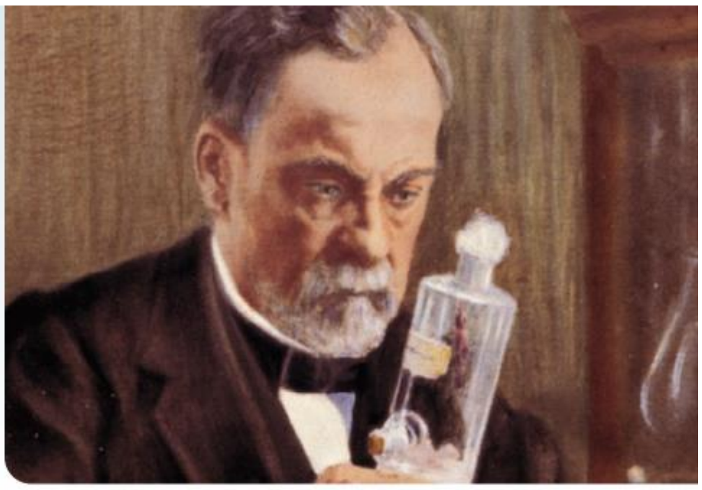
1 The History and Scope of Microbiology



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."

Preview

- Microbiology is defined not only by the size of its subjects but the techniques it uses to study them.
- Microorganisms include acellular entities (e.g., viruses), procarytic cells, and eucaryotic cells. Cellular microorganisms are found in all three domains of life: *Bacteria, Archaea, Eucarya*.
- 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. The development of these techniques in large part grew out of studies disproving the Theory of Spontaneous Generation and others establishing that microorganisms can cause disease.
- Microbiology is a large discipline; it has had and will continue to have a great impact on other areas of biology and general human welfare.

The importance of microorganisms can't be overemphasized. In terms of sheer number and mass—it is estimated that microbes contain 50% of the biological carbon and 90% of the biological nitrogen on Earth—they greatly exceed every other group of organisms on the planet. Furthermore, they are found everywhere: from geothermal vents in the ocean depths to the coldest arctic ice, to every person's skin. They are major contributors to the functioning of the biosphere, being indispensable for the cycling of the elements essential for life. They also are a source of nutrients at the base of all ecological food chains and webs. Most importantly, certain microorganisms carry out photosynthesis, rivaling plants in their role of capturing carbon dioxide and releasing oxygen into the atmosphere. Those microbes that inhabit humans also play important roles, including helping the body digest food and producing vitamins B and K. In addition, society in general benefits from microorganisms, as 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.

Although the majority of microorganisms play beneficial or benign roles, some harm humans and have 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, an arthropod-borne disease, struck Europe with brutal force, killing 1/3 of the population (about 25 million people) within four years. Over the next 80 years, the disease struck again and again, eventually wiping out 75% of the European population. The plague's effect was so great that some historians believe it changed European culture and prepared the way for the Renaissance. Today the struggle by microbiologists and others against killers like AIDS and malaria continues.

In this introductory chapter, we introduce the microbial world to provide a general idea of the organisms and agents that microbiologists study. Then we describe the historical development of the science of microbiology and its relationship to medicine and other areas of biology. Finally, we discuss the scope, relevance, and future of modern microbiology.

1.1 Members of the Microbial World

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

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

examined with a microscope, microbiology is concerned primarily with organisms and agents this small and smaller. However, some microorganisms, particularly some eucaryotic microbes, are visible without microscopes. For example, bread molds and filamentous algae are studied by microbiologists, yet are visible to the naked eye, as are the two bacteria *Thiomargarita* and *Epulopiscium*. Microbial Diversity & Ecology 3.1: Monstrous Microbes

The difficulty in setting the boundaries of microbiology has led to the suggestion of other criteria for defining the field. For instance, an important characteristic of microorganisms, even those that are large and multicellular, is that they are relatively simple in their construction, lacking highly differentiated cells and distinct tissues. Another suggestion, made by Roger Stanier, is that the field also be defined in terms of its techniques. Microbiologists usually first isolate a specific microorganism from a population and then culture it. Thus microbiology employs techniques—such as sterilization and the use of culture media—that are necessary for successful isolation and growth of microorganisms.

Microorganisms are diverse, and their classification has always been a challenge for microbial taxonomists. Their early descriptions as either plants or animals were too simple. For instance, some microbes are motile like animals, but also have cell walls and are photosynthetic like plants. Such microbes cannot be placed easily into one kingdom or another. Another important factor in classifying microorganisms is that some are composed of procaryotic cells and others of eucaryotic cells. **Procaryotic cells** [Greek *pro,* before, and *karyon,* nut or kernel; organisms with a primordial nucleus] have a much simpler morphology than eucaryotic cells and lack a true membrane-delimited nucleus. 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. These observations eventually led to the development of a classification scheme that divided organisms into five kingdoms: the *Monera, Protista, Fungi, Animalia,* and *Plantae.* Microorganisms (except for viruses, which are acellular and have their own classification system) were placed in the first three kingdoms.

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. The comparison of ribosomal RNA (rRNA), begun by Carl Woese in the 1970s, was instrumental in demonstrating that there are two very different groups of procaryotic organisms: *Bacteria* and *Archaea,* which had been classified together as *Monera* in the five-kingdom system. Later, studies based on rRNA comparisons suggested that *Protista* was not a cohesive taxonomic unit and that it should be divided into three or more kingdoms. These studies and others have led many taxonomists to conclude that the five-kingdom system is too simple. A number of alternatives have been suggested, but currently, most

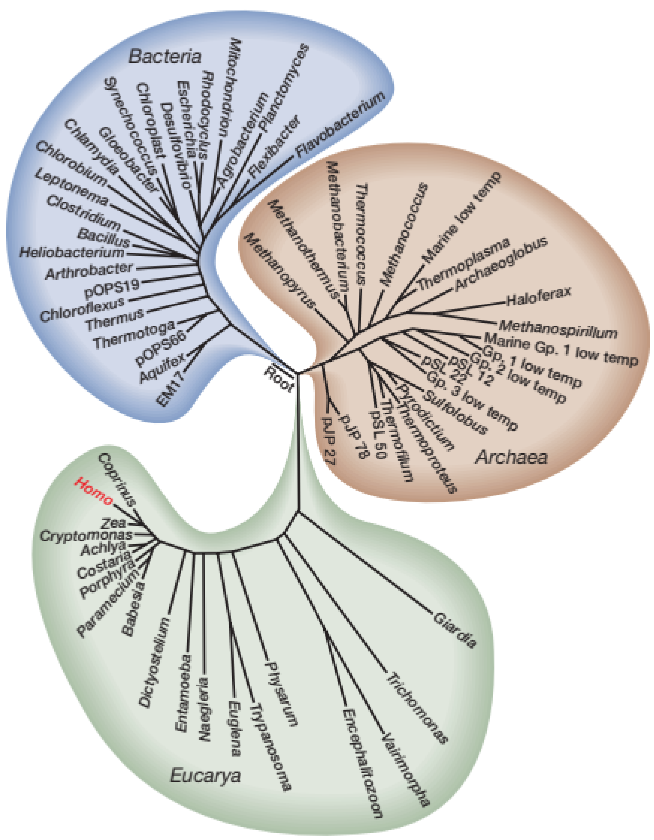


Figure 1.1 Universal Phylogenetic Tree. These evolutionary relationships are based on rRNA sequence comparisons. Man (Homo) is highlighted in red.

microbiologists believe that organisms should be divided among three domains: *Bacteria* (the true bacteria or eubacteria), *Archaea,*[1] and *Eucarya* (all eucaryotic organisms) (**figure 1.1**). This system, which we shall use here, and the results leading to it are discussed in chapter 19. A brief description of the three domains and of the microorganisms placed in them follows.

Bacteria[2] are procaryotes that are usually single-celled organisms. Most have cell walls that contain the structural molecule peptidoglycan. They are abundant in soil, water, and air and are also major inhabitants of our skin, mouth, and intestines. Some bacteria live in environments that have extreme temperatures,

[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.*
[2] In this text, the term bacteria (s., bacterium) will be used to refer to procaryotes that belong to domain *Bacteria,* and the term archaea (s., archaeon) will be used to refer to procaryotes that belong to domain *Archaea.* It should be noted that in some publications, the term bacteria is used to refer to all procaryotes. That is not the case in this text.

pH, or salinity. Although some bacteria cause disease, many play more beneficial roles such as cycling elements in the biosphere, breaking down dead plant and animal material, and producing vitamins. Cyanobacteria produce significant amounts of oxygen through the process of photosynthesis.

Archaea are procaryotes that are distinguished from *Bacteria* by many features, most notably their unique ribosomal RNA sequences. They also lack peptidoglycan in their cell walls and have unique membrane lipids. Some have unusual metabolic characteristics, such as the methanogens, which generate methane gas. Many archaea are found in extreme environments. Pathogenic archaea have not yet been identified.

Domain *Eucarya* includes microorganisms classified as protists or *Fungi*. Animals and plants are also placed in this domain. **Protists** are generally larger than procaryotes and include unicellular algae, protozoa, slime molds, and water molds. **Algae** are photosynthetic protists that together with the cyanobacteria produce about 75% of the planet's oxygen. They are also the foundation of aquatic food chains. **Protozoa** are unicellular, animal-like protists that are usually motile. Many free-living protozoa function as the principal hunters and grazers of the microbial world. They obtain nutrients by ingesting organic matter and other microbes. They can be found in many different environments and some are normal inhabitants of the intestinal tracts of animals, where they aid in digestion of complex materials such as cellulose. A few cause disease in humans and other animals. **Slime molds** are protists that are like protozoa in one stage of their life cycle, but are like fungi in another. In the protozoan phase, they hunt for and engulf food particles, consuming decaying vegetation and other microbes. **Water molds,** as their name implies, are found in the surface water of freshwater sources and moist soil. They feed on decaying vegetation such as logs and mulch. Some water molds have produced devastating plant infections, including the Great Potato Famine of 1846–1847. ***Fungi*** are a diverse group of microorganisms that range from unicellular forms (yeasts) to molds and mushrooms. Molds and mushrooms are multicellular fungi that form thin, threadlike structures called hyphae. They absorb nutrients from their environment, including the organic molecules that they use as a source of carbon and energy. Because of their metabolic capabilities, many fungi play beneficial roles, including making bread rise, producing antibiotics, and decomposing dead organisms. Other fungi cause plant diseases and diseases in humans and other animals.

Viruses are acellular entities that must invade a host cell in order to replicate. They are the smallest of all microbes (the smallest is 10,000 times smaller than a typical bacterium), but their small size belies their power—they cause many animal and plant diseases and have caused epidemics that have shaped human history. The diseases they cause include smallpox, rabies, influenza, AIDS, the common cold, and some cancers.

The development of microbiology as a science is described in sections 1.2 to 1.5. **Figure 1.2** presents a summary of some of the major events in this process and their relationship to other historical landmarks.

1. Describe the field of microbiology in terms of the size of its subject material and the nature of its techniques.
2. Describe and contrast procaryotic and eucaryotic cells.
3. Describe and contrast the five-kingdom classification system with the three-domain system. Why do you think viruses are not included in either system?

1.2 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. In 1665, the first drawing of a microorganism was published in Robert Hooke's *Micrographia*. However, the first person to publish extensive, accurate observations of microorganisms was the amateur microscopist Antony van Leeuwenhoek (1632–1723) of Delft, The Netherlands (**figure 1.3*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 1.3*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 in which the organisms appeared as bright objects against a dark background and made bacteria clearly visible (figure 1.3*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.

As important as Leeuwenhoek's observations were, the development of microbiology essentially languished for the next 200 years. Little progress was made primarily because microscopic observations of microorganisms do not provide sufficient information to understand their biology. For the discipline to develop, techniques for isolating and culturing microbes in the laboratory were needed. Many of these techniques began to be developed as scientists grappled with the conflict over the Theory of Spontaneous Generation. This conflict and the subsequent studies on the role played by microorganisms in causing disease ultimately led to what is now called the Golden Age of Microbiology.

1. Give some examples of the kind of information you think can be provided by microscopic observations of microorganisms.
2. Give some examples of the kind of information you think can be provided by isolating microorganisms from their natural environment and culturing them in the laboratory.

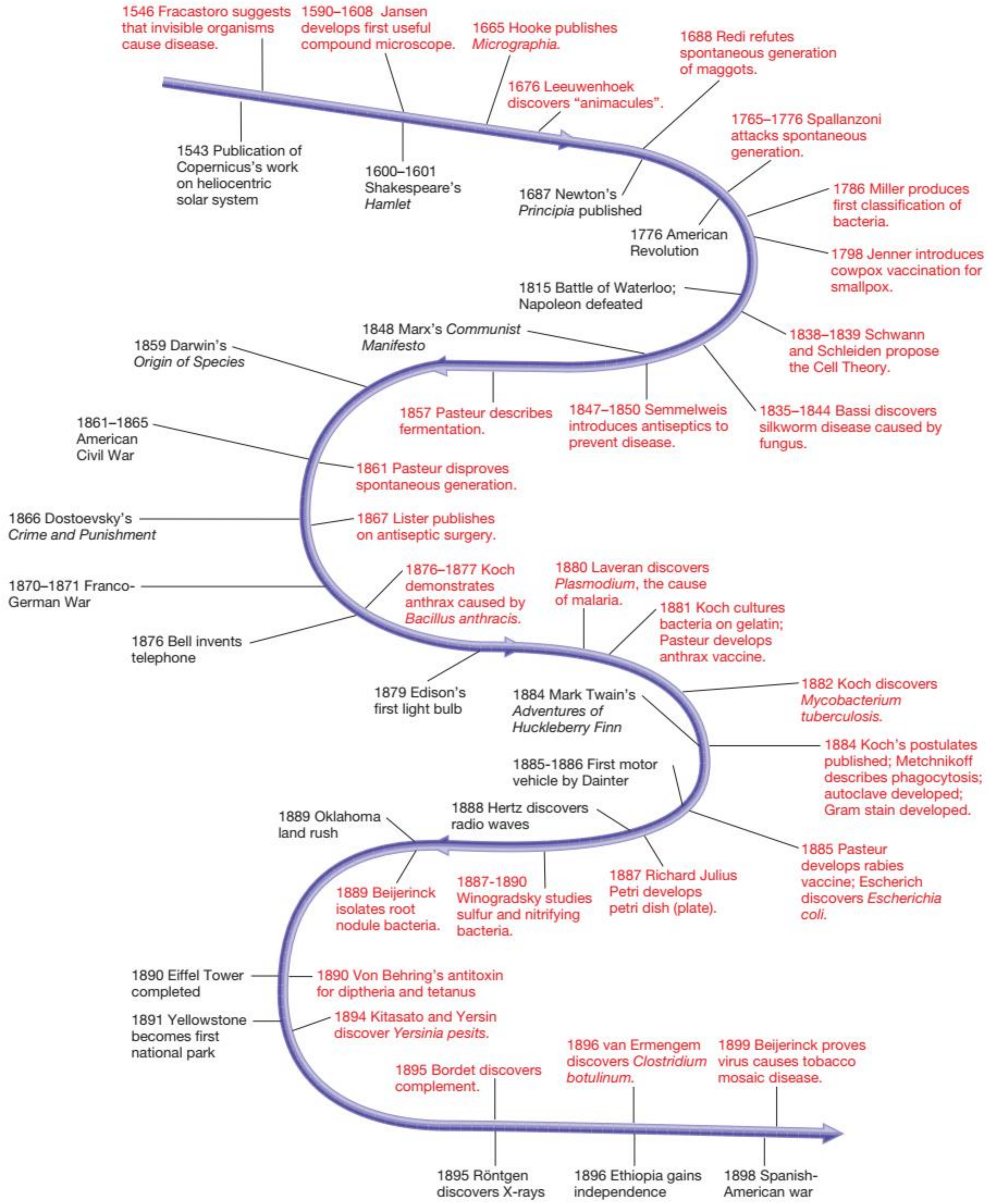


Figure 1.2(a) Some Important Events in the Development of Microbiology (1546–1899). Milestones in microbiology are marked in red; other historical events are in black.

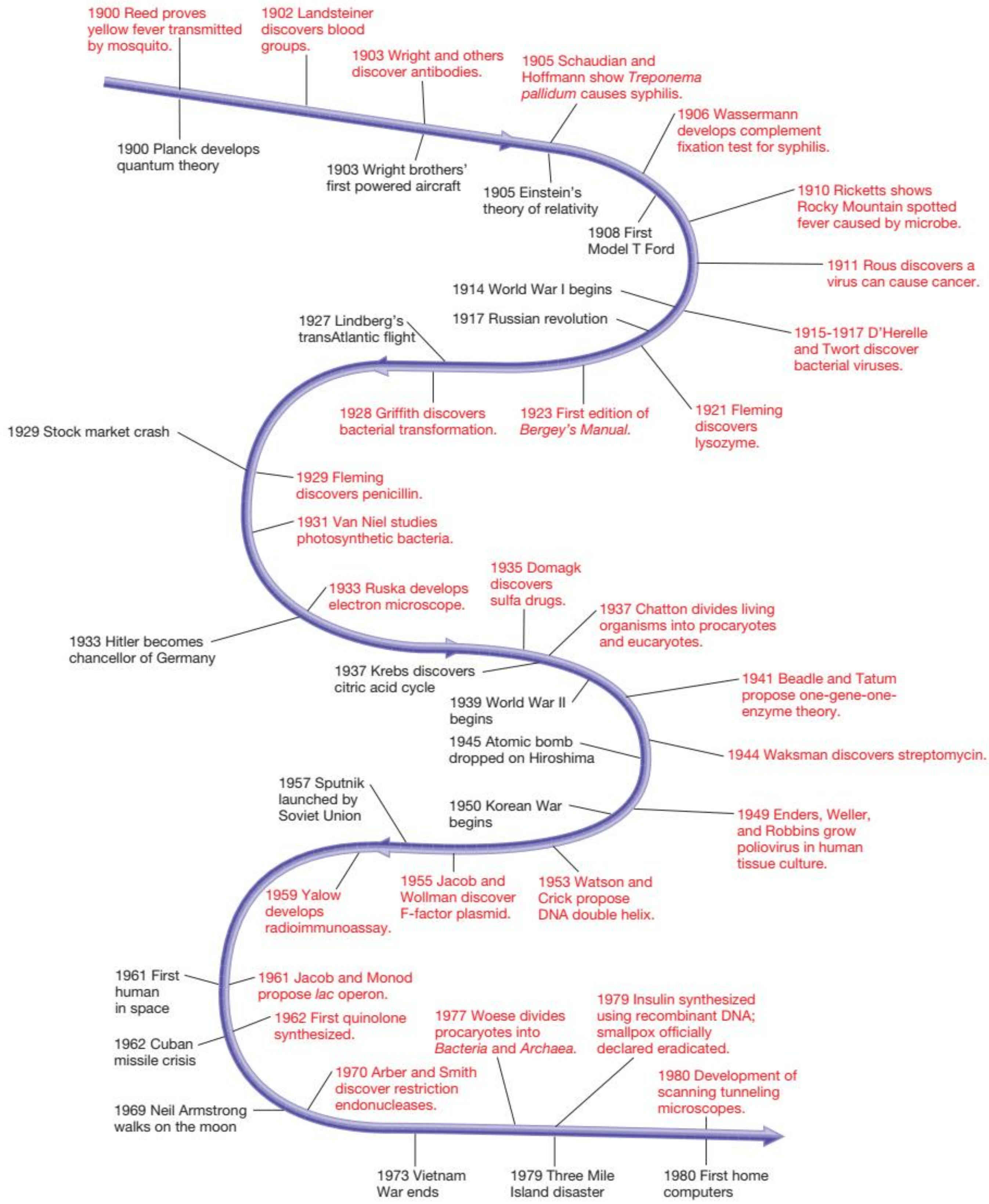


Figure 1.2(b) Some Important Events in the Development of Microbiology (1900–1980). Milestones in microbiology are marked in red; other historical events are in black.

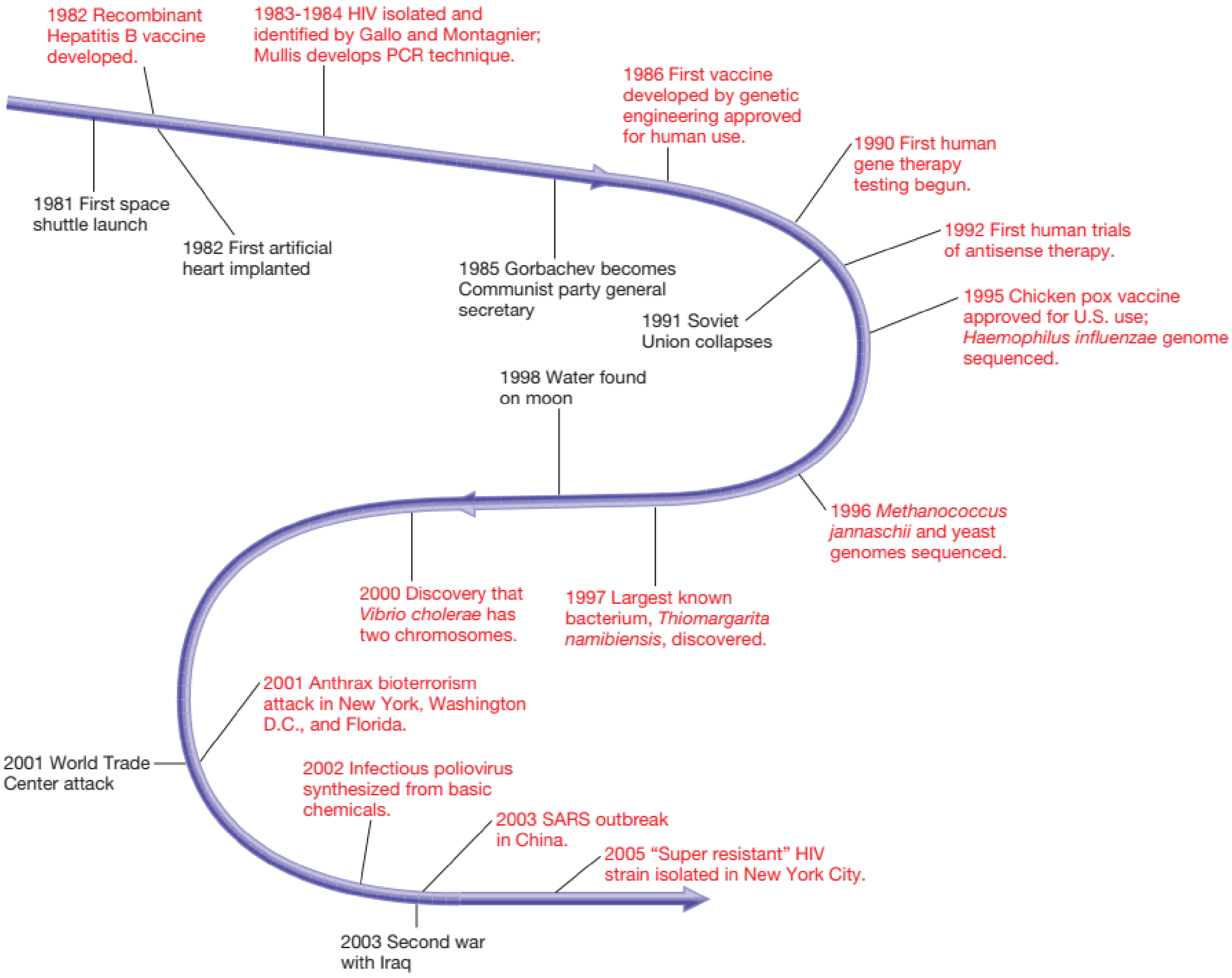


Figure 1.2(c) Some Important Events in the Development of Microbiology (1981–2005). Milestones in microbiology are marked in red; other historical events are in black.

1.3 The Conflict Over Spontaneous Generation

From earliest times, people had believed in **spontaneous generation**—that living organisms could develop from nonliving matter. Even 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. 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 1.4**) 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 occurred. 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 1.5**). 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.

Figure 1.3 Antony van Leeuwenhoek. **(a)** An oil painting of Leeuwenhoek (1632–1723). **(b)** A brass replica of the Leeuwenhoek microscope. Inset photo shows how it is held. **(c)** Leeuwenhoek's drawings of bacteria from the human mouth.

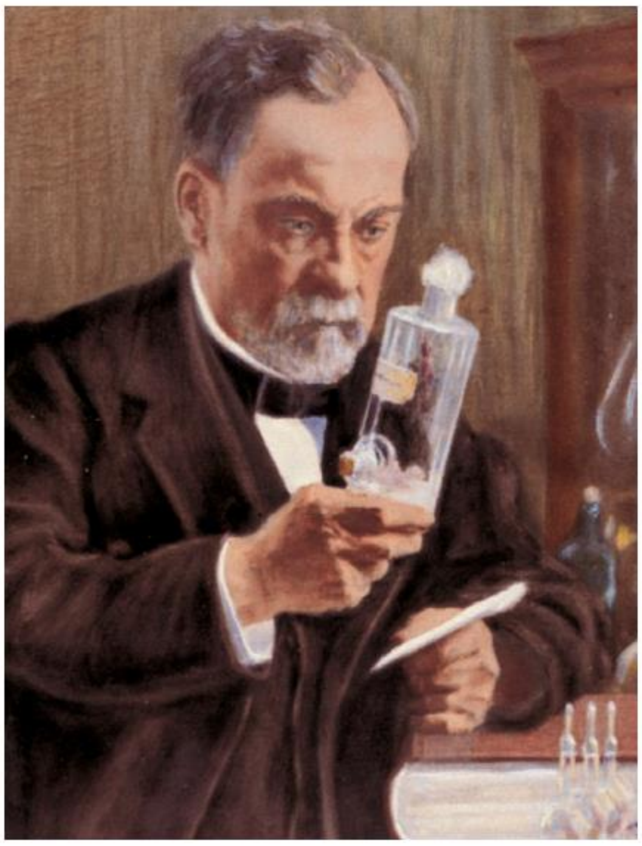


Figure 1.4 Louis Pasteur. Pasteur (1822–1895) working in his laboratory.

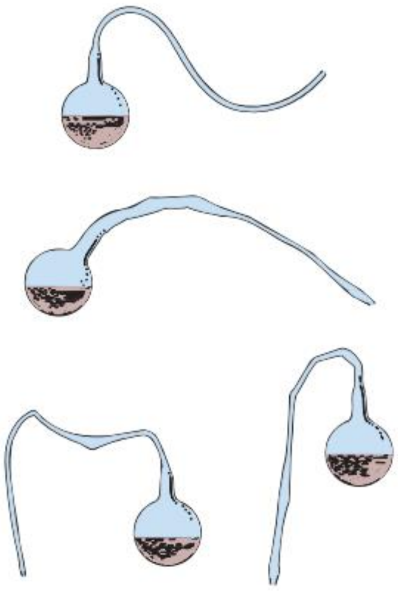


Figure 1.5 The Spontaneous Generation Experiment. Pasteur's swan neck flasks used in his experiments on the spontaneous generation of microorganisms. *Source: Annales Sciences Naturelle, 4th Series, Vol. 16, pp. 1–98, Pasteur, L., 1861, "Mémoire sur les Corpuscules Organisés Qui Existent Dans L'Atmosphère: Examen de la Doctrine des Générations Spontanées."*

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. The bacterial endospore (section 3.11)

1. How did Pasteur and Tyndall finally settle the spontaneous generation controversy?
2. Why was the belief in spontaneous generation an obstacle to the development of microbiology as a scientific discipline?

1.4 The Golden Age of Microbiology

Pasteur's work with swan neck flasks ushered in the Golden Age of Microbiology. Within 60 years (1857–1914), a number of disease-causing microbes were discovered, great strides in understanding microbial metabolism were made, and techniques for isolating and characterizing microbes were improved. Scientists also identified the role of immunity in preventing disease and controlling microbes, developed vaccines, and introduced techniques used to prevent infection during surgery.

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 among the four humors thought to be present in the body. The role of the four humors (blood, phlegm, yellow bile [choler], and black bile [melancholy]) in disease had been widely accepted since the time of the Greek physician Galen (129–199). Support for the idea that microorganisms cause disease—that is, 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 water mold, and in 1853, Heinrich de Bary showed that smut and rust fungi caused cereal crop diseases. Following his successes with the study of fermentation, Pasteur was asked by the French government to investigate the pèbrine 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.

Indirect evidence for the germ theory of 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 kills bacteria, also prevented wound infections.

Koch's Postulates

The first direct demonstration of the role of bacteria in causing disease came from the study of anthrax by the German physician Robert Koch (1843–1910). Koch (**figure 1.6**) 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 (**Techniques & Applications 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 endospores. When the isolated bacilli or their 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** (**table 1.1**). Koch's proof that *B. 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.

Although Koch used the general approach described in the postulates during his anthrax studies, he did not outline them fully until his work on the cause of tuberculosis (table 1.1). In 1884, he reported that this disease was caused by a rod-shaped bacterium, *Mycobacterium tuberculosis;* he was awarded the Nobel Prize in Physiology or Medicine in 1905 for his work. Koch's postulates quickly became the cornerstone of connecting many diseases to their causative agent. However, their use is at times not feasible (**Disease 1.2**). For instance, some organisms, like *Mycobacterium leprae,* the causative agent of leprosy, cannot be isolated in pure culture.

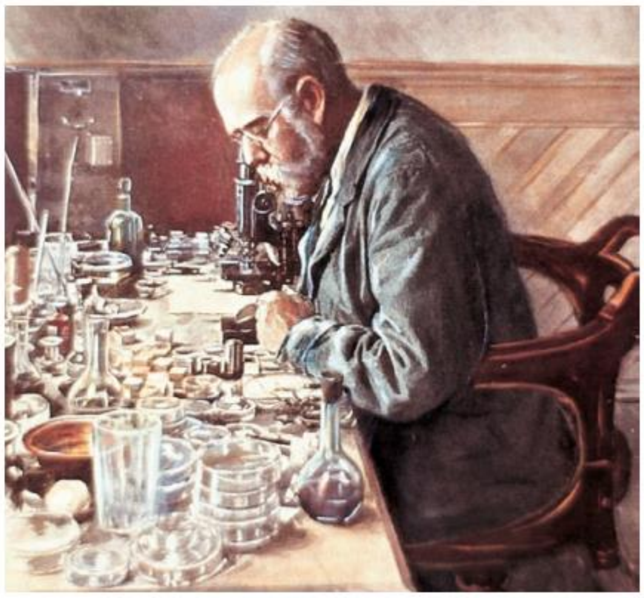


Figure 1.6 Robert Koch. Koch (1843–1910) examining a specimen in his laboratory.

The Development of Techniques for Studying Microbial Pathogens

During Koch's studies on bacterial diseases, it became necessary to isolate suspected bacterial pathogens in pure culture—a culture containing only one type of microorganism. At first Koch cultured bacteria on the sterile surfaces of cut, boiled potatoes, but this was unsatisfactory because the bacteria would not always grow well. Eventually he developed culture media using meat extracts and protein digests because of their similarity to body fluids. He first tried to solidify the media by adding gelatin. Separate bacterial colonies developed after the surface of the solidified medium had been streaked with a bacterial sample. The sample could also be mixed with liquefied gelatin medium.

Table 1.1 Koch's Application of His Postulates to Demonstrate that *Mycobacterium tuberculosis* is the Causative Agent of Tuberculosis.

Postulate	Experimentation
1. The microorganism must be present in every case of the disease but absent from healthy organisms.	Koch developed a staining technique to examine human tissue. *M. tuberculosis* cells could be identified in diseased tissue.
2. The suspected microorganisms must be isolated and grown in a pure culture.	Koch grew *M. tuberculosis* in pure culture on coagulated blood serum.
3. The same disease must result when the isolated microorganism is inoculated into a healthy host.	Koch injected cells from the pure culture of *M.tuberculosis* into guinea pigs. The guinea pigs subsequently died of tuberculosis.
4. The same microorganism must be isolated again from the diseased host.	Koch isolated *M. tuberculosis* from the dead guinea pigs and was able to again culture the microbe in pure culture on coagulated blood serum.

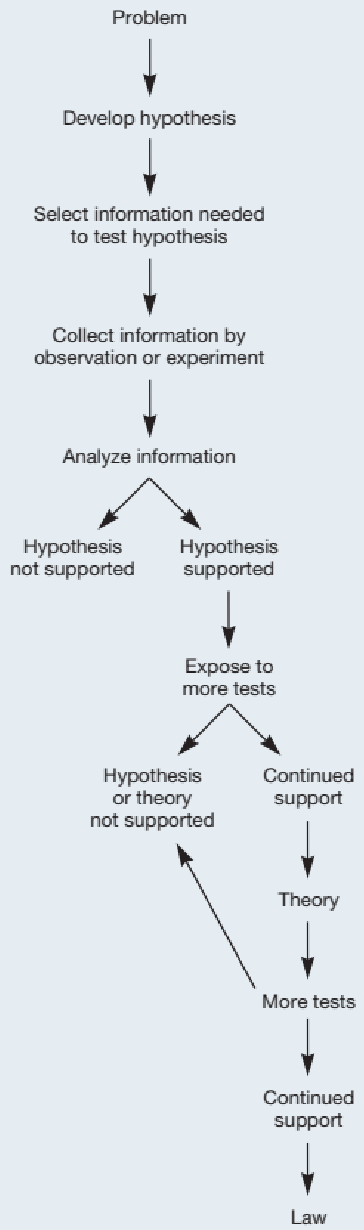
Techniques & Applications

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. Ultimately, if the support for a hypothesis or theory becomes very strong, it is considered to be a scientific law. Examples include the laws of thermodynamics discussed in section 8.3.



When the gelatin medium hardened, individual bacteria produced separate colonies. Despite its advantages, gelatin was not an ideal solidifying agent because it can be digested by many bacteria and melts at temperatures above 28°C. A better alternative was provided by Fannie Eilshemius Hesse, the wife of Walther Hesse, one of Koch's assistants (**figure 1.7**). 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. Furthermore, once melted, it did not solidify until it reached a temperature of 50°C, eliminating the need to handle boiling liquid and providing time for manipulation of the medium. Some of the media developed by Koch and his associates, such as nutrient broth and nutrient agar, are still widely

Disease

1.2 Koch's Molecular Postulates

Although the criteria that Koch developed for proving a causal relationship between a microorganism and a specific disease have been of great 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 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.

Figure 1.7 Fannie Eilshemius (1850–1934) and Walther Hesse (1846–1911). Fannie Hesse suggested to her husband Walther (a physican and bacteriologist) that he should try using agar in his culture medium when more typical media failed to meet his needs.

used. Another important tool developed in Koch's laboratory was a container for holding solidified media—the petri dish (plate), named after Richard Petri, who devised it. These developments directly stimulated progress in all areas of bacteriology. Culture media (section 5.7); Isolation of pure cultures (section 5.8)

Viral pathogens were also studied during this time. 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. Dimitri Ivanowski and Martinus Beijerinck (pronounced "by-a-rink") used the filter to study tobacco mosaic disease. They found that plant extracts and sap from diseased plants were infectious, even after being filtered with Chamberland's filter. Because the infectious agent passed through a filter that was designed to trap bacterial cells, the agent must be something smaller than a bacterium. Beijerinck proposed that the agent was a "filterable virus." Eventually viruses were shown to be tiny, acellular infectious agents. Early development of virology (section 16.1)

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 material from cowpox lesions to protect people against smallpox. 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. Control of epidemics: Vaccines and immunizations (section 36.8)

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 1.8**). He called these cells phagocytes and the process phagocytosis [Greek *phagein,* eating].

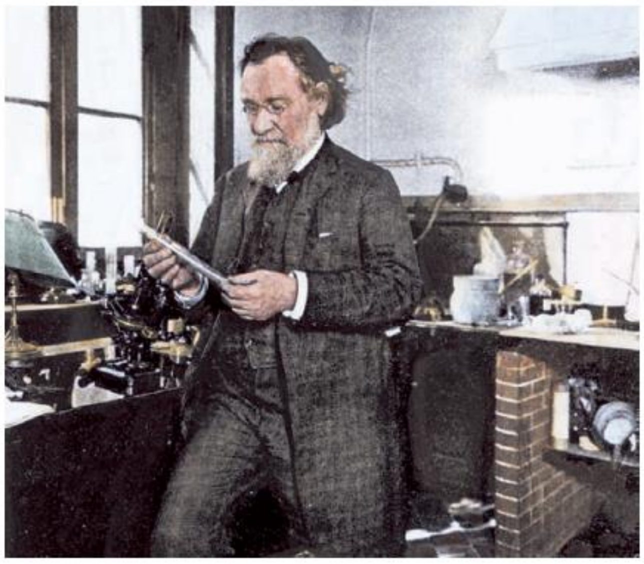


Figure 1.8 Elie Metchnikoff. Metchnikoff (1845–1916) shown here at work in his laboratory.

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 is a pure culture? Why are pure cultures important to Koch's postulates?
4. Would microbiology have developed more slowly if Fannie Hesse had not suggested the use of agar? Give your reasoning.
5. What are Koch's molecular postulates? Why are they important?
6. Some individuals can be infected by a pathogen yet not develop disease. In fact, some become chronic carriers of the pathogen. How does this observation impact Koch's postulates? How might the postulates be modified to account for the existence of chronic carriers?
7. Describe the scientific method in your own words. How does a theory differ from a hypothesis? Why is it important to have a control group?
8. How did von Behring and Metchnikoff contribute to the development of immunology?

1.5 The Development of Industrial Microbiology and Microbial Ecology

Although humans had unknowingly exploited microbes for thousands of years, industrial microbiology developed in large part from the work of Louis Pasteur and others on the alcoholic fermentations that yielded wine and other alcoholic beverages. In 1837, when Theodore Schwann and others proposed that yeast cells were responsible for the conversion of sugars to alcohol, 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; he believed that fermentations were carried out by living organisms. 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 that produced 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 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. Controlling food spoilage (section 40.3)

Microbial ecology developed when 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. The Russian microbiologist Sergei Winogradsky (1856–1953) 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 Beijerinck (1851–1931) 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 also developed the enrichment-culture technique and the use of selective media, which have been of such great importance in microbiology. Biogeochemical cycling (section 27.2); Culture media (section 5.7)

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

3. Leeuwenhoek is often referred to as the Father of Microbiology. However, many historians feel that Louis Pasteur, Robert Koch, or perhaps both, deserve that honor. Who do you think is the Father of Microbiology? Why?
4. Consider the discoveries described in sections 1.2 to 1.5. Which do you think were the most important to the development of microbiology? Why?

1.6 The Scope and Relevance of Microbiology

As the late scientist-writer Steven Jay Gould emphasized, we live in the Age of *Bacteria.* They were the first living organisms on our planet and live virtually everywhere life is possible. Furthermore, the whole biosphere depends on their activities, and they influence human society in countless ways. Because microorganisms play such diverse roles, 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. One indication of the importance of microbiology is the Nobel Prize given for work in physiology or medicine. About one-third of these have been awarded to scientists working on microbiological problems (*see inside front cover*).

Microbiology has both basic and applied aspects (**figure 1.9**). The basic aspects are concerned with the biology of microorganisms themselves and include such fields as bacteriology, virology, mycology (study of fungi), phycology or algology (study of algae), protozoology, microbial cytology and physiology, microbial genetics and molecular biology, microbial ecology, and microbial taxonomy. The applied aspects are concerned with practical problems such as disease, water and wastewater treatment, food spoilage and food production, and industrial uses of microbes. It is important to note that the basic and applied aspects of microbiology are intertwined. Basic research is often conducted in applied fields and applications often arise out of basic research. A discussion of some of the major fields of microbiology and the occupations they provide follows.

One of the most active and important fields in microbiology is medical microbiology, which deals with diseases of humans and animals. Medical microbiologists identify the agents causing infectious diseases and plan measures for their control and elimination. Frequently they are involved in tracking down new, unidentified pathogens such as the agent that causes variant Creutzfeldt-Jakob disease, (the human version of "mad cow disease") the hantavirus, the West Nile virus, and the virus responsible for SARS. These microbiologists also study the ways in which microorganisms cause disease. Arthropod-borne viral diseases (section 37.2); Microbial Diversity & Ecology 18.1: SARS: Evolution of a virus

Public health microbiology is closely related to medical microbiology. Public health microbiologists try to identify and 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. Techniques & Applications 32.2: Monoclonal Antibody Technology

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.

Microbial ecology is concerned with the relationships between microorganisms and the components of their living and nonliving habitats. Microbial ecologists study the global and local contributions of microorganisms to the carbon, nitrogen, and sulfur cycles. 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.

Scientists working in food and dairy microbiology try to prevent microbial spoilage of food and the transmission of foodborne diseases such as botulism and salmonellosis. 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. Microbiology of food (chapter 40)

In 1929, Alexander Fleming discovered that the fungus *Penicillium* produced what he called penicillin, the first antibiotic that could successfully control bacterial infections. Although it took World War II for scientists to learn how to mass produce it, scientists soon found other microorganisms capable of producing additional antibiotics as well as compounds such as citric acid, vitamin B_{12}, and monosodium glutamate. Today, industrial microbiologists use microorganisms to make products such as antibiotics, vaccines, steroids, alcohols and other solvents, vitamins, amino acids, and enzymes. Industrial microbiologists identify microbes of use to industry. They also engineer microbes with desirable traits and devise systems for culturing them and isolating the products they make.

Microbiologists working in microbial physiology and biochemistry study many aspects of the biology of microorganisms. They may study the synthesis of antibiotics and toxins, microbial energy production, the ways in which microorganisms survive harsh environmental conditions, microbial nitrogen fixation, and the effects of chemical and physical agents on microbial growth and survival.

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 structure and function. Microbial geneticists play an important role in applied microbiology because they develop techniques that are useful in agricultural microbiology, industrial microbiology, food and dairy microbiology, and medicine.

1. Briefly describe the major subdisciplines in microbiology.
2. Why do you think microorganisms are so useful to biologists as experimental models?
3. List all the activities or businesses you can think of in your community that are directly dependent on microbiology.

(a) Rita Colwell (b) R. G. E. Murray (c) Stanley Falkow (d) Martha Howe (e) Frederick Neidhardt (f) Jean Brenchley

Figure 1.9 Important Contributors to Microbiology. **(a)** Rita 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 Neidhardt has contributed to microbiology through his work on the regulation of *E. coli* physiology and metabolism, and by coauthoring advanced textbooks. **(f)** Jean 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.

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, microbiology has great 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? A discussion of some aspects of the future of microbiology follows.

Medical microbiology, public health microbiology, and immunology will continue to be areas of intense research. New infectious diseases are continually arising and old diseases are once again becoming widespread and destructive. AIDS, SARS, 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. They will also need to find ways to stop the spread of established infectious diseases, as well as the spread of multiple antibiotic resistance, which can render a pathogen resistant to current medical treatment. Microbiologists will also be called upon to create new drugs and vaccines, to study the association between infectious agents and chronic disease (e.g., autoimmune and cardiovascular diseases), and to further our understanding of host defenses and how pathogens interact with host cells. It will be necessary to use techniques in molecular biology and recombinant DNA technology to solve many of these problems.

Industrial microbiology and environmental microbiology also face many challenges and opportunities. 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 serve as sources of high-quality food and other practical products such as enzymes for industrial applications. They may also be used to degrade pollutants and toxic wastes and as vectors to treat diseases and enhance agricultural productivity. There also is a continuing need to protect food and crops from microbial damage.

The development of techniques, especially DNA-based techniques, that allow the study of microorganisms in their natural environment has greatly stimulated research in microbial ecology. Several areas of research will continue to be important. Understanding microbial diversity is one area that requires further research. It is estimated that less than 1% of Earth's microbes have been cultured. Greater efforts to grow previously uncultivated microbes will be required. Much work also needs to be done on microorganisms living in extreme environments. The discovery of new and unusual microorganisms may well lead to further advances in the development of new antimicrobial agents, industrial processes, and bioremediation. Another area of increasing interest to microbial ecologists is biofilms. Microbes often form biofilms on surfaces, and in doing so exhibit a physiology that differs from that observed when they live freely or planktonically. For instance, microbes in a biofilm are often more resistant to killing agents than they are when not in a biofilm. Biofilms are not only of interest to microbial ecologists; they can form on human tissues, on indwelling catheters, and on other man-made medical devices. In fact, microbial ecologists and medical microbiologists now understand that microorganisms are essential partners with higher organisms. Greater knowledge of the nature of these symbiotic relationships can help improve our appreciation of the living world. It also will lead to new approaches in treating infectious diseases in livestock and in humans.

The fields of genomics and proteomics have and will continue to have a tremendous impact on microbiology. The genomes of many microorganisms have 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 function and for providing insights into fundamental questions in biology, such as how complex cellular structures develop and how cells communicate with one another and respond to the environment. 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.

Perhaps the biggest challenge facing microbiologists will be to assess the implications of new discoveries and technological developments. The pace of these discoveries and developments is very rapid, and sometimes it is difficult for nonscientists to follow and assess them. Microbiologists will need to communicate a balanced view of both the positive and the negative long-term impacts of these developments on society.

Clearly, 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.

1. What do you think are the five most important research areas to pursue in microbiology? Give reasons for your choices.

Summary

1.1 Members of the Microbial World

a. Microbiology studies microscopic organisms that are often unicellular, or if multicellular, do not have highly differentiated tissues. The discipline is also defined by the techniques it uses—in particular, those used to isolate and culture microorganisms.

b. Procaryotic cells differ from eucaryotic cells in lacking a membrane-delimited nucleus, and in other ways as well.

c. Microbiologists divide organisms into three domains: *Bacteria*, *Archaea*, and *Eucarya*.

d. Domains *Bacteria* and *Archaea* consist of procaryotic microorganisms. The eucaryotic microbes (protists and fungi) are placed in *Eucarya*. Viruses are acellular entities that are not placed in any of the domains but are classified by a separate system.

1.2 The Discovery of Microorganisms

a. Antony van Leeuwenhoek was the first person to extensively describe microorganisms.

1.3 The Conflict Over Spontaneous Generation

a. Experiments by Redi and others disproved the theory of spontaneous generation in regard to larger organisms.

b. The spontaneous generation of microorganisms was disproved by Spallanzani, Pasteur, Tyndall, and others.

1.4 The Golden Age of Microbiology

a. 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.

b. Koch's postulates and molecular Koch's postulates are used to prove a direct relationship between a suspected pathogen and a disease.

c. Koch developed the techniques required to grow bacteria on solid media and to isolate pure cultures of pathogens.

d. Vaccines against anthrax and rabies were made by Pasteur; von Behring and Kitasato prepared antitoxins for diphtheria and tetanus.

e. Metchnikoff discovered some blood leukocytes could phagocytize and destroy bacterial pathogens.

1.5 The Development of Industrial Microbiology and Microbial Ecology

a. Pasteur showed that fermentations were caused by microorganisms and that some microorganisms could live in the absence of oxygen.

b. The role of microorganisms in carbon, nitrogen, and sulfur cycles was first studied by Winogradsky and Beijerinck.

1.6 The Scope and Relevance of Microbiology

a. In the twentieth century, microbiology contributed greatly to the fields of medicine, genetics, agriculture, food science, biochemistry, and molecular biology.

b. 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.

1.7 The Future of Microbiology

a. 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

algae 3	fungi 3	procaryotic cell 2	spontaneous generation 6
Archaea 3	Koch's postulates 9	protists 3	viruses 3
Bacteria 2	microbiology 1	protozoa 3	water molds 3
eucaryotic cell 1	microorganism 1	slime molds 3	

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 and 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 fulltime 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?

Learn More

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