**The Microbial World**

CHAPTER

1

# Summary

Chapter 1 introduces the study of microbiology for students, most of whom will have had little or no exposure to the subject. Consequently, you have a good opportunity to provide an overview of microbiology that demonstrates the important roles microorganisms (or *microbes*) play in human activities and in the ecology of the entire biosphere. The ability of microorganisms to exist independently in nature as free-living cells within larger microbial communities (Figure 1.1) confers enormous adaptive advantages over cells of macroorganisms, which are incapable of an independent existence.

# 1.1 | Microorganisms, Tiny Titans of the Earth

Describe to students ways in which microbiology serves as both a basic and applied biological science. From a basic perspective, the study of microorganisms in laboratory culture has been the primary means by which the fundamental genetic and biochemical properties of living cells have been revealed (Figure 1.2). From an applied perspective, microorganisms directly affect the quality of human life in both detrimental and beneficial ways. Although microorganisms are the causative agents of some of the most important human, animal, and plant diseases, they are also used for the industrial production of antibiotics, pharmaceuticals, and foods. Microbes are also increasingly being used for beneficial purposes as diverse as bioremediation of polluted sites, gene therapies for genetic diseases, and the production of biofuels. Microbiology is therefore a science of far-reaching scope, with applications that affect the quality of human life in a variety of ways.

You should also emphasize to students the importance of microorganisms in the emergence and maintenance of higher forms of life. From the production of molecular oxygen (by cyanobacteria) to the biogeochemical cycling of key elements, such as carbon, nitrogen, and sulfur, microorganisms play a major role in sustaining all life on the planet. Point out in your course introduction that for all the reasons summarized in this section, microbiology is the foundation of all biological sciences.

# 1.2 | Structure and Activities of Microbial Cells

Because microorganisms generally exist as free-living cells, it is important to discuss the characteristics of cells in general. Emphasize that all cells exhibit a nonrandom organization with a semipermeable membrane boundary that encompasses an internal system that is not in equilibrium with its environment. Point out that prokaryotic cells (i.e., all *Bacteria* and *Archaea*) do not contain membrane-bound, internal organelles as traditionally described for eukaryotic cells (the *Eukarya*; Figure 1.3). In particular, the organization of prokaryotic DNA as a single chromosome in an arrangement called a *nucleoid*, an aggregated mass of genetic material within the cytoplasm, is in stark contrast to the compartmentalized, multichromosomal configuration typically found in eukaryotes. However, despite the structural and morphological similarities of *Bacteria* and *Archaea*, make sure your students are aware early on that these groups of microorganisms have quite distinct evolutionary lineages and are, therefore, not closely related on a genetic level. This concept is discussed in more detail in Section 1.3.

The ability of cells to maintain a thermodynamic energy flow far from equilibrium defines what we refer to as a *living system*. All living systems display some form of enzyme-driven metabolism in which both energy-yielding (catabolic) and energy-consuming (anabolic) biochemical reactions are catalyzed concurrently. These chemical transformations allow for biosynthesis of new cell structures and, ultimately, cell division (microbial *growth*). Figure 1.4 shows the characteristics that define cellular life, some of which are universal (e.g., metabolism and evolution) and some of which occur only in some cells (e.g., differentiation and motility).

# 1.3 | Microorganisms and the Biosphere

Although Earth is believed to be about 4.6 billion years old, several lines of evidence indicate that the appearance of multicellular life did not occur until about 600 million years ago (Figure 1.5). By contrast, microbial cells (single-celled prokaryotes) likely appeared between 3.8 and 3.9 billion years ago and therefore were the *only* inhabitants of the planet for about 80% of its history! The appearance of multicellular life was necessarily preceded by the accumulation of significant amounts of molecular oxygen (O2) in the atmosphere, a phenomenon that resulted from the activity of cyanobacteria and the evolution of a chlorophyll molecule that could use water (H2O) as electron donor (see Chapter 13). Develop this concept for students in some detail at an early stage in the course to provide proper historical perspective of important events in cellular evolution. You may wish to start with the evidence supporting the hypothesis that the first self-replicating “entities” may have been RNA molecules, and the incorporation of these entities within lipid membranes may have represented the first cell type(s). This topic will be more thoroughly discussed in Chapter 13.

Discuss with your students some of the events that led to multicellular forms of life. The evolution of anaerobic phototrophic metabolisms likely began within 500 million years after the appearance of the first cells, followed nearly a billion years later by the evolution of cyanobacteria from these early phototrophs (Figure 1.6), thus starting the slow process of oxygenating the atmosphere and the evolutionary path to multicellularity.

Impress upon your students the enormous abundance of microorganisms in the biosphere; the total number of microbial cells on Earth is estimated to be about eight orders of magnitude greater than the number of stars in the universe! Emphasize that this vast quantity of microbial biomass sequesters more life-sustaining nutrients (e.g., carbon, nitrogen, and phosphorus) than all plants and animals combined (Figure 1.7).

Introduce the students to *microbial ecology*, the exciting subdiscipline of microbiology that focuses on the interactions and influence of microbial communities on the macroscopic world around them. The biochemical conversions and molecular interactions of microorganisms significantly control the balance and community structure of all living organisms in an ecosystem. In fact, the very existence of multicellular life depends upon the nutrient cycling activities of microorganisms. The effect of microbes on animals, plants, and global ecosystems is a topic interwoven throughout a number of chapters in the text. These concepts will come as a surprise for most students—who generally consider microorganisms “simple,” insignificant creatures that only cause disease—and will set the stage for more detailed study in future chapters.

Finally, point out that microorganisms can be found in even the most inhospitable environments by human standards. Students are often amazed at the extreme conditions in which microorganisms can thrive (Table 1.1). Point out that even in punishingly harsh ecosystems, such as in volcanic hot springs or freezing Antarctic lakes, microbes called *extremophiles* are able to exist or even thrive in conditions that define the physiochemical limits to life on Earth. These communities contain a reservoir of genetic diversity that is largely unexplored and that could benefit humankind in as yet unknown ways.

# 1.4 | The Impact of Microorganisms on Human Society

Students probably understand the importance of microorganisms as agents of infectious disease, and indeed, this has been a driving force in the development of microbiology as a distinct scientific discipline. The effects of microorganisms upon human affairs have been remarkable, particularly when one compares the foremost causes of death in the United States at the beginning of the twentieth century (before the widespread use of antibiotics and vaccines) with the major causes of death in this century (Figure 1.8). While the deleterious effects of pathogenic bacteria to human wellness should be discussed here, it is important to also stress the key beneficial roles microorganisms play in agriculture (Figure 1.9), in human digestive health (Figure 1.10), in the food and beverage industries (Figure 1.11), and in the growing fields of biotechnology and alternative and renewable energy (Figure 1.12). With this discussion, students should become aware of the tremendous influence of microorganisms in human society.

The genomes of microorganisms hold incredible potential for the production of useful and valuable products. The traditional methods of employing microorganisms for human use (e.g., fermentation) have evolved into the large-scale, commercial ventures of *industrial microbiology* and *biotechnology*. Whereas industrial microbiology exploits microbial activities in the production of large quantities of lower cost commodities, such as solvents and enzymes, the “biotech” industry uses genetically engineered microorganisms to produce smaller quantities of specialized products of high value, such as certain pharmaceuticals. An excellent example is the production of human insulin by *Escherichia coli*, an innovation that made the treatment of diabetes affordable for people from all economic backgrounds. In addition to producing a product directly, bacterial cells can be used as a vehicle to introduce specific genes into the genomes of other organisms for commercial production of a desired product. For example, genetic engineering of *Agrobacterium tumefaciens*, a plant pathogen that naturally transfers DNA directly into plant cells upon infection, has led to the use of plants for the production of human antibodies that have potential anticancer or antiviral properties.

One area of extensive research in industrial microbiology is the production of *biofuels*. These products are often used to supplement petroleum-based fuels and may include methane, ethanol, biodiesel, hydrogen (H2), and other fuels derived from microbial metabolisms. Microorganisms are also being used extensively to restore polluted natural areas in a process called *bioremediation*. By applying specific microorganisms (or nutrients to stimulate their growth) to polluted sites, contaminants such as pesticides, heavy metals, and crude oil and other hydrocarbons can all be cleaned successfully and thoroughly.

# 1.5–1.8 | Microscopy and the Origins of Microbiology

It is important to emphasize the historical roots of microbiology, particularly considering its rather recent beginnings as a systematic field of science in the nineteenth century and the fact that several new fields have arisen from this discipline (e.g., molecular biology and biotechnology). Lead the students on a chronological journey, beginning with Robert Hooke’s first description of a microorganism (a fungal mold) in 1665 (Figure 1.13) and Antoni van Leeuwenhoek’s microscope and publication of the first observation of bacteria in 1684 (Figure 1.14).

Following your introduction of the pioneers of microscopy, provide the students with an overview of microscopic methods in common usage today. The variety of methods available for observing microorganisms must be introduced early, as much of the presentation of structure–function relationships depends upon the excellent micrographs that appear throughout the book. Although details of microscopy are more easily introduced in the laboratory portion of the course, the material included here is pertinent to effective lecture presentation.

* + - Discuss the basic principles and components of the compound light microscope, including the relationships between resolution and magnification, and numerical aperture (Figure 1.15). Note that although *bright-field microscopy* is fine for visualizing pigmented cells (Figure 1.16), it is not an efficient tool for viewing unstained cells with no natural pigmentation, such as nonphototrophic bacteria.
    - This deficiency will lead to a discussion of various methods employed to increase contrast. Discuss the various simple dyes used to stain cells, most of which are positively charged, basic dyes capable of binding to negatively charged cell surfaces (e.g., methylene blue and crystal violet; Figure 1.17). Continue the discussion of *differential stains*, the most widely used of which is the Gram stain (Figure 1.18).
    - Students should understand that while staining procedures increase the contrast of cells against the background to make them more visible, they also kill cells and may distort their appearance. Discuss *phase-contrast microscopy* and *dark-field microscopy* (Figure 1.19), two tools that allow one to look at living cells without the need for staining.
    - *Fluorescence microscopy* is widely used in clinical diagnostic microbiology and environmental microbiology (Figure 1.20). Most students who enter the biotechnology industry or medical profession will work with fluorescent molecules (such as those used for fluorescence antibody staining methods). The variety and sensitivity of these molecules have increased dramatically over the past decade. This has allowed the development of a wide variety of nonradioactive alternatives to biological assays that are now routinely used in research.
    - Students should be interested in the micrographs from three-dimensional imaging of cells. Depending upon the level of the course, you may choose to discuss the principles of *differential interference contrast microscopy* (Figure 1.21) and *confocal scanning laser microscopy* (Figure 1.22). Lastly, show and discuss the micrographs obtained from *electron microscopy* (Figures 1.23 and 1.24). Note the differences between scanning electron microscopy (SEM), which provides an image of the external features of a specimen, and transmission electron microscopy (TEM), in which thin sections of the specimen show its detailed internal structure.

# 1.9 | Pasteur and Spontaneous Generation

As a young researcher, Louis Pasteur was formally trained as a chemist with expertise in the field of crystallography. His knowledge of the optical properties of crystals provided the basis for his early investigations into the biological sciences. Begin the discussion of his celebrated scientific achievements by pointing out that it was his observation that living organisms discriminate between optical isomers of metabolites during growth (Figure 1.25) that led him to begin a series of experiments that would result in the defeat of the centuries-old theory of spontaneous generation. Pasteur’s experimental design that finally disproved this theory introduced the now famous swan-necked flask (Figure 1.26). His work is a classic example of the effectiveness of using the scientific method and controlled experimentation to resolve questions rather than personal observation and interpretation, which often lead to unsubstantiated (and often erroneous) conclusions. Point out to students that disproving spontaneous generation resulted in the development of effective procedures for controlling microbial growth, including the familiar process of pasteurization. Outline the major career accomplishments of Pasteur, pointing out the fact that although his professional training pertained to chemistry rather than biology, his laboratory experimentation led him to the study of microorganisms.

* As a chemist, Pasteur’s work with optical isomers of amyl alcohol, a byproduct of alcohol fermentation by beets, led him to confirm that fermentation was not a strictly chemical phenomenon but that living yeast cells were required for the process. The outcome of his fermentation studies led him to the classic experiments that defeated spontaneous generation (Figure 1.26).
* Discuss the many other accomplishments of Pasteur, including the development of vaccines for anthrax, fowl cholera, and rabies. The dramatic results of his rabies vaccine, which saved a young boy, startled the scientific world and led the French government to build the Pasteur Institute in Paris in 1888 (Figure 1.27). Together with Robert Koch, whose scientific achievements are discussed next, Pasteur played a major role in establishing the field of medical microbiology.

# 1.10 | Koch, Infectious Diseases, and Pure Cultures

Explain to your students that for centuries many thought that some unknown agent from a diseased person could be transmitted to healthy people, but no real proof of this existed until the work of Robert Koch (Figure 1.28). Although Koch was 21 years younger than Pasteur, several of their greatest achievements were carried out around the same time during the late 1800s. The following list serves as an introduction to the works of Koch for your students.

* + - Koch’s work on the germ theory of disease (1876) was perhaps the most important stimulus for the development of medical microbiology. Given the focus on bioterrorism in recent years, students may find it interesting that Koch’s work leading to this important theory involved the study of the endospore-forming bacterium *Bacillus anthracis*, the causative agent of anthrax. Carefully discuss Koch’s postulates, the use of which has since led to discoveries for the prevention and cure of many infectious diseases (Figure 1.29). However, be sure to note that while Koch’s postulates are the “gold standard” for determining the causative agent of a disease, it is not always possible to carry them out in full, especially considering that many pathogenic bacteria resist laboratory culture. It is in these cases that genomics is proving useful in linking a causative agent to a particular disease in the absence of cultivable microorganisms.
    - Koch’s postulates require that the suspected organism be obtained in pure culture. Koch developed clever ways of obtaining pure cultures, including the use of solidifying agents such as gelatin and agar to obtain single colonies. Point out to students that a colony is a population of cells arising from a single cell, and thus each colony contains a single type of microorganism, an observation that Koch himself was quick to realize.
    - Ferdinand Cohn had also been active in his research during this time, and he and Koch regarded each other’s work with mutual respect. Several of Cohn’s innovations for preventing contamination of pure cultures were adopted by Koch and his associate Walter Hesse (Figure 1.30), thereby aiding many of Koch’s studies of pathogenic bacteria.
    - Koch was the first to isolate *Mycobacterium tuberculosis* and demonstrate that it was the causative agent of tuberculosis (Figure 1.31). Considering the huge number of individuals that succumbed to this disease during Koch’s time (about 15% of all reported deaths), the significance of this achievement could hardly be exaggerated. In addition to this, Koch discovered and characterized *Vibrio cholerae*, the causative agent of the severe diarrheal disease cholera.

# 1.11 | Discovery of Microbial Diversity

Continue your discussion of the historical roots of microbiology by introducing the work of Martinus Beijerinck and Sergei Winogradsky, both of whom are known for their study of microbial diversity. In the same way that Pasteur and Koch propelled the field of medical microbiology to the scientific mainstream, Beijerinck and Winogradsky became the chief figures of environmental microbiology. Both of these researchers were interested in the biogeochemical cycling of sulfur and nitrogen in the environment, including the oxidation of sulfur by both phototrophic and chemolithotrophic bacteria (Figure 1.32) and the reduction of atmospheric nitrogen to ammonia by nitrogen-fixing bacteria (Figure. 1.33). In addition, Beijerinck was the first to describe the existence of subcellular infectious agents of disease (viruses) through his study of tobacco mosaic disease.

* + - Martinus Beijerinck, a Dutchman, developed the concept of enrichment culture technique for the selective culture of specific types of microorganisms from natural samples. The use of selective culture media and incubation conditions was a major innovation for the isolation of novel strains of microorganisms, and the technique is still used extensively today. For example, enrichment cultures are routinely used to isolate novel bacteria for industrial and medical use, such as antibiotic-producing microorganisms from soil (see Chapter 16). It is important to describe the principle of this important technique to students since this concept will be especially relevant for certain laboratory exercises that usually accompany courses in general microbiology.
    - Sergei Winogradsky of Russia used enrichment culture to study bacteria in soil, particularly those that play a role in the cycling of nitrogen and sulfur. He isolated the first nitrogen-fixing bacterium, Clostridium pasteurianum, and developed the concept of bacterial fixation—an important ecological discovery. Winogradsky was also interested in bacteria that oxidize inorganic compounds for energy conservation, including the nitrifying and sulfur-oxidizing bacteria, and he showed that these organisms are autotrophic, using CO2 as their sole source of carbon. Winogradsky was perhaps the first scientist to recognize the metabolic significance of biogeochemical processes. Briefly discuss Winogradsky’s major discoveries: chemolithotrophy and nitrogen fixation.

# 1.12 | Molecular Basis of Life

The foundational work of early microbiologists in the late 1800s led to groundbreaking discoveries in the twentieth century as biologists, including Beijerinck’s protégé Albert Jan Kluyver, realized that microorganisms served as excellent models to elucidate the basic biochemistry of all cells. The principle mystery of living cells in the early 1900s was the identity of the molecule that carries hereditary information. Initially, protein was favored over DNA for this function because of the comparative simplicity of the latter⎯with just four nucleotide bases, DNA was viewed as incapable of accounting for the astounding diversity of life.

Frederick Griffith brought clarity to the issue with his classic experiment using two strains of *Streptococcus pneumoniae*: 1) an encapsulated strain that was highly virulent (called strain “S” because it produced smooth colonies), and 2) a nonencapsulated strain that was harmless (called strain “R” for the rough appearance of its colonies). Griffith was able to show that strain R could become virulent when mixed with killed cells of strain S, confirming that molecules carrying genetic information could be transferred from one cell to another (Figure 1.34). Avery, MacLeod, and McCarty took this line of experimentation further by isolating both proteins and DNA from strain S and subsequently showed that transformation of strain R took place in the presence of DNA, but not protein, from strain S. In this way, they provided the proof needed to confirm DNA as the genetic material in cells.

Just under 10 years later, in 1953, James Watson and Francis Crick would determine the now familiar double-helix structure of DNA with the use of X-ray diffraction images obtained by Rosalind Franklin. With the realization that hereditary information is encoded in the nucleotide base sequences of DNA, random mutations and natural election came together as the drivers of evolutionary change in living organisms.

# 1.13 | Woese and the Tree of Life

A discussion of Carl Woese and his groundbreaking use of comparative DNA sequencing to determine the evolutionary relationships of organisms will nicely round out your treatment of the history of microbiology. Even well into the 1960s, the best efforts to depict the evolutionary history of living organisms were woefully lacking because critical information that would ultimately be gleaned from DNA sequence analyses remained unrealized and unconsidered (Figure 1.35). Painstaking work by Woese in the 1970s changed all of this when he analyzed the sequences of ribosomal RNA (rRNA) genes from different microorganisms and concluded that a taxonomic hierarchy above kingdom⎯the *domain*⎯was necessary for an accurate depiction of the lineage of living organisms.

Discuss the methods now routinely used in the development and refinement of the phylogenetic tree of life (Figure 1.36a). Introduce to your students the enormous impact rRNA gene sequencing has had on our understanding of the classification of living organisms into three distinct domains⎯the *Bacteria*, *Archaea*, and *Eukarya* (Figure 1.36b)⎯and on our ever-expanding understanding of the remarkable extent of microbial diversity on Earth (Figure 1.37). Point out the following reasons why the analysis of rRNA gene sequences is ideal for phylogenetic studies:

* rRNA genes are present in all organisms.
* Their function is constant across all levels of life.
* Because functioning ribosomes are absolutely necessary, rRNA genes are highly conserved in all cells.
* rRNA gene sequences are long enough to provide statistically significant information.

# 1.14 | An Introduction to Microbial Life

The final section of the chapter provides an overview of the key factors that distinguish cells of the different domains of life and how these are further distinguished from acellular microbes⎯the viruses. Figure 1.38 beautifully compares the size relationships of a variety of microbial forms and components, from the uncommonly large bacterium *Epulopiscium fishelsoni* at a length of 600 μm (see also Figure 2.2) to individual proteins at the limit of resolution of an electron microscope  
(0.2 nm). Mention the following key points to conclude the chapter:

* The greatest diversity of *Bacteria* and *Archaea* has never been cultured in the laboratory, and such species are known only by their DNA sequences found in environmental samples.
* Although they both have a prokaryotic cell structure, *Bacteria* and *Archaea* are not closely related. On a molecular level, *Archaea* have much in common with the eukaryotes.
* The metabolic and physiological diversity of microorganisms, especially the *Bacteria* and *Archaea*, is astonishing, with species capable of aerobic respiration, anaerobic respiration, fermentation, and/or phototrophy inhabiting nearly every imaginable habitat on Earth.
* Microorganisms of the domain *Eukarya* include the fungi, protists (including the protozoa and algae), and helminths (roundworms and flatworms).
* The *Eukarya* lineage is evolutionarily younger than either the *Bacteria* or the *Archaea*.
* Because viruses are acellular and incapable of autonomous reproduction, most microbiologists consider them to be nonliving biological entities.
* Viral genomes may be either DNA or RNA, but not both. Whether DNA or RNA, their genomes may be single- or double-stranded. All cells have double-stranded DNA genomes.

## Answers to Chapter Review Questions

1. Arising from a single cell, a bacterial colony is a macroscopically visible population of genetically identical cells growing on the surface of a solid medium. The original cell is deposited on a suitable growth medium, and subsequent cell division and exponential growth lead to the formation of the visible mass of cells.
2. The Bacteria and Archaea consist of prokaryotic, generally free-living cells having a nucleoid rather than a membrane-bound nucleus. The Eukarya are eukaryotic cells (or multicellular organisms consisting of eukaryotic cells) that are generally significantly larger than prokaryotes and have a greater degree of complexity, with a defined nucleus and membrane-bound organelles, such as mitochondria, chloroplasts, and lysosomes. For cells that possess them, a cell wall is a rigid structure that defines the cell’s shape and surrounds the cell (cytoplasmic) membrane. Its composition is primarily polysaccharide, and its major function is to provide structural support and protect the cell from osmotic lysis. By contrast, the cell membrane is a thin structure composed of lipids that provides a semipermeable barrier to separate the cell’s interior from the external environment. Whereas all cells have a cell membrane, cell walls are not universal. They are found in most prokaryotes, algae, fungi, and plants, but animal cells and protozoa do not have cell walls.
3. The early Earth was a hot and sterile place. As the first forms of life, microbial cells are believed to have dominated Earth for most of its history. The evolution of cyanobacteria had a profound effect on the biology of the planet, as it resulted in the oxygenation of the atmosphere. For the 2 billion years before this event, only single-celled microorganisms capable of anaerobic metabolisms inhabited the planet. The evolution of a metabolism that could transfer electrons extracted from reduced organic carbon molecules to O2 as an electron acceptor (i.e., aerobic respiration) permitted the generation of much larger amounts of adenosine triphosphate (ATP) (due to the highly electropositive reduction potential of O2, described in Chapter 3) and the eventual evolution of the eukaryotic cell and multicellularity. Three domains of life exist today: the Bacteria, Archaea, and Eukarya. It is generally believed that all three domains are derived from a single ancestral cell, the last universal common ancestor.
4. Microorganisms carry out many important functions, with only a small percentage of bacteria (<1%) capable of causing disease. In fact, the human body has more bacterial cells on and in it than human cells, and while a small fraction of these microorganisms are potentially pathogenic, the vast majority are commensal species essential for maintaining proper human health. In addition, some bacteria fix nitrogen (N2) and are thus important in agriculture. Others are important in food and beverage production. Most microorganisms play crucial ecological roles in the environment.
5. Magnification is the optical enlargement of an object, whereas resolution is the ability to distinguish two objects as distinct and separate, especially in reference to microscopic observation. Although magnification can be increased virtually without limit, increases in resolution are limited by the physical properties of light.
6. Cells are stained to increase their contrast so that they can be more easily seen against the background. In bright-field microscopy, cationic (positively charged) dyes are used because they combine with negatively charged cellular constituents, such as nucleic acids, teichoic acids in the gram-positive cell wall, and acidic polysaccharides. Phase-contrast microscopy exploits the principle that different substances refract light differently. By amplifying these differences using a phase ring, living, unstained specimens can be quickly and easily viewed. Therefore, unlike bright-field microscopy, phase-contrast microscopes can be used to observe living specimens in wet mount preparations with good contrast against the lit background.
7. Fluorescence microscopes apply specific wavelengths of light to the specimen, which then fluoresces a different color based on its own natural pigments or on previous application of fluorescent stains. Confocal scanning laser microscopy takes this a step further by using a laser to cause multiple planes of focus throughout the specimen to fluoresce. The images produced from each layer can then be combined to provide a three-dimensional image. A differential interference contrast (DIC) microscope polarizes light, generating two distinct beams. When these beams recombine, they are not completely in phase, and therefore subtle differences within the cell are intensified. Because of this, specific cellular structures have a three-dimensional appearance, and harsh staining techniques become unnecessary. Imaging of this type is not possible with a bright-field microscope.
8. Whereas light microscopes use a beam of light and objective lenses to view the specimen, electron microscopes use an electron beam in a vacuum and electromagnets as lenses to visualize the specimen. Light microscopes can be used to view living cells, but harsh sample preparation methods prevent this in electron microscopy. However, at 0.2 nm, the resolution of electron microscopes is about 1000 times greater than that of light microscopes, and therefore much greater magnification of the specimen is possible using electron microscopy. Scanning electron microscopy is used to view the three-dimensional features of a cell.
9. Pasteur’s experiments using swan-necked flasks silenced the objection of vitalists who claimed that sealing the mouth of a flask blocked the availability of open air, which they viewed as being essential for spontaneous generation (they were entirely unaware of the existence of anaerobes). Pasteur definitively showed that, even in open air, putrefaction of the heated broth did not occur unless the flask was tipped such that the broth came in contact with dust particles trapped in the neck of the flask, thus proving that the microorganisms that contaminated the broth were carried in the air.
10. Koch’s postulates are a set of criteria for proving that a specific type of microorganism causes a specific disease. The main points are as follows: (1) The suspected microorganism must be present in all cases of disease and not present in healthy individuals; (2) the organism must be cultivated from diseased individuals and isolated in pure culture; (3) inoculation of a healthy individual with organisms from the pure culture must initiate disease in susceptible animals; and (4) the organism should be re-isolated from these experimental animals in pure culture and be identified as the same infecting organism as that found in the original diseased animal. Investigators still use these postulates as a guide to determine the causative agents of diseases and for developing successful treatments for the prevention and cure of many infectious diseases.
11. While Robert Koch was an eminent early medical microbiologist, Beijerinck and Winogradsky were responsible for developing the field of microbial ecology as it relates to the environment rather than the human body. Their primary contributions to microbiology were in the design and implementation of the enrichment culture technique and the isolation and characterization of bacteria responsible for cycling nitrogen and sulfur, including those having chemolithoautotrophic metabolisms. Before Winogradsky’s work, the use of carbon dioxide (CO2) as the sole source of carbon by nonphotosynthetic organisms was unknown. Winogradsky isolated and described the first nitrogen-fixing bacterium, the anaerobe Clostridium pasteurianum. Later, Beijerinck described the first aerobic nitrogen-fixing bacterium, Azotobacter chroococcum, from a soil sample.
12. The experiments that proved DNA to be the molecule of heredity were those of Griffith and Avery, MacLeod, and McCarty, described in Section 1.12 above.
13. The key insight in reconstructing the tree of life was Carl Woese’s realization that directly comparing the DNA sequences of a universal, highly conserved gene of sufficient length would provide an accurate depiction of evolutionary lineages. Based on this reconstruction, it is clear that the Bacteria are more closely related to Archaea than to Eukarya, since the Bacteria and Archaea diverge from one another while the Eukarya later diverge from the Archaea.
14. Answers will vary, but the bulleted list in Section 1.14 above contains several major distinguishing features of viruses, Bacteria, Archaea, and Eukarya.

## Answers to Application Questions

1. Before Pasteur’s experiments, and despite some evidence to the contrary, it was thought that microorganisms could arise spontaneously in or on the surface of a nutrient-containing medium and, by extension, in any environment. Pasteur showed that microorganisms must be carried into the environment from the outside. A medium that was initially sterile remained so until a living cell from outside was introduced. Pasteur’s work provided some of the basic concepts for the development of aseptic technique, as well as methods of food preservation, such as canning and pasteurization. Moreover, with this development, it became possible to think of microorganisms as the causative agents of disease.
2. Koch used the following methodology, which became known as Koch’s postulates, to determine the causative agent of a disease:
   1. The organism must be isolated from the diseased animal and grown in pure culture.
   2. The organism must not be present in healthy animals.
   3. When the microorganism is injected into a healthy animal, the disease must occur.
   4. The pathogenic agent must be re-isolated from the diseased animal.

Koch succeeded in isolating the organism we now know as *Mycobacterium tuberculosis* from every animal that died from the disease (postulates *a, b*). He injected cells of a pure culture of *M. tuberculosis* into healthy guinea pigs, and these guinea pigs developed systemic tuberculosis (postulate *c*). These microorganisms were then re-isolated from the newly diseased animals and were shown to be *M. tuberculosis* (postulate *d*). Thus, all four criteria were met.

1. The sudden elimination of all microorganisms from Earth would result in the rapid disappearance of animal and plant life for a number of reasons. Nitrogen-fixing bacteria provide the reduced nitrogen source for the nucleic acids and proteins found in plants and animals, and cessation of that process alone would eliminate higher organisms. In addition, photosynthesis by cyanobacteria would cease, causing a huge deficiency in both primary production (the conversion of inorganic forms of carbon, such as CO2, to organic forms that can be catabolized by animals) and the release of O2 to the atmosphere via photosynthesis. By contrast, the elimination of plants and animals would affect only a small percentage of microorganisms. Prokaryotes carry out a variety of aerobic and anaerobic metabolisms and molecular interconversions that are independent of higher organisms; therefore, prokaryotic life would persist.