore
- New Beneficial Microbes box on oil-eating microbes in the Gulf of Mexico
- Additional recent information concerning the presence of significant nitrogen fixation by deep-sea archaea associated in microbial communities with bacteria
- New figure legend question concerning food sterilization
- New Visualize It! question on nitrogen cycling
- New fill-in the blank Concept Map on microbial roles in food production

Reviewers for the Fourth Edition

I thank the hundreds of instructors and students who participated in reviews, class tests, and focus groups for earlier editions of the textbook. Your comments have informed this book from beginning to end, and I am deeply grateful. For the fourth edition, I extend my deepest appreciation to the following reviewers:

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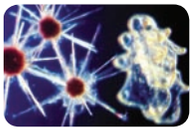

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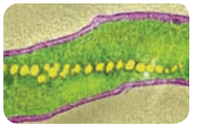

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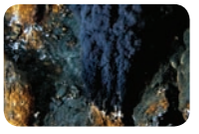

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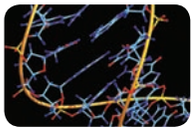

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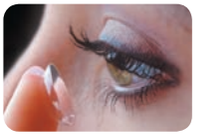

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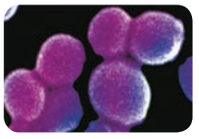

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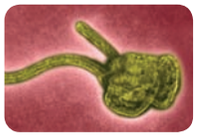

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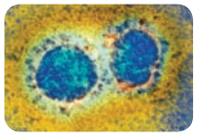

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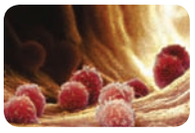

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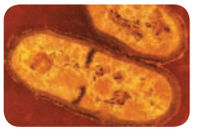

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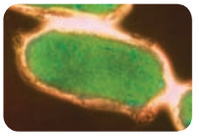

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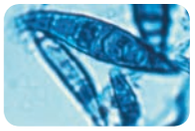

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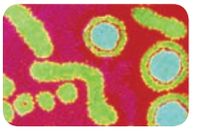

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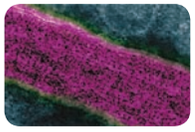

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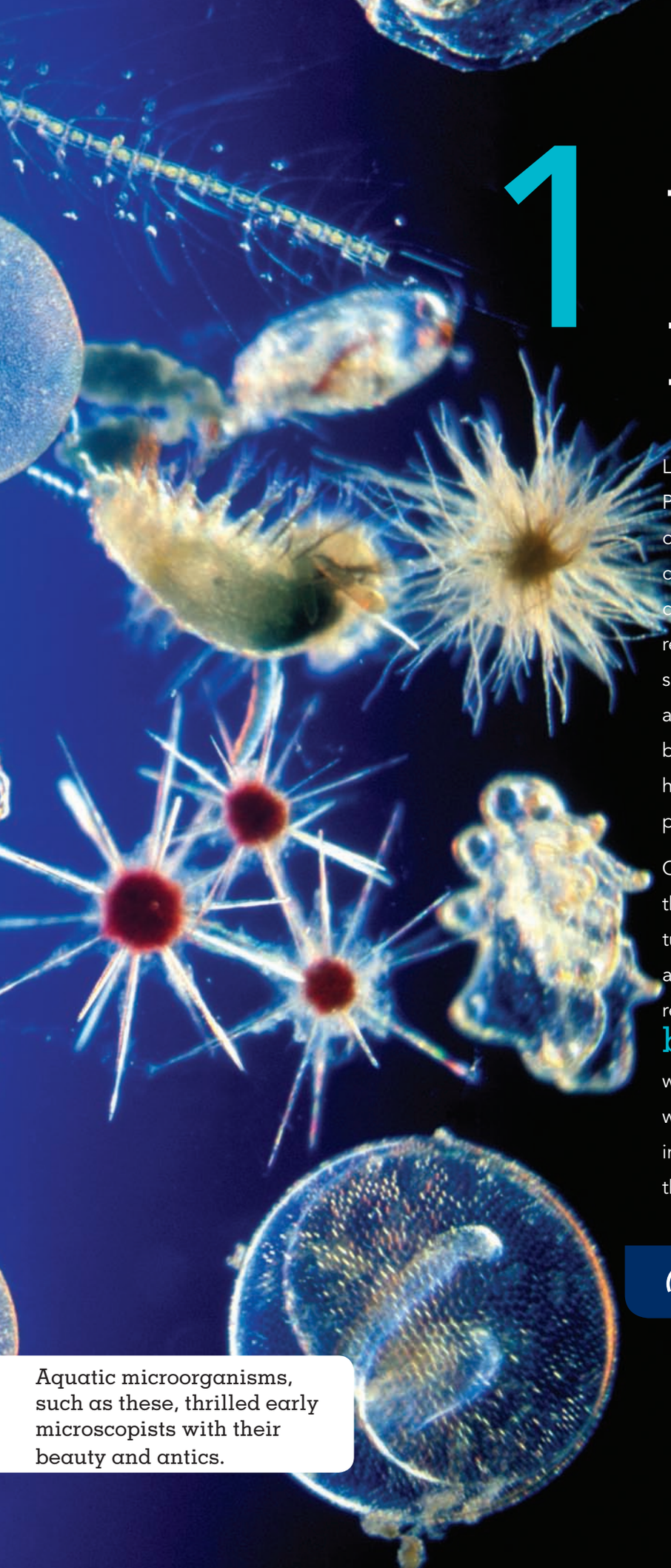


Aquatic microorganisms, such as these, thrilled early microscopists with their beauty and antics.

1 A Brief History of Microbiology

Life as we know it would not exist without microorganisms. Plants depend on **microorganisms** to help them obtain the nitrogen they need for survival. Animals such as cows and sheep need microbes in order to digest the many carbohydrates in their plant-based diets. **Ecosystems** rely on microorganisms to enrich soil, degrade wastes, and support life. We use microorganisms to make wine and cheese and to **develop** vaccines and antibiotics. The human body is home to trillions of microorganisms, many of which help keep us healthy. Microorganisms are an **essential** part our lives.

Of course, some microorganisms do cause harm to us, from the common cold to more serious **diseases** such as tuberculosis, malaria, and AIDS. The threats of bioterrorism and new or **reemerging** infectious diseases are real. This textbook explores all the roles—both harmful and **beneficial**—that microorganisms play in our lives, as well as their sophisticated structures and processes. We begin with a look at the history of microbiology, starting with the invention of crude microscopes that revealed, for the first time, the **existence** of this miraculous, miniature world.

Take the pre-test for this chapter online. Visit the MasteringMicrobiology Study Area.

Science is the study of nature that proceeds by posing questions about observations. Why are there seasons? What is the function of the nodules at the base of this plant? Why does this bread taste sour? What does plaque from between teeth look like when magnified? Why are so many crows dying this winter? What causes new diseases?

Many early written records show that people have always asked questions like these. For example, the Greek physician Hippocrates (ca. 460–ca. 377 B.C.) wondered whether there is a link between environment and disease, and the Greek historian Thucydides (ca. 460–ca. 404 B.C.) questioned why he and other survivors of the plague could have intimate contact with victims and not fall ill again. For many centuries, the answers to these and other fundamental questions about the nature of life remained largely unanswered. But about 350 years ago, the invention of the microscope began to provide some clues.

In this chapter we'll see how one man's determination to answer a fundamental question about the nature of life—What does life really look like?—led to the birth of a new science called *microbiology*. We'll then see how the search for answers to other questions, such as those concerning spontaneous generation, the reason fermentation occurs, and the cause of disease, prompted advances in this new science. Finally, we'll look briefly at some of the key questions microbiologists are asking today.

▲ **Figure 1.1 Antoni van Leeuwenhoek.** Leeuwenhoek reported the existence of protozoa in 1674 and of bacteria in 1676. *Why did Leeuwenhoek discover protozoa before bacteria?*

Figure 1.1 *Protozoa are generally larger than bacteria.*

The Early Years of Microbiology

The early years of microbiology brought the first observations of microbial life and the initial efforts to organize them into logical classifications.

What Does Life Really Look Like?

Learning Outcomes

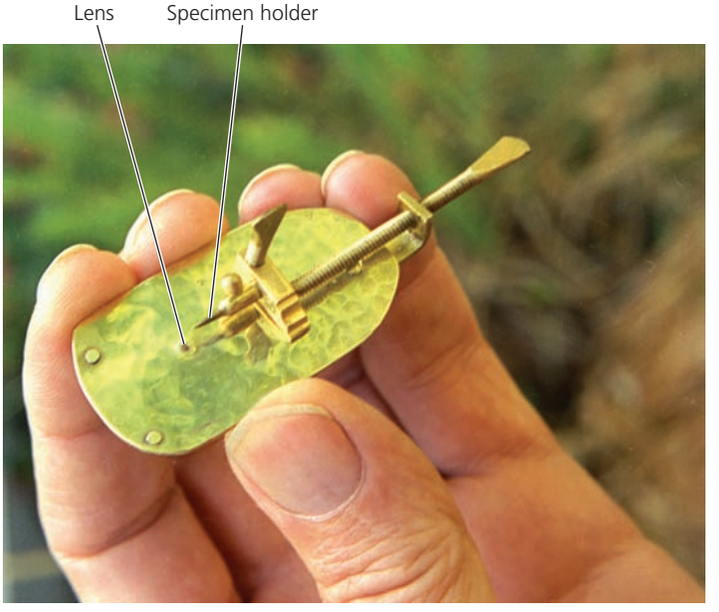
1.1 Describe the world-changing scientific contributions of Leeuwenhoek.

1.2 Define microbes in the words of Leeuwenhoek and as we know them today.

A few people have changed the world of science forever. We've all heard of Galileo, Newton, and Einstein, but the list also includes Antoni van Leeuwenhoek (lā´vĕn-huk;1632–1723), a Dutch tailor, merchant, and lens grinder, and the man who first discovered the bacterial world **(Figure 1.1)**.

Leeuwenhoek was born in Delft, the Netherlands, and lived most of his 90 years in the city of his birth. What set Leeuwenhoek apart from most other men of his generation was an insatiable curiosity coupled with an almost stubborn desire to do everything for himself. His journey to fame began simply enough, when as a cloth merchant he needed to examine the quality of cloth. Rather than merely buying one of the magnifying lenses already available, he learned to make glass lenses of his own **(Figure 1.2)**. Soon he began asking, "What does it really look like?" of everything in his world: the stinger of a bee, the brain of a fly, the leg of a louse, a drop of blood, flakes of his own skin. To find answers, he spent hours examining, reexamining, and recording every detail of each object he observed.

Making and looking through his simple microscopes, most really no more than magnifying glasses, became the overwhelming passion of his life. His enthusiasm and dedication are



▲ **Figure 1.2 Reproduction of Leeuwenhoek's microscope.** This simple device is little more than a magnifying glass with screws for manipulating the specimen, yet with it, Leeuwenhoek changed the way we see our world. The lens, which is convex on both sides, is about the size of a pinhead. The objective to be viewed was mounted either directly on the specimen holder or inside a small glass tube, which was then mounted on the specimen holder.

evident from the fact that he sometimes personally extracted the metal for his microscope from ore. Further, he often made a new microscope for each specimen, which remained mounted so that he could view it again and again. Then one day, he turned a lens onto a drop of water. We don't know what he expected to see, but certainly he saw more than he had anticipated. As he reported to the Royal Society of London[1] in 1674, he was surprised and delighted by

> some green streaks, spirally wound serpent-wise, and orderly arranged. . . . Among these there were, besides, very many little animalcules, some were round, while others a bit bigger consisted of an oval. On these last, I saw two little legs near the head, and two little fins at the hind most end of the body. . . . And the motion of most of these animalcules in the water was so swift, and so various, upwards, downwards, and round about, that 'twas wonderful to see.

Leeuwenhoek had discovered a previously unknown microbial world, which today we know to be populated with tiny animals, fungi, algae, and single-celled protozoa **(Figure 1.3)**. In a later report to the Royal Society, he noted that

> the number of these animals in the plaque of a man's teeth, are so many that I believe they exceed the number of men in a kingdom. . . . I found too many living animals therein, that I guess there might have been in a quantity of matter no bigger than the 1/100 part of a [grain of] sand.

From the figure accompanying this report and the precise description of the size of these organisms from between his teeth, we know that Leeuwenhoek was reporting the existence of bacteria. By the end of the 19th century, Leeuwenhoek's "beasties," as he sometimes dubbed them, were called **microorganisms,** and today we also know them as **microbes.** Both terms include all organisms that are too small to be seen without a microscope.

Because of the quality of his microscopes, his profound observational skills, his detailed reports over a 50-year period, and his report of the discovery of many types of microorganisms, Antoni van Leeuwenhoek was elected to the Royal Society in 1680. He and Isaac Newton were probably the most famous scientists of their time.

▲ **Figure 1.3 The microbial world.** Leeuwenhoek reported seeing a scene very much like this, full of numerous fantastic, cavorting creatures.

How Can Microbes Be Classified?

Learning Outcomes

1.3 List six groups of microorganisms.
1.4 Explain why protozoa, algae, and nonmicrobial parasitic worms are studied in microbiology.
1.5 Differentiate prokaryotic from eukaryotic organisms.

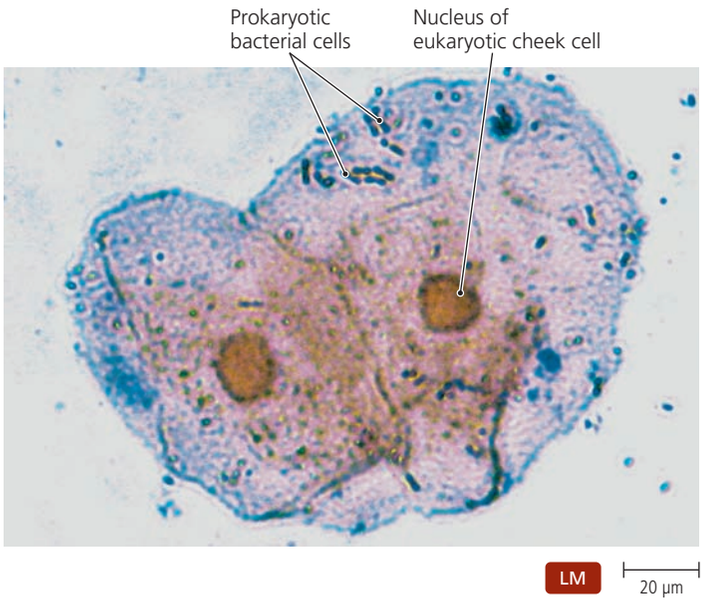
Shortly after Leeuwenhoek made his discoveries, the Swedish botanist Carolus Linnaeus (1707–1778) developed a **taxonomic system**—a system for naming plants and animals and grouping similar organisms together. For instance, Linnaeus and other scientists of the period grouped all organisms into either the animal kingdom or the plant kingdom. Today, biologists still use this basic system, but they have modified Linnaeus's scheme by adding categories that more realistically reflect the relationships among organisms. For example, scientists no longer classify yeasts, molds, and mushrooms as plants but instead as fungi. (We examine taxonomic schemes in more detail in Chapter 4.)

The microorganisms that Leeuwenhoek described can be grouped into six basic categories: bacteria, archaea, fungi, protozoa, algae, and small multicellular animals. The only type of microbes not described by Leeuwenhoek are *viruses,*[2] which are too small to be seen without an electron microscope. We briefly consider organisms in the first five categories in the following sections.

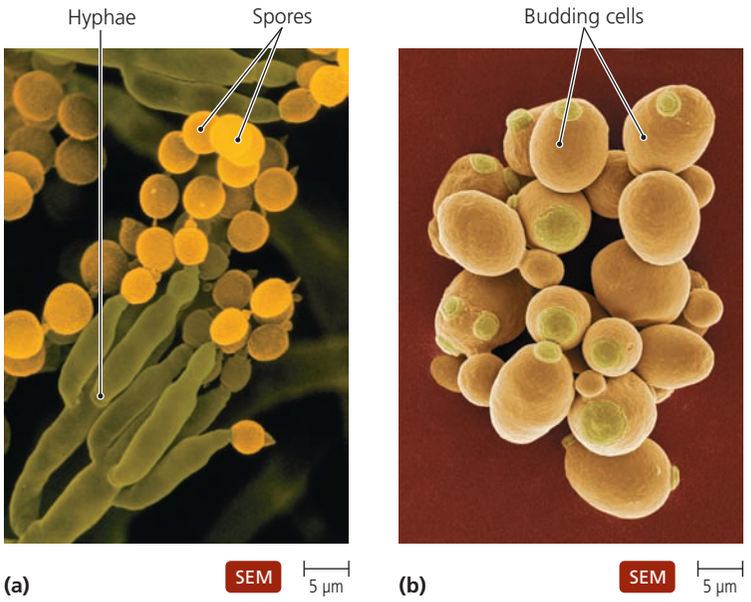
Bacteria and Archaea

Bacteria and **archaea** are **prokaryotic,**[3] meaning that they lack nuclei; that is, their genes are not surrounded by a membrane. Bacterial cell walls are composed of a polysaccharide called *peptidoglycan.* (Some bacteria, however, lack cell walls.) The cell walls of archaea lack peptidoglycan and instead are composed of other chemicals. Members of both groups reproduce asexually. (Chapters 3, 4, and 11 examine other differences between bacteria and archaea, and Chapters 19–21 discuss pathogenic [disease-causing] bacteria.)

[1]The Royal Society of London for the Promotion of Natural Knowledge, granted a royal charter in 1662, is one of the older and more prestigious scientific groups in Europe.
[2]Technically, viruses are not "organisms" because they neither replicate themselves nor carry on the chemical reactions of living things.
[3]From Greek *pro*, meaning "before," and *karyon*, meaning "kernel" (which in this case refers to the nucleus of a cell).



▲ **Figure 1.4 Cells of the bacterium *Streptococcus* (dark blue) and two human cheek cells.** Notice the size difference.



▲ **Figure 1.5 Fungi. (a)** The mold *Penicillium chrysogenum*, which produces penicillin, has long filamentous hyphae that intertwine to form its body. It reproduces by spores. **(b)** The yeast *Saccharomyces cerevisiae*. Yeasts are round to oval and typically reproduce by budding.

Most archaea and bacteria are much smaller than eukaryotic cells **(Figure 1.4)**. They live singly or in pairs, chains, or clusters in almost every habitat containing sufficient moisture. Archaea are often found in extreme environments, such as the highly saline and arsenic-rich Mono Lake in California, acidic hot springs in Yellowstone National Park, and oxygen-depleted mud at the bottom of swamps. No archaea are known to cause disease.

Though bacteria may have a poor reputation in our world, the great majority do not cause disease in animals, humans, or crops. Indeed, bacteria are beneficial to us in many ways. For example, bacteria (and fungi) degrade dead plants and animals to release phosphorus, sulfur, nitrogen, and carbon back into the air, soil, and water to be used by new generations of organisms. Without microbial recyclers, the world would be buried under the corpses of uncountable dead organisms. Without beneficial bacteria, our bodies would be much more susceptible to disease.

Fungi

Fungi (fŭn´jī)[4] cells are **eukaryotic;**[5] that is, each of their cells contains a nucleus composed of genetic material surrounded by a distinct membrane. Fungi are different from plants because they obtain their food from other organisms (rather than making it for themselves). They differ from animals by having cell walls.

Microscopic fungi include some molds and yeasts. **Molds** are typically multicellular organisms that grow as long filaments that intertwine to make up the body of the mold. Molds reproduce by sexual and asexual spores, which are cells that produce a new individual without fusing with another cell **(Figure 1.5a)**. The cottony growths on cheese, bread, and jams are molds. *Penicillium chrysogenum* (pen-i-sil´ē-ŭm krī-so´jĕn-ŭm) is a mold that produces penicillin.

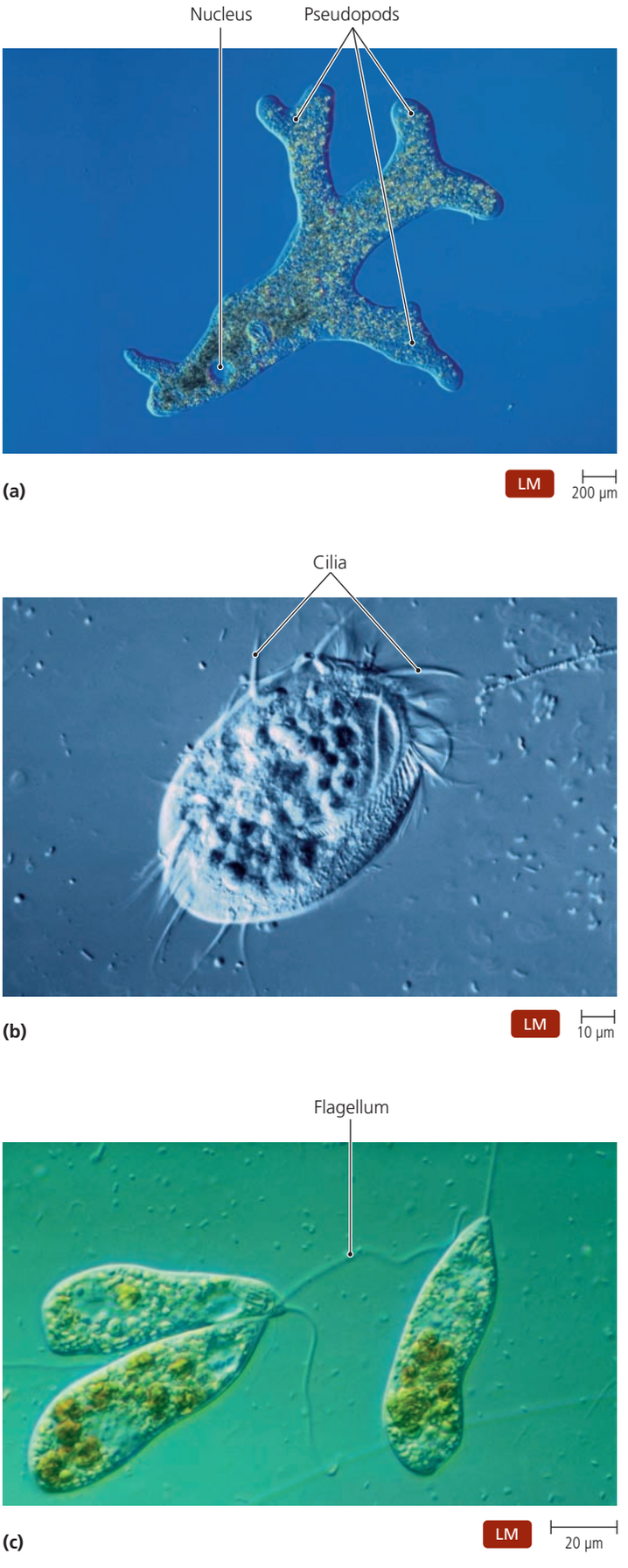
Yeasts are unicellular and typically oval to round. They reproduce asexually by *budding*, a process in which a daughter cell grows off the mother cell. Some yeasts also produce sexual spores. An example of a useful yeast is *Saccharomyces cerevisiae* (sak-ă-rō-mī´sēz se-ri-vis´ē-ī; **Figure 1.5b**), which causes bread to rise and produces alcohol from sugar (see **Beneficial Microbes: Bread, Wine, and Beer!** on p. 7). *Candida albicans* (kan´did-ă al´bi-kanz) is a yeast that causes most cases of yeast infections in women.

(Fungi and their significance in the environment, in food production, and as agents of human disease are discussed in Chapters 12 and 22.)

Protozoa

Protozoa are single-celled eukaryotes that are similar to animals in their nutritional needs and cellular structure. In fact, *protozoa* is Greek for "first animals," though scientists today classify them in their own groups rather than as animals. Most protozoa are capable of locomotion, and one way scientists categorize protozoa is according to their locomotive structures: *pseudopods*,[6] *cilia*,[7] or *flagella*.[8] Pseudopods are extensions of a cell that flow in the direction of travel **(Figure 1.6a)**. Cilia are numerous, short protrusions of a cell that beat rhythmically to propel the protozoan through its environment **(Figure 1.6b)**. Flagella are also extensions of a cell but are fewer, longer, and more whip-like than cilia **(Figure 1.6c)**. Some protozoa, such as the malaria-causing *Plasmodium* (plaz-mō´dē-ŭm), are nonmotile in their mature forms.

[4]Plural of the Latin *fungus*, meaning "mushroom."
[5]From Greek *eu*, meaning "true," and *karyon*, meaning "kernel."
[6]Plural Greek *pseudes*, meaning "false," and *podos*, meaning "foot."
[7]Plural of the Latin *cilium*, meaning "eyelid."
[8]Plural of the Latin *flagellum*, meaning "whip."



▶ **Figure 1.6 Locomotive structures of protozoa. (a)** Pseudopods are cellular extensions used for locomotion and feeding, as seen in *Amoeba proteus*. **(b)** Cilia are short, motile, hairlike extrusions, as seen in *Euplotes*. **(c)** Flagella are whiplike extensions that are less numerous and longer than cilia, as seen in *Peranema*. *How do cilia and flagella differ?*

Figure 1.6 *Cilia are short, numerous, and often cover the cell, whereas flagella are long and relatively few in number.*

Protozoa typically live freely in water, but some live inside animal hosts, where they can cause disease. Most protozoa reproduce asexually, though some are sexual as well. (Chapters 12 and 23 further examine protozoa.)

Algae

Algae[9] are unicellular or multicellular *photosynthetic* eukaryotes; that is, like plants, they make their own food from carbon dioxide and water using energy from sunlight. They differ from plants in the relative simplicity of their reproductive structures. Algae are categorized on the basis of their pigmentation and the composition of their cell walls.

Large algae, commonly called seaweeds and kelps, are common in the world's oceans. Chemicals from their gelatinous cell walls are used as thickeners and emulsifiers in many food and cosmetic products as well as in a hardening agent called *agar* in microbiological laboratory media.

Unicellular algae **(Figure 1.7)** are common in freshwater ponds, streams, and lakes and in the oceans as well. They are the major food of small aquatic and marine animals and provide most of the world's oxygen as a by-product of photosynthesis. The glasslike cell walls of diatoms provide grit for many polishing compounds. (Chapter 12 discusses other aspects of the biology of algae.)

CRITICAL **THINKING**

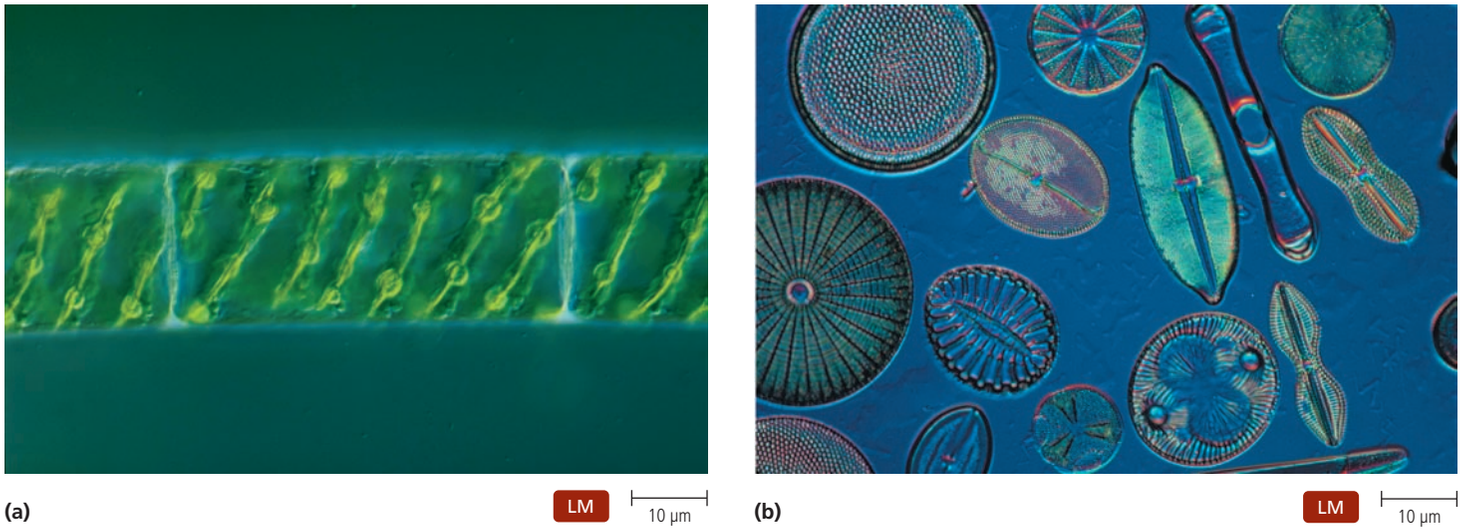
A few bacteria produce disease because they derive nutrition from human cells and produce toxic wastes. Algae do not typically cause disease. Why not?

Other Organisms of Importance to Microbiologists

Microbiologists also study parasitic worms, which range in size from microscopic forms **(Figure 1.8)** to adult tapeworms over 7 meters (approximately 23 feet) in length. Even though most of these worms are not microscopic as adults, many of them cause diseases that were studied by early microbiologists. Further, laboratory technicians diagnose infections of parasitic worms by finding microscopic eggs and immature stages in blood, fecal, urine, and lymph specimens. (Chapter 23 discusses parasitic worms.)

The only type of microbe that remained hidden from Leeuwenhoek and other early microbiologists was the virus,

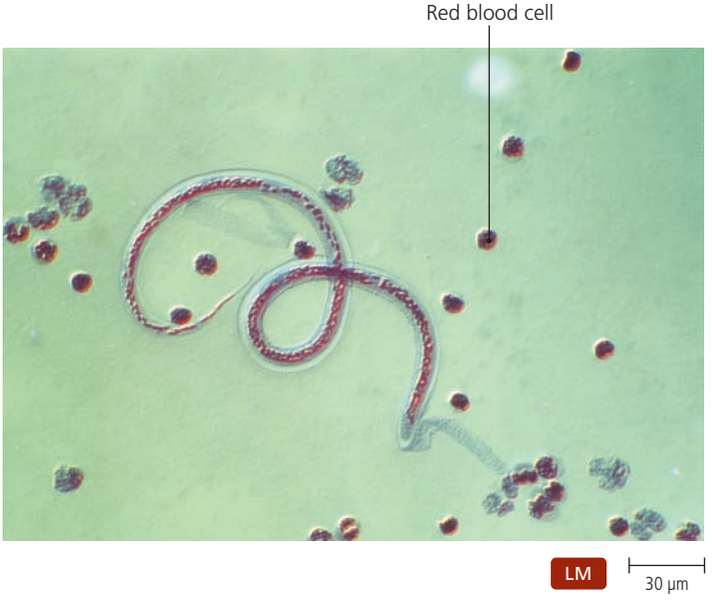
[9]Plural of the Latin *alga*, meaning "seaweed."



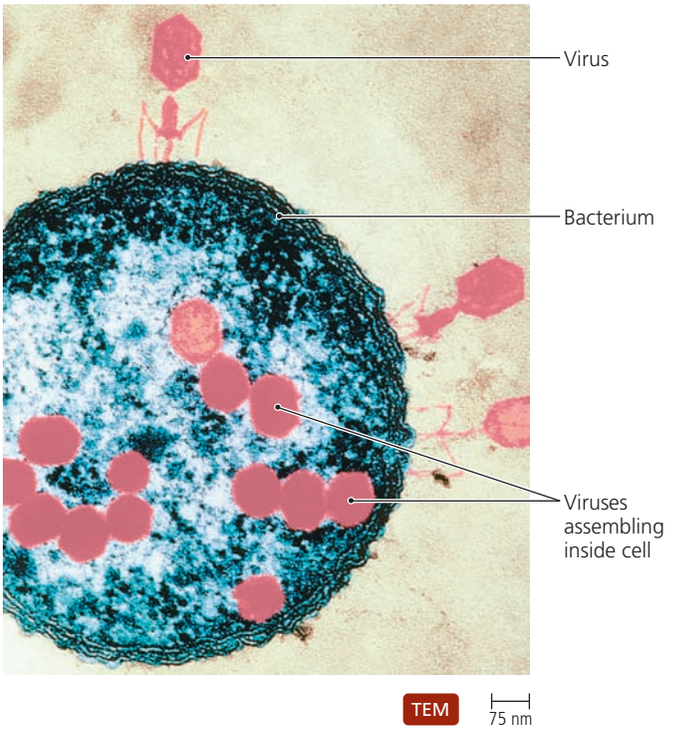
▲ **Figure 1.7 Algae. (a)** *Spirogyra.* These microscopic algae grow as chains of cells containing helical photosynthetic structures. **(b)** Diatoms. These beautiful algae have glasslike cell walls.

which is much smaller than the smallest prokaryote and is not visible by light microscopy **(Figure 1.9)**. Viruses could not be seen until the electron microscope was invented in 1932. All viruses are acellular (not composed of cells) obligatory parasites composed of small amounts of genetic material (either DNA or RNA) surrounded by a protein coat. (Chapter 13 examines the general characteristics of viruses, and Chapters 24 and 25 discuss specific viral pathogens.)

Leeuwenhoek first reported the existence of most types of microorganisms in the late 1600s, but microbiology did not develop significantly as a field of study for almost two centuries. There were a number of reasons for this delay. First, Leeuwenhoek was a suspicious and secretive man. Though he built over 400 microscopes, he never trained an apprentice, and he never sold or gave away a microscope. In fact, he never let *anyone*—not his family or such distinguished visitors as the czar of Russia—so much as peek through his very best instruments. When Leeuwenhoek died, the secret of creating superior microscopes was lost. It took almost 100 years for scientists to make microscopes of equivalent quality.



▲ **Figure 1.8 An immature stage of a parasitic worm in blood.**



▲ **Figure 1.9 A colorized electron microscope image of viruses infecting a bacterium.** Viruses, which are acellular obligatory parasites, are too small to be seen with a light microscope. Notice how small the viruses are compared to the bacterium.

Another reason that microbiology was slow to develop as a science is that scientists in the 1700s considered microbes to be curiosities of nature and insignificant to human affairs. But in the late 1800s, scientists began to adopt a new philosophy, one that demanded experimental evidence rather than mere acceptance of traditional knowledge. This fresh philosophical foundation, accompanied by improved microscopes, new laboratory techniques, and a drive to answer a series of pivotal questions, propelled microbiology to the forefront as a scientific discipline.

The Golden Age of Microbiology

Learning Outcome

1.6 List and answer four questions that propelled research in what is called the "Golden Age of Microbiology."

For about 50 years, during what is sometimes called the "Golden Age of Microbiology," scientists and the blossoming field of microbiology were driven by the search for answers to the following four questions:

- Is spontaneous generation of microbial life possible?
- What causes fermentation?
- What causes disease?
- How can we prevent infection and disease?

Competition among scientists who were striving to be the first to answer these questions drove exploration and discovery in microbiology during the late 1800s and early 1900s. These scientists' discoveries and the fields of study they initiated continue to shape the course of microbiological research today.

In the next sections we consider these questions and how the great scientists accumulated the experimental evidence that answered them.

Does Microbial Life Spontaneously Generate?

Learning Outcomes

1.7 Identify the scientists who argued in favor of spontaneous generation.

1.8 Compare and contrast the investigations of Redi, Needham, Spallanzani, and Pasteur concerning spontaneous generation.

1.9 List four steps in the scientific method of investigation.

A dry lake bed has lain under the relentless North African desert sun for eight long months. The cracks in the baked, parched mud are wider than a man's hand. There is no sign of life anywhere in the scorched terrain. With the abruptness characteristic of desert storms, rain falls in a torrent, and a raging flood of roiling water and mud crashes down the dry streambed and fills

BENEFICIAL MICROBES

BREAD, WINE, AND BEER!

Microorganisms play important roles in people's lives; for example, pathogens have undeniably altered the course of history. However, what may be the most important microbiological event—one that has had a greater impact on culture and society than that of any disease or epidemic—was the domestication of the yeast used by bakers and brewers. Its name, *Saccharomyces cerevisiae*, means "sugar fungus [that makes] beer."

The earliest record of the use of yeast comes from Persia (modern Iran), where archaeologists have found the remains of grapes and wine preservatives in pottery vessels more than 7000 years old. Brewing of beer likely started even earlier, its beginnings undocumented. The earliest examples of leavened bread are from Egypt and show that bread making was routine about 6000 years ago. Before that time, bread was unleavened and flat.

It is likely that making wine and brewing beer occurred earlier than the use of leavened bread because *Saccharomyces* is naturally found on grapes, which can begin to ferment while still on the vine. Historians hypothesize that early bakers may have exposed bread dough to circulating air, hoping that the invisible and inexplicable "fermentation principle" would inoculate the bread. Another hypothesis is that bakers learned to add small amounts of beer or wine to the bread, intentionally inoculating the dough with yeast. Of course, all those years before Leeuwenhoek and Pasteur, no one knew that the fermenting ingredient of wine was a living organism.

Besides its role in baking and in making alcoholic beverages, *S. cerevisiae* is an important tool for the study of cells. Scientists use yeast to delve into the mysteries of cellular function, organization, and genetics, making *Saccharomyces* the most intensely studied eukaryote. In fact, molecular biologists published the complete sequence of the genes of *S. cerevisiae* in 1996—a first for any eukaryotic cell.

Today, scientists are working toward using *S. cerevisiae* in novel ways. For example, some nutritionists and gastroenterologists are examining the use of *Saccharomyces* as a *probiotic*, that is, a microorganism intentionally taken to ward off disease and promote good health. Research suggests that the yeast helps treat diarrhea and colitis and may even help prevent these and other gastrointestinal diseases.

▲ **Figure 1.10 Redi's experiments.** When the flask remained unsealed, maggots covered the meat within a few days. When the flask was sealed, flies were kept away, and no maggots appeared on the meat. When the flask opening was covered with gauze, flies were kept away, and no maggots appeared on the meat, although a few maggots appeared on top of the gauze.

the lake. Within hours, what had been a lifeless, dry mudflat becomes a pool of water teeming with billions of shrimp; by the next day it is home to hundreds of toads. Where did these animals come from?

Many philosophers and scientists of past ages thought that living things arose via three processes: through asexual reproduction, through sexual reproduction, or from nonliving matter. The appearance of shrimp and toads in the mud of what so recently was a dry lake bed was seen as an example of the third process, which came to be known as *abiogenesis*,[10] or **spontaneous generation.** The theory of spontaneous generation as promulgated by Aristotle (384–322 B.C.) was widely accepted for over 2000 years because it seemed to explain a variety of commonly observed phenomena, such as the appearance of maggots on spoiling meat. However, the validity of the theory came under challenge in the 17th century.

Redi's Experiments

In the late 1600s, the Italian physician Francesco Redi (1626–1697) demonstrated by a series of experiments that when decaying meat was kept isolated from flies, maggots never developed, whereas meat exposed to flies was soon infested **(Figure 1.10)**. As a result of experiments such as these, scientists began to doubt Aristotle's theory and adopt the view that animals come only from other animals.

Needham's Experiments

The debate over spontaneous generation was rekindled when Leeuwenhoek discovered microbes and showed that they appeared after a few days in freshly collected rainwater. Though scientists agreed that larger animals could not arise spontaneously, they disagreed about Leeuwenhoek's "wee animalcules";

[10]From Greek *a*, meaning "not"; *bios*, meaning "life"; and *genein*, meaning "to produce."

HIGHLIGHT

"THE NEW NORMAL": THE CHALLENGE OF EMERGING AND REEMERGING DISEASES

Severe acute respiratory syndrome (SARS). Monkeypox. West Nile encephalitis. These and diseases like them are emerging diseases—ones that appear in a population for the first time. Among them are H1N1 influenza ("swine flu"); Nipah encephalitis, a highly fatal disease carried by pigs; and mosquito-borne chikungunya, which causes severe joint pain and sometimes death. Indeed, unfamiliar diseases have become "the new normal" for health care workers, according to the Centers for Disease Control and Prevention.

Meanwhile, diseases once thought to be near eradication, such as polio, whooping cough, and tuberculosis, have reemerged in troubling outbreaks. Other near-vanquished pathogens such as smallpox or anthrax may become potential weapons in bioterrorist attacks.

How do emerging and reemerging diseases arise? Some are introduced to humans as we move into remote jungles and contact infected animals, some are carried by insects whose range is spreading as climate changes, and some take advantage of the AIDS crisis, infecting immunocompromised patients. In other cases, previously harmless microbes acquire new genes that allow them to be infective and cause disease. Some emerging pathogens spread with the speed of jet planes carrying infected people around the globe, and still others arise when previously treatable microbes develop resistance to our antibiotics.

However they arise, scientists are monitoring emerging and reemerging diseases that may develop into the next generation of high-profile infectious diseases. Throughout this textbook, you will encounter many boxed discussions of such emerging and reemerging diseases.

Workers dumping poultry suspected of harboring avian influenza virus.

surely they did not have parents, did they? They must arise spontaneously.

The proponents of spontaneous generation pointed to the careful demonstrations of British investigator John T. Needham (1713–1781). He boiled beef gravy and infusions[11] of plant material in vials, which he then tightly sealed with corks. Some days later, Needham observed that the vials were cloudy, and examination revealed an abundance of "microscopical animals of most dimensions." As he explained it, there must be a "life force" that causes inanimate matter to spontaneously come to life because he had heated the vials sufficiently to kill everything. Needham's experiments so impressed the Royal Society that they elected him a member.

Spallanzani's Experiments

Then, in 1799, the Italian Catholic priest and scientist Lazzaro Spallanzani (1729–1799) reported results that contradicted Needham's findings. Spallanzani boiled infusions for almost an hour and sealed the vials by melting their slender necks closed. His infusions remained clear unless he broke the seal and exposed the infusion to air, after which they became cloudy with microorganisms. He concluded three things:

- Needham either had failed to heat his vials sufficiently to kill all microbes or had not sealed them tightly enough.
- Microorganisms exist in the air and can contaminate experiments.
- Spontaneous generation of microorganisms does not occur; all living things arise from other living things.

Although Spallanzani's experiments would appear to have settled the controversy once and for all, it proved difficult to dethrone a theory that had held sway for 2000 years, especially when so notable a man as Aristotle had propounded it. One of the criticisms of Spallanzani's work was that his sealed vials did not allow enough air for organisms to thrive; another objection was that his prolonged heating destroyed the "life force." The debate continued until the French chemist Louis Pasteur **(Figure 1.11)** conducted experiments that finally laid the theory of spontaneous generation to rest.

Pasteur's Experiments

Louis Pasteur (1822–1895) was an indefatigable worker who pushed himself as hard as he pushed others. As he wrote his sisters, "To *will* is a great thing dear sisters, for Action and Work usually follow Will, and almost always Work is accompanied by Success. These three things, Work, Will, Success, fill human existence. Will opens the door to success both brilliant and happy; Work passes these doors, and at the end of the journey Success comes to crown one's efforts." When his wife complained about his long hours in the laboratory, he replied, "I will lead you to fame."

[11] Infusions are broths made by heating water containing plant or animal material.

▲ **Figure 1.11 Louis Pasteur.** Often called the Father of Microbiology, he disproved spontaneous generation. In this depiction, Pasteur examines some bacterial cultures.

Pasteur's determination and hard work are apparent in his investigations of spontaneous generation. Like Spallanzani, he boiled infusions long enough to kill everything. But instead of sealing the flasks, he bent their necks into an S-shape, which allowed air to enter while preventing the introduction of dust and microbes into the broth **(Figure 1.12)**.

Crowded for space and lacking funds, he improvised an incubator in the opening under a staircase. Day after day he crawled on hands and knees into this incommodious space and examined his flasks for the cloudiness that would indicate the presence of living organisms. In 1861, he reported that his "swan-necked flasks" remained free of microbes even 18 months later. Because the flasks contained all the nutrients (including air) known to be required by living things, he concluded, "Never will spontaneous generation recover from the mortal blow of this simple experiment."

Pasteur followed this experiment with demonstrations that microbes in the air were the "parents" of Needham's microorganisms. He broke the necks off some flasks, exposing the liquid in them directly to the air, and he carefully tilted others so that the liquid touched the dust that had accumulated in their necks. The next day, all of these flasks were cloudy with microbes. He concluded that the microbes in the liquid were the progeny of microbes that had been on the dust particles in the air.

The Scientific Method

The debate over spontaneous generation led in part to the development of a generalized **scientific method** by which questions are answered through observations of the outcomes of carefully controlled experiments instead of by conjecture or according to the opinions of any authority figure. The scientific method, which provides a framework for conducting an investigation