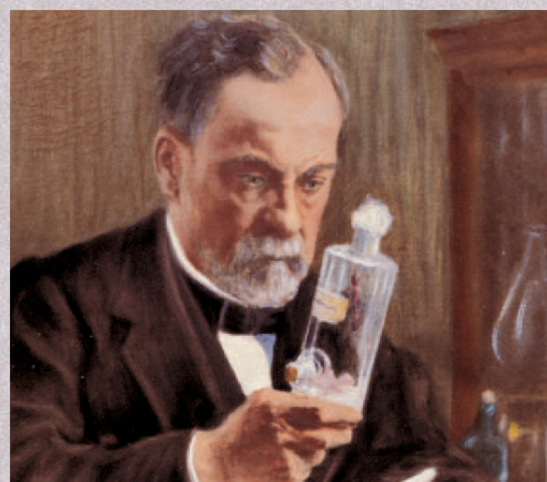
PART I

Introduction to Microbiology

CHAPTER 1

The History and Scope of Microbiology



Louis Pasteur, one of the greatest scientists of the nineteenth century, maintained that "Science knows no country, because knowledge belongs to humanity, and is a torch which illuminates the world."

Outline

Concepts

1. Microbiology is the study of organisms that are usually too small to be seen by the unaided eye; it employs techniques—such as sterilization and the use of culture media—that are required to isolate and grow these microorganisms.
2. Microorganisms are not spontaneously generated from inanimate matter but arise from other microorganisms.
3. Many diseases result from viral, bacterial, fungal, or protozoan infections. Koch's postulates may be used to establish a causal link between the suspected microorganism and a disease.
4. The development of microbiology as a scientific discipline has depended on the availability of the microscope and the ability to isolate and grow pure cultures of microorganisms.
5. Microorganisms are responsible for many of the changes observed in organic and inorganic matter (e.g., fermentation and the carbon, nitrogen, and sulfur cycles that occur in nature).
6. Microorganisms have two fundamentally different types of cells—procaryotic and eucaryotic—and are distributed among several kingdoms or domains.
7. Microbiology is a large discipline, which has a great impact on other areas of biology and general human welfare.

Dans les champs de l'observation, le hasard ne favorise que les esprits préparés.
(In the field of observation, chance favors only prepared minds.)
—Louis Pasteur

One can't overemphasize the importance of microbiology. Society benefits from microorganisms in many ways. They are necessary for the production of bread, cheese, beer, antibiotics, vaccines, vitamins, enzymes, and many other important products. Indeed, modern biotechnology rests upon a microbiological foundation. Microorganisms are indispensable components of our ecosystem. They make possible the cycles of carbon, oxygen, nitrogen, and sulfur that take place in terrestrial and aquatic systems. They also are a source of nutrients at the base of all ecological food chains and webs.

Of course microorganisms also have harmed humans and disrupted society over the millennia. Microbial diseases undoubtedly played a major role in historical events such as the decline of the Roman Empire and the conquest of the New World. In 1347 plague or black death (*see chapter 39*) struck Europe with brutal force. By 1351, only four years later, the plague had killed 1/3 of the population (about 25 million people). Over the next 80 years, the disease struck again and again, eventually wiping out 75% of the European population. Some historians believe that this disaster changed European culture and prepared the way for the Renaissance. Today the struggle by microbiologists and others against killers like AIDS and malaria continues. The biology of AIDS and its impact (pp. 878–84)

In this introductory chapter the historical development of the science of microbiology is described, and its relationship to medicine and other areas of biology is considered. The nature of the microbial world is then surveyed to provide a general idea of the organisms and agents that microbiologists study. Finally, the scope, relevance, and future of modern microbiology are discussed.

Microbiology often has been defined as the study of organisms and agents too small to be seen clearly by the unaided eye—that is, the study of **microorganisms.** Because objects less than about one millimeter in diameter cannot be seen clearly and must be examined with a microscope, microbiology is concerned primarily with organisms and agents this small and smaller. Its subjects are viruses, bacteria, many algae and fungi, and protozoa (*see table 34.1*). Yet other members of these groups, particularly some of the algae and fungi, are larger and quite visible. For example, bread molds and filamentous algae are studied by microbiologists, yet are visible to the naked eye. Two bacteria that are visible without a microscope, *Thiomargarita* and *Epulopiscium,* also have been discovered (*see p. 45*). The difficulty in setting the boundaries of microbiology led Roger Stanier to suggest that the field be defined not only in terms of the size of its subjects but also in terms of its techniques. A microbiologist usually first isolates a specific microorganism from a population and then cultures it. Thus microbiology employs techniques—such as sterilization and the use of culture media—that are necessary for successful isolation and growth of microorganisms.

The development of microbiology as a science is described in the following sections. **Table 1.1** presents a summary of some of the major events in this process and their relationship to other historical landmarks.

1.1 The Discovery of Microorganisms

Even before microorganisms were seen, some investigators suspected their existence and responsibility for disease. Among others, the Roman philosopher Lucretius (about 98–55 B.C.) and the physician Girolamo Fracastoro (1478–1553) suggested that disease was caused by invisible living creatures. The earliest microscopic observations appear to have been made between 1625 and 1630 on bees and weevils by the Italian Francesco Stelluti, using a microscope probably supplied by Galileo. However, the first person to observe and describe microorganisms accurately was the amateur microscopist Antony van Leeuwenhoek (1632–1723) of Delft, Holland (**figure 1.1*a***). Leeuwenhoek earned his living as a draper and haberdasher (a dealer in men's clothing and accessories), but spent much of his spare time constructing simple microscopes composed of double convex glass lenses held between two silver plates (figure 1.1*b*). His microscopes could magnify around 50 to 300 times, and he may have illuminated his liquid specimens by placing them between two pieces of glass and shining light on them at a 45° angle to the specimen plane. This would have provided a form of dark-field illumination (*see chapter 2*) and made bacteria clearly visible (figure 1.1*c*). Beginning in 1673 Leeuwenhoek sent detailed letters describing his discoveries to the Royal Society of London. It is clear from his descriptions that he saw both bacteria and protozoa.

1.2 The Conflict over Spontaneous Generation

From earliest times, people had believed in **spontaneous generation**—that living organisms could develop from nonliving matter. Even the great Aristotle (384–322 B.C.) thought some of the simpler invertebrates could arise by spontaneous generation. This view finally was challenged by the Italian physician Francesco Redi (1626–1697), who carried out a series of experiments on decaying meat and its ability to produce maggots spontaneously. Redi placed meat in three containers. One was uncovered, a second was covered with paper, and the third was covered with a fine gauze that would exclude flies. Flies laid their eggs on the uncovered meat and maggots developed. The other two pieces of meat did not produce maggots spontaneously. However, flies were attracted to the gauze-covered container and laid their eggs on the gauze; these eggs produced maggots.

Table 1.1 Some Important Events in the Development of Microbiology

Date	Microbiological History	Other Historical Events
1546	Fracastoro suggests that invisible organisms cause disease	Publication of Copernicus's work on the heliocentric solar system (1543)
1590–1608	Jansen develops first useful compound microscope	Shakespeare's *Hamlet* (1600–1601)
1676	Leeuwenhoek discovers "animalcules"	J. S. Bach and Handel born (1685)
1688	Redi publishes work on spontaneous generation of maggots	Isaac Newton publishes the *Principia* (1687)
		Linnaeus's *Systema Naturae* (1735)
		Mozart born (1756)
1765–1776	Spallanzani attacks spontaneous generation	
1786	Müller produces first classification of bacteria	French Revolution (1789)
1798	Jenner introduces cowpox vaccination for smallpox	Beethoven's first symphony (1800)
		The battle of Waterloo and the defeat of Napoleon (1815)
		Faraday demonstrates the principle of an electric motor (1821)
1838–1839	Schwann and Schleiden, the Cell Theory	England issues first postage stamp (1840)
1835–1844	Bassi discovers that silkworm disease is caused by a fungus and proposes that many diseases are microbial in origin	Marx's *Communist Manifesto* (1848)
1847–1850	Semmelweis shows that childbed fever is transmitted by physicians and introduces the use of antiseptics to prevent the disease	Velocity of light first measured by Fizeau (1849)
		Clausius states the first and second laws of thermodynamics (1850)
1849	Snow studies the epidemiology of a cholera epidemic in London	Graham distinguishes between colloids and crystalloids
		Melville's *Moby Dick* (1851)
		Otis installs first safe elevator (1854)
		Bunsen introduces the use of the gas burner (1855)
1857	Pasteur shows that lactic acid fermentation is due to a microorganism	
1858	Virchow states that all cells come from cells	Darwin's *On the Origin of Species* (1859)
1861	Pasteur shows that microorganisms do not arise by spontaneous generation	American Civil War (1861–1865)
		Mendel publishes his genetics experiments (1865)
		Cross-Atlantic cable laid (1865)
1867	Lister publishes his work on antiseptic surgery	Dostoevski's *Crime and Punishment* (1866)
1869	Miescher discovers nucleic acids	Franco-German War (1870–1871)
1876–1877	Koch demonstrates that anthrax is caused by *Bacillus anthracis*	Bell invents telephone (1876)
		Edison's first light bulb (1879)
1880	Laveran discovers *Plasmodium,* the cause of malaria	
1881	Koch cultures bacteria on gelatin	Ives produces first color photograph (1881)
	Pasteur develops anthrax vaccine	
1882	Koch discovers tubercle bacillus, *Mycobacterium tuberculosis*	First central electric power station constructed by Edison (1882)
1884	Koch's postulates first published	Mark Twain's *The Adventures of Huckleberry Finn* (1884)
	Metchnikoff describes phagocytosis	
	Autoclave developed	
	Gram stain developed	
1885	Pasteur develops rabies vaccine	First motor vehicles developed by Daimler (1885–1886)
	Escherich discovers *Escherichia coli,* a cause of diarrhea	
1886	Fraenkel discovers *Streptococcus pneumoniae,* a cause of pneumonia	
1887	Petri dish (plate) developed by Richard Petri	
1887–1890	Winogradsky studies sulfur and nitrifying bacteria	Hertz discovers radio waves (1888)
1889	Beijerinck isolates root nodule bacteria	Eastman makes box camera (1888)
1890	Von Behring prepares antitoxins for diphtheria and tetanus	
1892	Ivanowsky provides evidence for virus causation of tobacco mosaic disease	First zipper patented (1895)
1894	Kitasato and Yersin discover *Yersinia pestis,* the cause of plague	
1895	Bordet discovers complement	Röntgen discovers X rays (1895)
1896	Van Ermengem discovers *Clostridium botulinum,* the cause of botulism	
1897	Buchner prepares extract of yeast that ferments	Thomson discovers the electron (1897)
	Ross shows that malaria parasite is carried by the mosquito	Spanish-American War (1898)
1899	Beijerinck proves that a virus particle causes the tobacco mosaic disease	
1900	Reed proves that yellow fever is transmitted by the mosquito	Planck develops the quantum theory (1900)
1902	Landsteiner discovers blood groups	First electric typewriter (1901)

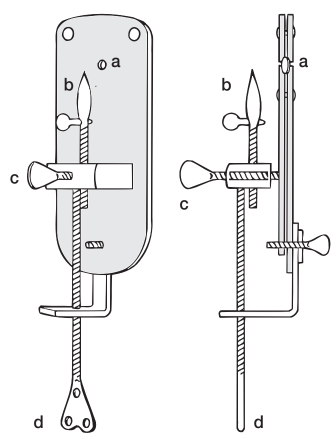
Table 1.1 Continued

Date	Microbiological History	Other Historical Events
1903	Wright and others discover antibodies in the blood of immunized animals	First powered aircraft (1903)
1905	Schaudinn and Hoffmann show *Treponema pallidum* causes syphilis	Einstein's special theory of relativity (1905)
1906	Wassermann develops complement fixation test for syphilis	
1909	Ricketts shows that Rocky Mountain spotted fever is transmitted by ticks and caused by a microbe (*Rickettsia rickettsii*)	First model T Ford (1908) Peary and Hensen reach North Pole (1909)
1910	Ehrlich develops chemotherapeutic agent for syphilis	Rutherford presents his theory of the atom (1911)
1911	Rous discovers a virus that causes cancer in chickens	Picasso and cubism (1912) World War I begins (1914)
1915–1917	D'Herelle and Twort discover bacterial viruses	Einstein's general theory of relativity (1916) Russian Revolution (1917)
1921	Fleming discovers lysozyme	
1923	First edition of *Bergey's Manual*	Lindberg's transatlantic flight (1927)
1928	Griffith discovers bacterial transformation	
1929	Fleming discovers penicillin	Stock market crash (1929)
1931	Van Niel shows that photosynthetic bacteria use reduced compounds as electron donors without producing oxygen	
1933	Ruska develops first transmission electron microscope	Hitler becomes chancellor of Germany (1933)
1935	Stanley crystallizes the tobacco mosaic virus Domagk discovers sulfa drugs	
1937	Chatton divides living organisms into procaryotes and eucaryotes	Krebs discovers the citric acid cycle (1937) World War II begins (1939)
1941	Beadle and Tatum, one-gene-one-enzyme hypothesis	
1944	Avery shows that DNA carries information during transformation Waksman discovers streptomycin	The insecticide DDT introduced (1944)
		Atomic bombs dropped on Hiroshima and Nagasaki (1945)
1946	Lederberg and Tatum describe bacterial conjugation	United Nations formed (1945) First electronic computer (1946)
1949	Enders, Weller, and Robbins grow poliovirus in human tissue cultures	
1950	Lwoff induces lysogenic bacteriophages	Korean War begins (1950)
1952	Hershey and Chase show that bacteriophages inject DNA into host cells Zinder and Lederberg discover generalized transduction	First hydrogen bomb exploded (1952) Stalin dies (1952) First commercial transistorized product (1952)
1953	Phase-contrast microscope developed Medawar discovers immune tolerance Watson and Crick propose the double helix structure for DNA	U.S. Supreme Court rules against segregated schools (1954)
1955	Jacob and Wollman discover the F factor is a plasmid Jerne and Burnet propose the clonal selection theory	Montgomery bus boycott (1955) Sputnik launched by Soviet Union (1957)
1959	Yalow develops the radioimmunoassay technique	Birth control pill (1960)
1961	Jacob and Monod propose the operon model of gene regulation	First humans in space (1961)
1961–1966	Nirenberg, Khorana, and others elucidate the genetic code	Cuban missile crisis (1962) Nuclear test ban treaty (1963)
1962	Porter proposes the basic structure for immunoglobulin G First quinolone antimicrobial (nalidixic acid) synthesized	Civil Rights March on Washington (1963) President Kennedy assassinated (1963) Arab-Israeli War (1967) Martin Luther King assassination (1968) Neil Armstrong walks on the moon (1969)
1970	Discovery of restriction endonucleases by Arber and Smith Discovery of reverse transcriptase in retroviruses by Temin and Baltimore	
1973	Ames develops a bacterial assay for the detection of mutagens Cohen, Boyer, Chang, and Helling use plasmid vectors to clone genes in bacteria	Salt I Treaty (1972) Vietnam War ends (1973)
1975	Kohler and Milstein develop technique for the production of monoclonal antibodies Lyme disease discovered	President Nixon resigns because of Watergate cover-up (1974)
1977	Recognition of archaea as a distinct microbial group by Woese and Fox	Panama Canal Treaty (1977)

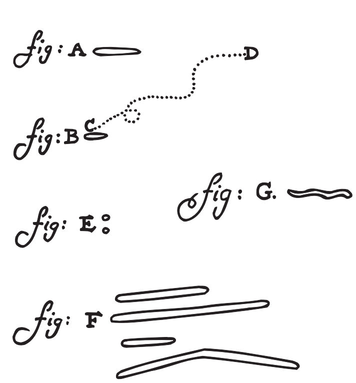
Table 1.1 Continued

Date	Microbiological History	Other Historical Events
	Gilbert and Sanger develop techniques for DNA sequencing	
1979	Insulin synthesized using recombinant DNA techniques	Hostages seized in Iran (1978)
	Smallpox declared officially eliminated	Three Mile Island disaster (1979)
1980	Development of the scanning tunneling microscope	Home computers marketed (1980)
1982	Recombinant hepatitis B vaccine developed	AIDS first recognized (1981)
1982–1983	Discovery of catalytic RNA by Cech and Altman	First artificial heart implanted (1982)
1983–1984	The human immunodeficiency virus isolated and identified by Gallo and Montagnier	Meter redefined in terms of distance light travels (1983)
	The polymerase chain reaction developed by Mullis	
1986	First vaccine (hepatitis B vaccine) produced by genetic engineering approved for human use	Gorbachev becomes Communist party general secretary (1985) Berlin Wall falls (1989)
1990	First human gene-therapy testing begun	Persian Gulf War with Iraq begins (1990)
		Soviet Union collapse; Boris Yeltsin comes to power (1991)
1992	First human trials of antisense therapy	
1995	Chickenpox vaccine approved for U.S. use	
	Haemophilus influenzae genome sequenced	
1996	*Methanococcus jannaschii* genome sequenced	Water found on the moon (1998)
	Yeast genome sequenced	
1997	Discovery of *Thiomargarita namibiensis,* the largest known bacterium	
	Escherichia coli genome sequenced	
2000	Discovery that *Vibrio cholerae* has two separate chromosomes	

(a)



(b)



(c)

Figure 1.1 Antony van Leeuwenhoek. Leeuwenhoek (1632–1723) and his microscopes. (**a**) Leeuwenhoek holding a microscope. (**b**) A drawing of one of the microscopes showing the lens, *a;* mounting pin, *b;* and focusing screws, *c* and *d.* (**c**) Leeuwenhoek's drawings of bacteria from the human mouth. (*b*) *Source: C. E. Dobell,* Antony van Leeuwenhoek and His Little Animals *(1932), Russell and Russell, 1958.*

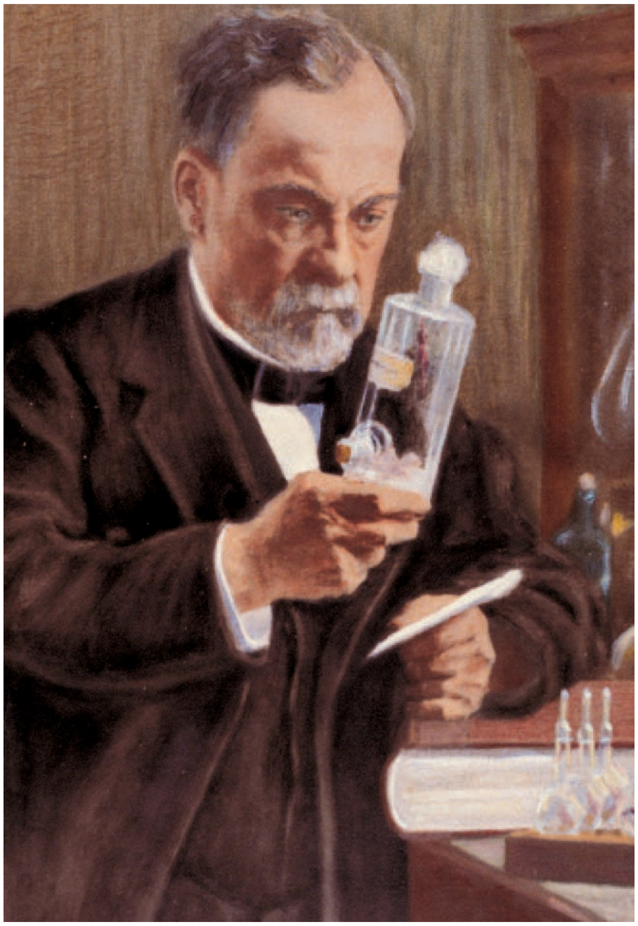


Figure 1.2 Louis Pasteur. Pasteur (1822–1895) working in his laboratory.

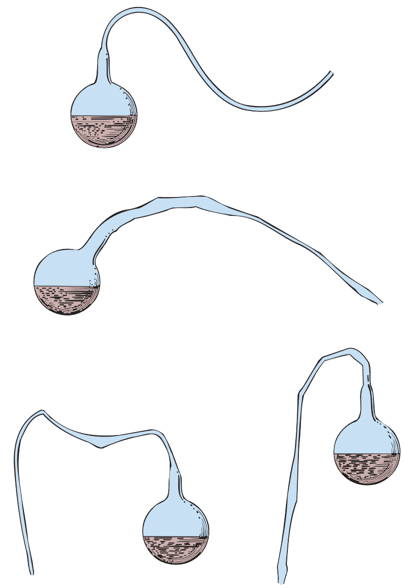


Figure 1.3 The Spontaneous Generation Experiment. Pasteur's swan neck flasks used in his experiments on the spontaneous generation of microorganisms. *Source:* Annales Sciences Naturelle, *4th Series, Vol. 16, pp.1–98, Pasteur, L., 1861, "Mémoire sur les Corpuscules Organisés Qui Existent Dans L'Atmosphère: Examen de la Doctrine des Générations Spontanées."*

Thus the generation of maggots by decaying meat resulted from the presence of fly eggs, and meat did not spontaneously generate maggots as previously believed. Similar experiments by others helped discredit the theory for larger organisms.

Leeuwenhoek's discovery of microorganisms renewed the controversy. Some proposed that microorganisms arose by spontaneous generation even though larger organisms did not. They pointed out that boiled extracts of hay or meat would give rise to microorganisms after sitting for a while. In 1748 the English priest John Needham (1713–1781) reported the results of his experiments on spontaneous generation. Needham boiled mutton broth and then tightly stoppered the flasks. Eventually many of the flasks became cloudy and contained microorganisms. He thought organic matter contained a vital force that could confer the properties of life on nonliving matter. A few years later the Italian priest and naturalist Lazzaro Spallanzani (1729–1799) improved on Needham's experimental design by first sealing glass flasks that contained water and seeds. If the sealed flasks were placed in boiling water for 3/4 of an hour, no growth took place as long as the flasks remained sealed. He proposed that air carried germs to the culture medium, but also commented that the external air might be required for growth of animals already in the medium. The supporters of spontaneous generation maintained that heating the air in sealed flasks destroyed its ability to support life.

Several investigators attempted to counter such arguments. Theodore Schwann (1810–1882) allowed air to enter a flask containing a sterile nutrient solution after the air had passed through a red-hot tube. The flask remained sterile. Subsequently Georg Friedrich Schroder and Theodor von Dusch allowed air to enter a flask of heat-sterilized medium after it had passed through sterile cotton wool. No growth occurred in the medium even though the air had not been heated. Despite these experiments the French naturalist Felix Pouchet claimed in 1859 to have carried out experiments conclusively proving that microbial growth could occur without air contamination. This claim provoked Louis Pasteur (1822–1895) to settle the matter once and for all. Pasteur (**figure 1.2**) first filtered air through cotton and found that objects resembling plant spores had been trapped. If a piece of the cotton was placed in sterile medium after air had been filtered through it, microbial growth appeared. Next he placed nutrient solutions in flasks, heated their necks in a flame, and drew them out into a variety of curves, while keeping the ends of the necks open to the atmosphere (**figure 1.3**). Pasteur then boiled the solutions for a few minutes and allowed them to cool. No growth took place even though the contents of the flasks were exposed to the air. Pasteur pointed out that no growth occurred because dust and germs had been trapped on the walls of the curved necks. If the necks were broken, growth commenced immediately. Pasteur had not only resolved the controversy by 1861 but also had shown how to keep solutions sterile.

The English physicist John Tyndall (1820–1893) dealt a final blow to spontaneous generation in 1877 by demonstrating that dust did indeed carry germs and that if dust was absent, broth remained sterile even if directly exposed to air. During the course of his studies, Tyndall provided evidence for the existence of exceptionally heat-resistant forms of bacteria. Working independently, the German botanist Ferdinand Cohn (1828–1898) discovered the existence of heat-resistant bacterial endospores (*see chapter 3*).

1. Describe the field of microbiology in terms of the size of its subject material and the nature of its techniques.
2. How did Pasteur and Tyndall finally settle the spontaneous generation controversy?

1.3 The Role of Microorganisms in Disease

The importance of microorganisms in disease was not immediately obvious to people, and it took many years for scientists to establish the connection between microorganisms and illness. Recognition of the role of microorganisms depended greatly upon the development of new techniques for their study. Once it became clear that disease could be caused by microbial infections, microbiologists began to examine the way in which hosts defended themselves against microorganisms and to ask how disease might be prevented. The field of immunology was born.

Recognition of the Relationship between Microorganisms and Disease

Although Fracastoro and a few others had suggested that invisible organisms produced disease, most believed that disease was due to causes such as supernatural forces, poisonous vapors called miasmas, and imbalances between the four humors thought to be present in the body. The idea that an imbalance between the four humors (blood, phlegm, yellow bile [choler], and black bile [melancholy]) led to disease had been widely accepted since the time of the Greek physician Galen (129–199). Support for the germ theory of disease began to accumulate in the early nineteenth century. Agostino Bassi (1773–1856) first showed a microorganism could cause disease when he demonstrated in 1835 that a silkworm disease was due to a fungal infection. He also suggested that many diseases were due to microbial infections. In 1845 M. J. Berkeley proved that the great Potato Blight of Ireland was caused by a fungus. Following his successes with the study of fermentation, Pasteur was asked by the French government to investigate the pébrine disease of silkworms that was disrupting the silk industry. After several years of work, he showed that the disease was due to a protozoan parasite. The disease was controlled by raising caterpillars from eggs produced by healthy moths.

Figure 1.4 Robert Koch. Koch (1843–1910) examining a specimen in his laboratory.

Indirect evidence that microorganisms were agents of human disease came from the work of the English surgeon Joseph Lister (1827–1912) on the prevention of wound infections. Lister impressed with Pasteur's studies on the involvement of microorganisms in fermentation and putrefaction, developed a system of antiseptic surgery designed to prevent microorganisms from entering wounds. Instruments were heat sterilized, and phenol was used on surgical dressings and at times sprayed over the surgical area. The approach was remarkably successful and transformed surgery after Lister published his findings in 1867. It also provided strong indirect evidence for the role of microorganisms in disease because phenol, which killed bacteria, also prevented wound infections.

The first direct demonstration of the role of bacteria in causing disease came from the study of anthrax (*see chapter 39*) by the German physician Robert Koch (1843–1910). Koch (**figure 1.4**) used the criteria proposed by his former teacher, Jacob Henle (1809–1885), to establish the relationship between *Bacillus anthracis* and anthrax, and published his findings in 1876 (**Box 1.1** briefly discusses the scientific method). Koch injected healthy mice with material from diseased animals, and the mice became ill. After transferring anthrax by inoculation through a series of 20 mice, he incubated a piece of spleen containing the anthrax bacillus in beef serum. The bacilli grew, reproduced, and produced spores. When the isolated bacilli or spores were injected into mice, anthrax developed. His criteria for proving the causal relationship between a microorganism and a specific disease are known as **Koch's postulates** and can be summarized as follows:

1. The microorganism must be present in every case of the disease but absent from healthy organisms.

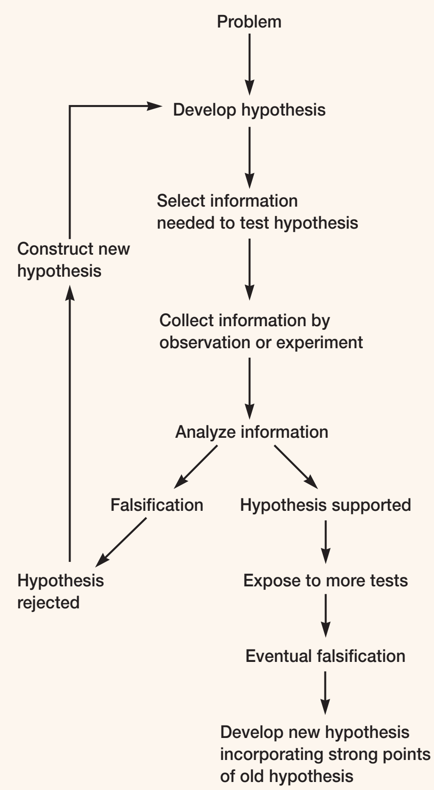
Box 1.1

The Scientific Method

Although biologists employ a variety of approaches in conducting research, microbiologists and other experimentally oriented biologists often use the general approach known as the scientific method. They first gather observations of the process to be studied and then develop a tentative **hypothesis**—an educated guess—to explain the observations (see **Box figure**). This step often is inductive and creative because there is no detailed, automatic technique for generating hypotheses. Next they decide what information is required to test the hypothesis and collect this information through observation or carefully designed experiments. After the information has been collected, they decide whether the hypothesis has been supported or falsified. If it has failed to pass the test, the hypothesis is rejected, and a new explanation or hypothesis is constructed. If the hypothesis passes the test, it is subjected to more severe testing. The procedure often is made more efficient by constructing and testing alternative hypotheses and then refining the hypothesis that survives testing. This general approach is often called the hypothetico-deductive method. One deduces predictions from the currently accepted hypothesis and tests them. In deduction the conclusion about specific cases follows logically from a general premise ("if . . . , then . . ." reasoning). Induction is the opposite. A general conclusion is reached after considering many specific examples. Both types of reasoning are used by scientists.

When carrying out an experiment, it is essential to use a control group as well as an experimental group. The control group is treated precisely the same as the experimental group except that the experimental manipulation is not performed on it. In this way one can be sure that any changes in the experimental group are due to the experimental manipulation rather than to some other factor not taken into account.

If a hypothesis continues to survive testing, it may be accepted as a valid theory. A **theory** is a set of propositions and concepts that provides a reliable, systematic, and rigorous account of an aspect of nature. It is important to note that hypotheses and theories are never absolutely proven. Scientists simply gain more and more confidence in their accuracy as they continue to survive testing, fit with new observations and experiments, and satisfactorily explain the observed phenomena.



The Hypothetico-Deductive Method. This approach is most often used in scientific research.

2. The suspected microorganism must be isolated and grown in a pure culture.
3. The same disease must result when the isolated microorganism is inoculated into a healthy host.
4. The same microorganism must be isolated again from the diseased host.

Although Koch used the general approach described in the postulates during his anthrax studies, he did not outline them fully until his 1884 publication on the cause of tuberculosis (**Box 1.2**).

Koch's proof that *Bacillus anthracis* caused anthrax was independently confirmed by Pasteur and his coworkers. They discovered that after burial of dead animals, anthrax spores survived and were brought to the surface by earthworms. Healthy animals then ingested the spores and became ill.

The Development of Techniques for Studying Microbial Pathogens

During Koch's studies on bacterial diseases, it became necessary to isolate suspected bacterial pathogens. At first he cultured bacteria on the sterile surfaces of cut, boiled potatoes. This was unsatisfactory because bacteria would not always grow well on potatoes. He then tried to solidify regular liquid media by adding gelatin. Separate bacterial colonies developed after the surface had been streaked with a bacterial sample. The sample could also be mixed with liquefied gelatin medium. When the gelatin medium hardened, individual bacteria produced separate colonies. Despite its advantages gelatin was not an ideal solidifying agent because it was digested by many bacteria and melted when the temperature rose above 28°C. A better alternative was provided by Fannie

Box 1.2

Molecular Koch's Postulates

Although the criteria that Koch developed for proving a causal relationship between and a microorganism and a specific disease have been of immense importance in medical microbiology, it is not always possible to apply them in studying human diseases. For example, some pathogens cannot be grown in pure culture outside the host; because other pathogens grow only in humans, their study would require experimentation on people. The identification, isolation, and cloning of genes responsible for pathogen virulence (*see p. 794*) have made possible a new molecular form of Koch's postulates that resolves some of these difficulties. The emphasis is on the virulence genes present in the infectious agent rather than on the agent itself. The molecular postulates can be briefly summarized as follows:

1. The virulence trait under study should be associated much more with pathogenic strains of the species than with nonpathogenic strains.
2. Inactivation of the gene or genes associated with the suspected virulence trait should substantially decrease pathogenicity.
3. Replacement of the mutated gene with the normal wild-type gene should fully restore pathogenicity.
4. The gene should be expressed at some point during the infection and disease process.
5. Antibodies or immune system cells directed against the gene products should protect the host.

The molecular approach cannot always be applied because of problems such as the lack of an appropriate animal system. It also is difficult to employ the molecular postulates when the pathogen is not well characterized genetically.

Eilshemius Hesse, the wife of Walther Hesse, one of Koch's assistants (**figure 1.5**). She suggested the use of agar as a solidifying agent—she had been using it successfully to make jellies for some time. Agar was not attacked by most bacteria and did not melt until reaching a temperature of 100°C. One of Koch's assistants, Richard Petri, developed the petri dish (plate), a container for solid culture media. These developments made possible the isolation of pure cultures that contained only one type of bacterium, and directly stimulated progress in all areas of bacteriology. Isolation of bacteria and pure culture techniques (pp. 106–10).

Figure 1.5 Fannie Eilshemius (1850–1934) and Walther Hesse (1846–1911). Fannie Hesse first proposed using agar in culture media.

Koch also developed media suitable for growing bacteria isolated from the body. Because of their similarity to body fluids, meat extracts and protein digests were used as nutrient sources. The result was the development of nutrient broth and nutrient agar, media that are still in wide use today.

By 1882 Koch had used these techniques to isolate the bacillus that caused tuberculosis. There followed a golden age of about 30 to 40 years in which most of the major bacterial pathogens were isolated (table 1.1).

The discovery of viruses and their role in disease was made possible when Charles Chamberland (1851–1908), one of Pasteur's associates, constructed a porcelain bacterial filter in 1884. The first viral pathogen to be studied was the tobacco mosaic disease virus (*see chapter 16*). The development of virology (pp. 362–63).

Immunological Studies

In this period progress also was made in determining how animals resisted disease and in developing techniques for protecting humans and livestock against pathogens. During studies on chicken cholera, Pasteur and Roux discovered that incubating their cultures for long intervals between transfers would attenuate the bacteria, which meant they had lost their ability to cause the disease. If the chickens were injected with these attenuated cultures, they remained healthy but developed the ability to resist the disease. He called the attenuated culture a vaccine [Latin *vacca,* cow] in honor of Edward Jenner because, many years earlier, Jenner had used vaccination with material from cowpox lesions to protect people against smallpox (*see section 16.1*). Shortly after this, Pasteur and Chamberland developed an attenuated anthrax vaccine in two ways: by treating cultures with potassium bichromate and by incubating the bacteria at 42 to 43°C. Vaccines and immunizations (pp. 764–68).

Pasteur next prepared rabies vaccine by a different approach. The pathogen was attenuated by growing it in an abnormal host, the rabbit. After infected rabbits had died, their brains and spinal cords were removed and dried. During the course of these studies, Joseph Meister, a nine-year-old boy who had been bitten by a rabid dog, was brought to Pasteur. Since the boy's death was certain in the absence of treatment, Pasteur agreed to try vaccination. Joseph was injected 13 times over the next 10 days with increasingly virulent preparations of the attenuated virus. He survived.

In gratitude for Pasteur's development of vaccines, people from around the world contributed to the construction of the Pasteur Institute in Paris, France. One of the initial tasks of the Institute was vaccine production.

After the discovery that the diphtheria bacillus produced a toxin, Emil von Behring (1854–1917) and Shibasaburo Kitasato (1852–1931) injected inactivated toxin into rabbits, inducing them to produce an antitoxin, a substance in the blood that would inactivate the toxin and protect against the disease. A tetanus antitoxin was then prepared and both antitoxins were used in the treatment of people.

The antitoxin work provided evidence that immunity could result from soluble substances in the blood, now known to be antibodies (humoral immunity). It became clear that blood cells were also important in immunity (cellular immunity) when Elie Metchnikoff (1845–1916) discovered that some blood leukocytes could engulf disease-causing bacteria (**figure 1.6**). He called these cells phagocytes and the process phagocytosis [Greek *phagein,* eating].

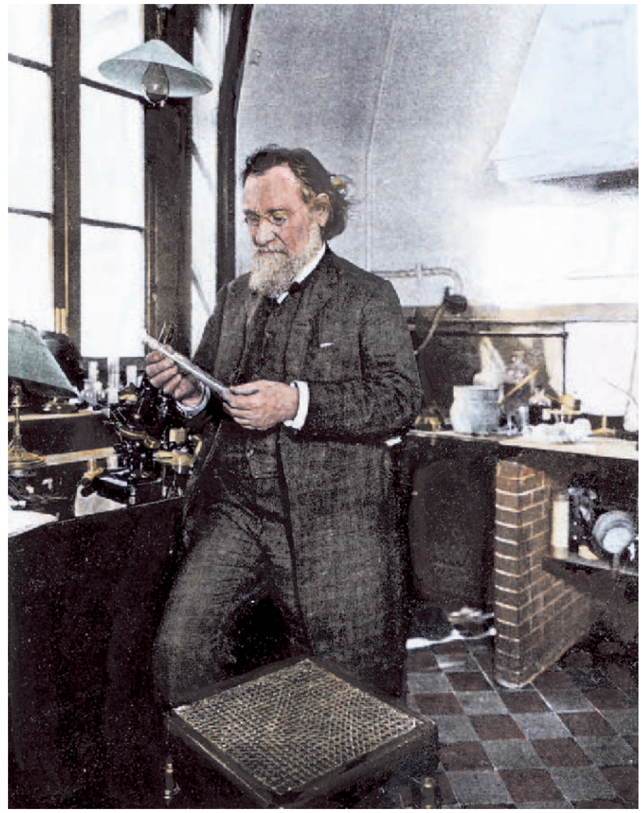


Figure 1.6 Elie Metchnikoff. Metchnikoff (1845–1916) shown here at work in his laboratory.

1. Discuss the contributions of Lister, Pasteur, and Koch to the germ theory of disease and to the treatment or prevention of diseases.
2. What other contributions did Koch make to microbiology?
3. Describe Koch's postulates. What are the molecular Koch's postulates and why are they important?
4. How did von Behring and Metchnikoff contribute to the development of immunology?

1.4 Industrial Microbiology and Microbial Ecology

Although Theodore Schwann and others had proposed in 1837 that yeast cells were responsible for the conversion of sugars to alcohol, a process they called alcoholic fermentation, the leading chemists of the time believed microorganisms were not involved. They were convinced that fermentation was due to a chemical instability that degraded the sugars to alcohol. Pasteur did not agree. It appears that early in his career Pasteur became interested in fermentation because of his research on the stereochemistry of molecules. He believed that fermentations were carried out by living organisms and produced asymmetric products such as amyl alcohol that had optical activity. There was an intimate connection between molecular asymmetry, optical activity, and life. Then in 1856 M. Bigo, an industrialist in Lille, France, where Pasteur worked, requested Pasteur's assistance. His business produced ethanol from the fermentation of beet sugars, and the alcohol yields had recently declined and the product had become sour. Pasteur discovered that the fermentation was failing because the yeast normally responsible for alcohol formation had been replaced by microorganisms producing lactic acid rather than ethanol. In solving this practical problem, Pasteur demonstrated that all fermentations were due to the activities of specific yeasts and bacteria, and he published several papers on fermentation between 1857 and 1860. His success led to a study of wine diseases and the development of pasteurization (*see chapter 7*) to preserve wine during storage. Pasteur's studies on fermentation continued for almost 20 years. One of his most important discoveries was that some fermentative microorganisms were anaerobic and could live only in the absence of oxygen, whereas others were able to live either aerobically or anaerobically. Fermentation (pp. 179–81); The effect of oxygen on microorganisms (pp. 127–29).

A few of the early microbiologists chose to investigate the ecological role of microorganisms. In particular they studied microbial involvement in the carbon, nitrogen, and sulfur cycles taking place in soil and aquatic habitats. Two of the pioneers in this endeavor were Sergei N. Winogradsky (1856–1953) and Martinus W. Beijerinck (1851–1931). Biogeochemical cycles (pp. 611–18).

The Russian microbiologist Sergei N. Winogradsky made many contributions to soil microbiology. He discovered that soil bacteria could oxidize iron, sulfur, and ammonia to obtain energy, and that many bacteria could incorporate CO_2 into organic matter much like photosynthetic organisms do. Winogradsky also isolated anaerobic nitrogen-fixing soil bacteria and studied the decomposition of cellulose.

Martinus W. Beijerinck was one of the great general microbiologists who made fundamental contributions to microbial ecology and many other fields. He isolated the aerobic nitrogen-fixing bacterium *Azotobacter;* a root nodule bacterium also capable of fixing nitrogen (later named *Rhizobium*); and sulfate-reducing bacteria. Beijerinck and Winogradsky developed the enrichment-culture technique and the use of selective media (*see chapter 5*), which have been of such great importance in microbiology.

1. Briefly describe the work of Pasteur on microbial fermentations.
2. How did Winogradsky and Beijerinck contribute to the study of microbial ecology?

1.5 Members of the Microbial World

Although the kingdoms of organisms and the differences between procaryotic and eucaryotic cells are discussed in much more detail later, a brief introduction to the organisms a microbiologist studies is given here. Comparison of procaryotic and eucaryotic cells (pp. 91–92).

Two fundamentally different types of cells exist. **Procaryotic cells** [Greek *pro,* before, and *karyon,* nut or kernel; organism with a primordial nucleus] have a much simpler morphology than eucaryotic cells and lack a true membrane-delimited nucleus. All bacteria are procaryotic. In contrast, **eucaryotic cells** [Greek *eu,* true, and *karyon,* nut or kernel] have a membrane-enclosed nucleus; they are more complex morphologically and are usually larger than procaryotes. Algae, fungi, protozoa, higher plants, and animals are eucaryotic. Procaryotic and eucaryotic cells differ in many other ways as well (*see chapter 4*).

The early description of organisms as either plants or animals clearly is too simplified, and for many years biologists have divided organisms into five kingdoms: the *Monera, Protista, Fungi, Animalia,* and *Plantae* (*see chapter 19*). Microbiologists study primarily members of the first three kingdoms. Although they are not included in the five kingdoms, viruses are also studied by microbiologists. Fungi (chapter 25); Algae (chapter 26); Protozoa (chapter 27); Introduction to the viruses (chapters 16–18)

In the last few decades great progress has been made in three areas that profoundly affect microbial classification. First, much has been learned about the detailed structure of microbial cells from the use of electron microscopy. Second, microbiologists have determined the biochemical and physiological characteristics of many different microorganisms. Third, the sequences of nucleic acids and proteins from a wide variety of organisms have been compared. It is now clear that there are two quite different groups of procaryotic organisms: Bacteria and Archaea. Furthermore, the protists are so diverse that it may be necessary to divide the kingdom *Protista* into three or more kingdoms. Thus many taxonomists have concluded that the five-kingdom system is too simple and have proposed a variety of alternatives (*see section 19.7*). The differences between Bacteria, Archaea, and the eucaryotes seem so great that many microbiologists have proposed that organisms should be divided among three domains: Bacteria (the true bacteria or eubacteria), Archaea[1], and Eucarya (all eucaryotic organisms). This system, which we shall use here, and the results leading to it are discussed in chapter 19.

1. Describe and contrast procaryotic and eucaryotic cells.
2. Briefly describe the five-kingdom system and give the major characteristics of each kingdom.

1.6 The Scope and Relevance of Microbiology

As the scientist-writer Steven Jay Gould emphasized, we live in the Age of Bacteria. They were the first living organisms on our planet, live virtually everywhere life is possible, are more numerous than any other kind of organism, and probably constitute the largest component of the earth's biomass. The whole ecosystem depends on their activities, and they influence human society in countless ways. Thus modern microbiology is a large discipline with many different specialties; it has a great impact on fields such as medicine, agricultural and food sciences, ecology, genetics, biochemistry, and molecular biology.

For example, microbiology has been a major contributor to the rise of molecular biology, the branch of biology dealing with the physical and chemical aspects of living matter and its function. Microbiologists have been deeply involved in studies on the genetic code and the mechanisms of DNA, RNA, and protein synthesis. Microorganisms were used in many of the early studies on the regulation of gene expression and the control of enzyme activity (*see chapters 8 and 12*). In the 1970s new discoveries in microbiology led to the development of recombinant DNA technology and genetic engineering. The mechanisms of DNA, RNA, and protein synthesis (chapters 11 and 12); Recombinant DNA and genetic engineering (chapter 14)

One indication of the importance of microbiology in the twentieth century is the Nobel Prize given for work in physiology or medicine. About 1/3 of these have been awarded to scientists working on microbiological problems (*see inside front cover*).

[1]Although this will be discussed further in chapter 19, it should be noted here that several names have been used for the Archaea. The two most important are archaeobacteria and archaebacteria. In this text, we shall use only the name Archaea for sake of clarity and consistency.

Figure 1.7 Some Well-Known Modern Microbiologists. This figure depicts a few microbiologists who have made significant contributions in different areas of microbiology. (**a**) Rita R. Colwell has studied the genetics and ecology of marine bacteria such as *Vibrio cholerae* and helped establish the field of marine biotechnology. (**b**) R. G. E. Murray has contributed greatly to the understanding of bacterial cell envelopes and bacterial taxonomy. (**c**) Stanley Falkow has advanced our understanding of how bacterial pathogens cause disease. (**d**) Martha Howe has made fundamental contributions to our knowledge of the bacteriophage Mu. (**e**) Frederick C. Neidhardt has contributed to microbiology through his work on the regulation of *E. coli* physiology and metabolism, and by coauthoring advanced textbooks. (**f**) Jean E. Brenchley has studied the regulation of glutamate and glutamine metabolism, helped found the Pennsylvania State University Biotechnology Institute, and is now finding biotechnological uses for psychrophilic (cold-loving) microorganisms.

Microbiology has both basic and applied aspects. Many microbiologists are interested primarily in the biology of the microorganisms themselves (**figure 1.7**). They may focus on a specific group of microorganisms and be called virologists (viruses), bacteriologists (bacteria), phycologists or algologists (algae), mycologists (fungi), or protozoologists (protozoa). Others are interested in microbial morphology or particular functional processes and work in fields such as microbial cytology, microbial physiology, microbial ecology, microbial genetics and molecular biology, and microbial taxonomy. Of course a person can be thought of in both ways (e.g., as a bacteriologist who works on taxonomic problems). Many microbiologists have a more applied orientation and work on practical problems in fields such as medical microbiology, food and dairy microbiology, and public health microbiology (basic research is also conducted in these fields). Because the various fields of microbiology are interrelated, an applied microbiologist must be familiar with basic microbiology. For example, a medical microbiologist must have a good understanding of microbial taxonomy, genetics, immunology, and physiology to identify and properly respond to the pathogen of concern.

What are some of the current occupations of professional microbiologists? One of the most active and important is medical microbiology, which deals with the diseases of humans and animals. Medical microbiologists identify the agent causing an infectious disease and plan measures to eliminate it. Frequently they are involved in tracking down new, unidentified pathogens such as the agent that causes variant creutzfeldt-Jacob disease, the hantavirus, and the virus responsible for AIDS. These microbiologists also study the ways in which microorganisms cause disease. Legionnaires' disease (pp. 901–2); Hantavirus pulmonary syndrome (p. 877); AIDS (pp. 878–84)

Public health microbiology is closely related to medical microbiology. Public health microbiologists try to control the spread of communicable diseases. They often monitor community food establishments and water supplies in an attempt to keep them safe and free from infectious disease agents.

Immunology is concerned with how the immune system protects the body from pathogens and the response of infectious agents. It is one of the fastest growing areas in science; for example, techniques for the production and use of monoclonal antibodies have developed extremely rapidly. Immunology also deals with practical health problems such as the nature and treatment of allergies and autoimmune diseases like rheumatoid arthritis. Monoclonal antibodies and their uses (section 32.3 and Box 36.2)

Many important areas of microbiology do not deal directly with human health and disease but certainly contribute to human welfare. Agricultural microbiology is concerned with the impact of microorganisms on agriculture. Agricultural microbiologists try to combat plant diseases that attack important food crops, work on methods to increase soil fertility and crop yields, and study the role of microorganisms living in the digestive tracts of ruminants such as cattle. Currently there is great interest in using bacterial and viral insect pathogens as substitutes for chemical pesticides.

The field of microbial ecology is concerned with the relationships between microorganisms and their living and nonliving habitats. Microbial ecologists study the contributions of microorganisms to the carbon, nitrogen, and sulfur cycles in soil and in freshwater. The study of pollution effects on microorganisms also is important because of the impact these organisms have on the environment. Microbial ecologists are employing microorganisms in bioremediation to reduce pollution effects.

Scientists working in food and dairy microbiology try to prevent microbial spoilage of food and the transmission of food-borne diseases such as botulism and salmonellosis (*see chapter 39*). They also use microorganisms to make foods such as cheeses, yogurts, pickles, and beer. In the future microorganisms themselves may become a more important nutrient source for livestock and humans.

In industrial microbiology microorganisms are used to make products such as antibiotics, vaccines, steroids, alcohols and other solvents, vitamins, amino acids, and enzymes. Microorganisms can even leach valuable minerals from low-grade ores.

Research on the biology of microorganisms occupies the time of many microbiologists and also has practical applications. Those working in microbial physiology and biochemistry study the synthesis of antibiotics and toxins, microbial energy production, the ways in which microorganisms survive harsh environmental conditions, microbial nitrogen fixation, the effects of chemical and physical agents on microbial growth and survival, and many other topics.

Microbial genetics and molecular biology focus on the nature of genetic information and how it regulates the development and function of cells and organisms. The use of microorganisms has been very helpful in understanding gene function. Microbial geneticists play an important role in applied microbiology by producing new microbial strains that are more efficient in synthesizing useful products. Genetic techniques are used to test substances for their ability to cause cancer. More recently the field of genetic engineering (*see chapter 14*) has arisen from work in microbial genetics and molecular biology and will contribute substantially to microbiology, biology as a whole, and medicine. Engineered microorganisms are used to make hormones, antibiotics, vaccines, and other products (*see chapter 42*). New genes can be inserted into plants and animals; for example, it may be possible to give corn and wheat nitrogen-fixation genes so they will not require nitrogen fertilizers.

1.7 The Future of Microbiology

As the preceding sections have shown, microbiology has had a profound influence on society. What of the future? Science writer Bernard Dixon is very optimistic about microbiology's future for two reasons. First, microbiology has a clearer mission than do many other scientific disciplines. Second, it is confident of its value because of its practical significance. Dixon notes that microbiology is required both to face the threat of new and reemerging human infectious diseases and to develop industrial technologies that are more efficient and environmentally friendly.

What are some of the most promising areas for future microbiological research and their potential practical impacts? What kinds of challenges do microbiologists face? The following brief list should give some idea of what the future may hold:

1. New infectious diseases are continually arising and old diseases are once again becoming widespread and destructive. AIDS, hemorrhagic fevers, and tuberculosis are excellent examples of new and reemerging infectious diseases. Microbiologists will have to respond to these threats, many of them presently unknown.
2. Microbiologists must find ways to stop the spread of established infectious diseases. Increases in antibiotic resistance will be a continuing problem, particularly the spread of multiple drug resistance that can render a pathogen impervious to current medical treatment.

Microbiologists have to create new drugs and find ways to slow or prevent the spread of drug resistance. New vaccines must be developed to protect against diseases such as AIDS. It will be necessary to use techniques in molecular biology and recombinant DNA technology to solve these problems.

3. Research is needed on the association between infectious agents and chronic diseases such as autoimmune and cardiovascular diseases. It may be that some of these chronic afflictions partly result from infections.
4. We are only now beginning to understand how pathogens interact with host cells and the ways in which diseases arise. There also is much to learn about how the host resists pathogen invasions.
5. Microorganisms are increasingly important in industry and environmental control, and we must learn how to use them in a variety of new ways. For example, microorganisms can (a) serve as sources of high-quality food and other practical products such as enzymes for industrial applications, (b) degrade pollutants and toxic wastes, and (c) be used as vectors to treat diseases and enhance agricultural productivity. There also is a continuing need to protect food and crops from microbial damage.
6. Microbial diversity is another area requiring considerable research. Indeed, it is estimated that less than 1% of the earth's microbial population has been cultured. We must develop new isolation techniques and an adequate classification of microorganisms, one which includes those microbes that cannot be cultivated in the laboratory. Much work needs to be done on microorganisms living in extreme environments. The discovery of new microorganisms may well lead to further advances in industrial processes and enhanced environmental control.
7. Microbial communities often live in biofilms, and these biofilms are of profound importance in both medicine and microbial ecology. Research on biofilms is in its infancy; it will be many years before we more fully understand their nature and are able to use our knowledge in practical ways. In general, microbe-microbe interactions have not yet been extensively explored.
8. The genomes of many microorganisms already have been sequenced, and many more will be determined in the coming years. These sequences are ideal for learning how the genome is related to cell structure and what the minimum assortment of genes necessary for life is. Analysis of the genome and its activity will require continuing advances in the field of bioinformatics and the use of computers to investigate biological problems.
9. Further research on unusual microorganisms and microbial ecology will lead to a better understanding of the interactions between microorganisms and the inanimate world. Among other things, this understanding should enable us to more effectively control pollution. Similarly, it has become clear that microorganisms are essential partners with higher organisms in symbiotic relationships. Greater knowledge of symbiotic relationships can help improve our appreciation of the living world. It also will lead to improvements in the health of plants, livestock, and humans.
10. Because of their relative simplicity, microorganisms are excellent subjects for the study of a variety of fundamental questions in biology. For example, how do complex cellular structures develop and how do cells communicate with one another and respond to the environment?
11. Finally, microbiologists will be challenged to carefully assess the implications of new discoveries and technological developments. They will need to communicate a balanced view of both the positive and negative long-term impacts of these events on society.

The future of microbiology is bright. The microbiologist René Dubos has summarized well the excitement and promise of microbiology:

> How extraordinary that, all over the world, microbiologists are now involved in activities as different as the study of gene structure, the control of disease, and the industrial processes based on the phenomenal ability of microorganisms to decompose and synthesize complex organic molecules. Microbiology is one of the most rewarding of professions because it gives its practitioners the opportunity to be in contact with all the other natural sciences and thus to contribute in many different ways to the betterment of human life.

Summary

1. Microbiology may be defined in terms of the size of the organisms studied and the techniques employed.
2. Antony van Leeuwenhoek was the first person to describe microorganisms.
3. Experiments by Redi and others disproved the theory of spontaneous generation in regard to larger organisms.
4. The spontaneous generation of microorganisms was disproved by Spallanzani, Pasteur, Tyndall, and others.
5. Support for the germ theory of disease came from the work of Bassi, Pasteur, Koch, and others. Lister provided indirect evidence with his development of antiseptic surgery.
6. Koch's postulates and molecular Koch's postulates are used to prove a direct relationship between a suspected pathogen and a disease.
7. Koch developed the techniques required to grow bacteria on solid media and to isolate pure cultures of pathogens.
8. Vaccines against anthrax and rabies were made by Pasteur; von Behring and Kitasato prepared antitoxins for diphtheria and tetanus.
9. Metchnikoff discovered some blood leukocytes could phagocytize and destroy bacterial pathogens.
10. Pasteur showed that fermentations were caused by microorganisms and that some microorganisms could live in the absence of oxygen.

11. The role of microorganisms in carbon, nitrogen, and sulfur cycles was first studied by Winogradsky and Beijerinck.
12. Procaryotic cells differ from eucaryotic cells in lacking a membrane-delimited nucleus, and in other ways as well.
13. The Archaea are so different that many microbiologists divide organisms into three domains: *Bacteria, Archaea,* and *Eucarya.*
14. In the twentieth century microbiology has contributed greatly to the fields of biochemistry and genetics. It also has helped stimulate the rise of molecular biology.
15. There is a wide variety of fields in microbiology, and many have a great impact on society. These include the more applied disciplines such as medical, public health, industrial, food, and dairy microbiology. Microbial ecology, physiology, biochemistry, and genetics are examples of basic microbiological research fields.
16. Microbiologists will be faced with many exciting and important future challenges such as finding new ways to combat disease, reduce pollution, and feed the world's population.

Key Terms

eucaryotic cell *11*
hypothesis *8*
Koch's postulates *7*
microbiology *2*
microorganism *2*
procaryotic cell *11*
spontaneous generation *2*
theory *8*

Questions for Thought and Review

1. Why was the belief in spontaneous generation an obstacle to the development of microbiology as a scientific discipline?
2. Describe the major contributions of the following people to the development of microbiology: Leeuwenhoek, Spallanzani, Fracastoro, Pasteur, Tyndall, Cohn, Bassi, Lister, Koch, Chamberland, von Behring, Metchnikoff, Winogradsky, and Beijerinck.
3. Would microbiology have developed more slowly if Fannie Hesse had not suggested the use of agar? Give your reasoning. What is a pure culture?
4. Why do you think viruses are not included in the five-kingdom or three domain systems?
5. Why are microorganisms so useful to biologists as experimental models?
6. What do you think were the most important discoveries in the development of microbiology? Why?
7. List all the activities or businesses you can think of in your community that are directly dependent on microbiology.
8. Describe in your own words the scientific method. How does a theory differ from a hypothesis? Why is it important to have a control group?
9. What do you think are the five most important research areas to pursue in microbiology? Give reasons for your choices.

Critical Thinking Questions

1. Consider the impact of microbes on the course of world history. History is full of examples of instances or circumstances under which one group of people lost a struggle against another. In fact, when examined more closely, the "losers" often had the misfortune of being exposed to, more susceptible to, or unable to cope with an infectious agent. Thus, weakened in physical strength or demoralized by the course of a devastating disease, they were easily overcome by human "conquerors."
   a. Choose an example of a battle or other human activity such as exploration of new territory and determine the impact of microorganisms, either indigenous or transported to the region, on that activity.
   b. Discuss the effect that the microbe(s) had on the outcome in your example.
   c. Suggest whether the advent of antibiotics, food storage or preparation technology, or sterilization technology would have made a difference in the outcome.
2. Vaccinations against various childhood diseases have contributed to the entry of women, particularly mothers, into the full-time workplace.
   a. Is this statement supported by data—comparing availability and extent of vaccination with employment statistics in different places or at different times?
   b. Before vaccinations for measles, mumps, and chickenpox, what was the incubation time and duration of these childhood diseases? What impact would such diseases have on mothers with several elementary schoolchildren at home if they had full-time jobs and lacked substantial child care support?
   c. What would be the consequence if an entire generation of children (or a group of children in one country) were not vaccinated against any diseases? What do you predict would happen if these children went to college and lived in a dormitory in close proximity with others who had received all of the recommended childhood vaccines?

Additional Reading

General

American Society for Microbiology. 1999. Celebrating a century of leadership in microbiology. *ASM News* 65(5).

Baker, J. J. W., and Allen, G. E. 1968. *Hypothesis, prediction, and implication in biology.* Reading, Mass.: Addison-Wesley.

Beck, R. W. 2000. *A chronology of microbiology in historical context.* Washington, D.C.: ASM Press.

Brock, T. D. 1961. *Milestones in microbiology.* Englewood Cliffs, N.J.: Prentice-Hall.

Bulloch, W. 1979. *The history of bacteriology.* New York: Dover.

Chung, K.-T.; Stevens, Jr., S. E.; and Ferris, D. H. 1995. A chronology of events and pioneers of microbiology. *SIM News* 45(1):3–13.

Clark, P. F. 1961. *Pioneer microbiologists of America.* Madison: University of Wisconsin Press.

Collard, P. 1976. *The development of microbiology.* New York: Cambridge University Press.

de Kruif, P. 1937. *Microbe hunters.* New York: Harcourt, Brace.

Gabriel, M. L., and Fogel, S., editors. 1955. *Great experiments in biology.* Englewood Cliffs, N.J.: Prentice-Hall.

Geison, G. L. 1995. *The private science of Louis Pasteur.* Princeton, N.J.: Princeton University Press.

Hellemans, A., and Bunch, B. 1988. *The timetables of science.* New York: Simon and Schuster.

Hill, L. 1985. Biology, philosophy, and scientific method. *J. Biol. Educ.* 19(3):227–31.

Lechevalier, H. A., and Solotorovsky, M. 1965. *Three centuries of microbiology.* New York: McGraw-Hill.

McNeill, W. H. 1976. *Plagues and peoples.* Garden City, N.Y.: Anchor Press/Doubleday.

Ruestow, E. G. 1996. *The microscope in the Dutch republic: The shaping of discovery.* New York: Cambridge University Press.

Singer, C. 1959. *A history of biology,* 3d ed. New York: Abelard-Schuman.

Singleton, P., and Sainsbury, D. 1995. *Dictionary of microbiology and molecular biology,* 3d ed. New York: John Wiley and Sons.

Staley, J. T.; Castenholz, R. W.; Colwell, R. R.; Holt, J. G.; Kane, M. D.; Pace, N. R.; Salyers, A. A.; and Tiedje, J. M. 1997. *The microbial world: Foundation of the biosphere.* Washington, D.C.: American Academy of Microbiology.

Stanier, R. Y. 1978. What is microbiology? In *Essays in microbiology,* J. R. Norris and M. H. Richmond, editors, 1/1–1/32. New York: John Wiley and Sons.

Summers, W. C. 2000. History of microbiology. In *Encyclopedia of microbiology,* vol. 2, J. Lederberg, editor, 677–97. San Diego: Academic Press.

1.1 The Discovery of Microorganisms

Dobell, C. 1960. *Antony van Leeuwenhoek and his "little animals."* New York: Dover.

Ford, B. J. 1981. The Van Leeuwenhoek specimens. *Notes and Records of the Royal Society of London* 36(1):37–59.

Ford, B. J. 1998. The earliest views. *Sci. Am.* 278(4):50–53.

1.2 The Conflict over Spontaneous Generation

Drews, G. 1999. Ferdinand Cohn, a founder of modern microbiology. *ASM News* 65(8):547–53.

Dubos, R. J. 1950. *Louis Pasteur: Free lance of science.* Boston: Little, Brown.

Strick, J. E. 1997. New details add to our understanding of spontaneous generation controversies. *ASM News* 63(4):193–98.

Vallery-Radot, R. 1923. *The life of Pasteur.* New York: Doubleday.

1.3 The Role of Microorganisms in Disease

Brock, T. D. 1988. *Robert Koch: A life in medicine and bacteriology.* Madison, Wis.: Science Tech Publishers.

Fredricks, D. N., and Relman, D. A. 1996. Sequence-based identification of microbial pathogens: A reconsideration of Koch's postulates. *Clin. Microbiol. Rev.* 9(1):18–33.

Hesse, W. 1992. Walther and Angelina Hesse—early contributors to bacteriology. *ASM News* 58(8):425–28.

Hitchens, A. P., and Leikind, M. C. 1939. The introduction of agar-agar into bacteriology. *J. Bacteriol.* 37(5):485–93.

Silverstein, A. M. 1989. *A history of immunology.* San Diego: Academic Press.

1.4 Industrial Microbiology and Microbial Ecology

Chung, K.-T., and Ferris, D. H. 1996. Martinus Willem Beijerinck (1851–1931): Pioneer of general microbiology. *ASM News* 62(10):539–43.

1.7 The Future of Microbiology

Dixon, B. 1997. Microbiology present and future. *ASM News* 63(3):124–25.

Young, P. 1997. American academy of microbiology outlines basic research priorities. *ASM News* 63(10):546–50.