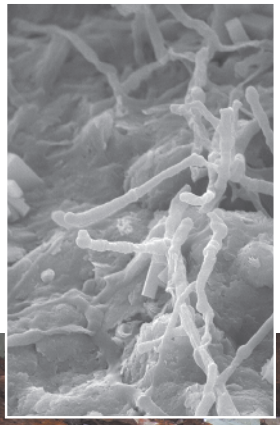
CHAPTER 1

Scope and History of Microbiology

You'll Die in 30 Minutes Here! What's a Microbiologist Doing Here?

M.N. Spilde & P.J. Boston



Come with geomicrobiologist Dr. Penny Boston, deep under the Chihuahuan Desert of Mexico, to a cave full of giant gypsum crystals weighting over 5 tons each. A pump has drained out the scalding hot water in which they were immersed for over 100 million years. The cave sits atop a chamber of volcanic magma which heats it to 50°C (120°F) and 99% humidity. Even with special suits, breathing apparatus and ice packs, a human can survive here for less than 30 minutes. But Penny Boston has found over 80 kinds of bacteria living inside fluid in the crystals. All never seen on Earth before. She has done their DNA and they are unique. Each drop of fluid also holds over 200 million bacteriophages, a type of virus that eats bacteria! As the crystals grew, defects in their latticework of molecules left small holes which filled with the water bathing them—hot and full of microbes. Further growth closed these holes, preserving the bacteria and viruses alive in the fluid. Most of them are still alive! They are living fossils over a half million years old! They are not dormant. They have a superslow metabolism, extra food just kills them. Red colored bacteria growing on the 80 to 85-billion-year-old limestone walls of the cave are also alive.



Scientists(left) in ice-cooled suits explore Mexico's deadly hot Cave of Crystals.
(C. Oscar Necoechea - S/F [speleoresearch and film])

These microbes are living in what is called the "deep hot biosphere," like what much of the early Earth was like.

They need no light, no organic matter; they eat the minerals they are entombed with. Are there similar conditions on other planets or their moons that could also have given rise to life? Europa, a moon of Jupiter, in covered with ice but has a 60-mile-deep ocean of liquid nitrogen, which sits on volcanic heat. It certainly is deep and hot—perhaps it is a "deep hot biosphere." Titan and Enceladus, moons of Saturn, also have such places. Most of the time that there has been life on Earth, it has been solely bacteria and viruses. Perhaps we'll find more kinds of microbes

COPYRIGHTED MATERIAL

elsewhere in the universe. Studying the ones found in the Crystal Cave may give us a better idea of what to look for.

More surprises lie ahead. Oh! The things we'll learn in this course—it's going to be a real adventure!

To read about more cave explorations by Dr. Penny Boston and Dr. Diana Northrup, see Chapter 26.

CHAPTER MAP

Follow the Chapter Map to help you pinpoint the core concepts in the chapter.

WHY STUDY MICROBIOLOGY? 2

Microbes in the Environment and Human Health 2 • Insight into Life Processes 3 • We Are the Planet of Bacteria 4

SCOPE OF MICROBIOLOGY 4

The Microbes 4 • The Microbiologists 6

HISTORICAL ROOTS 9

THE GERM THEORY OF DISEASE 11

Early Studies 11 • Pasteur's Further Contributions 12 • Koch's Contributions 13 • Work Toward Controlling Infections 14

EMERGENCE OF SPECIAL FIELDS OF MICROBIOLOGY 15

Immunology 15 • Virology 16 • Chemotherapy 17 • Genetics and Molecular Biology 19

TOMORROW'S HISTORY 19

Genomics 22

Almost one-half of children under the age of 10 died of infectious disease prior to the last century.

"It's just some 'bug' going around." You have heard that from others or said it yourself when you have been ill for a day or two. Indeed, the little unidentified illnesses we all have from time to time and attribute to a "bug" are probably caused by viruses, the tiniest of all *microbes*. Other groups of **microorganisms**—bacteria, fungi, protozoa, and some algae—also have disease-causing members. Before studying microbiology, therefore, we are likely to think of microbes as germs that cause disease. Health scientists are concerned with just such microbes and with treating and preventing the diseases they cause. Yet less than 1% of known microorganisms cause disease, so focusing our study of microbes exclusively on disease gives us too narrow a view of microbiology.

WHY STUDY MICROBIOLOGY?

Microbes in the Environment and Human Health

If you were to dust your desk and shake your dust cloth over the surface of a medium designed for growing microorganisms, after a day or so you would find a variety of organisms growing on that medium. If you were to cough onto such a medium or make fingerprints on it, you would later find a different assortment of microorganisms growing on the medium. When you have a sore throat and your physician orders a throat culture, a variety of organisms will be present in the culture—perhaps including the one that is causing your sore throat. Thus, microorganisms have a close association with humans. They are in us, on us, and nearly everywhere around us (**Figure 1.1**). One reason for studying microbiology is that *microorganisms are*

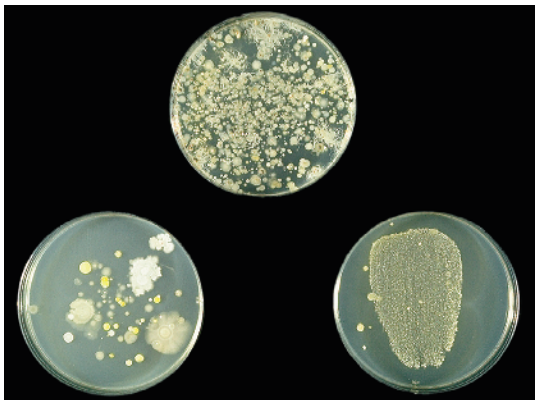


FIGURE 1.1 A simple experiment shows that microorganisms are almost everywhere in our environment. Soil was added to nutrient agar, a culture medium (dish on top); another dish with agar was exposed to air (bottom left); and a tongue print was made on an agar surface (bottom right). After 3 days of incubation under favorable conditions, abundant microbial growth is easily visible in all three dishes. *(Courtesy Jacquelyn G. Black)*

part of the human environment and are therefore important to human health.

Microorganisms are essential to the web of life in every environment. Many microorganisms in the ocean and in bodies of fresh water capture energy from sunlight and store it in molecules that other organisms use as food. Microorganisms decompose dead organisms, waste material from living organisms, and even some kinds of industrial wastes. They make nitrogen available to plants.

These are only a few of the many examples of how microorganisms interact with other organisms and help maintain the balance of nature. The vast majority of microorganisms are directly or indirectly beneficial, not only to other organisms, but also to humans. They form essential links in many food chains that produce plants and animals that humans eat. Aquatic microbes serve as food for small macroscopic animals that, in turn, serve as food for fish and shellfish that humans eat. Certain microorganisms live in the digestive tracts of grazing animals such as cattle and sheep and aid in their digestive processes. Without these microbes, cows could not digest grass, and horses would get no nourishment from hay. Humans occasionally eat microbes, such as some algae and fungi, directly. Mushrooms, for instance, are the macroscopic reproductive bodies of masses of microscopic fungi. Biochemical reactions carried out by microbes also are used by the food industry to make pickles, sauerkraut, yogurt and other dairy products, fructose used in soft drinks, and the artificial sweetener aspartame. Fermentation reactions in microorganisms are used in the brewing industry to make beer and wine, and in baking to leaven dough.

A bacterium may weigh approximately 0.00000000001 grams, yet collectively microbes constitute about 60% of the Earth's biomass.

One of the most significant benefits that microorganisms provide is their ability to synthesize *antibiotics*, substances derived from one microorganism that kill or restrict the growth of other microorganisms. Therefore, microorganisms can be used to cure diseases as well as cause them. Finally, microorganisms are the major tools of genetic engineering. Several products important to humans, such as interferon and growth hormones, can now be produced economically by microbes because of genetic engineering.

New organisms are being engineered to degrade oil spills, to remove toxic materials from soil, and to digest explosives that are too dangerous to handle. They will be major tools in cleaning up our environment. Other organisms will be designed to turn waste products into energy. Still other organisms will receive desirable genes from other types of organisms—for example, crop plants will be given bacterial genes that produce nitrogen-containing compounds needed for plant growth. The citizen of today, and even more so of tomorrow, must be scientifically literate, understanding many microbial products and processes.

Although only certain microbes cause disease, learning how such diseases are transmitted and how to diagnose, treat, and prevent them is of great importance in a health-science career. Such knowledge will help those of you who pursue such a career to care for patients and avoid becoming infected yourself.

Insight into Life Processes

Another reason for studying microbiology is that such study *provides insight into life processes in all life-forms.* Biologists in many different disciplines use ideas from microbiology and use the organisms themselves. Ecologists draw on principles of microbiology to understand how matter is decomposed and made available for continuous recycling. Biochemists use microbes to study metabolic pathways—sequences of chemical reactions in living organisms. Geneticists use microbes to study how hereditary information is transferred and how such information controls the structure and functions of organisms.

Microorganisms are especially useful in research for at least three reasons:

1. Compared to other organisms, microbes have relatively simple structures. It is easier to study most life processes in simple unicellular organisms than in complex multicellular ones.
2. Large numbers of microorganisms can be used in an experiment to obtain statistically reliable results at a reasonable cost. Growing a billion bacteria costs less than maintaining 10 rats. Experiments with large numbers of microorganisms give more reliable results than do those with small numbers of organisms with individual variations.
3. Because microorganisms reproduce very quickly, they are especially useful for studies involving the transfer of genetic information. Some bacteria can undergo three cell divisions in an hour, so the effects of gene transfer can quickly be followed through many generations.

By studying microbes, scientists have achieved remarkable success in understanding life processes and disease control. For example, within the last few decades,

CLOSE UP

We Are Not Alone

"We are outnumbered. The average human contains about 10 trillion cells. On that average human are about 10 times as many microorganisms, or 100 trillion microscopic beings. . . . As long as they stay in balance and where they belong, [they] do us no harm. . . . In fact, many of them provide some important services to us. [But] most are opportunists, who if given the opportunity of increasing growth or invading new territory, will cause infection."

—Robert J. Sullivan, 1989

vaccines have nearly eradicated several dreaded childhood diseases—including measles, polio, German measles, mumps, and chickenpox. Smallpox, which once accounted for 1 out of every 10 deaths in Europe, has not been reported anywhere on the planet since 1978. Much has also been learned about genetic changes that lead to antibiotic resistance and about how to manipulate genetic information in bacteria. Much more remains to be learned. For example, how can vaccines be made available on a worldwide basis? How can the development of new antibiotics keep pace with genetic changes in microorganisms? How will increased world travel continue to affect the spread of infections? Will the continued encroachment of humans into virgin jungles result in new, emerging diseases? Can a vaccine or effective treatment for acquired immunodeficiency syndrome (AIDS) be developed? How can we fight Ebola and Zika? Therein lie some of the challenges for the next generation of biologists and health scientists.

We Are the Planet of Bacteria

The full extent and importance of bacteria to our planet is just now being revealed. Deep drilling projects have discovered bacteria living at depths that no one had believed possible. At first their presence was attributed to contaminated drilling materials from the surface. But now several careful studies have confirmed populations of bacteria truly native to depths such as 1.6 km in France, 4.2 km in Alaska, and 5.2 km in Sweden. It seems that no matter how far down we drill, we always find bacteria living there. But, as we approach the hot interior of the Earth, temperature increases with depth. The Alaskan bacteria were living at 110°C! Evidence has accumulated that there is a "**deep hot biosphere**," as named by American scientist Thomas Gold. This region of microbial life may extend down as far as 10 km below our "surface biosphere." At places along the border between these two biospheres, materials such as oil, hydrogen sulfide (H_2S), and methane (CH_4) are upwelling, carrying along with them bacteria from deep inside our planet. Scientists now speak of a "continuous subcrustal culture" of bacteria filling a deep hot zone lying beneath the entire Earth's surface. The mass of bacteria in the surface biosphere by far exceeds the total weight of all other living things. Add to this the weight of all the bacteria living inside the deep hot biosphere, and it is apparent that our Earth is truly "the planet of bacteria."

The cave shown in the photo at the beginning of this chapter is one of those places along the border between the two biospheres. In this book we will examine bacteria at other borderland sites (for example, black hot smoking vents located deep at the ocean bottom; cold seeps higher up in the ocean on the continental shelves; and boiling mud pots such as those at Yellowstone National Park in the United States and at the Kamchatka peninsula of Russia). And, of course, we will take a closer look at those fascinating caves shown at the beginning of this chapter.

SCOPE OF MICROBIOLOGY

Microbiology is the study of **microbes**, organisms so small that a microscope is needed to study them. We consider two dimensions of the scope of microbiology: (1) the variety of kinds of microbes, and (2) the kinds of work microbiologists do.

The Microbes

The major groups of organisms studied in microbiology are bacteria, algae, fungi, viruses, and protozoa (**Figure 1.2a–e**). All are widely distributed in nature. For example, a recent study of bee bread (a pollen-derived nutrient eaten by worker bees) showed it to contain 188 kinds of fungi and 29 kinds of bacteria. Most microbes consist of a single cell. (Cells are the basic units of structure and function in living things; they are discussed in ▶ Chapter 5.) Viruses, tiny acellular entities on the borderline between the living and the nonliving, behave like living organisms when they gain entry to cells. They, too, are studied in microbiology. Microbes range in size from small viruses 20 nm in diameter to large protozoans 5 mm or more in diameter. In other words, the largest microbes are as much as 250,000 times the size of the smallest ones! (Refer to ▶ Appendix A for a review of metric units.)

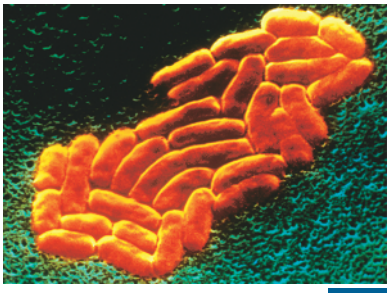
500 bacteria, each 1 μm (1/1000 of a millimeter) long, would fit end-to-end across the dot above the letter "i."

Bacteria

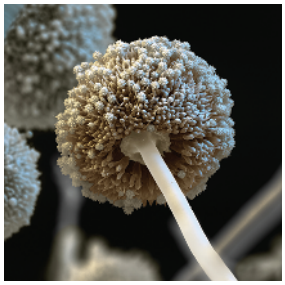
Among the great variety of microorganisms that have been identified, bacteria probably have been the most thoroughly studied. The majority of **bacteria** (singular: *bacterium*) are single-celled organisms with spherical, rod, or spiral shapes, but a few types form filaments. Most are so small they can be seen with a light microscope only under the highest magnification. Although bacteria are cellular, they do not have a cell nucleus, and they lack the membrane-enclosed intracellular structures found in most other cells. Many bacteria absorb nutrients from their environment, but some make their own nutrients by photosynthesis or other synthetic processes. Some are stationary, and others move about. Bacteria are widely distributed in nature, for example, in aquatic environments and in decaying matter. And some occasionally cause diseases.

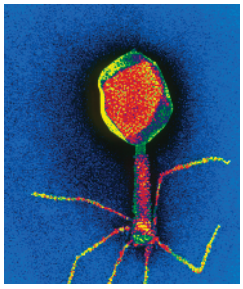
Archaea

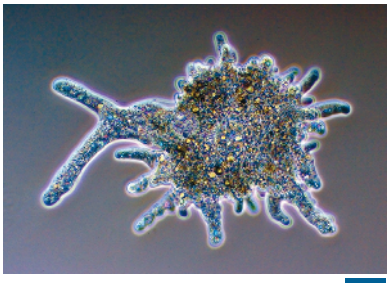
Very similar to bacteria are the group known as Archaea. They and the bacteria belong in the same Kingdom, called the Monera. A new category of classification, the **Domain**, has been erected as being higher than Kingdom. There are 3 Domains: Bacteria, Archaea, and Eukarya, which are discussed in ▶ Chapter 10. Like Bacteria, the Archaea are single celled and do not have a nucleus. However, they are genetically and metabolically very


(a) SEM


(b) SEM


(c) SEM


(d) TEM


(e) LM


(f) SEM

FIGURE 1.2 Typical microorganisms (enhanced with artificial coloring). **(a)** Several *Klebsiella pneumoniae* cells, a bacterium that can cause pneumonia in humans (5, 821X). *(Science Photo Library/Science Source)* **(b)** *Micrasterius*, a green alga that lives in fresh water. *(SciencePR/Oxford Scientific/Getty)* **(c)** Spore bearing fruiting bodies of the fungus *Aspergillus niger*. *(Eye of Science/Science Source Images)* **(d)** Bacteriophages (viruses that infect bacteria; 35,500X). *(Eye of Science/Science Source)* **(e)** *Amoeba*, a protozoan (183X). *(Roland Birke/Photolibrary/Getty)* **(f)** Head of the tapeworm *Acanthrocirrus retirostris* (189X). At the top of the head are hooks and suckers that the worm uses to attach to a host's intestinal tissues. *(Department of Zoology, University of Hull/SPL/Science Source)*

different. Many Archaea are extremophiles, preferring to live in environments having extreme temperatures, pH, salinities, and hydrostatic and osmotic pressures. Their lipids, cell walls, and flagella differ considerably from those of Bacteria. Archaea are not proven to cause disease in humans; in fact, many are very important in ruminant animal digestive tracts.

In contrast to bacteria, several groups of microorganisms consist of larger, more complex cells that have a cell nucleus. They include algae, fungi, and protozoa, all of which can easily be seen with a light microscope.

Algae

Many **algae** (al′je; singular: *alga*) are single-celled microscopic organisms, but some marine algae are large, relatively complex, multicellular organisms. Unlike bacteria, algae have a clearly defined cell nucleus and numerous membrane-enclosed intracellular structures. All algae photosynthesize their own food as plants do, and many can move about. Algae are widely distributed in both fresh water and oceans. Because they are so numerous and because they capture energy from sunlight in the food they make, algae are an important source of food for other organisms. Algae are of little medical importance; only one species, *Prototheca*, has been found to cause disease in humans. Having lost its chlorophyll, and therefore the ability to produce its own food, it now makes meals of humans.

Fungi

Like algae, many **fungi** (fun′ji; singular: *fungus*), such as yeasts and some molds, are single-celled microscopic organisms. Some, such as mushrooms, are multicellular, macroscopic organisms. Fungi also have a cell nucleus and intracellular structures. All fungi absorb ready-made nutrients from their environment. Some fungi form extensive networks of branching filaments, but the organisms themselves generally do not move. Fungi are widely distributed in water and soil as decomposers of dead organisms. Some are important in medicine either as agents of diseases such as ringworm and vaginal yeast infections or as sources of antibiotics.

Viruses

Viruses are acellular entities too small to be seen with a light microscope. They are composed of specific chemical substances—a nucleic acid and a few proteins (▶ Chapter 3). Indeed, some viruses can be crystallized and stored in a

container on a shelf for years, but they retain the ability to invade cells. Viruses replicate themselves and display other properties of living organisms only when they have invaded cells. Many viruses can invade human cells and cause disease. Even smaller acellular agents of disease are **viroids** (nucleic acid without a protein coating), and **prions** (protein without any nucleic acid). Viroids have been shown to cause various plant diseases, whereas prions cause mad cow disease and related disorders. New information about prions has just exploded in the last 2 years. We will explore this in ▶ Chapter 11.

Protozoa

Protozoa (pro-to-zo′ah; singular: *protozoan*) also are single-celled, microscopic organisms with at least one nucleus and numerous intracellular structures. A few species of amoebae are large enough to be seen with the naked eye, but we can study their structure only with a microscope. Many protozoa obtain food by engulfing or ingesting smaller microorganisms. Most protozoa can move, but a few, especially those that cause human disease, cannot. Protozoa are found in a variety of water and soil environments, as well as in animals such as malaria-carrying mosquitoes.

Helminths and Arthropods

In addition to organisms properly in the domain of microbiology, in this text we consider some macroscopic *helminths* (worms) (**Figure 1.2f**) and *arthropods* (insects and similar organisms). The helminths have microscopic stages in their life cycles that can cause disease, and the arthropods can transmit these stages, as well as other disease-causing microbes.

Taxonomy

We will learn more about the classification of microorganisms in ▶ Chapter 10. For now it is important to know only that cellular organisms are referred to by two names: their *genus* and *species* names. For example, a bacterial species commonly found in the human gut is called *Escherichia coli*, and a protozoan species that can cause severe diarrhea is called *Giardia intestinalis*. The naming of viruses is less precise. Some viruses, such as herpesviruses, are named for the group to which they belong. Others, such as polioviruses, are named for the disease they cause.

Disease-causing organisms and the diseases they cause in humans are discussed in detail in ▶ Chapters 20–25. Hundreds of infectious diseases are known to medical science. Some of the most important—those diseases that physicians should report to the U.S. Centers for Disease Control and Prevention (CDC)—are listed in **Table 1.1**. The CDC is a federal agency that collects data about diseases and about developing ways to control them.

The Microbiologists

Microbiologists study many kinds of problems that involve microbes. Some study microbes mainly to find out more about a particular type of organism—the life stages of a particular fungus, for example. Other microbiologists are interested in a particular kind of function, such as the metabolism of a certain sugar or the action of a specific gene. Still others focus directly on practical problems, such as how to purify or synthesize a new antibiotic or how to make a vaccine against a particular disease. Quite often the findings from one project are useful in another, as when agricultural scientists use information from microbiologists to control pests and improve crop yields, or when environmentalists attempt to maintain natural food chains and prevent damage to the environment. Some fields of microbiology are described in **Table 1.2**.

Microbiologists work in a variety of settings (**Figure 1.3**). Some work in universities, where they are likely to teach, do research, and train students to do research. Microbiologists in both university and commercial laboratories are helping to develop the microorganisms used in genetic engineering. Some law firms are hiring microbiologists to help with the complexities of patenting new genetically engineered organisms. These organisms can be used in such important ways as cleaning up the environment (*bioremediation*), controlling insect pests, improving foods, and fighting disease. Many microbiologists work in health-related positions. Some work in clinical laboratories, performing tests to diagnose diseases or determining which antibiotics will cure a particular disease. A few microbiologists develop new clinical tests. Others work in industrial laboratories to develop or manufacture antibiotics, vaccines, or similar biological products. Still others, concerned with controlling the spread of infections and related public health matters, work in hospitals or government labs.

From the point of view of health scientists, today's research is the source of tomorrow's new technologies. Research in *immunology* is greatly increasing our knowledge of how microbes trigger host responses and how the microbes escape these responses. It also is contributing to the development of new vaccines and to the treatment of immunologic disorders. Research in *virology* is improving our understanding of how viruses cause infections and how they are involved in cancer. Research in *chemotherapy* is increasing the number of drugs available to treat infections and is also improving our knowledge of how these drugs work. Finally, research in *genetics* is providing new information about the transfer of genetic information and, especially, about how genetic information acts at the molecular level.



and CHECK

1. List three reasons to study microbiology.
2. What is the difference between microbiology and bacteriology?
3. What is the difference between etiology and epidemiology?
4. List five bacterial diseases and five viral diseases.

TABLE 1.1 Nationally Notifiable Infectious Conditions by Cause* (U.S. 2017, CDC)

Bacterial Diseases

Anthrax
Botulism
- Foodborne; infant; other (wound and unspecified)

Brucellosis
Chancroid
Chlamydia trachomatis infections
Cholera
Diphtheria
Ehrlichiosis/Anaplasmosis
- *Ehrlichia chaffeensis*; *Ehrlichia euringii*; *Anaplasma phagocytophilum*; undetermined

Gonorrhea
Haemophilus influenzae (invasive disease)
Hansen's disease (leprosy)
Hemolytic uremic syndrome, post-diarrheal
Legionellosis
Leptospirosis
Listeriosis
Lyme disease
Meningococcal disease
Pertussis (whooping cough)
Plague
Psittacosis
Q Fever
- Acute; chronic

Salmonellosis
Shiga toxin-producing *Escherichia coli* (STEC)
Shigellosis
Spotted Fever Rickettsiosis
Streptococcal toxic-shock syndrome
Streptococcus pneumoniae (invasive disease)
Syphilis
- Primary; secondary; latent; early latent; late latent; latent, unknown duration; neurosyphilis; late, non-neurological; stillbirth; congenital

Tetanus
Toxic-shock syndrome (other than *Streptococcus*)
Tuberculosis
Tularemia
Typhoid fever
Vancomycin-intermediate *Staphylococcus aureus* (VISA)
Vancomycin-resistant *Staphylococcus aureus* (VRSA)
Vibriosis

Viral Diseases

AIDS has been reclassified as HIV stage III
Arboviral neuroinvasive and nonneuroinvasive diseases
- California serogroup virus disease
- Eastern equine encephalitis virus disease
- Powassan virus disease
- St. Louis encephalitis virus disease
- West Nile virus disease
- Western equine encephalitis virus disease

Dengue
- Dengue fever; dengue hemorrhagic fever; dengue shock syndrome

Hantavirus pulmonary syndrome
Hepatitis
- A, acute; B, acute; B, chronic; B virus, perinatal infection; C, acute; C, chronic

HIV infection
- AIDS has been reclassified as HIV stage III
- HIV infection, adult/adolescent (age >= 13 years)
- HIV infection, child (age >= 18 months and <13 years)
- HIV infection, pediatric (age <18 months)

Influenza-associated pediatric mortality
Measles
Mumps
Novel influenza A virus infections, e.g., H1N1
Poliomyelitis, paralytic
Poliomyelitis infection, nonparalytic
Rabies (animal, human)
Rubella
Rubella, congenital syndrome
Severe Acute Respiratory Syndrome-associated Coronavirus (SARS-CoV) disease
Smallpox
Varicella morbidity (chickenpox, shingles)
Varicella (deaths only)
Viral Hemorrhagic Fever due to:
- Ebola virus; Marburg virus; Arenavirus; Crimean-Congo Hemorrhagic Fever virus; Lassa virus; lujo virus; New World arenaviruses (Gunarito, Machupo, Junin, and Sabia viruses)

Yellow Fever

Algal Diseases

None

Fungal Diseases

Coccidioidomycosis

Protozoan Disease

Babesiosis
Cryptosporidiosis
Cyclosporiasis
Giardiasis
Malaria

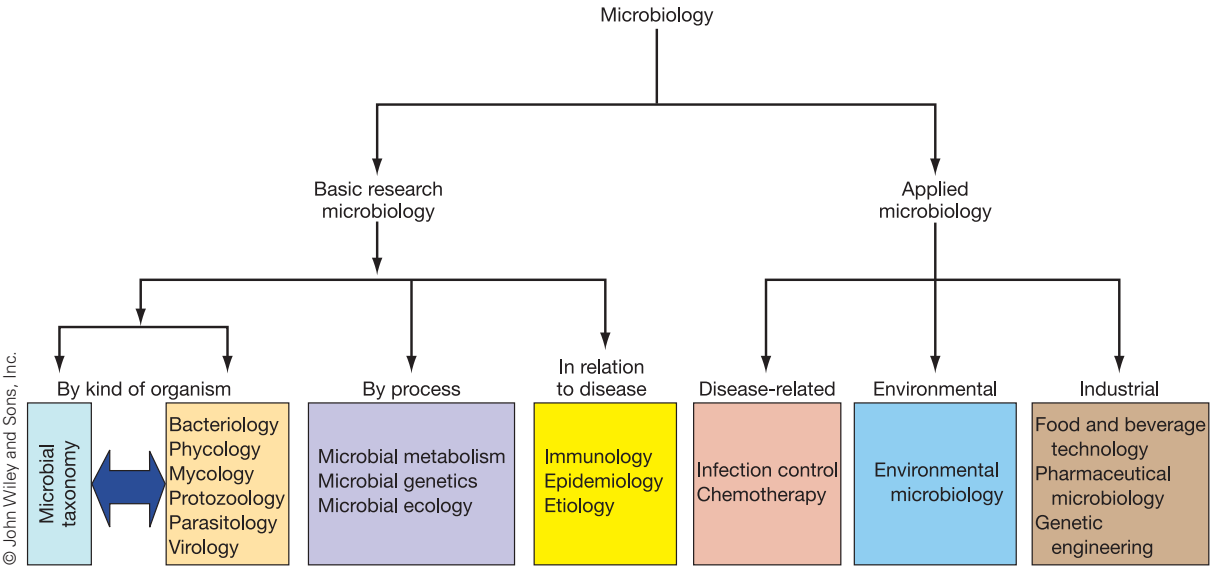
Helminth Disease

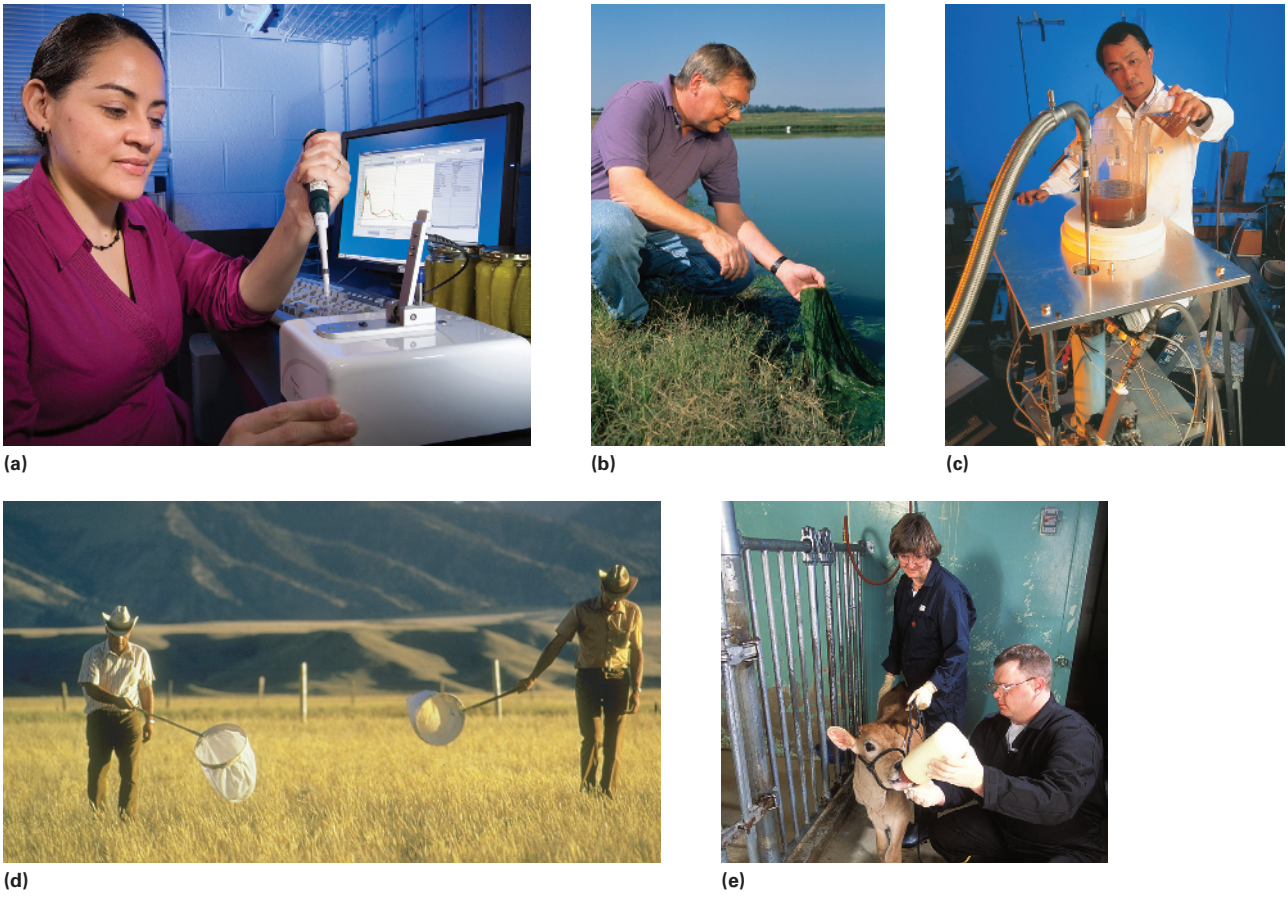
Trichinosis

*Infectious disease reporting varies by state. This table lists most of the diseases commonly reported to the U.S. Centers for Disease Control and Prevention (CDC) as of 2017.

TABLE 1.2 Fields of Microbiology 1

Field (Pronunciation)	Examples of What Is Studied
Microbial taxonomy	Classification of microorganisms
Fields According to Organisms Studied	
Bacteriology (bak″ter-e-ol′o-je)	Bacteria
Phycology (fi-kol′o-je)	Algae (*phyco*, "seaweed")
Mycology (mi-kol′o-je)	Fungi (*myco*, "a fungus")
Protozoology (pro″to-zo-ol′o-je)	Protozoa (*proto*, "first"; *zoo*, "animal")
Parasitology (par″a-si-tol′o-je)	Parasites
Virology (vi-rol′o-je)	Viruses
Fields According to Processes or Functions Studied	
Microbial metabolism	Chemical reactions that occur in microbes
Microbial genetics	Transmission and action of genetic information in microorganisms
Microbial ecology	Relationships of microbes with each other and with the environment
Health-Related Fields	
Immunology (im″u-nol′o-je)	How host organisms defend themselves against microbial infection
Epidemiology (epi-i-de-me-ol′o-je)	Frequency and distribution of diseases
Etiology (e-te-ol′-o-je)	Causes of disease
Infection control	How to control the spread of nosocomial (nos-o-ko′me-al), or hospital-acquired, infections
Chemotherapy	The development and use of chemical substances to treat diseases
Fields According to Applications of Knowledge	
Food and beverage technology	How to protect humans from disease organisms in fresh and preserved foods
Environmental microbiology	How to maintain safe drinking water, dispose of wastes, and control environmental pollution
Industrial microbiology	How to apply knowledge of microorganisms to the manufacture of fermented foods and other products of microorganisms
Pharmaceutical microbiology	How to manufacture antibiotics, vaccines, and other health products
Genetic engineering	How to use microorganisms to synthesize products useful to humans





(a) **(b)** **(c)** **(d)** **(e)**

FIGURE 1.3 **Microbiology is used in diverse careers.** These careers include such activities as **(a)** food microbiologist examines cucumber brine *(Peggy Greb/Courtesy USDA);* **(b)** algae being examined by agricultural microbiologist *(Peggy Greb/Courtesy USDA);* **(c)** using bacteria to decontaminate toxic wastes *(Science Photo Library/Science Source);* **(d)** using beating nets to survey for ticks that can spread disease to livestock and humans *(Courtesy United States Department of Agriculture);* **(e)** veterinary microbiologists vaccinate a calf orally. *(Stephen Ausmus/Courtesy USDA)*

HISTORICAL ROOTS

Many of the ancient Mosaic laws found in the Bible about basic sanitation have been used through the centuries and still contribute to our practices of preventive medicine. In Deuteronomy, Chapter 13, Moses instructed the soldiers to carry spades and bury solid waste matter. The Bible also refers to leprosy and to the isolation of lepers. Although in those days the term *leprosy* probably included other infectious and noninfectious diseases, isolation did limit the spread of the infectious diseases.

The Greeks anticipated microbiology, as they did so many things. The Greek physician Hippocrates, who lived around 400 B.C., set forth ethical standards for the practice of medicine that are still in use today. Hippocrates was wise in human relations and also a shrewd observer. He associated particular signs and symptoms with certain illnesses and realized that diseases could be transmitted from one person to another by clothing or other objects. At about the same time, the Greek historian Thucydides observed that people who had recovered from the plague could take care of plague victims without danger of getting the disease again.

The Romans also contributed to microbiology, as early as the first century B.C. The scholar and writer Varro proposed that tiny invisible animals entered the body through the mouth and nose to cause disease. Lucretius, a philosophical poet, cited "seeds" of disease in his *De Rerum Natura (On the Nature of Things)*.

Bubonic plague, also called the Black Death, appeared in the Mediterranean region around 542 A.D., where it reached epidemic proportions and killed millions. In 1347 the plague invaded Europe along the caravan routes and sea lanes from central Asia, affecting Italy first, then France, England, and finally northern Europe. Although no accurate records were kept at that time, it is estimated that tens of millions of people in Europe died during this and successive waves of plague over the next 300 years. The Black



FIGURE 1.4 ***The Triumph of Death*** **by Pieter Brueghel the Elder.** What were people thinking and feeling when Brueghel painted this picture in the mid-sixteenth century, a time when outbreaks of plague were still common in many parts of Europe? The painting dramatizes the swiftness and inescapability of death for people of all social and economic classes. *(Pieter Brueghel the Elder (1528–1569), Flemish, Trionfolo della Morte, Painting, Prado, Madrid, Spain/Scala/Art Resource, NY)*

Death was a great leveler—it killed rich and poor alike (**Figure 1.4**). The wealthy fled to isolated summer homes but carried plague-infected fleas with them in unwashed hair and clothing. In the mid-fourteenth century (1347–1351) plague alone wiped out 25 million people—one-fourth of the population of Europe and neighboring regions—in just 5 years. In the vicinity of the Sedlec Monastery near Prague, over 30,000 people died of plague in 1 year. Their bones are now displayed in an ossuary (**Figure 1.5**).

Until the seventeenth century, the advance of microbiology was hampered by the lack of appropriate tools to observe microbes. Around 1665, the English scientist Robert Hooke built a compound microscope (one in which light passes through two lenses) and used it to observe thin slices of cork. He coined the term *cell* to describe the orderly arrangement of small boxes that he saw because they reminded him of the cells (small, bare rooms) of monks. However, it was Anton van Leeuwenhoek (**Figure 1.6**), a Dutch cloth merchant and amateur lens grinder, who first made and used lenses to observe living microorganisms. The lenses Leeuwenhoek made were of excellent quality; some gave magnifications up to 300X and were remarkably free of distortion. Making these lenses and looking through them were the passions of his life. Everywhere he looked he found what he called "animalcules." He found them in stagnant water, in sick people, and even in his own mouth.

Over the years Leeuwenhoek observed all the major kinds of microorganisms—protozoa, algae, yeast, fungi, and bacteria in spherical, rod, and spiral forms. He once wrote, "For my part I judge, from myself (howbeit I clean my mouth like I've already said), that all the people living in our United Netherlands are not as many as the living animals that I carry in my own mouth this very day." Starting in the 1670s he wrote numerous letters to the Royal Society in London and pursued his studies until his death in 1723 at the age of 91. Leeuwenhoek refused to sell his microscopes to others and so failed to foster the development of microbiology as much as he could have.

After Leeuwenhoek's death, microbiology did not advance for more than a century. Eventually microscopes

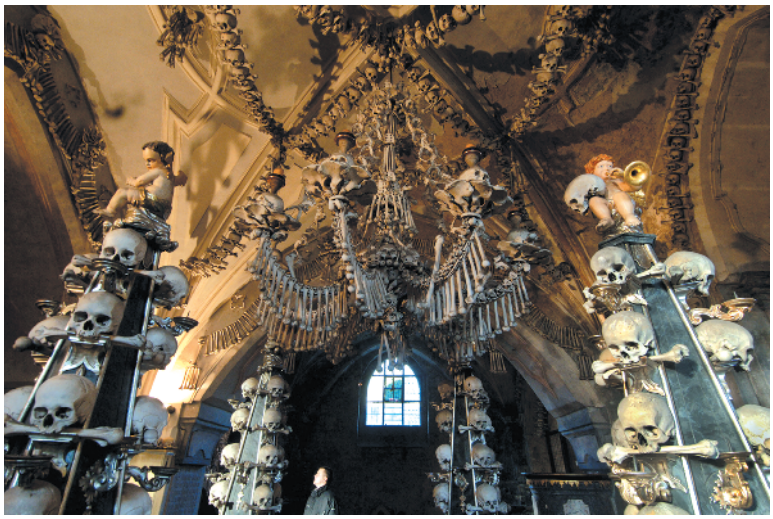


FIGURE 1.5 Ossuary (bone display) at the Sedlec Monastery, located near Prague. Most of the bones are from victims of the plague outbreak of 1347–1351, in which more than 30,000 people died. *(Michal Cizek/AFP/Getty/NewsCom)*



FIGURE 1.6 Anton van Leeuwenhoek (1632–1723). He is shown holding one of his simple microscopes. *(Bettmann/Getty Images)*

became more widely available, and progress resumed. Several workers discovered ways to stain microorganisms with dyes to make them more visible.

The Swedish botanist Carolus Linnaeus developed a general classification system for all living organisms. The German botanist Matthias Schleiden and the German zoologist Theodor Schwann formulated the **cell theory**, which states that cells are the fundamental units of life and carry out all the basic functions of living things. Today this theory still applies to all cellular organisms, but not to viruses.

THE GERM THEORY OF DISEASE

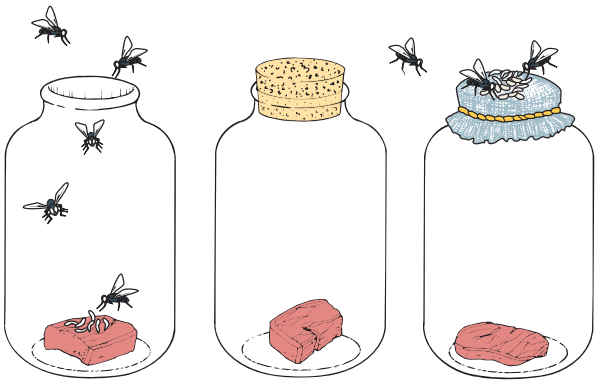
The **germ theory of disease** states that microorganisms (germs) can invade other organisms and cause disease. Although this is a simple idea and is generally accepted today, it was not widely accepted when formulated in the mid-nineteenth century. Many people believed that broth, left standing, turned cloudy because of something about the broth itself. Even after it was shown that microorganisms in the broth caused it to turn cloudy, people believed that the microorganisms, like the "worms" (fly larvae, or maggots) in rotting meat, arose from nonliving things, a concept known as **spontaneous generation**. Widespread belief in spontaneous generation, even among scientists, hampered further development of the science of microbiology and the acceptance of the germ theory of disease. As long as they believed that microorganisms could arise from nonliving substances, scientists saw no purpose in considering how diseases were transmitted or how they could be controlled. Dispelling the belief in spontaneous generation took years of painstaking effort.

Early Studies

For as long as humans have existed, some probably have believed that living things somehow originated spontaneously from nonliving matter. Aristotle's theories about his four "elements"—fire, earth, air, and water—seem to have suggested that nonliving forces somehow contributed to the generation of life. Even some naturalists believed that rodents arose from moist grain, beetles from dust, and worms and frogs from mud. As late as the nineteenth century, it seemed obvious to most people that rotting meat gave rise to "worms."

In the late seventeenth century, the Italian physician Francesco Redi devised a set of experiments to demonstrate that if pieces of meat were covered with gauze so that flies could not reach them, no "worms" appeared in the meat, no matter how rotten it was (**Figure 1.7**). Maggots did, however, hatch from fly eggs laid on top of the gauze. Despite the proof that maggots did not arise spontaneously, some scientists still believed in spontaneous generation—at least that of microorganisms. Lazzaro Spallanzani, an Italian cleric and scientist, was more skeptical. He boiled broth infusions containing organic (living or previously living) matter and sealed the flasks to demonstrate that no organisms would develop spontaneously in them. Critics did not accept this as disproof of spontaneous generation. They argued that boiling drove off oxygen (which they thought all organisms required) and that sealing the flasks prevented its return.

Several scientists tried different ways of introducing air to counter this criticism. Schwann heated air before introducing it into flasks, and other scientists filtered air



© John Wiley and Sons, Inc.

FIGURE 1.7 Redi's experiments refuting the spontaneous generation of maggots in meat. When meat is exposed in an open jar, flies lay their eggs on it, and the eggs hatch into maggots (fly larvae). In a sealed jar, however, no maggots appear. If the jar is covered with gauze, maggots hatch from eggs that the flies lay on top of the gauze but still no maggots appear on the meat.



FIGURE 1.8 An actual "swan-necked" flask that Pasteur used in refuting the theory of spontaneous generation. Although air could enter the flasks, microbes became trapped in the curved necks and never reached the contents. The contents, therefore, remained sterile—and still are today, in the museum—despite their exposure to the air. *(Charles O'Rear/Getty Images)*

through chemicals or cotton plugs. All these methods prevented the growth of microorganisms in the flasks. But the critics still argued that altering the air prevented spontaneous generation.

Even nineteenth-century scientists of some stature continued to argue vociferously in favor of spontaneous generation. They believed that an organic compound previously formed by living organisms contained a "vital force" from which life sprang. The force, of course, required air, and they believed that all the methods of introducing air somehow changed it so that it could not interact with the force.

The proponents of spontaneous generation were finally defeated, mainly by the work of the French chemist Louis Pasteur and the English physicist John Tyndall. When the French Academy of Science sponsored a competition in 1859 "to try by well-performed experiment to throw new light on the question of spontaneous generation," Pasteur entered the competition.

During the years Pasteur worked in the wine industry, he had established that alcohol was produced in wine only if yeast was present, and he learned a lot about the growth of microorganisms. Pasteur's experiment for the competition involved his famous "swan-necked" flasks (**Figure 1.8**). He boiled *infusions* (broths of foodstuffs) in flasks, heated the glass necks, and drew them out into long, curved tubes open at the end. Air could enter the flasks without being subjected to any of the treatments that critics had claimed destroyed its effectiveness. Airborne microorganisms could also enter the necks of the flasks, but they became trapped in the curves of the neck and never reached the infusions. The infusions from Pasteur's experiments remained sterile unless a flask was tipped so that the infusion flowed into the neck and then back into the flask. This manipulation allowed microorganisms trapped in the neck to wash into the infusion, where they could grow and cause the infusion to become cloudy. In another experiment Pasteur filtered air through three cotton plugs. He then immersed the plugs in sterile infusions, demonstrating that growth occurred in the infusions from organisms trapped in the plugs.

Tyndall delivered another blow to the idea of spontaneous generation when he arranged sealed flasks of boiled infusion in an airtight box. After allowing time for all dust particles to settle to the bottom of the box, he carefully removed the covers from the flasks. These flasks, too, remained sterile. Tyndall had shown that air could be sterilized by settling, without any treatment that would prevent the "vital force" from acting.

Both Pasteur and Tyndall were fortunate that the organisms present in their infusions at the time of boiling were destroyed by heat. Others who tried the same experiments observed that the infusions became cloudy from growth of microorganisms. We now know that the infusions in which growth occurred contained heat-resistant or spore-forming microorganisms, but at the time, the growth of such organisms was seen as evidence of spontaneous generation. Still, the works of Pasteur and Tyndall successfully disproved spontaneous generation to most scientists of the time. Recognition that microbes must be introduced into a medium before their growth can be observed paved the way for further development of microbiology—especially for development of the germ theory of disease.

Pasteur's Further Contributions

Louis Pasteur (**Figure 1.9**) was such a giant among nineteenth-century scientists working in microbiology that we must consider some of his many contributions. Born



FIGURE 1.9 Louis Pasteur in his laboratory. The first rabies vaccine, developed by Pasteur, was made from the dried spinal cords of infected rabbits. *(Hulton Archive/Getty)*

in 1822, the son of a sergeant in Napoleon's army, Pasteur worked as a portrait painter and a teacher before he began to study chemistry in his spare time. Those studies led to posts in several French universities as professor of chemistry and to significant contributions to the wine and silkworm industries. He discovered that carefully selected yeasts made good wine, but that mixtures of other microorganisms competed with the yeast for sugar and made wine taste oily or sour. To combat this problem, Pasteur developed the technique of pasteurization (heating wine to 56°C in the absence of oxygen for 30 minutes) to kill unwanted organisms. While studying silkworms, he identified three different microorganisms, each of which caused a different disease. His association of specific organisms with particular diseases, even though in silkworms rather than in humans, was an important first step in proving the germ theory of disease.

Despite personal tragedy—the deaths of three daughters and a cerebral hemorrhage that left him with permanent paralysis—Pasteur went on to contribute to the development of vaccines. The best known of his vaccines is the rabies vaccine, made of dried spinal cord from rabbits infected with rabies, which was tested in animals. When a 9-year-old boy who had been severely bitten by a rabid dog was brought to him, Pasteur administered the vaccine, but only after a long night of soul searching. He was not a physician, and had never practiced medicine before. The boy, who had been doomed to die, survived and became the first person to be immunized against rabies. Later, during World War II, the then grown-up boy was killed by German soldiers for refusing to give them access to Pasteur's tomb so that they could desecrate his bones.

In 1894 Pasteur became director of the Pasteur Institute, which was built for him in Paris. Until his death in 1895, he guided the training and work of other scientists at the institute. Today the Pasteur Institute is a thriving research center—an appropriate memorial to its founder.

Koch's Contributions

Robert Koch (**Figure 1.10**), a contemporary of Pasteur, finished his medical training in 1872 and worked as a

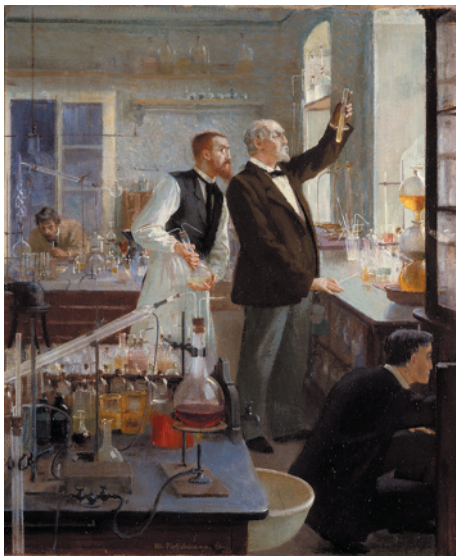


FIGURE 1.10 Robert Koch in his laboratory with his assistants. Koch formulated four postulates for linking a given organism to a specific disease. *(SuperStock)*

physician in Germany throughout most of his career. After he bought a microscope and photographic equipment, he spent most of his time studying bacteria, especially those that cause disease. Koch identified the bacterium that causes anthrax, a highly contagious and lethal disease in cattle and sometimes in humans. He recognized both actively dividing cells and dormant cells (spores) and developed techniques for studying them *in vitro* (outside a living organism).

Koch also found a way to grow bacteria in *pure cultures*—cultures that contained only one kind of organism. He tried streaking bacterial suspensions on potato slices and then on solidified gelatin. But gelatin melts at incubator (body) temperature; even at room temperature, some microbes liquefy it. Finally, Angelina Hesse (**Figure 1.11**), the American wife of one of Koch's colleagues, suggested that Koch add agar (a thickener used in cooking) to his bacteriological media. This created a firm surface over which microorganisms could be spread very thinly—so thinly that some individual organisms were separated from all others. Each individual organism then multiplied to make a colony of thousands of descendants. Koch's technique of preparing pure cultures is still used today.

Koch's outstanding achievement was the formulation of four postulates to associate a particular organism with a specific disease. **Koch's Postulates**, which provided scientists with a method of establishing the germ theory of disease, are as follows:

1. The specific causative agent must be found in every case of the disease.
2. The disease organism must be isolated in pure culture.



FIGURE 1.11 Angelina and Walther Hesse. The American wife of Koch's assistant suggested solidifying broths with agar as an aid to obtaining pure cultures. She had used it to solidify broths in her kitchen, and we still use it in our labs today. *(From ASM News 47(7)392, 1961. Reproduced with permission of American Society for Microbiology.)*

3. Inoculation of a sample of the culture into a healthy, susceptible animal must produce the same disease.
4. The disease organism must be recovered from the inoculated animal.

Implied in Koch's postulates is his one organism–one disease concept. The postulates assume that an infectious disease is caused by a single organism, and they are directed toward establishing that fact. This concept also was an important advance in the development of the germ theory of disease.

After obtaining a laboratory post at Bonn University in 1880, Koch was able to devote his full time to studying microorganisms. He identified the bacterium that causes tuberculosis, developed a complex method of staining this organism, and disproved the idea that tuberculosis was inherited. He also guided the research that led to the isolation of *Vibrio cholerae*, the bacterium that causes cholera.

In a few years Koch became professor of hygiene at the University of Berlin, where he taught a microbiology course believed to be the first ever offered. He also developed *tuberculin*, which he hoped would be a vaccine against tuberculosis. Because he underestimated the difficulty of killing the organism that causes tuberculosis, the use of tuberculin resulted in several deaths from that disease. Although tuberculin was unacceptable as a vaccine, its development laid the groundwork for a skin test to diagnose tuberculosis. After the vaccine disaster, Koch left Germany. He made several visits to Africa, at least two visits to Asia, and one to the United States.

In the remaining 15 years of his life, his accomplishments were many and varied. He conducted research on malaria, typhoid fever, sleeping sickness, and several other diseases. His studies of tuberculosis won him the Nobel Prize for Physiology and Medicine in 1905, and his work in Africa and Asia won him great respect on those continents.

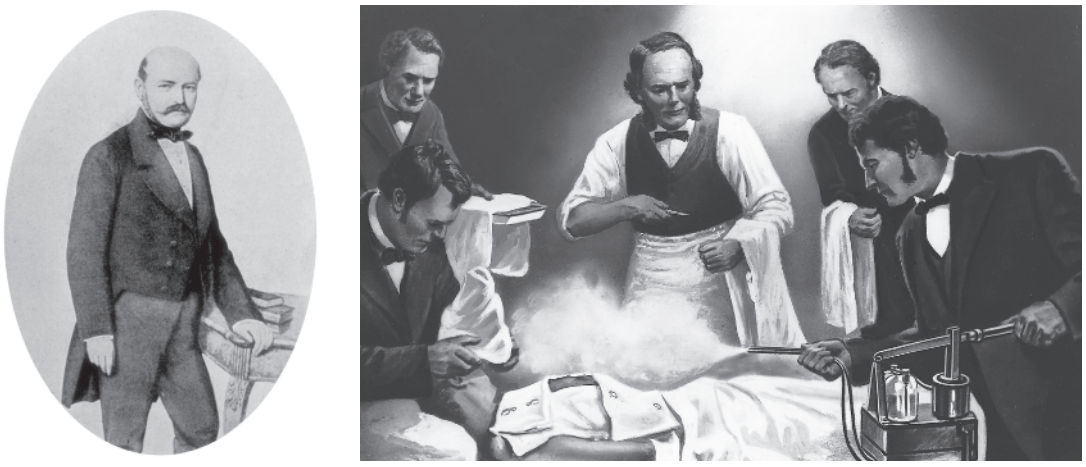
Work Toward Controlling Infections

Like Koch and Pasteur, two nineteenth-century physicians, Ignaz Philipp Semmelweis of Austria and Joseph Lister of England, were convinced that microorganisms caused infections (**Figure 1.12**). Semmelweis recognized a connection between autopsies and puerperal (childbed) fever. Many physicians went directly from performing autopsies to examining women in labor without so much as washing their hands. When Semmelweis attempted to encourage more sanitary practices, he was ridiculed and harassed until he had a nervous breakdown and was sent to an asylum. Ultimately, he suffered the curious irony of succumbing to an infection caused by the same organism that produces puerperal fever. In 1865, Lister, who had read of Pasteur's work on pasteurization and Semmelweis's work on improving sanitation, initiated the use of dilute carbolic acid on bandages and instruments to reduce infection. Lister, too, was ridiculed, but with his imperturbable temperament, resolute will, and tolerance of hostile criticism, he was able to continue his work. His methods, the first *aseptic techniques*, were proven effective by the decrease in surgical wound infections in his surgical wards. At age 75, some 37 years after he had introduced the use of carbolic acid, Lister was awarded the Order of Merit for his work in preventing the spread of infection. He is considered the father of antiseptic surgery.

CLOSE UP

What's in the Last Drop?

During the nineteenth century, French and German scientists were fiercely competitive. One area of competition was the preparation of pure cultures. Koch's reliable method of preparing pure cultures from colonies on solid media allowed German microbiologists to forge ahead. The French microbiologists' method of broth dilution, though now often used to count organisms (▶ Chapter 7), hampered their progress. They added a few drops of a culture to fresh broth, mixed it, and added a few drops of the mixture to more fresh broth. After several successive dilutions, they assumed that the last broth that showed growth of microbes had contained a single organism. Unfortunately, the final dilution often contained more than one organism, and sometimes the organisms were of different kinds. This faulty technique led to various fiascos, such as inoculating animals with deadly organisms instead of vaccinating them.



(a) (b)

FIGURE 1.12 Two nineteenth-century pioneers in the control of infections. (a) Ignaz Philipp Semmelweis, who died in an asylum before his innovations were widely accepted, depicted on a 1965 Austrian postage stamp *(Universal Images Group/Getty Images);* **(b)** Joseph Lister, performing surgery using a spray of carbolic acid above the surgical field, successfully carried on Semmelweis's work toward achieving aseptic techniques. *(Bettmann/Getty Images)*

STOP and CHECK

1. What similarities and differences do you see when comparing past epidemics of plague with today's AIDS epidemic?
2. State the germ theory of disease. Try to think of an explanation of disease causation that would be contrary to the germ theory.
3. How did Pasteur's experiment with "swan-necked" flasks disprove the theory of spontaneous generation?
4. Why was the French microbiologists' method of broth dilution inadequate for obtaining pure cultures of organisms?

EMERGENCE OF SPECIAL FIELDS OF MICROBIOLOGY

Pasteur, Koch, and most other microbiologists considered to this point were generalists interested in a wide variety of problems. Certain other contributors to microbiology had more specialized interests, but their achievements were no less valuable. In fact, those achievements helped establish the special fields of immunology, virology, chemotherapy, and microbial genetics—fields that are today prolific research areas. Selected fields of microbiology are defined in Table 1.2.

Immunology

Disease depends not only on microorganisms invading a host but also on the host's response to that invasion. Today, we know that the host's response is in part a response of the immune system.

The ancient Chinese knew that a person scarred by smallpox would not again get the disease. They took dried scabs from lesions of people who were recovering from the disease and ground them into a powder that they sniffed. As a result of inhaling weakened organisms, they acquired a mild case of smallpox but were protected against subsequent infection.

Smallpox was unknown in Europe until the Crusaders carried it back from the Near East in the twelfth century. By the seventeenth century, it was widespread. In 1717 Lady Mary Ashley Montagu, wife of the British ambassador to Turkey, introduced a kind of immunization to England. A thread was soaked in fluid from a smallpox vesicle (blister) and drawn through a small incision in the arm. This technique, called *variolation*, was used at first by only a few prominent people, but eventually it became widespread.

Pocahontas died of smallpox in 1617, in England.

In the late eighteenth century, Edward Jenner realized that milkmaids who got cowpox did not get smallpox, and he inoculated his own son with fluid from a cowpox blister. He later similarly inoculated an 8-year-old and subsequently inoculated the same child with smallpox. The child remained healthy. The word *vaccinia (vacca*, the Latin name for "cow") gave rise both to the name of the virus that causes cowpox and to the word *vaccine.* In the early 1800s Jenner received grants amounting to a total of 30,000 British pounds to extend his work on vaccination. Today, those grants would be worth more than $1 million. They may have been the first grants for medical research.

Calves—shaved, inoculated, and covered with cowpox lesions—were led house to house by entrepreneurs who offered vaccination during American colonial times.

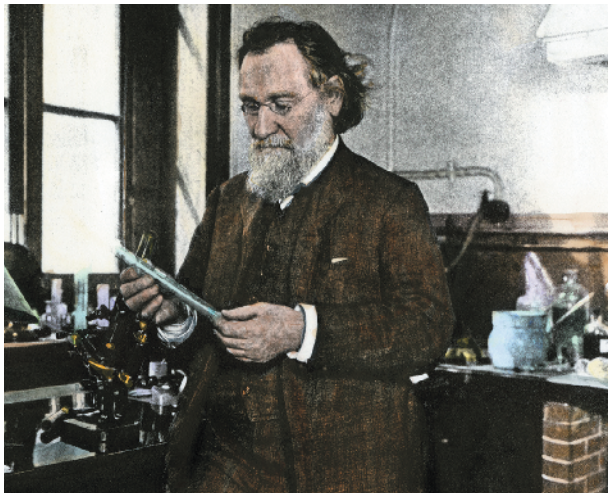


FIGURE 1.13 Elie Metchnikoff. Metchnikoff was one of the first scientists to study the body's defenses against invading microorganisms. *(North Wind Picture Archives/Alamy)*

Pasteur contributed significantly to the emergence of immunology with his work on vaccines for rabies and cholera. In 1879, when Pasteur was studying chicken cholera, his assistant accidentally used an old chicken cholera culture to inoculate some chickens. The chickens did not develop disease symptoms. When the assistant later inoculated the same chickens with a fresh chicken cholera culture, they remained healthy. Although he had not planned to use the old culture first, Pasteur did realize that the chickens had been immunized against chicken cholera. He reasoned that the organisms must have lost their ability to produce disease but retained their ability to produce immunity. This finding led Pasteur to look for techniques that would have the same effect on other organisms. His development of the rabies vaccine was a successful attempt.

Along with Jenner and Pasteur, the nineteenth-century Russian zoologist Elie Metchnikoff was a pioneer in immunology (**Figure 1.13**). In the 1880s many scientists believed that immunity was due to noncellular substances in the blood. Metchnikoff discovered that certain cells in the body would ingest microbes. He named those cells *phagocytes*, which literally means "cell eating." The identification of phagocytes as cells that defend the body against invading microorganisms was a first step in understanding immunity. Metchnikoff also developed several vaccines. Some were successful, but unfortunately some infected the recipients with the microorganisms against which they were supposedly being immunized. A few of his subjects acquired gonorrhea and syphilis from his vaccines. Metchnikoff had used the French method of obtaining supposedly "pure" cultures.

Virology

The science of virology emerged after that of bacteriology because viruses could not be recognized until certain techniques for studying and isolating larger particles such as bacteria had been developed. When Pasteur's collaborator Charles Chamberland developed a porcelain filter to remove bacteria from water in 1884, he had no idea that any kind of infectious agent could pass through the filter. But researchers soon realized that some filtrates (materials that passed through the filters) remained infectious even after the bacteria were filtered out. The Dutch microbiologist Martinus Beijerinck determined why such filtrates were infectious and was thus the first to characterize viruses. The term *virus* had been used earlier to refer to poisons and to infectious agents in general. Beijerinck used the term to refer to specific *pathogenic* (disease-causing) molecules incorporated into cells. He also believed that these molecules could borrow for their own use existing metabolic and replicative mechanisms of the infected cells, known as *host cells*.

Further progress in virology required development of techniques for isolating, propagating, and analyzing viruses. The American scientist Wendell Stanley crystallized tobacco mosaic virus in 1935, showing that an agent with properties of a living organism also behaved as a chemical substance (**Figure 1.14**). The crystals consisted of protein and ribonucleic acid (RNA). The nucleic acid was soon shown to be important in the infectivity of viruses. Viruses were first observed with an electron microscope in 1939. From that time both chemical and microscopic studies were used to investigate viruses.

By 1952 the American biologists Alfred Hershey and Martha Chase had demonstrated that the genetic material of some viruses is another nucleic acid, deoxyribonucleic acid (DNA). In 1953 the American postdoctoral student James Watson and the English biophysicist Francis Crick determined the structure of DNA. The stage was set for rapid advances in understanding how DNA functions as

CLOSE UP

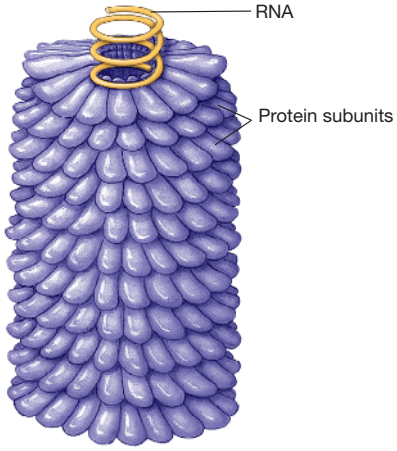
A "Thorny" Problem

Metchnikoff's personal life played a role in his discovery of phagocytes. A widower, when he remarried he took in his 16-year-old wife's dozen younger brothers and sisters as part of the marriage agreement. On one occasion, Metchnikoff left under his microscope a starfish he was studying before he went to lunch with his wife. While the starfish was unattended, the mischievous children poked thorns into it. Metchnikoff was enraged to discover his mutilated starfish, but he looked through the microscope before he discarded his ruined specimen. He was amazed to discover that cells of the starfish had gathered around the thorns. After further study, he identified those cells as *leukocytes* (white blood cells) and found that they could devour foreign particles. He coined the word *phagocytosis* (*phago:* "to eat"; *cyte:* "cell") to describe this process. He realized that this process was a major mechanism of body defense, for which he received the Nobel Prize in 1908, and has become known as the father of immunology. Incidentally, he never did have any children of his own.



Omikron/Science Source

(a)



(b)

FIGURE 1.14 The tobacco mosaic virus. **(a)** Electron micrograph of tobacco mosaic virus (magnification approx. 617,000X). *(Omikron/Science Source)* **(b)** The structure of the tobacco mosaic virus. A helical core of RNA is surrounded by a coat that consists of repeating protein units. The structure of the particles is so regular that the viruses can be crystallized.

genetic material both in viruses and in cellular organisms. Since the 1950s hundreds of viruses have been isolated and characterized. Although much remains to be learned about viruses, tremendous progress has been made in understanding their structure and how they function.

Chemotherapy

The Greek physician Dioscorides compiled *Materia Medica* in the first century A.D. This five-volume work listed a number of substances derived from medicinal plants still in use today—digitalis, curare, ephedrine, and morphine—along with a number of herbal medications. Credit for bringing herbal medicine to the United States is given to many groups of settlers, but Native Americans used many medicinal plants before the arrival of Europeans in the Americas. Many so-called primitive peoples still use herbs extensively, and some pharmaceutical companies finance expeditions into the Amazon Basin and other remote areas to investigate the uses the natives make of the plants around them.

During the Middle Ages virtually no advances were made in the use of chemical substances to treat diseases. Early in the sixteenth century the Swiss physician Aureolus Paracelsus used metallic chemical elements to treat diseases—antimony for general infections and mercury for syphilis. In the mid-seventeenth century Thomas Sydenham, an English physician, introduced cinchona tree bark to treat malaria. This bark, which we now know contains quinine, had been used to treat fevers in Spain and South America. In the nineteenth century morphine was extracted from the opium poppy and used medicinally to alleviate pain.

Paul Ehrlich, the first serious researcher in the field of chemotherapy (**Figure 1.15**), received his doctoral degree from the University of Leipzig, Germany, in 1878. His discovery that certain dyes stained microorganisms but not animal cells suggested that the dyes or other chemicals might selectively kill microbial cells. This led him to search for the "magic bullet," a chemical that would destroy specific bacteria without damaging surrounding tissues. Ehrlich coined the term *chemotherapy* and headed the world's first institute concerned with the development of drugs to treat disease.

Early in the twentieth century the search for the magic bullet continued, especially among scientists at Ehrlich's institute. After testing hundreds of compounds (and numbering each compound), Ehrlich found compound 418 (arsenophenylglycine) to be effective against sleeping sickness and compound 606 (Salvarsan) to be effective against syphilis. For 40 years Salvarsan remained the best available treatment for this disease. In 1922 Alexander Fleming, a Scottish physician, discovered that lysozyme, an enzyme found in tears, saliva, and sweat, could kill bacteria. Lysozyme was the first body secretion shown to have chemotherapeutic properties.

The development of **antibiotics** began in 1917 with the observation that certain bacteria (actinomycetes) stopped

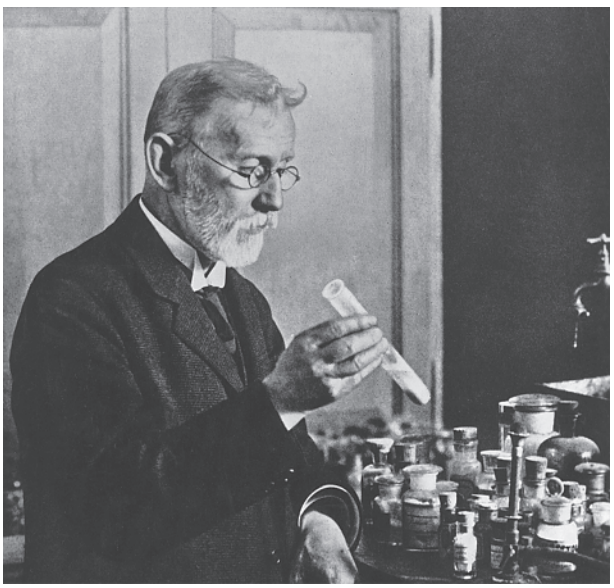


FIGURE 1.15 Paul Ehrlich. Ehrlich was a pioneer in the development of chemotherapy for infectious disease. *(Bettmann/Getty Images)*

PUBLIC HEALTH

Swamp Air or Mosquitoes?

During the American effort to dig the Panama Canal in 1905, yellow fever struck the men as they struggled in the swamps. Yellow fever was a terrible and fatal disease. As Paul de Kruif put it in *Microbe Hunters*, "when folks of a town began to turn yellow and hiccup and vomit black, by scores, by hundreds, every day—the only thing to do was to get up and get out of that town." The entire canal project was in jeopardy because of the disease, and the physician Walter Reed was assigned the task of controlling the disease. Reed listened to the advice of Dr. Carlos Finlay y Barres of Havana, Cuba, who for years had claimed that yellow fever was carried by mosquitoes. Reed ignored those who called Dr. Finlay a theorizing old fool and insisted yellow fever was due to swamp air. Several people, including James Carroll, Reed's longtime associate, volunteered to be bitten by mosquitoes known to have bitten yellow fever patients. Although Carroll survived after his heart had nearly stopped, most of the other volunteers died. Jesse Lazear, a physician working with Reed, was accidentally bitten by mosquitoes while working with patients. He began to show symptoms in 5 days and was dead in 12 days. Thus, it became clear that mosquitoes carried the yellow fever agent. Similar experiments in which volunteers slept on sheets filthy with vomitus of yellow fever patients demonstrated that bad air, contaminated water, sheets, and dishes were not involved. Later Carroll passed blood from yellow fever victims through a porcelain filter and used the filtrate to inoculate three people who had not had yellow fever. How he got their cooperation is not known, but it is known that two of them died of yellow fever. The agent that passed through the porcelain filter was eventually identified as a virus.

the growth of other bacteria. In 1928 Fleming (**Figure 1.16**) observed that a colony of *Penicillium* mold contaminating a culture of *Staphylococcus* bacteria had prevented growth of bacteria adjacent to itself. Although Fleming was not the first to observe this phenomenon, he was the first to recognize its potential for countering infections. However, purification of sufficient quantities of the substance he called *penicillin* proved to be very difficult. The great need for such a drug during World War II, money from the Rockefeller Institute, and the hard work of the German biochemist Ernst Chain, the Australian pathologist Howard Florey, and researchers at Oxford University accomplished the task. Penicillin became available as a safe and versatile chemotherapeutic agent for use in humans.

While this work was going on, sulfa drugs were being developed. In 1935, prontosil rubrum, a reddish dye containing a sulfonamide chemical group, was used in treating streptococcal infections. Further study showed that sulfonamides were converted in the body to sulfanilamides; much subsequent work was devoted to developing drugs containing sulfanilamide. The German chemist Gerhard Domagk played an important role in this work, and one of the drugs, prontosil, saved the life of his daughter. In 1939 he was awarded a Nobel Prize for his work, but Hitler refused to allow him to make the trip to receive it. Extensions of Domagk's work led to the development of isoniazid, an effective agent against tuberculosis. Both sulfa drugs and isoniazid are still used today.

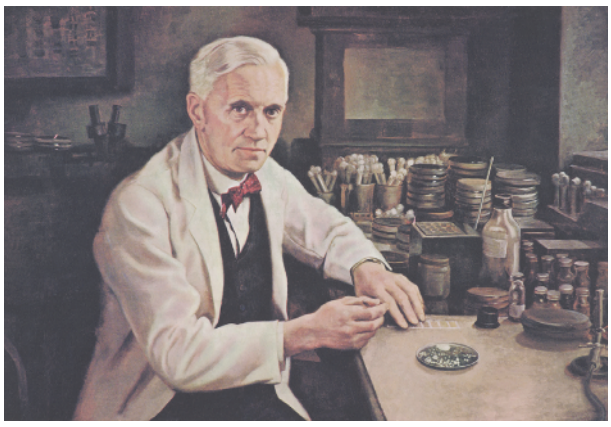


FIGURE 1.16 Alexander Fleming. Fleming discovered the antibacterial properties of penicillin. *(Science Source/Getty Images)*

The development of antibiotics resumed with the work of Selman Waksman, who was born in Ukraine and moved to the United States in 1910. Inspired by the 1939 discovery, by the French microbiologist Rene Dubos, of tyrothricin, an antibiotic produced by soil bacteria, Waksman examined soil samples from all over the world for growth-inhibiting microorganisms or their products. He coined the term *antibiotic* in 1941 to describe actinomycin and other products he isolated. Both tyrothricin and actinomycin proved to be too toxic for general use as antibiotics. After repeated efforts, Waksman isolated the less toxic drug streptomycin in 1943. Streptomycin constituted a major breakthrough in the treatment of tuberculosis. In the same decade Waksman and others isolated neomycin, chloramphenicol, and chlortetracycline.

Examining soil samples proved to be a good way to find antibiotics, and explorers and scientists still collect soil samples for analysis. The more common antibiotic-producing organisms are rediscovered repeatedly, but the possibility of finding a new one always remains. Even the sea has yielded antibiotics, especially from the fungus *Cephalosporium acremonium*. The Italian microbiologist Giuseppe Brotzu noted the absence of disease organisms in seawater where sewage entered, and he determined that an antibiotic must be present. Cephalosporin was subsequently purified, and a variety of cephalosporin derivatives are now available for treating human diseases.

The fact that many antibiotics have been discovered does not stop the search for more. As long as there are untreatable infectious diseases, the search will continue. Even when effective treatment becomes available,

it is always possible that a better, less toxic, or cheaper treatment can be found. Of the many chemotherapeutic agents currently available, none can cure viral infections. Consequently, much of today's drug research is focused on developing effective antiviral agents.

Genetics and Molecular Biology

Modern genetics began with the rediscovery in 1900 of Gregor Mendel's principles of genetics. Even after this significant event, for nearly three decades little progress was made in understanding how microbial characteristics are inherited. For this reason, microbial genetics is the youngest branch of microbiology. In 1928 the British scientist Frederick Griffith discovered that previously harmless bacteria could change their own nature and become capable of causing disease. The remarkable thing about this discovery is that live bacteria were shown to acquire heritable traits from dead ones. During the early 1940s, Oswald Avery, Maclyn McCarty, and Colin MacLeod of the Rockefeller Institute in New York City demonstrated that the change was produced by DNA. After that finding came the crucial discovery of the structure of DNA by James Watson and Francis Crick. This breakthrough ushered in the modern era of molecular genetics.

About the same time, the American geneticists Edward Tatum and George Beadle used genetic variations in the mold *Neurospora* to demonstrate how genetic information controls metabolism. In the early 1950s the American geneticist Barbara McClintock discovered that some genes (units of inherited information) can move from one location to another on a chromosome. Before McClintock's work, genes were thought to remain stationary. Her revolutionary discovery forced geneticists to revise their thinking about genes.

More recently, scientists have discovered the genetic basis that underlies the human body's ability to make an enormous diversity of *antibodies*, molecules that the immune system produces to combat invading microbes and their toxic products. Within cells of the immune system, genes are shuffled about and spliced together in various combinations, allowing the body to make millions of different antibodies, including some that can protect us from threats that the body has never previously encountered.

TOMORROW'S HISTORY

Today's discovery is tomorrow's history. In an active research field such as microbiology, it is impossible to present a complete history. Some of the microbiologists omitted from this discussion are listed in **Table 1.3.** The period represented there, 1874–1917, is called the Golden Age of Microbiology. You may find that many of the terms used to describe these scientists' accomplishments are unfamiliar, but you will become familiar with them as you pursue the study of microbiology. Since 1900, Nobel Prizes have been awarded annually to outstanding scientists, many of whom were in the fields of physiology or medicine (**Table 1.4**). In some years the prize has been shared by several scientists, although the scientists may have made independent contributions. Refer to Tables 1.3 and 1.4 as you begin to study each new area of microbiology.

You can see from Table 1.4 that microbiology has been in the forefront of research in medicine and biology for several decades and probably never more so than today. One reason is the renewed focus on infectious disease brought about by the advent of AIDS. Another is the dramatic progress in genetic engineering that has been made in the past two decades. Microorganisms have been and continue to be an essential part of the genetic engineering revolution. Most of the key discoveries that led to our present understanding of genetics emerged from research with microbes. Today scientists are attempting to redesign microorganisms for a variety of purposes (as we will see in ▶ Chapter 9). Bacteria have been converted into factories that produce drugs, hormones, vaccines, and a variety of biologically important compounds. And microbes, viruses in particular, are often the vehicle by which scientists insert new genes into other organisms. Such techniques are beginning to enable us to produce improved varieties of plants and animals such as pest-resistant crops and may even enable us to correct genetic defects in human beings.

In September 1990, a 4-year-old girl became the first gene-therapy patient. She had inherited a defective gene that crippled her immune system. Doctors at the National Institutes of Health (NIH) inserted a normal copy of the gene into some of her white blood cells in the laboratory and then injected these gene-treated cells back into her body, where, it was hoped, they would restore her immune system. Critics were worried that a new gene randomly inserted into the girl's white blood cells could damage other genes and cause cancer. The experiment was a success and she enjoys good health today.

New information is constantly being discovered and sometimes supersedes earlier findings. Occasionally, new discoveries lead almost immediately to the development of medical applications, as occurred with penicillin and as will most certainly occur when a cure or vaccine for AIDS is discovered. However, old ideas such as spontaneous generation and old practices such as unsanitary measures in medicine can take years to replace. Many new bioethics problems will require considerable thought. Decisions about AIDS testing and reporting, transplants, cloning, environmental cleanup, and related issues will not come easily or quickly. Because of the wealth of prior knowledge, it is likely that you will learn more about microbiology in a single course than many pioneers learned in a lifetime. Yet, those pioneers deserve great credit because they worked with the unknown and had few people to teach them.

The future of microbiology holds exciting developments. One area involves use of **bacteriophages**, viruses that attack and kill specific kinds of bacteria. Yes, "the big fleas do have little fleas to bite them, and so on ad infinitum." Using phages to treat bacterial infections was developed back in the 1920s and 1930s in Eastern Europe and the Soviet Union. With the discovery of antibiotics in the 1940s, use of phages fell into disfavor and never really

TABLE 1.3 The Golden Age of Microbiology: Early Microbiologists and Their Achievements

Year	Investigator	Achievement
1874	Billroth	Discovery of round bacteria in chains
1876	Koch	Identification of *Bacillus anthracis* as causative agent of anthrax
1878	Koch	Differentiation of staphylococci
1879	Hansen	Discovery of *Mycobacterium leprae* as causative agent of leprosy
1880	Neisser	Discovery of *Neisseria gonorrhoeae* as causative agent of gonorrhea
1880	Laveran and Ross	Identification of life cycle of malarial parasites in red blood cells of infected humans
1880	Eberth	Discovery of *Salmonella typhi* as causative agent of typhoid fever
1880	Pasteur and Sternberg	Isolation and culturing of pneumonia cocci from saliva
1881	Koch	Animal immunization with attenuated anthrax bacilli
1882	Leistikow and Loeffler	Cultivation of *Neisseria gonorrhoeae*
1882	Koch	Discovery of *Mycobacterium tuberculosis* as causative agent of tuberculosis
1882	Loeffler and Schutz	Identification of *Actinobacillus* that causes the animal disease glanders
1883	Koch	Identification of *Vibrio cholerae* as causative agent of cholera
1883	Klebs	Identification of *Corynebacterium diphtheriae* and toxin as causative agent of diphtheria
1884	Loeffler	Culturing of *Corynebacterium diphtheriae*
1884	Rosenbach	Pure culturing of streptococci and staphylococci
1885	Escherich	Identification of *Escherichia coli* as a natural inhabitant of the human gut
1885	Bumm	Pure culturing of *Neisseria gonorrhoeae*
1886	Flugge	Staining to differentiate bacteria
1886	Fraenckel	*Streptococcus pneumoniae* related to pneumonia
1887	Weichselbaum	*Neisseria meningitidis* related to meningitis
1887	Bruce	Identification of *Brucella melitensis* as causative agent of brucellosis in cattle
1887	Petri	Invention of culture dish
1888	Roux and Yersin	Discovery of action of diphtheria toxin
1889	Charrin and Roger	Discovery of agglutination of bacteria in immune serum
1889	Kitasato	Discovery that *Clostridium tetani* produces tetanus toxin
1890	Pfeiffer	Identification of Pfeiffer bacillus, *Haemophilus influenzae*
1890	von Behring and Kitasato	Immunization of animals with diphtheria toxin
1892	Ivanovski	Discovery of filterability of tobacco mosaic virus
1894	Roux and Kitasato	Identification of *Yersinia pestis* as causative agent of bubonic plague
1894	Pfeiffer	Discovery of bacteriolysis in immune serum
1895	Bordet	Discovery of alexin (complement) and hemolysis
1896	Widal and Grunbaum	Development of diagnostic test based on agglutination of typhoid bacilli by immune serum
1897	van Ermengem	Discovery of *Clostridium botulinum* as causative agent of botulism
1897	Kraus	Discovery of precipitins
1897	Ehrlich	Formulation of side-chain theory of antibody formation
1898	Shiga	Discovery of *Shigella dysenteriae* as causative agent of dysentery
1898	Loeffler and Frosch	Discovery of filterability of virus that causes foot-and-mouth disease
1899	Beijerinck	Discovery of intracellular reproduction of tobacco mosaic virus
1901	Bordet and Gengou	Identification of *Bordetella pertussis* as causative agent of whooping cough; development of complement fixation test
1901	Reed and colleagues	Identification of virus that causes yellow fever
1902	Portier and Richet	Work on anaphylaxis
1903	Remlinger and Riffat-Bey	Identification of virus that causes rabies
1905	Schaudinn and Hoffmann	Identification of *Treponema pallidum* as causative agent of syphilis
1906	Wasserman, Neisser, and Bruck	Development of Wasserman reaction for syphilis antibodies
1907	Asburn and Craig	Identification of virus that causes dengue fever
1909	Flexner and Lewis	Identification of virus that causes poliomyelitis
1915	Twort	Discovery of viruses that infect bacteria
1917	d'Herelle	Independent rediscovery of viruses that infect bacteria (bacteriophages)

made it into Western medical practice. However, in Soviet countries phage therapy is preferred over use of antibiotics even today. Soviet troops carried color-coded packets of various phages with them, specific for the bacterial diseases they were likely to encounter. When the first few men came down with disease "A," everyone was instructed to open the "red" packet and consume it; for disease "B," the "blue packet," etc., and thus epidemic outbreaks were

TABLE 1.4 Nobel Prize Awards for Research Involving Microbiology

Year of Prize	Prize Winner	Topic Studied
1901	von Behring	Serum therapy against diphtheria
1902	Ross	Malaria
1905	Koch	Tuberculosis
1907	Laveran	Protozoa and the generation of disease
1908	Ehrlich and Metchnikoff	Immunity
1913	Richet	Anaphylaxis
1919	Bordet	Immunity
1928	Nicolle	Typhus exanthematicus
1939	Domagk	Antibacterial effect of prontosil
1945	Fleming, Chain, and Florey	Penicillin
1951	Theiler	Vaccine for yellow fever
1952	Waksman	Streptomycin
1954	Enders, Weller, and Robbins	Cultivation of polio virus
1958	Lederberg	Genetic mechanisms
	Beadle and Tatum	Transmission of hereditary characteristics
1959	Ochoa and Kornberg	Chemical substances in chromosomes that play a role in heredity
1960	Burnet and Medawar	Acquired immunological tolerance
1962	Watson, Crick, and Wilkins	Structure of DNA
1965	Jacob, Lwoff, and Monod	Regulatory mechanisms in microbial genes
1966	Rous	Viruses and cancer
1968	Holley, Khorana, and Nirenberg	Genetic code
1969	Delbruck, Hershey, and Luria	Mechanism of virus infection in living cells
1972	Edelman and Porter	Structure and chemical nature of antibodies
1975	Baltimore, Temin, and Dulbecco	Interactions between tumor viruses and genetic material of the cell
1976	Blumberg and Gajdusek	New mechanisms for the origin and dissemination of infectious diseases
1978	Smith, Nathans, and Arber	Restriction enzymes for cutting DNA
1980	Benacerraf, Snell, and Dausset	Immunological factors in organ transplants
1980	Berg	Recombinant DNA
1984	Milstein, Köhler, and Jerne	Immunology
1987	Tonegawa	Genetics of antibody diversity
1988	Black, Elion, and Hitchings	Principles of drug therapy
1989	Bishop and Varmus	Genetic basis of cancer
1990	Murray, Thomas, and Corey	Transplant techniques and drugs
1993	Mullis	Polymerase chain reaction method to amplify (copy) DNA
1993	Smith	Method to splice foreign components into DNA
1993	Sharp and Roberts	Genes can be discontinuous
1996	Doherty and Zinkernagel	Recognition of virus-infected cells by immune defenses
1997	Prusiner	Prions
2005	Marshall and Warren	*Helicobacter pylori* causes stomach ulcers
2006	Fire and Mello	Interference RNA
2008	Zur Hausen	HPV causes cervical cancer
2008	Barré-Sinoussi and Montagnier	HIV
2011	Beutler and Huoffman	Activation of innate immunity
2011	Steinman	Discovery of dendritic cell and its role in adaptive immunity
2015	Campbell and Omura	Therapy against roundworm parasites (avermectin)
2015	Tu	Therapy against malaria (artemisinin)
2015	Lindahl, Modrich, and Sancor	Repair of DNA mutations
2016	Ohsumi	Autophagy in baker's yeast

prevented. Schoolchildren were also given such packets. Today, in the West, we are struggling against bacteria that have developed resistance to antibiotics—some against every known antibiotic—leading scientists to reexamine the usefulness of phages. In 2010, phage-based vaccines were patented for use in Japan.

Agricultural and food problems have recently been shown to have phage-mediated solutions. *Listeria monocytogenes*, a foodborne pathogen that can live and grow at refrigerator temperatures, causes bacterial diarrhea that is fatal in 20% of its cases. A phage has been shown to control growth of *Listeria* on cut apples and melons better

than chemical sanitizers, or washing. Herds of animals can also be protected with phages. The U.S. Department of Agriculture, in January 2007, approved use of a spray or wash containing phages targeted against *E. coli* O157:H7, to be applied to live animals prior to slaughter. This bacterium causes an often-fatal hemorrhagic dysentery. Removing it from hides before slaughter will help prevent its getting into products such as ground beef and will help to keep our food supply safe. Tests of effectiveness of phages on human diseases are underway now. Hopefully we will soon have replacements for antibiotics.

Unfortunately another thing the future holds for us is the threat of bioterrorism. Perhaps phages will be able to help us there, too, as we have already isolated phages that can destroy many strains of anthrax, for example. Other terrorist diseases and methods of their use and control will be discussed in ▶ Chapter 16, as well as in relevant parts of the chapters on disease. The words of Dr. Ken Alibek, former head of the Soviet secret germ warfare program, in his book, *Biohazard* (1999), should send shivers through us, "Our factory could turn out two tons of anthrax a day in a process as reliable and efficient as producing tanks, trucks, cars, or Coca-Cola." He also explains, "It would take only five kilograms of the Anthrax 836 developed at the Kazakhstan base to infect half the people living in a square kilometer of territory."

Farmers fighting weeds in their fields may soon have help from the U.S. Department of Agriculture. Scientists there (**Figure 1.17**) are searching for specialist microbes that will selectively attack weed seeds in soil, causing them to rot and die, without the use of chemical sprays.

Genomics

Microbial genetic techniques have made possible a colossal scientific undertaking: the Human Genome Project. Its purpose is to identify the location and chemical sequence of all the genes in the human genome—that is, all the genetic material in the human species. Begun in 1990, it was to be completed by 2005, at a cost of approximately $3 billion. Amazingly, it was finished in May 2000, ahead of schedule and under budget! Another surprise was the finding that humans have just over 25,000 genes, instead of estimates that ranged up to 142,000 genes. In February 2001 reports were published in separate scientific journals by the two rival groups that had completed the project: Dr. J. Craig Venter, then president of Celera Genomics (Rockville, Maryland) in *Science*, and in *Nature* by Dr. Eric Lander, representing the International Human Genome Sequencing Consortium, a group of academic centers funded mainly by the NIH and the Wellcome Trust of London. The 3 billion base pairs in the human genome do not all code for useful genes. An estimated 75% of them code for "junk DNA." However, scientists believe that we will eventually discover uses for what we now consider "junk."

Work on the Human Genome Project was based on techniques that were first developed for sequencing microbial genomes, which are smaller and easier to work with. Over 80,000 microbial genomes have been sequenced so far. A great surprise was finding that some bacteria have two or three chromosomes instead of the single one that was thought to be all any bacterium could have. It is interesting that 113 genes, and possibly scores more, have come to the human genome directly from bacteria. Venter has sequenced the mouse genome and reports that humans have only 300 genes not found in the mouse. The functions of 41.7% of human genes are still unknown. Says Venter, "The secrets of life are all spelled out for us in the genome, we just have to learn how to read it." Help reading the human genome is coming in great part from experiments using microbes.

Venter has synthesized the first totally artificial virus (a bacteriophage) and in 2010 created a synthetic bacteria. These feats of genetic engineering are discussed in ▶ Chapters 8 and 9. Venter also hopes to create new bacteria that will produce oil and natural gas, thereby relieving our dependence on fossil fuels, replacing them with clean energy.

In the next chapter (Chapter 2), we will investigate the powerful effects of the communities of microbes that live in and on you, and how a disturbance in their population can cause serious health problems. Did your mother have you by Caesarean section delivery? Does that make you more prone to develop diabetes? See what Chapter 2 has to say.

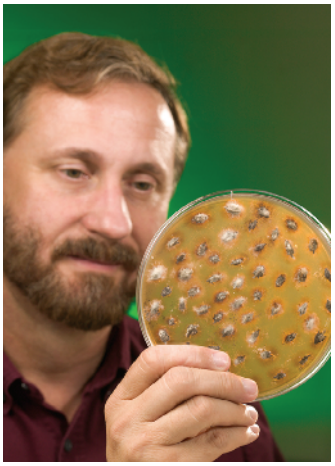


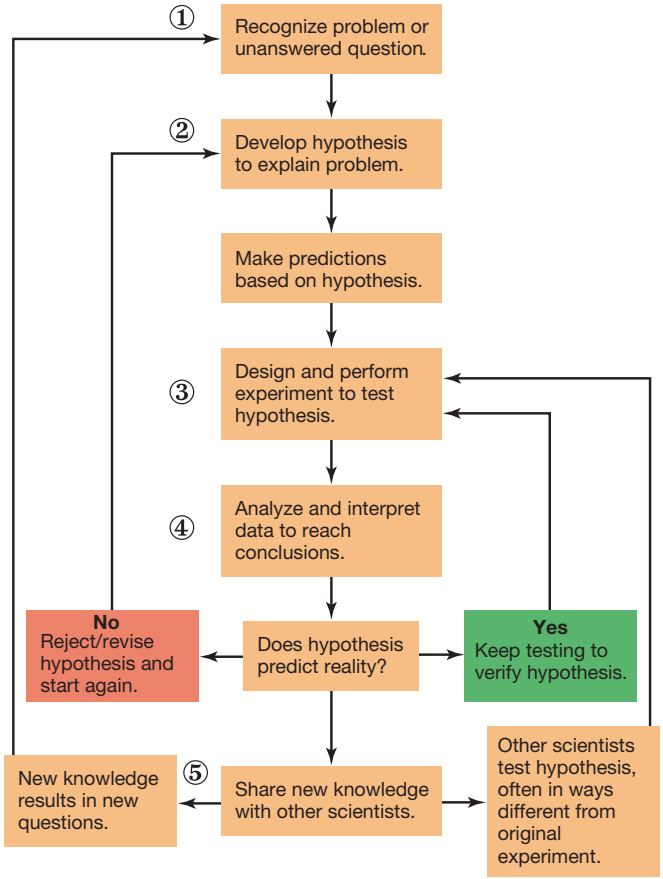
FIGURE 1.17 Let microbes kill weed seeds. U.S. Department of Agriculture scientist holds a culture of giant ragweed *(Ambrosia trifida)* seeds embedded in agar, some overgrown with soil microorganisms. He is investigating how and why some weed seeds escape decay by these organisms. *(Peggy Greb/Courtesy USDA)*

STOP and CHECK

1. What were the scientific contributions of Jenner, Metchnikoff, Ehrlich, Fleming, McClintock, and Venter?
2. When was the Golden Age of Microbiology? What types of discoveries were mostly made during this period?
3. What is the Human Genome Project? How has microbiology been associated with it?

CLOSE UP

The Scientific Method



© John Wiley and Sons, Inc.



poba/Getty Images

The main steps of The Scientific Method are outlined here.

RETRACING OUR JOURNEY

WHY STUDY MICROBIOLOGY?

- Microorganisms are part of the human environment and are therefore important to human health and activities.
- The study of microorganisms provides insight into life processes in all forms of life.

SCOPE OF MICROBIOLOGY

The Microbes

- **Microbiology** is the study of all **microorganisms (microbes)** in the microscopic range. These include **bacteria, algae, fungi, viruses, viroids, prions,** and **protozoa**.

The Microbiologists

- Immunology, virology, chemotherapy, and genetics are especially active research fields of microbiology.
- Microbiologists work as researchers or teachers in university, clinical, and industrial settings. They do basic research in the biological sciences; help to perform or devise diagnostic tests; develop and test antibiotics and vaccines; work to control infection, protect public health, and safeguard the environment; and play important roles in the food and beverage industries.

HISTORICAL ROOTS

- The ancient Greeks, Romans, and Jews all contributed to early understandings of the spread of disease.

- Diseases such as bubonic plague and syphilis caused millions of deaths because of the lack of understanding of how to control or treat the infections.
- The development of high-quality lenses by Leeuwenhoek made it possible to observe microorganisms and later to formulate the **cell theory**.

THE GERM THEORY OF DISEASE

- The **germ theory of disease** states that microorganisms (germs) can invade other organisms and cause disease.

Early Studies

- Progress in microbiology and acceptance of the germ theory of disease required that the idea of **spontaneous generation** be refuted. Redi and Spallanzani demonstrated that organisms did not arise from nonliving material. Pasteur, with his swan-necked flasks, and Tyndall, with his dust-free air, finally dispelled the idea of spontaneous generation.

Pasteur's Further Contributions

- Pasteur also studied wine making and disease in silkworms and developed the first rabies vaccine. His association of particular microbes with certain diseases furthered the establishment of the germ theory.

Koch's Contributions

- Koch developed four postulates that aided in the definitive establishment of the germ theory of disease. **Koch's postulates** are as follows:
- The specific causative agent must be found in every case of the disease.
- The disease organism must be isolated in pure culture.
- Inoculation of a sample of the culture into a healthy, susceptible animal must produce the same disease.
- The disease organism must be recovered from the inoculated animal.
- Koch also developed techniques for isolating organisms, identified the bacillus that causes tuberculosis, developed tuberculin, and studied various diseases in Africa and Asia.

Work Toward Controlling Infections

- Lister and Semmelweis contributed to improved sanitation in medicine by applying the germ theory and using aseptic technique.

EMERGENCE OF SPECIAL FIELDS OF MICROBIOLOGY

Immunology

- Immunization was first used against smallpox; Jenner used fluid from cowpox blisters to immunize against it.
- Pasteur developed techniques to weaken organisms so they would produce immunity without producing disease.

Virology

- Beijerinck characterized viruses as pathogenic molecules that could take over a host cell's mechanisms for their own use.
- Reed demonstrated that mosquitoes carry the yellow fever agent, and several other investigators identified viruses in the early twentieth century. The structure of DNA—the genetic material in many viruses and in all cellular organisms—was discovered by Watson and Crick.
- Techniques for isolating, propagating, and analyzing viruses were developed. Viruses could then be observed and in many cases crystallized, and their nucleic acids could be studied.

Chemotherapy

- Substances derived from medicinal plants were virtually the only source of chemotherapeutic agents until Ehrlich began a systematic search for chemically defined substances that would kill bacteria.
- Fleming and his colleagues developed penicillin, and Domagk and others developed sulfa drugs.
- Waksman and others developed streptomycin and other antibiotics derived from soil organisms.

Genetics and Molecular Biology

- Griffith discovered that previously harmless bacteria could change their nature and become capable of causing disease. This genetic change was shown by Avery, McCarty, and MacLeod to be due to DNA. Tatum and Beadle studied biochemical mutants of *Neurospora* to show how genetic information controls metabolism.

TOMORROW'S HISTORY

- Microbiology has been at the forefront of research in medicine and biology, and microorganisms continue to play a critical role in genetic engineering and gene therapy.
- Bacteriophage viruses may be able to cure diseases, and help ensure food safety.

Genomics

- The Human Genome Project has identified the location and sequence of all bases in the human genome. Microbes and microbiological techniques have contributed to this work.
- Over 80,000 bacterial genomes have been sequenced completely. A few have two or three instead of one chromosome.

TERMINOLOGY CHECK

algae *(p. 5)*
antibiotics *(p. 17)*
bacteria *(p. 4)*
bacteriophage *(p. 19)*
cell theory *(p. 11)*
deep hot biosphere *(p. 4)*
Domain *(p. 4)*
fungi *(p. 5)*
germ theory of disease *(p. 11)*
Koch's Postulates *(p. 13)*
microbe *(p. 4)*
microbiology *(p. 4)*
microorganism *(p. 2)*
prion *(p. 6)*
protozoa *(p. 6)*
spontaneous generation *(p. 11)*
viroid *(p. 6)*
viruses *(p. 5)*