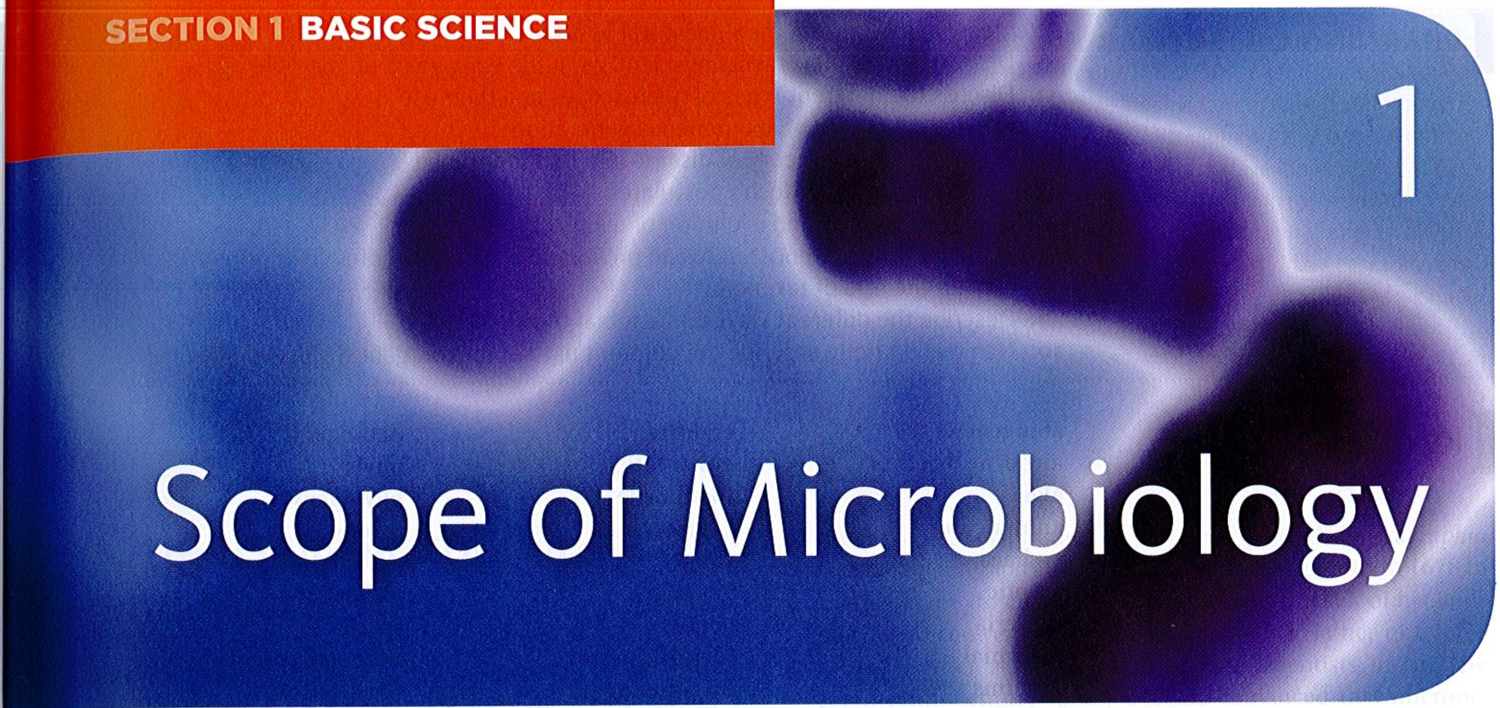
SECTION 1 BASIC SCIENCE



# 1 Scope of Microbiology

## OUTLINE

**Origins of Microbiology and Microscopy**
- Microscopy and Its Founding Fathers
- Types of Microscopes
- Spontaneous Generation
- Pasteurization
- Germ Theory of Disease

**Origin and Evolution of Microorganisms**
- Origin
- Evolution

**Classification of Microorganisms**
- Prokaryotes versus Eukaryotes
- Bacteria, Archaea, and Eukaryotes
- Viruses
- Prions
- Viroids
- Taxonomy

**Microorganisms in Health and Disease**
- Microbes in the Environment
- Normal Flora
- Pathogens

**Applied Microbiology**
- Microorganisms in Food Production
- Microorganisms in the Production of Alcoholic Beverages
- Treatment of Water Supplies
- Microbes and the Production of Pharmaceutical Agents
- Microbes in Agriculture
- Bioremediation
- Microbes, Biomass, and Energy
- Microbial Forensics

## LEARNING OBJECTIVES

*After reading this chapter, the student will be able to:*

1. Describe the achievements of scientists in the early years of microbiology
2. Discuss the different types of microscopes and their use
3. Explain the theory of spontaneous generation
4. Discuss the germ theory of disease and its significance
5. Name and describe Koch's postulates
6. Discuss the origin and evolution of microorganisms
7. Describe the differences between prokaryotes and eukaryotes
8. Explain the role of taxonomy
9. Describe the different ways of microbial transmission
10. Name and briefly describe the different uses of microorganisms in everyday life

## KEY TERMS

| | | |
|---|---|---|
| **abiogenesis** | **endospores** | **pathogenic** |
| **airborne diseases** | **eukaryotic** | **phyla** |
| **algae** | **foodborne diseases** | **phylogeny** |
| **animalcules** | **fungi** | **prions** |
| **archaea** | **genera** | **prokaryotes** |
| **aseptic** | **genus** | **protozoans** |
| **bacteria** | **identification** | **species** |
| **binomial** | **immunology** | **stereomicroscopes** |
| **biofilms** | **Koch's postulates** | **sterilization** |
| **bioremediation** | **light microscopes** | **stromatolites** |
| **classification** | **mutualism** | **synergism** |
| **commensalism** | **nomenclature** | **taxa** |
| **compound microscope** | **normal flora** | **taxonomy** |
| **dissection microscopes** | **parasitism** | **viroids** |
| **domains** | **pasteurization** | **waterborne disease** |
| **electron microscopes** | | |

## WHY YOU NEED TO KNOW

### HISTORY

To see where we are going from where we are, we must know where we have been. Measles, whooping cough, mumps, polio, cholera, influenza, rheumatic fever, pneumonia, diphtheria, tuberculosis, typhoid fever, meningitis, leprosy, syphilis, gonorrhea, tetanus, anthrax, the common cold, chickenpox, smallpox, rabies, encephalitis, malaria, dysentery, numerous epidemics and pandemics, and too many others to list have been with humankind from approximately 4000 BCE to the present. Humankind has lived with diseases and been able to survive with varying degrees of success. Knowledge of the nature of disease has been slow and difficult to obtain. Rational use of this knowledge to alleviate the distress caused by disease has been even slower. Technological advances were and are needed to cope with the problems. For example, cholera—a killer of epidemic proportions—was linked to a public water source by John Snow, an English physician in the mid-1800s. He removed the pump handle, preventing access to the water, and the epidemic ended—ingenious, but serendipitous and of limited use.

### IMPACT

The advancement of microbiology began with Robert Hooke's (1635-1703) observations utilizing the then new compound microscope that could magnify objects 20 to 30 times. Later, Antony van Leeuwenhoek (1632-1723), using his skills at lens grinding and the use of light, improved the resolving power of the microscope to 200 times. His were among the first observations of bacteria and, arguably, the beginning of microbiology. Bacteria were eventually recognized as causative agents of disease. These observations and others led Pasteur and Koch in the late 1800s to develop the germ theory of disease, an understanding that boosted disease prevention and treatment significantly. For example, Robert Koch in 1883 microscopically identified the causative pathogen for cholera *(Vibrio cholerae)*, which he had grown on a plate of agar. After acceptance of the germ theory of disease, new pathogens were reported on the average of every year and a half. Technological advancements in light microscopy and the development of electron microscopy permitted visualization of pathogens or their shadows, allowing assessment of the effectiveness of treatment. Although it was deduced that the causative agent for the influenza pandemic of 1918 was a filterable agent, it was the advent of electron microscopy that allowed visualization of the virus in a rare lung sample from a victim. More recent advances in the biotechnology of genetic analysis have provided information on the nature of the functions of hemagglutinin and neuraminidase, viral coat proteins of the influenza virus. It is hoped that this information will direct investigations for methods of protection from another potential influenza pandemic.

### THE FUTURE

Microbiology not only affords the detailed study of recognized pathogenic microorganisms but is invaluable in the identification of new pathogens in emerging diseases. With the reemergence of diseases previously considered eradicated, such as measles, along with a dramatic increase in strains of drug-resistant microorganisms, the challenges facing microbiologists are growing in number and complexity. Infectious microorganisms found in healthcare facilities such as *Staphylococcus aureus*, enterococci, *Pseudomonas aeruginosa*, and *Clostridium difficile* are becoming increasingly resistant to antibiotics that have long been effective against these organisms. These resistant microorganisms have been christened "superbugs," and their potential effects on human health are alarming. Cephalosporins in many cases are the last line of defense. Clearly, much work lies ahead if we wish to bias the balance in favor of survival.



# Origins of Microbiology and Microscopy

Microbiology is the study of microorganisms, using a variety of techniques for purposes of visualization, identification, and study of their function. The science of microbiology originated with the invention and development of the microscope. Microscopy allowed humans to magnify objects and microorganisms not detectable by the naked eye. Technological advances then have led to the improvement of microscopes, which became an essential investigative tool for biology in general and for the study of cells, tissues, and microorganisms (Figure 1.1) in particular.

## Microscopy and Its Founding Fathers

The development of microscopes started in the sixteenth century and evolved through time into a sophisticated tool used routinely in many branches of science. To this day all different types of microscopes continue to be improved and new ones are being developed.

**Zaccharias** and **Hans Janssen,** a father-and-son team of Dutch eyeglass makers (around the year 1590), found that optical images could be enlarged and viewed using different lenses. The first microscope they produced was a **compound microscope** consisting of a simple tube with lenses at each end. Depending on the size of the diaphragm, the part of the microscope that regulates the amount of light striking the specimen, the magnification of objects under view ranged from 3 times (or ×3) to 9 times (or ×9).

**Antony van Leeuwenhoek** (1632-1723), another native of Holland, is considered to be the father of microscopy and is believed to be the first to observe live bacteria and protozoans. He was fascinated by the power of lenses, which made it possible to observe what the naked human eye could not see. The microscope he used contained only one convex objective lens and is now called a *simple microscope.* His interest in science and his native curiosity led him to some of the most important observations of biology. Van Leeuwenhoek was able to see small life forms that he called **"animalcules"** (little animals). Throughout the years he observed bacteria, protozoans, blood cells, sperm cells, microscopic nematodes, rotifers, and more. Much of his inspiration came from Hooke's *Micrographia* (see next paragraph). He published his observations in 1678 in a letter to the Royal Society of London. As a result of his findings, van Leeuwenhoek is referred to as "the father of microbiology." After some early skepticism, scientists in the late seventeenth century finally became convinced that microorganisms did, in fact, exist. Van Leeuwenhoek did not comment further on the origin of the microorganisms nor did he relate them to any diseases. The definitive relationship between microbes and disease was established later by Hooke, Pasteur, Koch, and others in what became known as the *germ theory of disease.*

**Robert Hooke** (1635-1703), an English scientist with remarkable engineering abilities and an interest in many aspects of science, greatly improved the design and capabilities of the compound light microscope. With his microscope he observed insects, sponges, bryozoans, foraminifera, bird feathers, and plant cells. He published his observations with magnificent drawings in the book *Micrographia.* He was requested by the Royal Society of London to confirm van Leeuwenhoek's finding of animalcules and succeeded in doing so.

Table 1.1 lists some significant events in the history of microbiology.

## Types of Microscopes

With advances in technology, continued development of microscopes for specific uses continues and many kinds of microscopes are now available to scientists. A brief overview and description of light and electron microscopes currently used in teaching, service, and research laboratories follows.

All **light microscopes** use visible light to illuminate, and optical lenses to observe, enlarged images of specimens. They are classified as either simple or compound. A simple light microscope, such as van Leeuwenhoek's, has a single magnifying lens and a visible light source and can magnify objects approximately 266 times (×266).

A *compound light* microscope also uses visible light, usually provided by an electric source, but uses multiple lenses for magnification.

- The lens or lenses close to the eye are called *ocular lenses* and are located in the headpiece of the microscope.

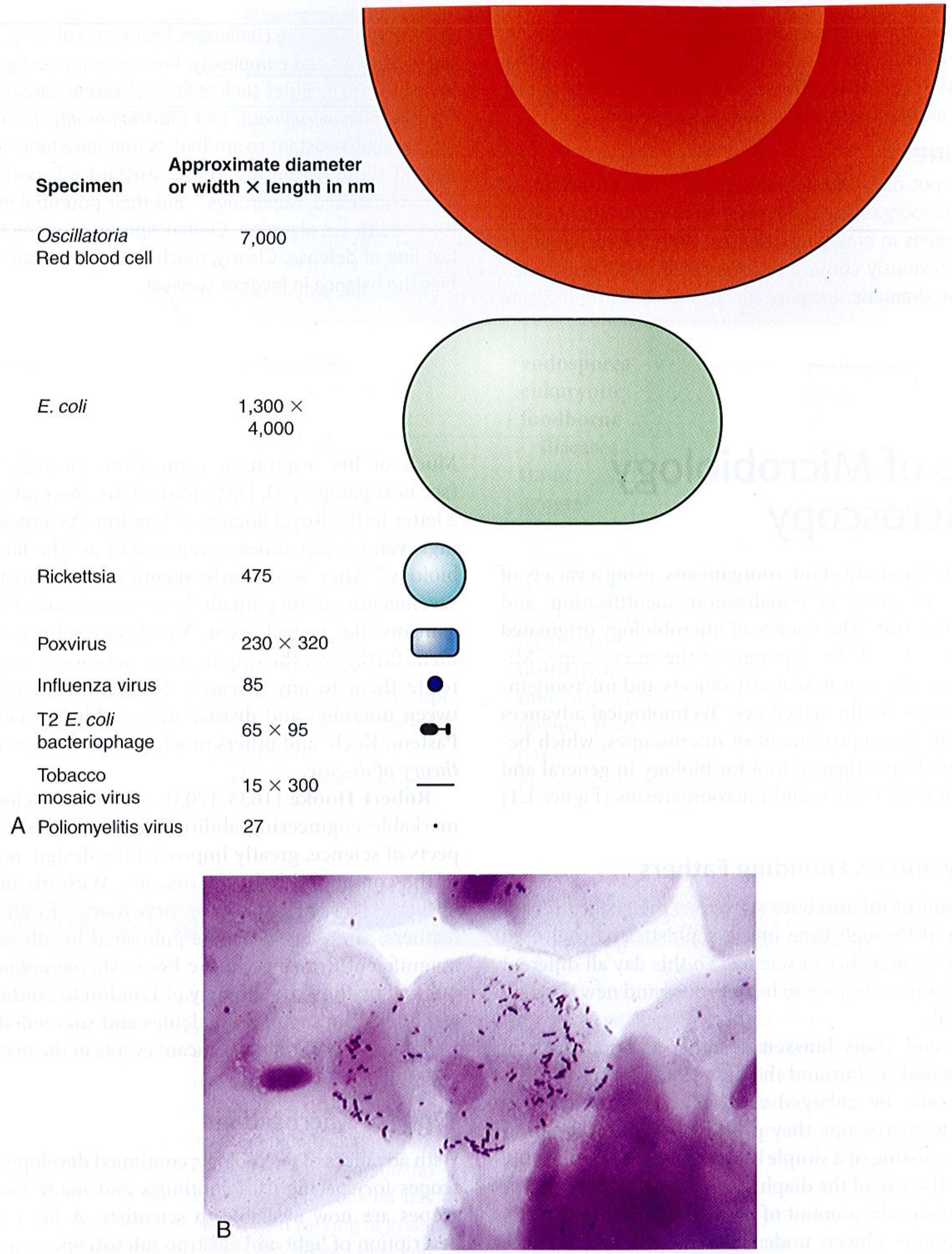



**FIGURE 1.1 A,** Comparison of cell sizes. This drawing shows relative size comparisons of bacteria, eukaryotic cells, and viruses. Although a few bacteria are actually larger than some eukaryotic cells, the vast majority are much smaller. **B,** Cheek cell with resident bacteria. This photo shows a human cheek cell with resident bacteria, which are stained and appear as dark purple spheres or rods. Note that the bacteria are even much smaller than the nucleus in the center of the cell. (Approximate magnification: ×1000.)

- The lenses closer to the specimen are located in the body of the microscope and are referred to as *objective lenses.* Each lens has its own magnifying power, and the final magnification of a compound microscope is the product of the enlarging power of the ocular lens multiplied by the power of the objective lens. Most often the ocular lenses, either single (monocular) or in pairs (binocular), magnify by a power of 10 (×10).
- The objective lenses are mounted on a revolving nosepiece and usually magnify ×4 or ×5, ×10, ×40, and ×100. In



**TABLE 1.1 Significant Events in Microbiology**

| Name | Year | Event |
|---|---|---|
| Zaccharias and Hans Janssen | 1590 | Invention of the first compound microscope |
| Robert Hooke | 1660 | Explores living and nonliving matter with a compound microscope |
| Francesco Redi | 1668 | Experiments to disprove spontaneous generation |
| Antony van Leeuwenhoek | 1676 | Observes bacteria and protozoan "animalcules" with simple microscope |
| Francesco Redi | 1688 | Published experiments on spontaneous generation of maggots |
| Lazzaro Spallanzani | 1776 | Conducts further experiments to disprove spontaneous generation |
| Edward Jenner | 1796 | Introduction of smallpox vaccination |
| Ignaz Semmelweis | 1847-1850 | First use of antiseptics to reduce hand-borne disease |
| Louis Pasteur | 1857 | Proves that fermentation is caused by microorganisms; introduces pasteurization |
| Louis Pasteur | 1861 | Completes experiments that show without doubt that spontaneous generation does not occur |
| Joseph Lister | 1867 | Antiseptic surgery—begins the trend toward modern aseptic techniques |
| Robert Koch | 1876-1877 | Studies anthrax in cattle and implicates *Bacillus anthracis* as causative agent |
| Louis Pasteur | 1881 | Develops anthrax vaccine for animals |
| Robert Koch | 1882 | Identifies causative agent of tuberculosis |
| Robert Koch | 1884 | Describes his postulates |
| Paul Ehrlich | 1891 | Proposes that antibodies are responsible for immunity |
| Alexander Fleming | 1929 | Publishes paper describing penicillin and its effect on gram-positive bacteria |
| Francois Jacob and Jacques Monod | 1960 | Proposes the operon concept for bacterial gene action |
| Craig Venter, Hamilton Smith, Claire Fraser | 1995 | Produce the first complete genome sequence of a microorganism: *Haemophilus influenzae* |

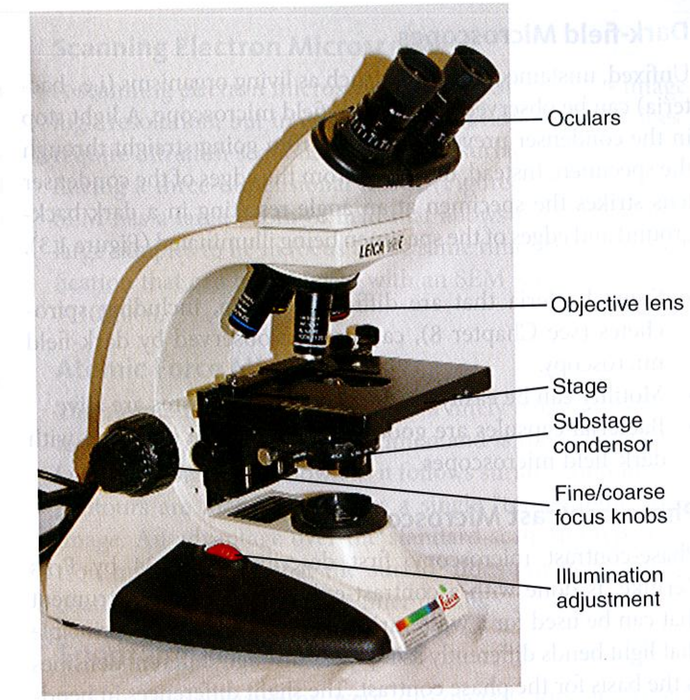



**FIGURE 1.2 Bright-field microscopy.** Bright-field microscopes vary somewhat in appearance based on brands, special features, or attached equipment, but they all have the same basic parts as labeled in the photo.

general, compound microscopes can magnify an object up to 1000 times (i.e., an ocular lens with a magnification of 10 times the objective lens with a power of ×100 = ×1000).
- The specimens for compound light microscopy can either be visualized as whole (i.e., bacteria and other microorganisms) or are specially prepared for viewing with a given type of microscope. After specific dehydration procedures larger specimens are cut into 1- to 10-µm sections. Both smear preparations, for single cells, and sections are usually stained for better visual images (see Chapter 8). Photographs taken through a microscope are referred to as *photomicrographs* or *micrographs.*

**Dissection microscopes** and **stereomicroscopes** are low-power microscopes designed for observing larger objects such as insects, worms, plants, or any objects that may have to be dissected for further observation. These microscopes provide three-dimensional images to determine surface structures and specific locations on a specimen.

## Bright-field Microscopes

The bright-field microscope, a type of compound microscope, can be used to examine small specimens and some of their details.

- The microscope exhibits a background brighter than the observed specimen and is dependent on altering the light path (refraction) only.
- Most specimens require staining for optimal observation.
- Bright-field microscopes are most commonly used to observe sectioned and stained tissues, organs, and microorganisms (Figure 1.2).

## Dark-field Microscopes

Unfixed, unstained specimens such as living organisms (i.e., bacteria) can be observed with a dark-field microscope. A light stop in the condenser prevents the light from going straight through the specimen. Instead, only light from the edges of the condenser lens strikes the specimen at an angle resulting in a dark background and edges of the specimen being illuminated (Figure 1.3).

- Some bacteria that are difficult to stain, including spirochetes (see Chapter 8), can best be observed by dark-field microscopy.
- Motility can be easily observed as the organisms are alive.
- Bacterial capsules are good candidates to be observed with dark-field microscopes.

## Phase-contrast Microscopes

Phase-contrast microscopy, first described in 1934 by Frits Zernike, is done with a contrast-enhancing optical instrument that can be used for a wide variety of applications. The principle that light bends differently as it passes through different densities is the basis for the phase contrast. The slight differences in bending as light goes through the different densities is exaggerated by shifting the phase of the light. The difference between light and dark areas produces contrast, which the phase shift increases.

- It produces high-contrast images of transparent specimens such as living plant and animal cells, microorganisms, and thin tissue slices (Figure 1.4).
- Light interference patterns, along with the phase shift, are also used to increase contrast in a Nomarski microscope.

This type of instrument is ideally suited for the observation of cytoplasmic streaming, motility, and the dynamic states of cell organelles. Cell division and phagocytosis are examples of processes well suited for phase-contrast microscopy. The development of video technology enabled the recording and demonstration of these processes.

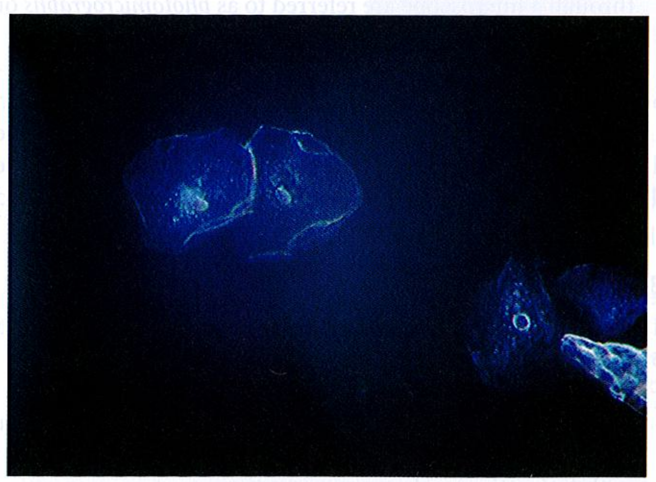


**FIGURE 1.3 Dark-field microscopy.** The dark-field microscope illuminates from the side rather than directly through the specimen. These cheek cells are in a living state and did not require any staining. (Approximate magnification: ×400.)

**FIGURE 1.4 Phase-contrast microscopy.** The phase-contrast microscope enhances the contrast of the image by shifting the phase of the light. These cheek cells are in a living state and did not require any staining. (Approximate magnification: ×400.)

## Fluorescence Microscopes

If a specimen can emit light (fluoresce) of one color when illuminated by ultraviolet radiation, fluorescence microscopy may be the method of choice. Fluorescence microscopes are used to visualize specimens that contain natural fluorescent substances such as chlorophyll or those stained with a fluorescent dye such as fluorescein, auramine, or rhodamine B (Figure 1.5). Fluorescence microscopy is an important and widely used tool in the diagnosis of infectious disease and in studies in microbial ecology. Fluorescence techniques are applied to identify specific antibodies, which are proteins produced in response to antigens (see Chapter 13). By attaching a fluorescent dye to these protein molecules, labeled antibodies are created that can be visualized, monitored, and studied.

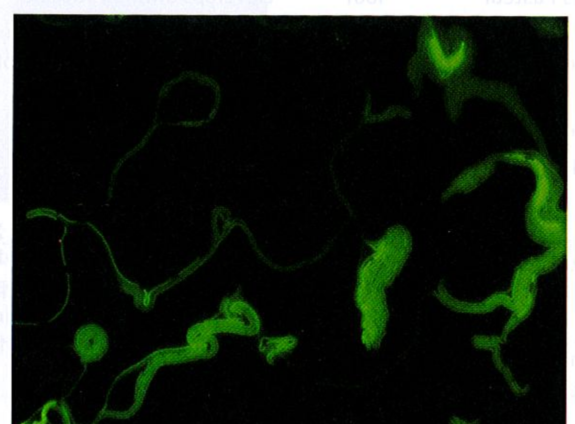


**FIGURE 1.5 Fluorescence microscopy.** The fluorescence microscope uses an ultraviolet lamp to illuminate specimens stained with fluorescent stain. This is a fluorescence microscopy photo of a modified strain of *Pseudomonas.* (Approximate magnification: ×1000.) Courtesy Dr. Larry Halverson, Iowa State University.



## Confocal Microscopes

In 1955 Marvin Minsky invented confocal microscopy, which was patented in 1957. Confocal microscopes have several advantages over the conventional optical microscopes, such as creating sharper images of specimens that would appear blurred with the use of a conventional compound microscope. In a confocal imaging system a single point of light focuses sequentially across a specimen, avoiding most of the unwanted scattered light that usually obscures an image when the entire specimen is illuminated at one time. An image is built by visualizing different planes of the layers of the specimen as the stage of the microscope is moved up and down.

## Electron Microscopes

**Electron microscopes** (EMs) are sophisticated instruments of the twentieth century that use a beam of electrons rather than light as the source of energy to visualize specimens. Magnetic fields instead of optical lenses are used to focus the electron beam. This allows much better resolution of the image than is possible with the light microscope. Specimens for EM studies require more extensive preparation, expensive laboratory equipment, and specially trained personnel than are required for the preparation of specimens used for light microscopy.

Bacteria can be visualized by light microscopy, but their detailed structure or specifics of their attachment to hosts are best seen by electron microscopy. Although most viruses are not visible by light microscopy, their effects on cells and tissues are. Investigations of specimen surfaces use scanning electron microscopy, whereas studies of the interior of cells and tissues use transmission electron microscopy.

## Transmission Electron Microscopes

In a transmission electron microscope (TEM) the electron beam travels through an ultrathin sectioned specimen (approximately 100 nm in thickness) and provides a two-dimensional image of the cell or other object. The resolving power of a TEM is approximately 0.002 μm, which is 100 times greater than can be achieved with a light microscope. The usual magnification of a TEM ranges from ×500,000 to ×1,000,000. Pictures taken of images created with an electron microscope are called *electron micrographs* (Figure 1.6).

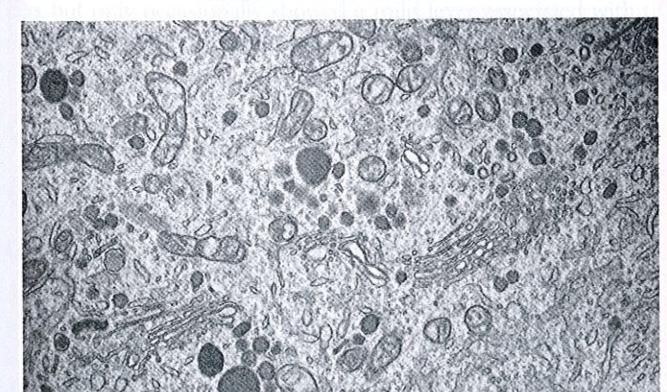


**FIGURE 1.6 Transmission electron microscopy.** This is a TEM image of a neuron.

## Scanning Electron Microscopes

A scanning electron microscope (SEM) also provides images of high resolution, but in contrast to the TEM, the SEM does not require ultrathin sections. It scans the surface of an object, producing a three-dimensional image (Figure 1.7). Moreover, the SEM has a large depth of field that allows the surface areas of large samples to be in focus at the same time. The usual magnification that can be achieved with an SEM ranges from ×10 to ×100,000.

## Atomic Force Microscopes

The atomic force microscope uses a finely balanced stylus that touches the surface of the specimen and is moved over its surface, traveling up and down as it follows surface contours. These contours are then compiled as a single "topographic" type of image. An advantage over the standard scanning type of electron microscope is that the surface does not need a metallic coating, which may obscure finer details.

## Spontaneous Generation

Before microorganisms were discovered and described by Antony van Leeuwenhoek, life was hypothesized to develop from nonliving matter **(abiogenesis).** Maggots, for example, were believed to arise spontaneously from rotting meat. The big question among scientists at that time was whether living organisms were produced spontaneously by decay and fermentation—or whether they caused decay and fermentation—raising the basic dilemma of "which comes first, the chicken or the egg?"

One of the first serious attacks on this controversial spontaneous generation theory came in 1668 from **Francesco Redi,** an Italian physician. He believed that maggots developed from fly eggs. In his experiments he put meat into different flasks, kept some of them exposed to the atmosphere and flies, and the rest sealed. As he expected, maggots developed only in the open flasks where flies had access to the meat.

At about the same time, van Leeuwenhoek's invention of the first microscope allowed him to observe small organisms,

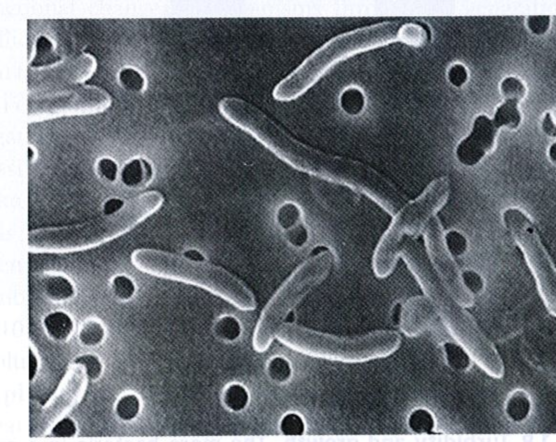


**FIGURE 1.7 Scanning electron microscopy.** Photo of an SEM image of the bacteria *Campylobacter* passing through the pores of a filter. (Approximate magnification: ×5000.) Courtesy Dr. James Dickson, Iowa State University.

which he called "animalcules." He further showed that when hay was placed into a mixture of water and soil and allowed to incubate for a few days, new creatures in the hay infusion could be observed. Yet these findings were interpreted by some scientists of the time as being additional proof of spontaneous generation.

This debate over the existence of spontaneous generation continued for centuries and in 1745 an English clergyman, **John Needham,** claimed victory for spontaneous generation with his experimental observations. He boiled chicken and corn broth, put it into a flask, and sealed it. After a few days cloudiness appeared in the broth, which is evidence of microbial growth. However, **Lazzaro Spallanzani,** an Italian priest, questioned Needham's interpretation and suggested that microorganisms could have entered the flask from the air before the tube was sealed. In his own experiments he also placed chicken broth in a flask, sealed it, and then drew off the air before boiling the broth. As a result no microorganisms grew, which he interpreted as evidence that spontaneous generation did not exist. However, proponents of spontaneous generation held that Spallanzani only proved that spontaneous generation could not occur in the absence of air.

**Louis Pasteur** (1822-1895), a French chemist, finally ended the controversy. In 1861 he designed and conducted a definitive experiment in which he boiled meat broth in a flask and then heated the neck of the flask until it could be bent into an S shape (swan-necked flask). Thus air could enter the flask but microorganisms trapped in the S-shaped neck were unable to reach the broth. There was no evidence of microbial growth. However, when he tilted the flask so that the broth could reach the organisms in the neck, the broth gradually became cloudy due to microbial growth (Figure 1.8).

In 1877 **John Tyndall** (1820-1893) demonstrated that microorganisms exist in dust. In a set of experiments similar to Pasteur's, but in the absence of dust, the broths remained sterile even when exposed to air. In addition, Tyndall provided evidence of the existence of heat-resistant bacteria. **Ferdinand Cohn** (1828-1898), independent of Tyndall's studies, also described heat-resistant bacterial **endospores** (see Chapter 3).

**FIGURE 1.8 Turbidity and growth.** The more bacteria in a container of broth, the cloudier or more turbid it is. The clear flask on the left is uninoculated and contains no bacteria. The turbid (cloudy) flask on the right contains a culture that has grown for 18 hours and contains an enormous number of bacteria.

## Pasteurization

In addition to his other findings Louis Pasteur invented the process we know as **pasteurization** (Box 1.1). The Emperor Napoleon III asked Pasteur to investigate the spoiling of wine that caused a negative impact on the production of wine, damaging the French wine industry. Pasteur discovered that wine spoilage (wine disease) is caused by microorganisms. He "pasteurized" the wine by heating it to 55° C for several minutes, which killed enough microorganisms to prevent the wine from spoiling. Pasteurization does not kill all microorganisms, but reduces the number of viable organisms so they are less likely to cause spoilage or disease. **Sterilization** (see Chapter 8), on the other hand, kills all microorganisms, including their endospores.

## Germ Theory of Disease

During the time when the existence of spontaneous generation was being debated, several physicians began to suspect that microorganisms not only could cause spoilage and decay but might also play a role in infectious disease. Two physicians in different parts of the world, one in the United States and one in Vienna, significantly contributed to the concept that microorganisms had a role in infectious diseases. **Oliver Wendell Holmes** (1809-1894) in the United States showed that death following childbirth was often caused by material on the hands of midwives or physicians. **Ignaz Semmelweis** (1818-1865) in Vienna observed that women in the maternity ward became infected after being examined by physicians or students who came directly from the autopsy room or from examining infected patients. Semmelweis also noted that these students' and physicians' hands stunk from putrefaction and that they did not wash their hands before examining patients in the maternity wards. He also suspected that microorganisms were responsible for the odor. Consequently, he required everyone to wash their hands in a solution of chlorine before entering the maternity ward and examining patients. The result was a drastic decline in the death rate on the wards.

**BOX 1.1 Contributions of Louis Pasteur**

The contributions of Louis Pasteur to the emerging field of microbiology include the following:

- Different microorganisms produce different fermentation products
- Pasteurization
- Infectious agents cause disease
- Germ theory of disease, in contrast to spontaneous generation
- Minimization of the spread of pathogens
    - Development of vaccines to prevent several diseases in chickens, sheep, cattle, pigs, and humans

Because of all these achievements, Pasteur is referred to as the father of *modern* microbiology.



**BOX 1.2 Koch's Postulates**

Koch's postulates state the following:

- The microbe must be present in every animal with the disease, and absent in healthy.
- The microbe can be isolated and grown in pure culture outside of the host.
- The cultured microorganism must cause the same disease in inoculated animals.
- The same microorganism must then be isolated from the inoculated animal.

**Joseph Lister** (1827-1912) studied the observations of Semmelweis and hypothesized that airborne microbes might play a role in postsurgical infections as well. He discovered that applying carbolic acid to dressings and using an aerosol of carbolic acid on the surgical field significantly decreased the number of wound infections. He was the first physician to introduce the use of **aseptic** techniques.

The germ theory of disease proposed by Louis Pasteur and **Robert Koch** (1843-1910) is based on the existence of infectious microorganisms. Although Pasteur was convinced that microbes caused disease in humans, he was never able to link a specific microbe with a particular disease. Koch's investigations focused on anthrax, an infectious disease that seriously affects animal herds and humans brought in contact with the microorganism. Koch also discovered that anthrax produced endospores that persist after the death of the exposed animals. Moreover, he proved that these spores can survive and later develop into the active anthrax microbe and infect other animals. This established that a specific organism caused a specific disease. After many years of experimentation he developed what we now know as **Koch's postulates** (Box 1.2), which set forth the conditions that should identify an organism as the specific cause of a specific disease.

**Immunology** begins with the work of **Edward Jenner** (1749-1823). Jenner observed that milkmaids who had contracted cowpox did not become infected with smallpox, a major killer during the eighteenth century. He applied material from cowpox lesions, containing the vaccinia virus, to small incisions or puncture wounds made in human arms. These human subjects did not develop smallpox but only occasionally showed a mild fever associated with the disease. With this procedure Jenner proved that immunity against smallpox could be achieved through vaccination.

# Origin and Evolution of Microorganisms

## Origin

Although earth formed about 4.5 billion years ago, life on earth probably has been present for most of the planet's history and likely began remarkably early, between 3.5 and 4 billion years ago. Although no one knows precisely how or when life began, scientists tend to agree on several things:

- In earliest times the earth was dominated by volcanoes, a lifeless ocean, and a turbulent atmosphere.
- Intensive chemical activity occurred and the ocean received organic matter from land, atmosphere, and meteorites.
- Several elements formed key molecules such as sugars, amino acids, and nucleotides, all of which are building blocks for living organisms (see Chapter 2).

The origin of microorganisms is described in geological time. The rich fossil record of prokaryotic life suggests that microbes were perhaps the first living things on earth. Microbes affect human life from birth to death and are found in almost every environment on earth. **Stromatolites** (layered mound-shaped deposits along ancient seashores) represent some of the oldest microbial communities. Evolution of plants and animals as we know them has occurred in the last 550 million years.

**LIFE APPLICATION**

**Inside a Glacier**

Scientists at Pennsylvania State University have found live microbes almost 2 miles deep inside a glacier in Greenland; including some never before seen species. The majority of microbes the scientists discovered in the ice core sample from the glacier were less than 1 μm in size, and they estimate that at least 80% of these microbes were still alive.

Scientists from the University of California, Berkeley, also conducted a study of methane-producing bacteria frozen in Greenland's glacier. Methane is a greenhouse gas present in the atmospheres of both Earth and Mars, and the finding of methanogenic (methane-producing) archaea in the extreme environment of a glacier suggests that such methanogens, and therefore the potential for life, might also be present on Mars.

## Evolution

Evolution is an important concept that involves all living things, including microorganisms. Evolution implies that living things gradually change over millions of years, resulting in structural and functional changes of organisms throughout generations. The millions of different species on earth and their successful adaptation to different habitats are indicators of the evolutionary process.

For the first three quarters of evolutionary history the only organisms on earth were microscopic and mostly unicellular. Fossils of **prokaryotes** go back 3.5 to 4 billion years followed by **eukaryotic** life approximately 2.2 billion years ago. Eukaryotic cells are larger and more complex than prokaryotic cells and scientists believe that eukaryotes have evolved from prokaryotic symbiotic communities. It is estimated that there are 5 million to 100 million species of organisms living on earth today. The evolutionary relationship between organisms is the subject of **phylogeny.** Today, the phylogenetic relationship between organisms can be determined by nucleic acid and nucleotide sequencing. Results from ribosomal ribonucleic acid (rRNA) sequencing identify three evolutionarily distinct cell lines that are classified as bacteria, archaea, and eukarya. These three distinct lineages, based on the origin of cell lines, are referred to as **domains** (Figure 1.9).

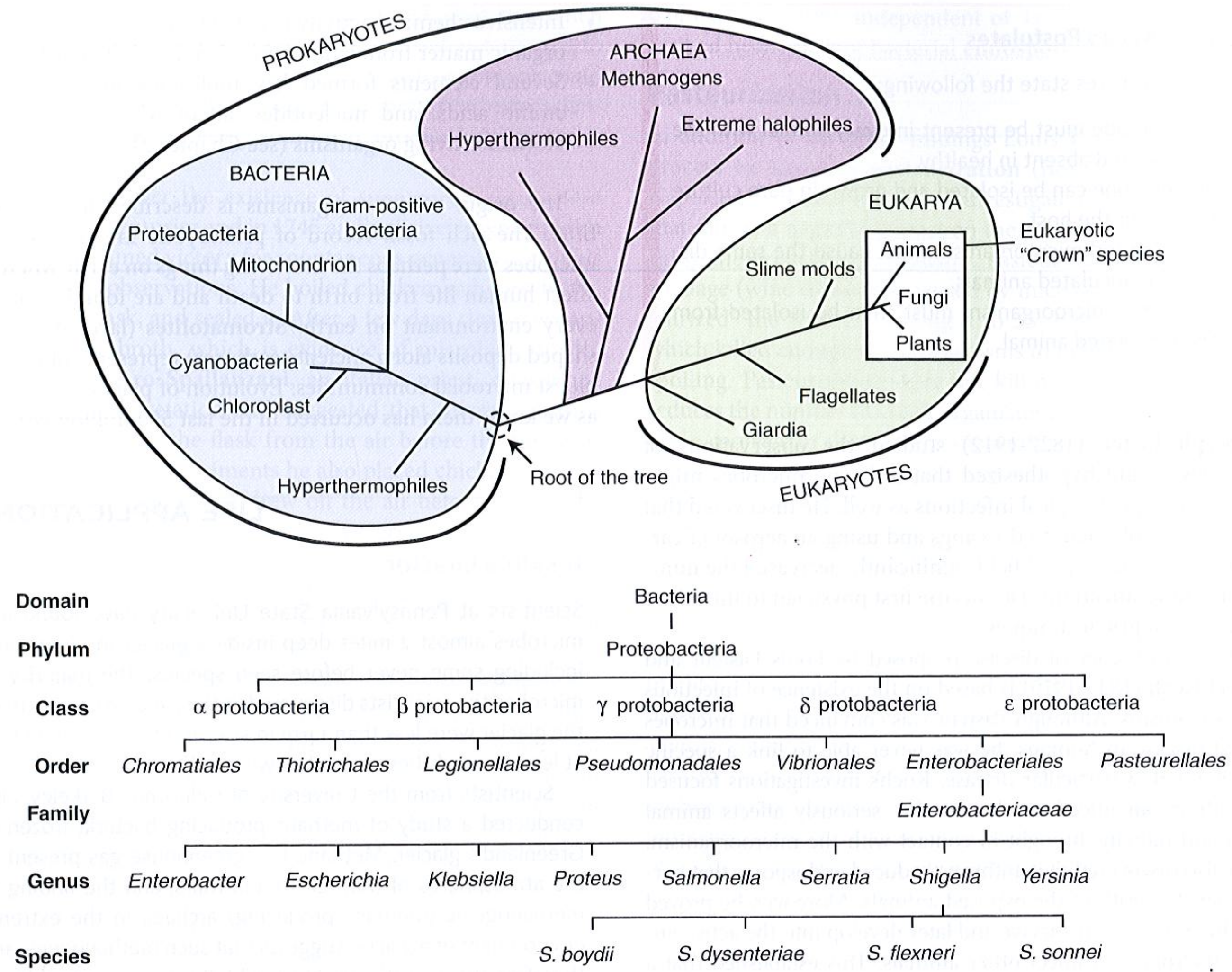



**FIGURE 1.9 Kingdoms of life.** These charts show the present organization and hierarchy of the kingdoms of life. The bacteria *Shigella sonnei* has been traced from kingdom to the level of species to illustrate its place in the overall scheme.

# Classification of Microorganisms

To better understand the different types of microorganisms (microbes), they are grouped or classified in various ways. Microbes are diverse, and, in terms of numbers, most of the diversity of life on earth is represented by microbes. Further detailed description and discussion of the individual groups of microorganisms is provided in Chapters 4, 5, and 6.

## Prokaryotes versus Eukaryotes

Differentiation between prokaryotes (*pro,* before; *karyon,* nucleus) and eukaryotes (*eu,* good or with; *karyon,* nucleus) was greatly advanced as more sophisticated microscopes became available, allowing scientists to identify membrane-bound structures in unicellular organisms. Significantly, scientists discovered that some unicellular organisms contained membrane-bound organelles such as a nucleus, whereas others did not. The cells without membrane-bound organelles and therefore without a nucleus were classified as prokaryotes and the ones with membrane-bound organelles and a nucleus were designated as eukaryotes. All bacterial cells are prokaryotic, whereas algae, fungi, and protozoans are eukaryotes (see Chapter 3).

## Bacteria, Archaea, and Eukaryotes

Bacteria, archaea, and eukaryotes are examples of diversity, and the root of microbial diversity is evolution. Within **bacteria** several evolutionary branches are present, including some **pathogenic** (disease-causing) bacteria, occurring in soil and water, in animal digestive tracts and skin, and in many other environments. Also, different bacteria require different sources of energy such as light, organic chemicals, inorganic chemicals, or combinations thereof.

**Archaea** are a group of single-celled microorganisms, similar to bacteria because they are also prokaryotes, but evolutionarily different. Many are characterized by their preference to live in extreme environments such as hot springs, glaciers, or highly salty environments (see Chapter 4).



Eukaryotic microorganisms include algae, fungi, and protozoans (see Chapter 6). **Algae** are a large and diverse group of simple organisms, ranging from unicellular to multicellular forms, containing chlorophyll that is needed for photosynthesis. They are common in aquatic bodies (both freshwater and saltwater) and in all types of soil, but have limited medical significance. **Fungi** include yeasts, molds, and mushrooms, which are not microbes. Their energy sources are organic compounds found in soil and water. Fungi play a major role in the breakdown of dead organic matter in a variety of environments. However, some fungi are pathogenic and can cause disease in humans and animals. **Protozoans** are colorless, mobile organisms feeding on other organisms for their energy source. In general, protozoans are free-living microorganisms and several of them are also pathogenic to humans and animals.

## Viruses

Viruses are noncellular, submicroscopic particles (visible only by electron microscopy) and consist of nucleic acid surrounded by a protein coat. They are metabolically inert, incapable of carrying out biosynthetic functions, and they are dependent on host machinery for their multiplication. Viruses are classified according to the type of nucleic acid (ribonucleic acid [RNA] or deoxyribonucleic acid [DNA]) they contain. Many viruses are pathogenic and can be classified on the basis of their host as animal viruses, plant viruses, and bacterial viruses (see Chapter 5).

## Prions

**Prions** (*pro*teinaceous *in*fectious particles) are not cellular organisms, nor are they viruses. Prions lack nucleic acids; they are normal proteins of animal tissues that can misfold during protein synthesis and become an infectious agent (see Chapter 5).

## Viroids

**Viroids** are plant pathogens that can cause serious economic problems. They are much smaller than viruses (15 to 100 nm) and consist of a small single-stranded, naked RNA circle. Viroids cannot produce proteins and do not have a protein coat (see Chapter 5).

**MEDICAL HIGHLIGHTS**

### Transmissible Spongiform Encephalopathies

Prions are associated with a group of diseases called *transmissible spongiform encephalopathies,* for example, bovine spongiform encephalopathy, also referred to as *"mad cow" disease.* A research group at the University of California led by Stanley B. Prusiner purified the infectious material, confirmed that the agent was a protein, and named it "prion." In 1997, Prusiner received the Nobel Prize for this research.

## Taxonomy

The formal system of organizing, classifying, and naming of living organisms is called **taxonomy.** Taxonomy sorts organisms on the basis of mutual similarities into nonoverlapping groups called **taxa.** The goal of taxonomy is **classification, nomenclature,** and **identification** for clarification and ease of reference.

- Classification is the assignment of organisms into taxa based on similarities.
- Nomenclature deals with the rules for naming organisms.
- Identification is the process of specifying, identifying, and recording the traits of organisms.
- The current system of taxonomy began with a Swedish botanist, Carolus Linnaeus (1707-1778). He provided a system that standardized the naming and classification of organisms on the basis of common characteristics. Linnaeus's system groups similar species into genera, genera sharing common features into families, similar families into orders, orders into classes, classes into **phyla,** and phyla into kingdoms (see Figure 1.9). In addition, in 1990, Carl Woese (1928-) and his colleagues introduced a three-domain system of taxonomy (archaea, bacteria, and eukarya) based on genetic rather than morphological similarities (see Figure 1.9).

All these categories in the Linnaeus system are taxa, which are hierarchic, starting with the **species** and **genera,** followed by successive taxa, each with a broader description than the preceding one. All names in the taxa are Latin or Latinized.

Linnaeus assigned each species a descriptive name of its genus and a specific name for the species. This method of assigning the scientific name is called the **binomial** (two-name) system of nomenclature. A **genus** is the name of an organism and is often abbreviated by a single capital letter, whereas the species name is never abbreviated. For example, *Escherichia coli* can be abbreviated to *E. coli.* A genus typically contains several species, and a particular species can be further subdivided into different strains. For instance, there are several species in the genus *Bacillus,* such as *B. subtilis* and *B. cereus.*

The taxonomic resource for bacteria is *Bergey's Manual of Systematic Bacteriology,* the second edition of which was published in five volumes. In addition to the number of keys for bacterial identification, newer versions of the manual also contain some molecular sequencing information for various bacterial groups. An outline of *Bergey's Manual* can be found in Appendix A of this book.

# Microorganisms in Health and Disease

Microbes form various mutualistic relationships with different organisms, and many of these are important to human well-being. *Microbial ecology* is the study of the interrelationship between microbes and their environment. Microbes are everywhere in the environment and generally have an impact in maintaining ecosystems.

## Microbes in the Environment

Microorganisms in nature are often organized into complex communities of different organisms called **biofilms.** A biofilm consists of surface-associated microbial cells enclosed in an extracellular polymeric substance matrix. Antony van Leeuwenhoek observed and described layers of microorganisms on

**TABLE 1.2 Microorganisms Associated with Biofilms on Medical Devices**

| Device | Microorganisms |
|---|---|
| Hip prosthesis | Coagulase-negative staphylococci<br>*Enterococcus* spp.<br>*Pseudomonas aeruginosa*<br>*Staphylococcus aureus* |
| Intrauterine device | *Candida albicans*<br>Coagulase-negative staphylococci<br>*Enterococcus* spp.<br>*Staphylococcus aureus* |
| Prosthetic heart valve | Coagulase-negative staphylococci<br>*Enterococcus* spp.<br>*Staphylococcus aureus* |
| Urinary catheter | Coagulase-negative staphylococci<br>*Enterococcus* spp.<br>*Klebsiella pneumoniae*<br>*Pseudomonas aeruginosa* |
| Venous catheter | *Candida albicans*<br>Coagulase-negative staphylococci<br>*Enterococcus* spp.<br>*Klebsiella pneumoniae*<br>*Pseudomonas aeruginosa*<br>*Staphylococcus aureus* |
| Voice prosthesis | *Candida albicans*<br>Coagulase-negative staphylococci |

tooth surfaces, representing a microbial biofilm (see Chapter 3). However, detailed, high-magnification observations of microbial biofilms did not occur until electron microscopy became available. Biofilms are found on a variety of surfaces, such as medical devices (Table 1.2), industrial water system piping, natural aquatic systems, and foods, and also on living tissues. An established biofilm structure provides a perfect environment for the exchange of genetic material between different organisms in the biofilm community.

Free-living fungi and bacteria decompose organic matter and return minerals and other nutrients to the soil. Decomposition is dependent on microbes, as is the cycling of elements. Examples of nutrient cycling in nature that requires microorganisms are the nitrogen, carbon, oxygen, sulfur, and phosphorus cycles.

Ecological interactions between organisms in a community, including **mutualism, commensalism, synergism,** and **parasitism,** are classified according to the degree of benefit or harm they pose to one another. In mutualism both organisms benefit, in commensalism the waste product of a microbe provides useful nutrients for another organism, in synergism two organisms are dependent on each other to break down a nutrient that neither breaks down alone, and in parasitism one organism benefits and the other is harmed (see Chapter 12).

## Normal Flora

Blood, lymph, cerebrospinal fluid, and internal organs are sterile. Before birth the entire healthy body is free of microbes. However, starting at birth microorganisms contaminate some of these previously sterile environments; for example, bacteria and other microorganisms colonize the mucous membranes of the upper respiratory tract, the digestive tract, and the surface of the skin. Microorganisms regularly found at any anatomic site in healthy humans and not causing infection or disease are referred to as the **normal flora.** The normal flora provides protection against pathogens by preventing attachment to the host tissue and by competing for the same nutrients. If successful, the pathogens cannot flourish or colonize and therefore are unable to reach sufficient numbers to cause infection (see Chapter 12).

## Pathogens

The human body is in continual contact with microorganisms, and, if a particular microbe is pathogenic, colonizes, and is present in sufficient numbers, this interaction can lead to disease. Diseases caused by communicable microorganisms are referred to as *infectious diseases.* Control of these diseases can be achieved by application of the aseptic technique, the development and use of antimicrobial drugs, and immunization. These practices have drastically reduced the death rate from infectious diseases in the United States. However, death from infectious diseases in developing countries remains extremely high, due in large part to inadequate healthcare and poor sanitation. Infectious diseases may be transmitted by direct or indirect contact and can be acute, subacute, or chronic. Within communities they can be epidemic, pandemic, endemic, or sporadic (see Chapter 12).

Infectious diseases in economically important species, such as domestic animals, are of great concern to agriculture. Not only is there an economic impact, but there is also the possibility that the disease can become *zoonotic,* meaning transmissible from the primary animal host to humans. Some typical examples are rabies and yellow fever. Fortunately, many of these infectious diseases can be prevented by vaccination and compliance with regulated practices of public health and hygiene.

**Foodborne diseases** result from consuming food that is contaminated with different pathogenic species of bacteria, viruses, parasites, or microbial toxins. At greater risk are young children, the elderly population, pregnant women and their fetuses, and anyone with a weakened or compromised immune system. Treatments vary depending on the severity of the illness and range from fluid and electrolyte replacement to hospitalization for severe conditions. In general, foodborne diseases can be prevented by the following measures:

- Washing hands with hot, soapy water before food preparation, after using the bathroom, and after changing diapers
- Keeping raw meat, poultry, seafood, and their juices away from prepared, ready-to-eat foods
- Cooking foods thoroughly at high enough temperatures to kill harmful bacteria and destroy their toxins
- Refrigerating foods within 2 hours of cooking (cold temperatures slow bacterial growth and multiplication)
- Cleaning sufficiently all surfaces on which food is to be prepared

**Waterborne disease** is the general term used to describe diseases acquired from contaminated water supplies, resulting



in four fifths of all illness in developing countries and a high infant death rate. Major floods contribute to large cholera epidemics, such as occurred in West Bengal, and have caused serious outbreaks in 1968, 1984, 1992, 1998, and 2000. Increased risk factors for waterborne diseases are multiple and include but are not limited to travel, living in rural areas, poverty, geographic location, the immediate environment (flooding, feces, overcrowding, and poor sanitation), inadequate personal health and hygiene, and poor water treatment practices and facilities. Floodwaters also often contain raw sewage, silt, oil, and chemical wastes. Parasites, viruses, and bacteria are readily transmitted by floodwaters. To reduce exposure to waterborne disease, the following recommendations should be practiced:

- Do not drink, swim, bathe, or play in floodwaters.
- Keep children away from floodwaters.
- Do not expose cuts or open wounds to floodwater.
- Monitor the quality of water supplies frequently.
- Keep the water clean and avoid using contaminated water.
- Drink water that has been purified by boiling or treatment with chlorine.
- Rinse fruits and vegetables with clean water.
- Avoid swallowing water from lakes, rivers, or swimming pools.
- Clean food preparation areas with clean water before use.
- Wash hands if exposed.

The Centers for Disease Control and Prevention (CDC) estimates that approximately 900,000 people in the United States become ill each year from drinking contaminated water. The lack of clean water and sanitary waste disposal, especially in underdeveloped countries, coupled with inadequate personal health and hygiene practices, is likely responsible for over 12 million deaths per year.

**Airborne diseases** are transmitted from infected people by coughing, sneezing, or talking. Pathogens are in small mucous saliva particles suspended as aerosols. Movements and directions of air currents play an important role in the spread of airborne respiratory diseases such as tuberculosis, legionellosis, and influenza. The public healthcare issues related to travel and airborne diseases are also discussed in Chapter 24.

# Applied Microbiology

*Applied microbiology* is the human use of microorganisms to improve certain aspects of life. Microbes are used in this

**HEALTHCARE APPLICATION**

**Foodborne Diseases and Waterborne Diseases**

| Disease | Organism | Transmission | Symptoms |
|---|---|---|---|
| Cholera | *Vibrio cholerae* | Ingestion of contaminated water, raw or partially cooked fish or shellfish | Sudden onset of vomiting, watery diarrhea, rapid dehydration, acidosis, possible circulatory collapse and death |
| *E. coli* infection | *Escherichia coli* | Uncooked meat or other food contaminated by fecal material; swimming in contaminated water | Severe bloody diarrhea, abdominal cramps |
| Shigellosis | *Shigella* spp. | Hand-to-mouth contact with feces from infected people or animals; contaminated foods | Diarrhea, fever, stomach cramps |
| Salmonellosis | *Salmonella choleraesuis, S. enteritidis* | Contaminated poultry, eggs, and meat; fecal-oral route | Gastroenteritis, enteric fever, septicemia |
| Hepatitis (inflammation of the liver) | Hepatitis A virus | Contaminated food, water contaminated with human feces | Fever, anorexia, nausea, abdominal discomfort |
| Gastroenteritis | Norwalk virus | Ingestion of contaminated seafood, handling of contaminated food; person-to-person transmission by fecal-oral route | Watery diarrhea, vomiting |
| Dysentery | *Shigella* | Contaminated water, contaminated milk | Abdominal pain, watery diarrhea, fever, blood and mucus almost always present in stool |
| Leptospirosis | *Leptospira interrogans* | Exposure to urine, contaminated water from infected animals | High fever, severe headache, chills, and vomiting; kidney and liver failure |
| Typhoid fever (enteric fever) | *Salmonella typhi* | Water contaminated with feces and urine from carriers | Septicemia |
| Giardiasis | *Giardia lamblia* | Water contaminated by feces of infected person or animal | Diarrhea, abdominal cramps |

HEALTHCARE APPLICATION

**Airborne Diseases**

| Disease | Organism | Transmission | Signs/Symptoms |
|---|---|---|---|
| Influenza | Influenza viruses | Aerosols | Fever, chills, headache, muscle aches |
| Tuberculosis | *Mycobacterium tuberculosis* | Aerosols | Range from asymptomatic to fever, cough, fatigue, lack of appetite, weight loss, pulmonary hemorrhage |
| Legionellosis | *Legionella pneumophila* | Aerosols from humidifiers, air conditioning equipment | Atypical pneumonia, fever, cough, difficulty in breathing, chest pain |
| Psittacosis | *Chlamydia psittaci* | Bird to human by aerosols | Headache, fever, nonproductive cough, occasional septicemia |
| Common cold | Rhinoviruses, adenoviruses, coronaviruses, and other viruses | Aerosols | Slight fever, headache, sore throat, coughing, sneezing, nasal discharge |
| Mumps | Paramyxovirus | Airborne droplets or contact with saliva of infected person | Swelling of parotid glands, fever, headache, generalized muscle aches |

capacity by the food, pharmaceutical, and agricultural industries and in forensics and other endeavors. Application of new technologies in genetic engineering has further increased the industrial use of microbes.

## Microorganisms in Food Production

Many nonpathogenic microorganisms occur naturally in food, are beneficial, and are used as starter cultures to produce foods such as vinegar, sauerkraut, pickles, fermented milks, yogurt, cheese, and bread (Box 1.3). For example, vinegar is made from foods containing starch or smaller sugars. The production requires a two-way fermentation process that begins with apple juice or other raw materials, to which the yeast *Saccharomyces cerevisiae* is added to speed up the fermentation process. In the second stage, cultures of acetic acid bacteria such as *Acetobacter aceti* are added to this alcoholic liquid, converting the alcohol into acetic acid. In the United States most vinegar is produced from apples and is named apple cider vinegar.

Sauerkraut is another product made by fermentation of shredded cabbage in the presence of salt. The shredded cabbage is tightly packed in an anaerobic environment, where it becomes dehydrated. The addition of salt promotes the growth of lactic acid bacteria.

Dairy products such as butter, buttermilk, sour cream, cottage cheese, and yogurt are produced from fermented milk. Raw milk contains the fermentable sugar lactose in addition to several acid-producing microorganisms. The natural microflora of raw milk is often inefficient, uncontrollable, and can produce unpredictable results. Moreover, these organisms are destroyed during heat treatments needed to pasteurize milk. Therefore starter cultures that can provide a more controlled and predictable fermentation process are added to produce the desired products. The function of the lactic acid starters is the production of lactic acid from lactose of the milk. Other starter cultures may provide flavor, aroma, and alcohol production.

**BOX 1.3 Some Food Products Produced with the Use of Microorganisms**

- Bread
- Butter
- Buttermilk
- Cheese
- Cottage cheese
- Pickles
- Sauerkraut
- Sour cream
- Vinegar
- Yogurt

LIFE APPLICATION

### Dairy Bacteria

Different starter cultures used in the production of dairy products are selected specifically to result in a desired product. For example, *Propionibacterium shermanii* is used in the production of the Swiss cheeses, *Brevibacterium linens* is responsible for the flavor of Limburger cheese, and molds (*Penicillium* species) are used in the production of Camembert, Roquefort, and Stilton cheeses. *Lactobacillus acidophilus*, *L. bulgaricus*, *L. lactis*, and *L. helveticus* are used in starter cultures for the production of buttermilk, yogurt, Emmental, and Italian cheeses. The manufacturing of cultured buttermilk, sour cream, cottage cheese, and ripened cream butter uses *Leuconostoc citrovorum* and *Leuconostoc dextranicum*.

## Microorganisms in the Production of Alcoholic Beverages

Wine is produced by yeast fermentation of carbohydrates in freshly harvested grapes, peaches, berries, pears, and other fruits or plants (even dandelions). In beer production barley or other grain is used as the source of fermentable carbohydrate. Beer and wine generally are limited to about 15% alcohol because yeast fails to ferment beyond this point. Beverages higher in alcohol content are produced through distillation and are called *spirits* or *liquor.*

LIFE APPLICATION

### Distilled Beverages

The source of material to be fermented for the production of distilled liquors *(spirits)* varies greatly and often determines the name of the resulting beverage. For example, the source of ale is barley and its distilled beverage is Scotch whisky, whereas Bourbon whisky is distilled from corn beer, which is the result of corn fermentation. Brandy and cognac are distilled from wine, which is produced by the fermentation of fruit, most often grapes. Beverages high in alcohol and with added flavorings (e.g., Grand Marnier) are referred to as *liqueurs.*

## Treatment of Water Supplies

Good health depends on a clean, drinkable water supply. It must be free of pathogens, toxins, odor, color, and bad taste to achieve these standards. The microbial content of drinking water should be constantly monitored to make sure the water is free of infectious agents. Most water purity assays are focused on the detection of fecal material, which would indicate the presence of pathogens. Wells, reservoirs, and other water resources can be analyzed for the presence of indicator bacteria that can be readily identified by routine laboratory procedures.

## Microbes and the Production of Pharmaceutical Agents

Louis Pasteur observed the inhibition of microorganisms by products formed by other microbes. This phenomenon is called *antibiosis.* The discovery of the penicillin-producing mold *Penicillium* by Alexander Fleming in the 1920s started the successful search for other antibiotic-producing microorganisms. Today, with the help of genetic engineering and recombinant DNA technology, not only antibiotics and semisynthetic antibiotics but also various hormones and other drugs are available on the market (see Chapter 11).

## Microbes in Agriculture

Agricultural microbiology focuses on the relationships between microbes and domesticated plants and animals. Farmers use microbes and their products in a variety of ways, particularly for crop management via recombinant DNA biotechnology (see Chapter 25). Plant microbiology involves the management of plant disease, soil fertility, and nutritional interactions. For example, the availability of nitrogen in the soil is essential for the growth of crops, and therefore bacteria involved in the nitrogen cycle of the soil are essential (see Soil Microbiology in Chapter 23). On the animal side, agricultural microbiology deals with the management and prevention of infectious diseases in farm animals, as well as other associations animals have with microorganisms.

## Bioremediation

**Bioremediation** is the process of using microorganisms to clean up toxic, hazardous, or unmanageable compounds by degrading them to harmless compounds. Some microbes performing these tasks have been genetically engineered to clean up specific wastes or pollutants. For example, genetically engineered petroleum-digesting bacteria assist in the cleanup of oil spills. Another form of bioremediation that has long been in use is the treatment of water and sewage. Reclamation procedures for treatment of polluted water to convert it to drinkable water are becoming more important worldwide because of the rapid dwindling of clean freshwater sources. Microorganisms have also been used to aid in the reclamation of soil that has been contaminated with the explosive trinitrotoluene (TNT).

## Microbes, Biomass, and Energy

By a process referred to as *bioconversion,* microorganisms can convert biomass such as organic matter and human, agricultural, and industrial waste into alternative fuels, including ethanol, methane, and hydrogen.

Ethanol produced during fermentation is one of the simplest alternative fuel sources to produce and can be mixed with gasoline to make gasohol. Although at present crops such as corn are used, it would be more economical to use crop wastes (e.g., corn stalks).

Some communities already use landfills as immense sources of methane, where methanogens convert wastes into methane by the process of fermentation. Methane gas can be piped through natural gas lines and used as an energy source in common households.

The concept of using microbes to power fuel cells is not new; however, in 2003 scientists found a way to generate electricity by feeding bacteria common sugars and other carbohydrates. In this study bacteria were grown on graphite electrodes within a fuel cell. When the organisms were fed sugars, they generated electrons and transferred them to the graphite electrodes. This flow of electrons generated electricity that the battery could store. The generation of power by microbes has led many scientists to develop microbial fuel cells. In 2005 Pennsylvania State University environmental engineers and scientists at Ion Power Inc. (in New Castle, Delaware) published the first process that shows bacteria can retrieve four times the amount of hydrogen out of a biomass than would typically be generated by fermentation alone.

## Microbial Forensics

Microbial forensics is a relatively new field applied to solving bioterrorism cases, medical negligence, and outbreaks of foodborne diseases. Use of microorganisms as weapons is not a new idea. They can be the weapon of choice in terrorists' activities, such as in the anthrax attacks of 2001. Forensic cases also have been reported about human immunodeficiency virus–infected people intentionally infecting others. Microbes are also of interest in cases of medical negligence, in which medical personnel are implicated in postsurgical or other hospital-acquired infections due to inadequate or relaxed hygiene practices. In the case of outbreaks of foodborne diseases or intentional food contamination, it is critical to trace the infecting microbe to the source, either the company or person(s) of origin. Microbial forensics is becoming an essential part of data collection and their interpretation. Inquiries should stand up to the review of scientists and healthcare professionals, as well as to the scrutiny of judges and juries.

## Summary

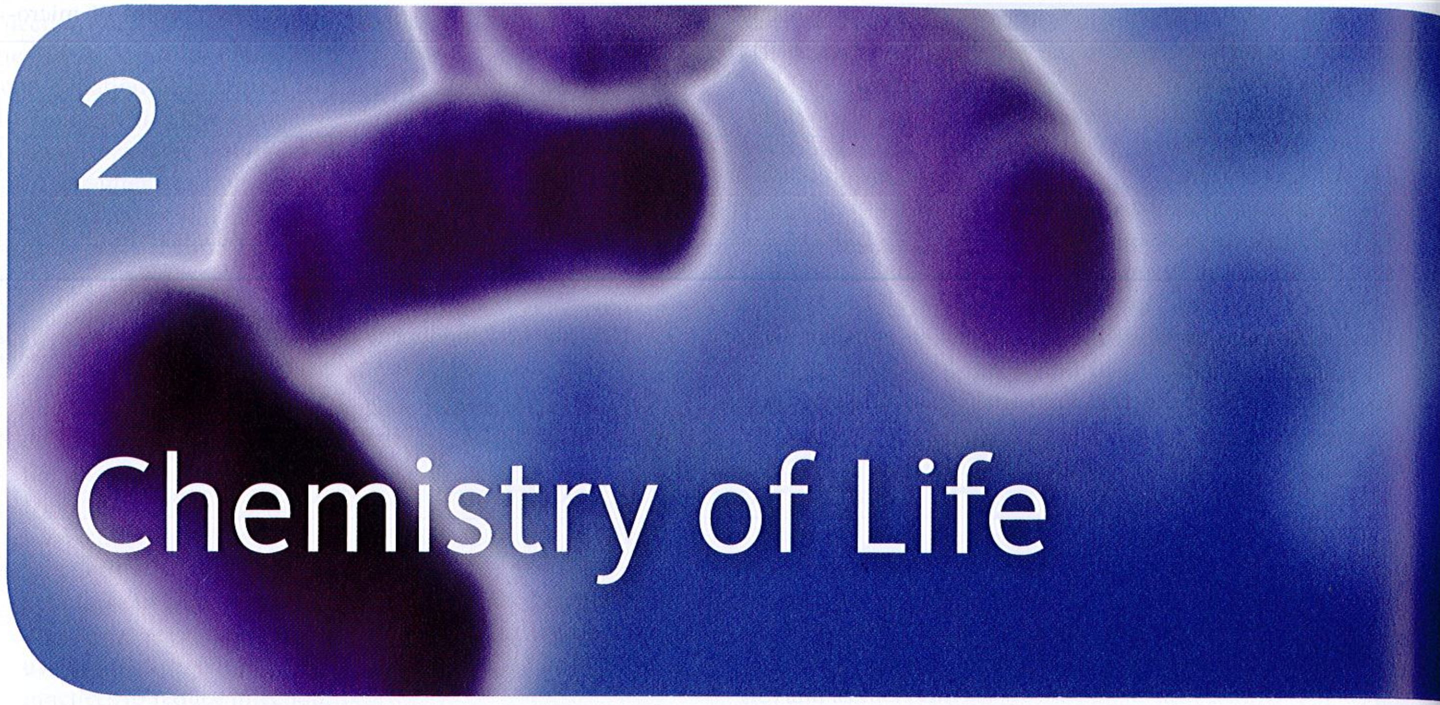
- The invention of microscopes made it possible to observe details of organisms and microorganisms that are invisible to the naked eye.
- Different types of microscopes developed over the years have been and continue to be specialized to perform different functions.
- Spontaneous generation was disproved and the germ theory of disease developed. This promoted vaccination and the use of aseptic techniques in surgery, among other hygienic practices to reduce infection.
- The first life on earth is dated at 3.5 to 4 billion years ago, and is believed to be represented by prokaryotes, which are still found in every environment on earth today. Through evolution, eukaryotic life appeared approximately 2.2 billion years ago.
- Microorganisms include bacteria, viruses, prions, algae, fungi, and protozoans.
- Taxonomy is the classification, nomenclature, and identification of living organisms. Classification starts with domain, followed by kingdom, phylum, class, order, family, and genus.
- Microbes occur in every environment, may build relationships with other organisms, and often form biofilms. Biofilms represent problems for many industries, including healthcare.
- Microorganisms are routinely found in and on humans without causing an infection or disease. These organisms are part of the normal flora.
- Transmission of infectious diseases can be airborne, waterborne, foodborne, or through direct contact.
- Microorganisms play a major role in the production of food, alcoholic beverages, and pharmaceuticals. They are also used in water treatment, agriculture, bioremediation, and forensics and as fuel cells.

## Review Questions

1. One type of microscope that provides a three-dimensional image of a specimen is the:
   a. Phase-contrast microscope
   b. Transmission electron microscope
   c. Bright-field microscope
   d. Scanning electron microscope
2. One type of microscope capable of observing living microorganisms is the:
   a. Bright-field microscope
   b. Phase-contrast microscope
   c. Fluorescence microscope
   d. Electron microscope
3. Which scientist is most responsible for ending the controversy about spontaneous generation?
   a. John Needham
   b. Joseph Lister
   c. Louis Pasteur
   d. Robert Koch
4. Fossils of prokaryotes go back __________ billion years.
   a. 4 to 5
   b. 3.5 to 4
   c. 2.5 to 3
   d. 2.2 to 2.7
5. Which of the following is not a microorganism?
   a. Bacterium
   b. Algae
   c. Insect
   d. Fungus
6. The correct descending order of the taxonomic categories is:
   a. Species, domain, phylum, kingdom, order, division, class, genus
   b. Domain, kingdom, phylum, class, family, order, genus, species
   c. Domain, kingdom, phylum, class, order, family, genus, species
   d. Kingdom, domain, phylum, order, class, family, genus, species
7. Complex communities of microorganisms on surfaces are called:
   a. Colonies
   b. Biofilms
   c. Biospheres
   d. Flora
8. A relationship between organisms in which the waste product of one provides nutrients for another is called:
   a. Mutualism
   b. Competition
   c. Synergism
   d. Commensalism
9. Which of the following sites of the human body does not have a normal flora?
   a. Intestine
   b. Skin
   c. Vagina
   d. Blood



10. Which of the following industries use(s) microorganisms?
    a. Chemical
    b. Wine
    c. Cheese
    d. All of the above
11. All bacteria are __________ cells based on presence or absence of cellular structures.
12. Cells that contain a nucleus are __________ cells.
13. The taxonomic resource for information on bacteria is the __________.
14. The proteins implicated in spongiform encephalopathy are __________.
15. The cleanup of different industrial waste is referred to as __________.
16. Name and briefly describe the different types of microscopes.
17. Describe Koch's postulates.
18. Compare and contrast prokaryotic and eukaryotic cells.
19. Describe how foodborne diseases can be prevented.
20. Describe the role of microorganisms in food production.



# 2 Chemistry of Life

## OUTLINE



## LEARNING OBJECTIVES

*After reading this chapter, the student will be able to:*

1. Define/describe matter, element, atom, and ion
2. Define/describe the atomic nucleus and define atomic weight, neutron, proton, electron, valence, and isotope
3. Name, describe, and rank the different types of chemical bonds
4. Describe the different types of chemical reactions
5. Define the rules of chemical notation
6. Discuss acid-base balance and the pH scale
7. Discuss the properties of water and define solvent, solute, solution, hypertonic, hypotonic, isotonic, hydrophilic, and hydrophobic
8. Describe the common properties of all organic molecules
9. Name the monomers of carbohydrates and describe the structure and function of disaccharides and polysaccharides



## LEARNING OBJECTIVES

10. Describe the structures and functions of amino acids, peptides, and proteins
11. Name and describe the structures and functions of the different lipids
12. Describe the structures of nucleic acids and nucleotides; name and discuss the function of the different nucleic acids and explain complementary base pairing

## KEY TERMS

**acids**
**adenosine triphosphate (ATP)**
**anabolism**
**anions**
**atomic nucleus**
**atomic number**
**atomic weight**
**atoms**
**bases**
**catabolism**
**cations**
**chemical bond**
**chemical compounds**
**chemical formula**
**cholesterol**
**covalent bonds**
**dehydration synthesis**
**deoxyribonucleic acid (DNA)**
**disaccharides**
**electrolyte**
**electrons**
**elements**
**endergonic**
**exchange reactions**
**exergonic**
**hydrogen bonds**
**hydrolysis**
**hydrophilic**
**hydrophobic**
**hypertonic**
**hypotonic**
**ionic bonds**
**ions**
**isotonic**
**isotopes**
**matter**
**molecules**
**monosaccharides**
**neutrons**
**nonpolar**
**oxidation**
**phospholipids**
**pH scale**
**polar**
**polysaccharides**
**prostaglandins**
**protons**
**radioactivity**
**redox**
**reduction**
**ribonucleic acid (RNA)**
**shells**
**solutes**
**solution**
**solvent**
**steroids**
**synthesis**
**triglycerides**
**valence electrons**
**van der Waals forces**

## WHY YOU NEED TO KNOW

### HISTORY

Before Aureolus Paracelsus (Philippus Theophrastus Bombastus von Hohenheim; 1493-1541 CE) the principles of Western medical practice evolved primarily from witchcraft, folk remedies, and religious mysticism. An organized step forward had occurred earlier with the Greek physician Hippocrates (460-377 BCE), known as *the father of medicine*, and Galen (131-201 CE), another Greek physician who practiced during the Roman era. Hippocrates accepted some of the rational concepts of his predecessors and taught that the body has natural resources to respond to disease and injury. Moreover, he believed that recovery from injury or disease is best implemented by getting the body in a condition to heal itself by using fresh air, water, and healthy food from nature, with limited intervention in the form of massages, purges, enemas, therapies, or drugs. His concepts of medical practice were based on a balance of the so-called "four humors": blood, phlegm, black bile, and yellow bile, to which Galen added the four "elements": earth, air, fire, and water. In addition, Galen introduced numerous medicaments called "galenicals," some of which are still in use today (e.g., Galen's cerate or cold cream) and an alcoholic extract called *tincture of opium* to alleviate pain.

Paracelsus in a much later era did not ascribe to these theories. Although he had studied medicine he did not obtain a degree to practice and was more interested in chemistry and alchemy. He proposed that the body was made up of chemicals and that disease was an imbalance of these chemicals that could be treated and/or corrected by the use of chemicals. He favored simple chemicals rather than complex compound chemical mixtures. He also prepared alcohol extracts or tinctures. Furthermore, he understood that the dose of a chemical was an important factor in determining effects that ranged from therapeutic to lethal, writing, "All things are poisons, for there is nothing without poisonous qualities. It is only the dose which makes a thing a poison." Successful, effective modern medical therapeutics stem from this chemical concept.

### IMPACT

The realization and acceptance of the role of body chemistry in health and disease have affected and shaped the understanding of current medical practice and of rational drug development. This understanding extends to the body's native physiological responses and to its responses to drugs. For example, according to the concept of chemical molecular structure (CMS), the configuration of specialized molecules on some cells complements or recognizes and fits the configuration of certain molecules (receptors) on other cells in a lock-and-key fashion. When this chemical recognition coupling

*Continued*

occurs it may initiate or interfere with a cascade of events that lead to a particular response. This chemical communication, modified by the genetic chemical directions given our cells, is the foundation for responses to our individual internal and external environments. If drugs such as antibiotics are administered, their degree of effectiveness is determined by how well the CMS of the antibiotic complements or fits the molecules of the receptor for that antibiotic. Thus the administration of an antibiotic, its distribution via the blood vascular system to its site of action, and the response are all understood through knowledge of chemistry. Microbiology is understood by understanding its chemistry within the network of the chemistry of life.

**FUTURE**

Improved technologies in chemistry, biochemistry, molecular biology, and biotechnology (including genetic engineering), coupled with investigative advances in other disciplines such as computer science, have significantly improved the development of new drugs for existing and emerging infectious diseases.

The role of the drug discovery chemist has changed significantly, although the same goals remain to find and test novel molecules that can reach and act on disease targets.

Therapies for current, emerging, and future infectious diseases are not only possible but are forthcoming as new technologies yield insights into their chemistry and the chemistry of the body.

# Atoms and Ions

All cells and organisms are made up of chemicals, and understanding the basic chemical principles is essential to understanding the structure and function of all organisms.

## Elements

Knowledge of the chemistry of life begins with an understanding of those chemical principles that govern the processes occurring in **matter.** *Matter* is defined as anything that occupies space and has mass. It can be in liquid, gaseous, or solid form and is composed of **elements,** the smallest particles of which are **atoms.** Elements cannot be broken down further by natural forces. Oxygen, carbon, hydrogen, nitrogen, phosphorus, and sulfur are some of the elements most commonly found in living cells (Table 2.1). Although these chemical elements usually do not exist in free form, they do occur in combinations called **chemical compounds.** The shorthand expression of a chemical compound is its **chemical formula.** For example, the chemical formula of table salt or sodium chloride is NaCl (see Chemical Notations later in this chapter).

## Atomic Model

All atoms have the same fundamental structure consisting of a center, or atomic nucleus, and surrounding shells (Figure 2.1). Due to the different numbers of subatomic particles, each element has its own characteristic atomic structure. Located in the center of the atom is the **atomic nucleus,** which consists of positively charged particles called **protons** and particles without charge called **neutrons.** The **atomic weight** (atomic mass) of an atom is equal to the sum of protons and neutrons. The **atomic number** indicates the number of protons in the atomic nucleus. Surrounding the atomic nucleus in shells are negatively charged particles called **electrons.** Electrons travel around the nucleus at high speed and occupy positions in a volume of space called an *orbital* or *electron cloud.* These orbitals form an energy level also referred to as **shells,** in which the electrons usually remain.

**TABLE 2.1** Common Elements in Living Organisms

| Element | Symbol | Atomic Number | Atomic Weight |
|---|---|---|---|
| Hydrogen | H | 1 | 1 |
| Carbon | C | 6 | 12 |
| Nitrogen | N | 7 | 14 |
| Oxygen | O | 8 | 16 |
| Sodium | Na | 11 | 23 |
| Magnesium | Mg | 12 | 24.3 |
| Phosphorus | P | 15 | 31 |
| Sulfur | S | 16 | 32.1 |
| Chlorine | Cl | 17 | 35.5 |
| Potassium | K | 19 | 39.1 |
| Calcium | Ca | 20 | 40.1 |
| Iron | Fe | 26 | 55.8 |
| Cobalt | Co | 27 | 58.9 |
| Copper | Cu | 29 | 63.5 |
| Zinc | Zn | 30 | 65.4 |
| Iodine | I | 53 | 126.9 |

The nucleus of a given atom is surrounded by successive shells spaced further and further away from the nucleus. The energy level of electrons increases with the distance of their shells from the nucleus. The innermost (first) shell can be occupied by up to two electrons within one orbital, the second shell by up to eight within four orbitals, and each consecutive shell can potentially hold more electrons. However, most elements with biological significance need eight electrons to fill the outermost shell. The shells always fill sequentially from the inside out: two electrons in the first shell, eight in the next, and so on. For example, carbon with 6 electrons carries 2 electrons in the first shell and 4 in the second shell, and sodium with 11 electrons has 2 electrons in the first shell, 8 in the second, and 1 in the third shell (Figure 2.2).

In general, the number of protons and electrons of an atom are equal, making the atom an electrically neutral unit. The stability of



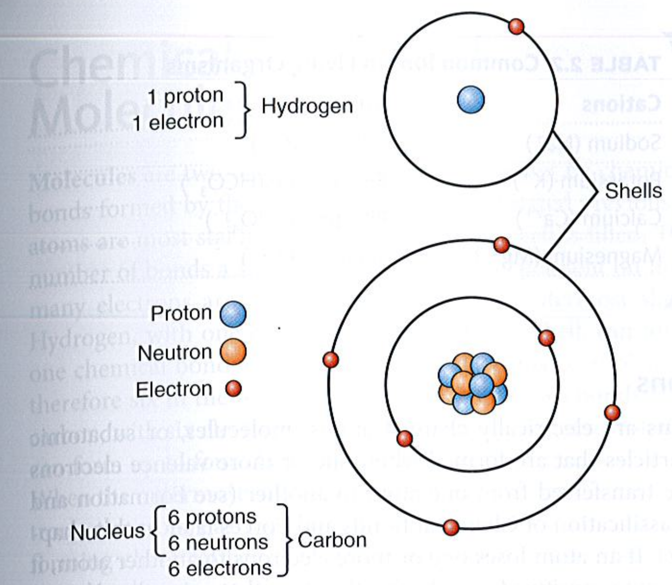



**FIGURE 2.1 Models of atomic structure.** These simplified diagrams of hydrogen and carbon atoms show the atomic nucleus containing protons and neutrons in the center of the atom, and the electrons in the surrounding shells.

an atom depends on the number of electrons in the outermost shell. An atom is most stable if the outermost shell is filled to its capacity. Hydrogen is the simplest element, with the atomic number of 1, and therefore has one electron in the outermost shell. Helium, with the atomic number of 2, has two electrons in the outermost shell. This shell is fully occupied and is stable. Helium atoms will not react with each other and also cannot combine with atoms of other elements. Helium is therefore called an *inert gas.*

If the outermost shell is not complete, the atom can participate in a chemical reaction and form a **chemical bond.** Electrons in the outermost shell of an atom that are available for chemical bonding are called **valence electrons.** These electrons determine what kind of chemical bonds, if any, the atom can form.

**Isotopes** are atoms with the same number of protons but a different number of neutrons. The atomic number of isotopes is unchanged because the number of protons remains the same and only the atomic weight is different. For example, the element hydrogen has two isotopes (Figure 2.3):

- Hydrogen (one proton)
- Deuterium (one proton and one neutron)
- Tritium (one proton and two neutrons)

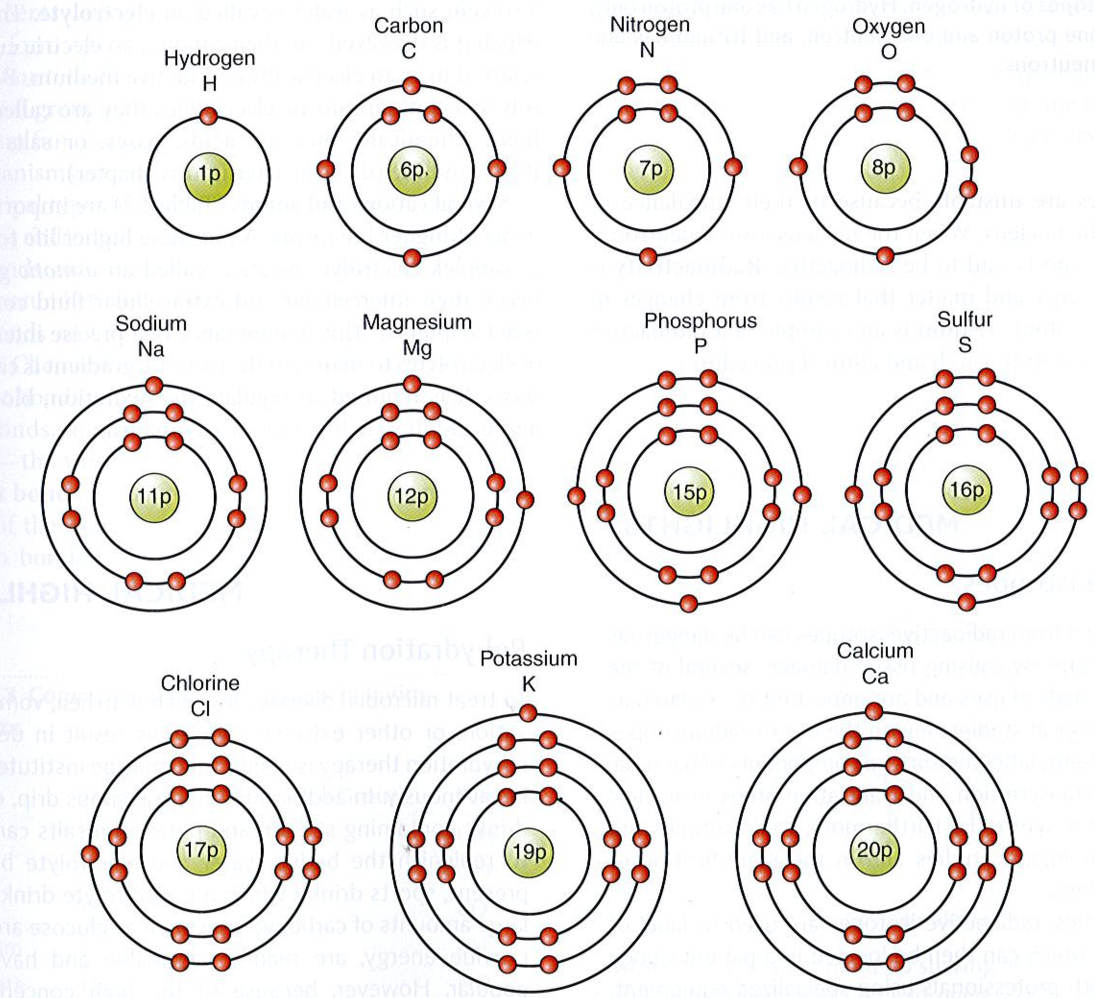



**FIGURE 2.2 Atomic structure of several different atoms important in living systems.** The diagrams illustrate that the number of protons equals the number of electrons in each of these atoms, which are found in association with living systems.

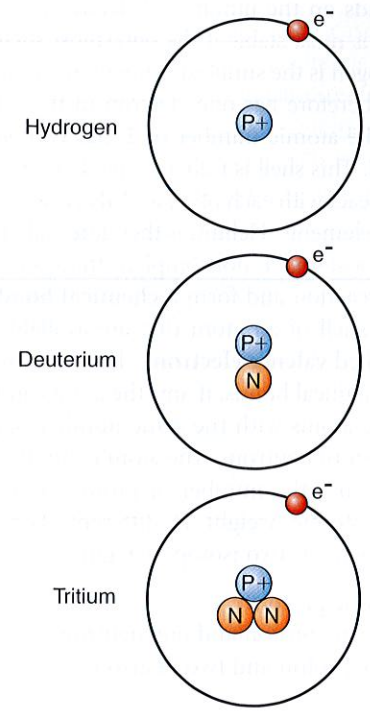



**FIGURE 2.3** **Isotopes of hydrogen.** Hydrogen has one proton only, deuterium has one proton and one neutron, and tritium has one proton and two neutrons.

Radioisotopes are unstable because of their imbalance of energy within the nucleus. When the nucleus loses a neutron it gives off energy and is said to be radioactive. **Radioactivity** is the release of energy and matter that results from changes in the nucleus of an atom. Tritium is an example of a radioactive isotope that is used in research and clinical procedures.

**MEDICAL HIGHLIGHTS**

### Radioactive Isotopes

Though radiation from radioactive isotopes can be dangerous to living organisms by causing tissue damage, several of the isotopes have medical uses and are important to research as well. Microbiological studies rely on the use of radioisotopes to identify proteins, label the surface components of bacteria, and trace the transcription and translation steps in nucleic acid and protein synthesis. Furthermore, radioisotopes are used in immunological studies and in the identification of specific pathogens.

In therapeutics, radioactive isotopes are used to label or tag molecules, which can then be located in a patient's body tissues by health professionals using specialized equipment. This methodology enables physicians to locate damaged tissue for more precisely focused treatment, including the targeting and killing of certain cancers.

**TABLE 2.2 Common Ions in Living Organisms**

| Cations | Anions |
|---|---|
| Sodium ($Na^+$) | Chloride ($Cl^-$) |
| Potassium ($K^+$) | Bicarbonate ($HCO_3^-$) |
| Calcium ($Ca^{2+}$) | Phosphate ($PO_4^{3-}$) |
| Magnesium ($Mg^{2+}$) | Sulfate ($SO_4^{2-}$) |

## Ions

**Ions** are electrically charged atoms, molecules, or subatomic particles that are formed when one or more valence electrons are transferred from one atom to another (see Formation and Classification of Chemical Bonds and Forces later in this chapter). If an atom loses one or more electrons to another atom, it becomes positive (+), whereas the atom that gains the electron becomes negative (−). Positively charged ions are called **cations,** and in an electric field move toward the negative pole, the cathode. Negatively charged ions, referred to as **anions,** move toward the positive pole, or anode, of an electric field.

A substance that dissociates into free ions when dissolved in a solvent such as water is called an **electrolyte.** The solvent in which it is dissolved can then conduct an electric current and is referred to as an electrically conductive medium. Because these solvents contain ions or electrolytes they are called *ionic solutions.* Chemically they are acids, bases, or salts (see Acids, Bases, and the pH Scale later in this chapter).

Several cations and anions (Table 2.2) are important components of higher life forms. All of these higher life forms require a complex electrolyte balance, called an *osmotic gradient,* between their intercellular and extracellular fluid compartments (see Chapter 3). This maintenance of a precise internal balance of electrolytes to maintain the osmotic gradient is called *homeostasis.* It is required to regulate the hydration, blood pH, and nerve and muscle function of an organism.

**MEDICAL HIGHLIGHTS**

### Rehydration Therapy

To treat microbial diseases in which diarrhea, vomiting, starvation, or other extreme conditions result in dehydration, rehydration therapy is needed and may be instituted orally or intravenously. In addition to an intravenous drip, electrolyte drinks containing sodium and potassium salts can be taken to replenish the body's water and electrolyte balance. At present, sports drinks, which are electrolyte drinks to which large amounts of carbohydrates such as glucose are added to provide energy, are readily obtainable and have become popular. However, because of the high concentration of sugar in these drinks, they are not recommended for regular use by children. Instead, specially formulated pediatric electrolyte solutions are available.



# Chemical Bonds and Molecules

**Molecules** are two or more atoms linked together by chemical bonds formed by their valence electrons. As stated previously, atoms are most stable when their outermost shell is filled. The number of bonds a single atom can have is dependent on how many electrons are needed to complete the outermost shell. Hydrogen, with one electron in the outermost shell, can form one chemical bond; oxygen, with eight electrons (2 + 6) (and therefore six in the outermost shell), can form two bonds; and carbon, with six electrons (2 + 4) (four in the outermost shell), can form up to four chemical bonds to fill the outermost shell. When the outermost shell is not completely occupied with electrons, the atom has the tendency to interact with other atoms forming chemical bonds to achieve higher stability. These atoms then become stable and cannot react with others.

## Formation and Classification of Chemical Bonds and Forces

Molecules made from atoms of different elements are called *compounds.* Compounds have different properties than the individual elements within the compound. Groups of atoms that consistently form specific entities within compounds are referred to as *functional groups.* Some molecules have more than one functional group, which may differ from one another. The most common functional groups found in molecules important to living organisms are shown in Table 2.3.

The principal types of chemical bonds formed by the interactions of atoms and/or molecules are covalent bonds, ionic bonds, hydrogen bonds, and van der Waals forces. Covalent and hydrogen bonds occur between atoms to form a molecule, whereas hydrogen bonds and van der Waals forces are intermolecular connections. Chemical bonds vary in their strength, but, in general, covalent bonds are considered the strongest bond, followed by ionic bonds, hydrogen bonds, and—with the weakest connection—the van der Waals forces.

**Covalent bonds** result from a sharing of electrons between two atoms of the same element or between atoms of different elements. In bonds between identical atoms such as oxygen and hydrogen, the electrons are shared equally by each atom. Covalent bonds usually are the strongest chemical bonds. Because the electrons are equally distributed, the resulting molecule is nonpolar and the bond is called a **nonpolar** covalent bond (Figure 2.4, *A*). Carbon atoms play a significant role in large organic molecules because they form stable nonpolar covalent bonds with each other. This stable framework is the backbone of organic carbon-based molecules, providing the chemical foundation of organic chemistry and of life.

The covalent bonds between atoms of two different-sized elements are **polar** covalent bonds, in which the electrons are unequally distributed because they are pulled toward the larger atom. As a result, one end of the molecule becomes more negative compared with the other end (Figure 2.4, *B*). Oxygen, nitrogen, and phosphorus atoms have a tendency to form polar covalent bonds. Polar covalent bonds are somewhat weaker than nonpolar covalent bonds. Coordinate covalent bonds, such as occurs in the formation of the ammonium ion from ammonia, are formed when both electrons are from one atom. The molecule no living organism can exist without is water, in which the atoms hydrogen and oxygen are held together by polar covalent bonds. Some properties of water result from this type of bond.

Depending on the number of electrons shared, molecules can be formed from a single covalent bond by sharing one pair of electrons, such as the bond between hydrogen atoms. Single covalent bonds are indicated by one solid line (H—H). Double covalent bonds are formed by sharing two pairs of electrons, as

**TABLE 2.3 Common Functional Groups in Living Organisms**

| Functional Group | Formula | Functional Group | Formula |
|---|---|---|---|
| Acetyl | $CH_3$ | Ethyl | $C_2H_5$ |
| Aldehyde | CHO | Hydroxyl | OH |
| Amino | $NH_2$ | Keto | CO |
| Ammonium | $NH_4$ | Methyl | $CH_3$ |
| Bicarbonate | $HCO_3$ | Nitrate | $NO_3$ |
| Carbonate | $CO_3$ | Phosphate | $PO_4$ |
| Carboxyl | COOH | Sulfate | $SO_4$ |

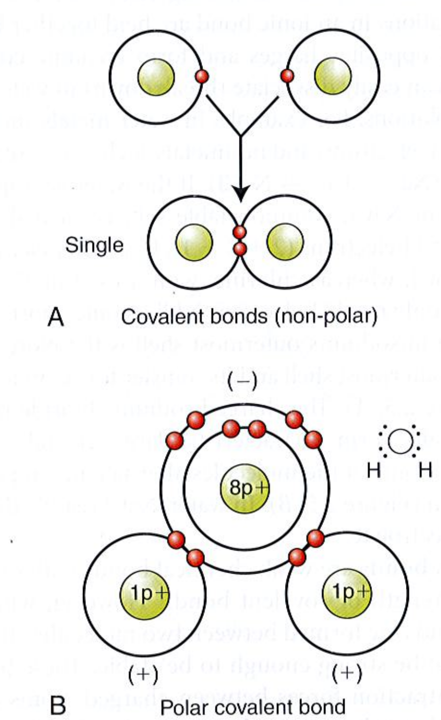



**FIGURE 2.4** **Covalent bonds: a sharing of electrons.** **A,** Nonpolar covalent bond between two hydrogen atoms ($H_2$), formed by an equal sharing of electrons. **B,** Polar covalent bond between an oxygen atom and two hydrogen atoms ($H_2O$), formed by an unequal sharing of electrons.

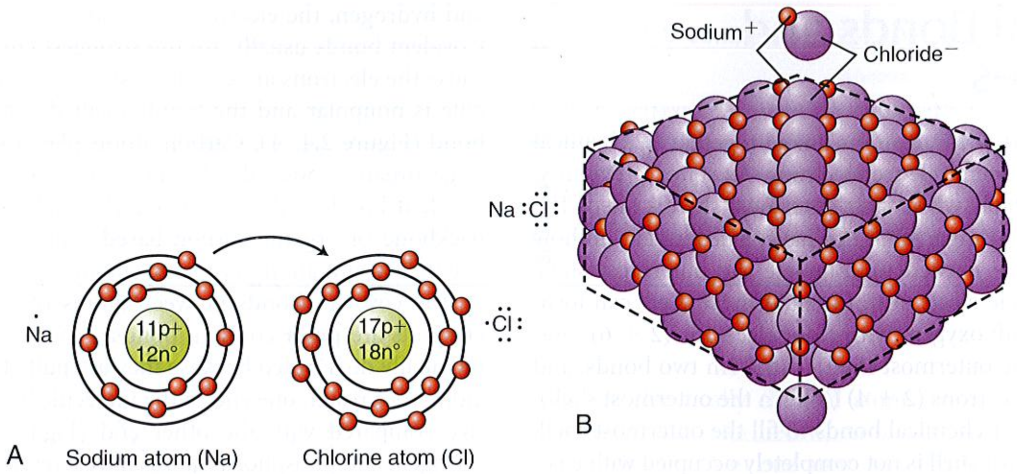



**FIGURE 2.5 Ionic bond. A,** The electron in the outermost shell of the sodium atom is transferred to the chlorine atom, resulting in a sodium (+) and a chloride (−) ion. **B,** Crystal of sodium chloride (table salt). The sodium and chloride ions alternate in a definite, regular, geometric pattern.

seen between oxygen atoms. These bonds are indicated by two solid lines (O=O). Triple covalent bonds may occur through the sharing of three pairs of electrons, such as between nitrogen atoms. These bonds are identified by three solid lines (N≡N).

**Ionic bonds** are formed when one or more electrons from one atom are transferred to another. If an atom loses one electron in the process it will have a charge of +1; if two electrons are lost the charge will be +2, because the protons in the nucleus will be unbalanced by the remaining electrons. The resulting anions and cations in an ionic bond are held together by attraction of their opposite charges and form an ionic compound. Ionic bonds can easily dissociate (break down) in water to form electrolyte solutions. For example, in water metals such as $Na^+$ readily give up electrons, and nonmetals such as $Cl^-$ readily take up electrons ($Na^+ + Cl^- \rightarrow NaCl$). If the water is evaporated, a solid crystal of NaCl, common table salt, is formed. Sodium, with a total of 11 electrons (2 + 8 + 1), has only 1 electron in its outermost shell, whereas chlorine, with a total of 17 electrons (2 + 8 + 7), only needs 1 electron to fill its outermost shell. The only electron in sodium's outermost shell is therefore attracted to chlorine's outermost shell and its transfer forms an ionic compound (Figure 2.5, *A*). The charged sodium chloride molecules and other salts form characteristic large crystal structures in which the atoms of the molecules alternate in a regular, geometric pattern (Figure 2.5, *B*). In water, NaCl readily dissociates to form an electrolyte.

**Hydrogen bonds** are weak chemical bonds with only about 5% of the strength of covalent bonds. However, when many hydrogen bonds are formed between two molecules, the resulting union can be strong enough to be stable. These bonds are formed by attraction forces between charged atoms within a large molecule or between adjacent molecules (Figure 2.6). Hydrogen bonds always involve a hydrogen atom with a slightly positive charge and an oxygen or nitrogen atom with a slightly negative charge. Although hydrogen bonds do not form molecules they can alter the shapes of molecules or hold together different molecules. Examples of hydrogen bonds include

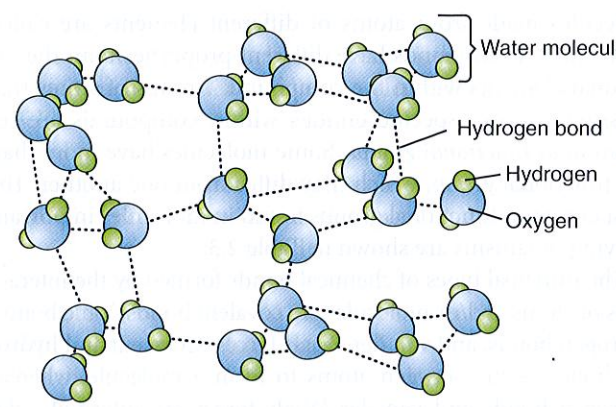



**FIGURE 2.6 Hydrogen bond between water molecules.** The positively charged hydrogen portion of one water molecule is slightly attracted to the negatively charged oxygen portion of another water molecule. Hydrogen bonds are always indicated by dotted lines.

bonds between water molecules, acetic acid molecules, amino acid molecules, and nucleic acid molecules. Hydrogen bonds are always indicated by dotted lines (- - -). The attraction created by hydrogen bonds keeps water in the liquid state over a wide range of temperatures.

**Van der Waals forces** are the weakest of the intermolecular forces in all chemical reactions. The van der Waals force of attraction is inversely proportional to the seventh power of the interatomic distance, whereas the force of an ionic bond diminishes as the square of the distance. Therefore a very slight increase in the interatomic distance between atoms markedly reduces the van der Waals force of attraction. In terms of the lock-and-key concept of reactants and their receptors, such as between antigens and antibodies and between drugs and their receptors, van der Waals forces determine the final molecular arrangements that define selectivity and specificity properties. In other words, if there is an interaction (selectivity), how well it fits (specificity) is a function of the van der Waals forces. Van der Waals forces explain how a spider can hang upside down



from a ceiling and why a gecko can hang by one toe from a glass surface. For drug-receptor interactions, ionic bonds based on electrostatic attraction probably come into play as the drug approaches the immediate vicinity of the receptor, followed by additional attraction based on van der Waals forces. Covalent bonding is more of a factor in longer lasting drug actions.

## Types of Chemical Reactions

Pathways of chemical reactions trace metabolic activities in living organisms. Within these pathways, several chemical reactions occur that are essential for the survival of living organisms, including microbes. These reactions are as follows:

- Dehydration synthesis (condensation)
- Hydrolysis (decomposition)
- Endergonic (energy-requiring) reactions
- Exergonic (energy-producing) reactions
- Oxidation and reduction (redox)
- Exchange reactions

**Dehydration synthesis,** or condensation, is the formation of a larger compound (polymer) from smaller ones (monomers). Monomers are the unit molecules (building blocks) of these larger molecules, called *polymers*. These reactions require specific enzymes and the removal of water from the reactants; that is, a hydroxyl group (OH) is removed from one monomer and combined with a hydrogen (H) from the other (Figure 2.7). Enzymes (see Chapter 3) are biological catalysts and function to speed up the rate of chemical reactions without changing themselves. The **synthesis** of new compounds within a cell occurs during **anabolism,** which uses energy provided by **catabolism** (see Cellular Metabolism in Chapter 3). An example of synthesis is the production of complex sugars from simple sugars (see Carbohydrates later in this chapter).

$$\text{Glucose} + \text{glucose} \rightarrow \text{maltose}$$

$$\text{Monomer} + \text{monomer} \rightarrow \text{polymer}$$

**Hydrolysis** (decomposition) breaks down large molecules (polymers) into their unit molecules (monomers). An example of hydrolysis is the breakdown of nutrient molecules such as carbohydrates, proteins, and lipids into smaller molecules during the digestive process. Hydrolysis is the reverse of dehydration synthesis and occurs during the metabolic process of catabolism. This complex reaction of breaking down polymers requires water, and the resulting monomers can be used in cellular metabolism for the generation of energy.

Reactions that yield energy are called **exergonic** reactions. Reactions that use energy are **endergonic** reactions. Hydrolysis that occurs during catabolism is an exergonic reaction and it releases energy. Endergonic reactions require energy such as occurs in the dehydration synthesis of nutrient molecules during anabolism.

**Redox** (*red*uction-*ox*idation) reactions are chemical reactions in which atoms have their oxidation number (oxidation state) changed. An oxidation reaction does not occur without a reduction reaction happening at the same time. **Oxidation** is loss of electrons and is a common reaction in the production of cellular energy. The transfer of electrons is catalyzed by enzymes within the metabolic pathways. The electrical state of an atom is identified as the oxidation state or by its oxidation number. Atoms are neutral and their oxidation state is therefore zero. The oxidation state changes because of a loss or gain of electrons. Loss of electrons results in a positive oxidation state of an atom and a gain of electrons results in a negative oxidation state. Oxidation reactions are indicated as follows, using calcium as an example:

$$Ca \rightarrow Ca^{2+}\ 2e^-$$

Oxidation-reduction (electron transfer) reactions are coupled. In other words, oxidation reactions occur with reduction reactions (Figure 2.8). **Reduction** is the gain of electrons. It is also catalyzed by enzymatic reactions and is written as follows:

$$O + 2e^- \rightarrow O^{2-}$$

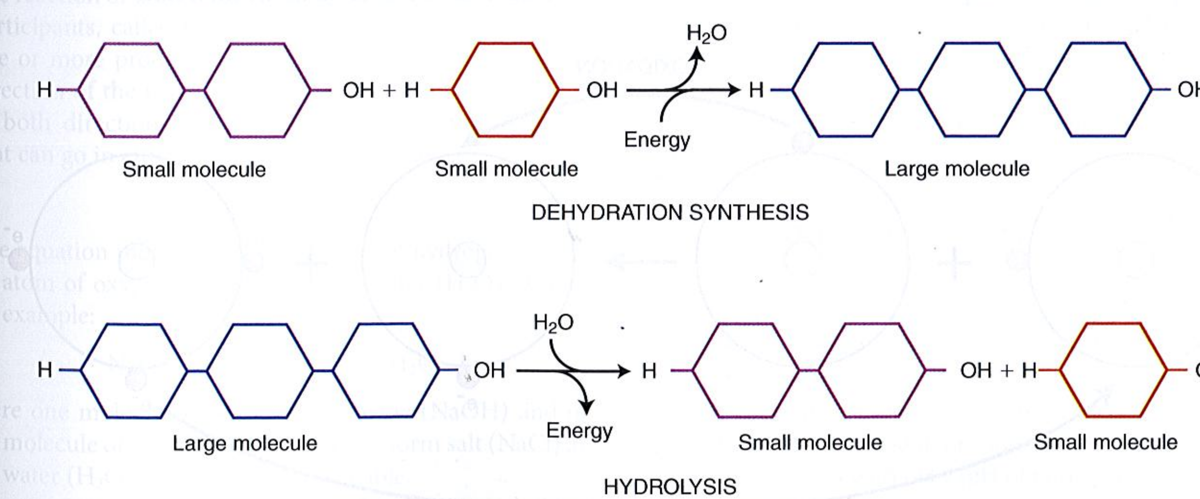



**FIGURE 2.7 Dehydration synthesis and hydrolysis.** Dehydration synthesis is an anabolic reaction (requires energy) producing polymers (large molecules) from monomers (small molecules) by removing water. Hydrolysis is a catabolic reaction (releasing energy) in which polymers are broken down into monomers. These reactions require water.

LIFE APPLICATION

### Bleach

The decolorizing action of bleaching agents is partly due to their ability to remove the electrons that are activated by light to produce the various colors. Most chemical bleaches contain sodium hypochlorite (NaOCl) or hydrogen peroxide ($H_2O_2$), which are both oxidizing agents. Hypochlorite ($OCl^-$) is reduced to chloride ions and hydroxyl ions:

$$OCl^- + 2e^- + HOH \rightarrow Cl^- + 2OH^-$$

Bleaches are sometimes combined with other compounds that are different from bleaches but are capable of absorbing wavelengths of ultraviolet light, which is invisible to the human eye. They convert these wavelengths to visible blue or blue-green light that is reflected, making the fabric appear brighter.

**Exchange reactions** transfer the same molecules in a reaction but in a different combination. In other words, the components of the reaction and the products remain the same but their combination results in a different product:

$$AB + CD \rightarrow AD + CB$$

The balancing of chemical activities in living cells is a continuous, dynamic process. In general, however, breaking of bonds releases more energy than is required for synthesis. A living cell continuously undergoes hydrolysis and synthesis for the purpose of energy production such as occurs in cells during the breakdown of complex molecules (catabolism). Chemical bonds contain potential energy that is released when the bonds are broken. This energy is captured and used in reactions for the essential functions of a cell. Catabolic reactions are primarily oxidation reactions and are divided into different pathways, namely, glycolysis, the Krebs cycle (citric acid cycle), and the electron transport chain (see Chapter 3).

LIFE APPLICATION

### Nitrogen Fixation

Nitrogen, the most abundant element in the atmosphere of the earth, is a vital element in many of the compounds essential to living systems. For example, in all green plants nitrogen is a primary nutrient. However, it must be modified by a process called nitrogen fixation before it can be used. Nitrogen fixation is a complex process that reduces nitrogen to nitrogen compounds such as ammonia, nitrate, and nitrogen dioxide. This process is performed by different nitrogen-fixing bacteria present in soil. Nitrogen fixation involves a number of oxidation-reduction reactions that occur sequentially to yield ammonium ions. Also, nitrogen in the form of ammonia is used by living systems in the synthesis of many organic compounds. Ammonia can be formed by the process of nitrification, during which nitrates and nitrites released by decaying organic matter are converted to ammonium ions by nitrifying bacteria, again present in the soil. This process can also be achieved by a series of oxidation-reduction reactions. Denitrifying bacteria, acting on ammonia and nitrates produced by decay, recycle these compounds to free nitrogen to complete what is called the *nitrogen cycle*.

## Chemical Notations

Chemical compounds and reactions are shown by "chemical shorthand," or *chemical notation*. The rules of the chemical notation are as follows (Box 2.1):

- The abbreviation of an element represents one atom of that element and is its chemical symbol.
- The number before the chemical symbol is the number of atoms; the number before the chemical formula is the number of molecules.
- The subscript after the chemical symbol of an element shows the number of that atom in the molecule.

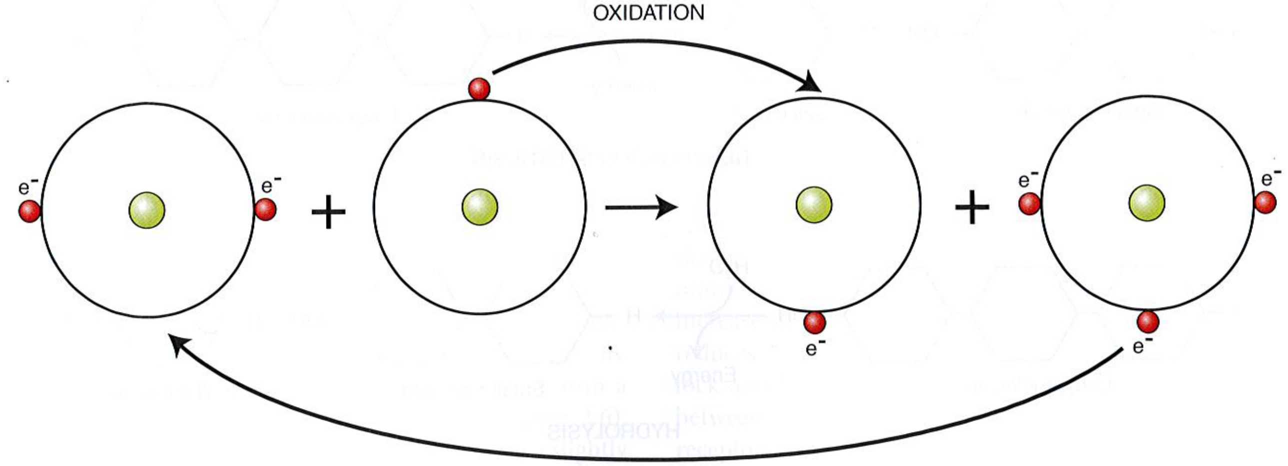



**FIGURE 2.8 Oxidation-reduction (electron transfer) reactions.** Oxidation refers to the loss of electrons, whereas reduction involves gaining electrons.



**BOX 2.1 Chemical Notation**

**SYMBOLS**

H: An atom of hydrogen
O: An atom of oxygen
C: An atom of carbon
N: An atom of nitrogen

**PREFIXES**

2H: Two individual atoms of hydrogen
2O: Two individual atoms of oxygen
3C: Three individual atoms of carbon
4N: Four individual atoms of nitrogen

**NUMBER OF ATOMS IN A MOLECULE**

$H_2$: A molecule with two hydrogen atoms
$O_2$: A molecule with two oxygen atoms
$C_6$: A molecule with six carbon atoms
$N_4$: A molecule with four nitrogen atoms

**SUPERSCRIPTS**

$Na^+$: A sodium atom that has lost one electron, resulting in a positively charged ion
$Cl^-$: A chlorine atom that has gained one electron, resulting in a negatively charged ion
$K^+$: A potassium ion
$Ca^{2+}$: A calcium atom that has lost two electrons

**CHEMICAL EQUATIONS**

**Balanced**

$2H + O \rightarrow H_2O$
$2H_2 + O_2 \rightarrow 2H_2O$
$NaOH + HCl \rightarrow NaCl + H_2O$

**Unbalanced**

$H_2O_2 \rightarrow H_2O$
$2NaOH + HCl \rightarrow NaCl + H_2O$

- The reaction of the chemicals describes the interaction of the participants, called the *reactants*. Chemical reactions form one or more products. Arrows in the formula indicate the direction of the reaction, from reactant to product. Arrows in both directions indicate a reversible chemical reaction that can go in either direction. For example:

$$2H + O \rightarrow H_2O$$

The equation indicates that two atoms of hydrogen and one atom of oxygen combine to form water ($H_2O$). Another example:

$$NaOH + HCl \leftrightarrow NaCl + H_2O$$

Here one molecule of sodium hydroxide (NaOH) and one molecule of hydrochloric acid (HCl) form salt (NaCl) and water ($H_2O$); the reaction is reversible.

- A superscript of plus or minus after the atomic symbol indicates an ion. A single plus or minus shows the charge of an ion. If more than one electron has been lost or gained, the charge of the ions is shown with a number before the plus or minus sign.
- Chemical reactions do not form or destroy atoms; they just rearrange them into new combinations. In any given chemical equation the number of atoms of each element must be the same on both sides, resulting in a balanced equation.

# Inorganic Compounds

Inorganic compounds consist of molecules that do not contain carbon, with the exception of a few molecules that are classified as inorganic compounds although they contain carbon, such as carbon dioxide ($CO_2$) and carbon monoxide (CO).

## Acids, Bases, and the pH Scale

Some chemical compounds dissociate in water as ions, carry an electric current, and display an electrical charge. Substances that release hydrogen ions ($H^+$) are **acids**, and those that release hydroxyl ions ($OH^-$) are **bases**. The strength of acids and bases is determined by the hydrogen ion concentration of the water in which they dissociate. The higher the hydrogen ion concentration in the solution the more acidic the solution is. A low hydrogen ion concentration of a solution indicates a basic solution (Box 2.2).

This acidity or alkalinity of a solution is measured by the **pH scale** ("potential hydrogens"). It is a chemical symbol that ranges from 0 to 14 and is the negative logarithm of the hydrogen ion concentration (Figure 2.9). A solution that has a neutral pH is one in which the concentrations of $H^+$ and $OH^-$ ions are equal ($10^{-7}$ M), and the chemical symbol for this negative logarithm is pH 7. The point of neutrality is standardized as the pH of pure water at 25° C. As the $H^+$ concentration increases, the $OH^-$ concentration decreases and vice versa. A change of 1 unit on the pH scale (the negative logarithm scale) represents a ten fold change in the hydrogen ion concentration. The higher the hydrogen ion concentration of a solution, the lower the number on the pH scale and the more acid the solution; the higher the number on the scale, the higher the hydroxyl ion concentration and the more basic (alkaline) the solution.

Chemical reactions in a living cell respond to slight changes in the pH of their environment. The majority of microbes, as well as human cells, survive better in a neutral or slightly basic environment. This sensitivity of microbes to changes in pH is

**BOX 2.2 Definitions**

**Acid:** A molecule that can release $H^+$ into a solution; it is called a "proton donor"
**Base:** A molecule that can combine with $H^+$ and removes it from the solution; it is called a "proton acceptor"
**pH:** The negative logarithm of the $H^+$ concentration of a solution, indicated in pH units on a pH scale that runs from 0 to 14 with pH 7 (pH of pure water at 25° C) as neutral
**Buffer:** A system of molecules and ions that stabilize the pH of a solution by resisting changes in the $H^+$ concentration of the solution

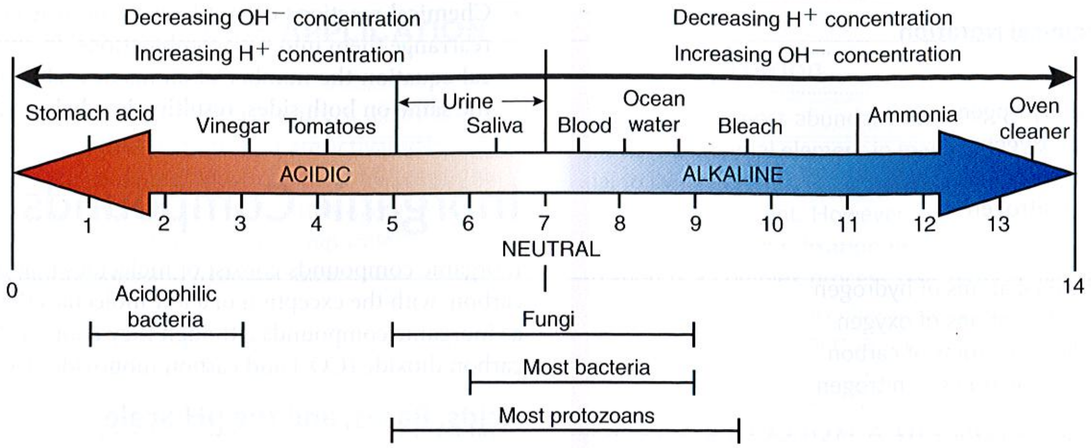



**FIGURE 2.9 The pH scale. With increasing hydrogen ion concentrations a solution becomes more acidic and the pH value decreases. When the hydrogen ion concentration decreases the solution becomes more basic and the pH value increases.**

used in the control of microbial growth and in food preservation (see Chapter 7). However, as mentioned in Chapter 1, some microorganisms are found to exist successfully in all environments, such as sulfur-oxidizing bacteria, which prefer a very acidic environment, and yeast, which flourishes under slightly acidic conditions.

## Buffers

Buffers are chemicals that can absorb hydrogen or hydroxyl ions and therefore resist changes in the pH of a solution. In a living organism buffers are essential to maintain the pH necessary for the survival of its cells. A common endogenous buffer in cells is the bicarbonate ($HCO_3^-$) ion. Bicarbonate picks up hydrogen ions ($H^+$), forming carbonic acid ($H_2CO_3$):

$$HCO_3^- + H^+ \leftrightarrow H_2CO_3$$

This reaction is readily reversible so that adjustments in the pH can be made to maintain the required pH in a given environment. All biological fluids, both intracellular and extracellular, are heavily buffered.

## Salts

Substances that dissociate in water and normally do not release hydrogen or hydroxyl ions are known as *normal salts.* Natron is a salt, composed of a mixture of sodium bicarbonate (common baking soda), sodium carbonate (soda ash), a small amount of sodium chloride (table salt), and sodium sulfate. Natron is called *impure salt* because it has lost its saltiness. Salts that contain a hydroxide ion are basic salts and salts that contain a hydrogen ion are acid salts. Salts are formed when acids and bases react. Solutions of salts in water are called *electrolytes* and they conduct electricity.

Salts are formed by chemical reactions between a base and an acid or between a metal and an acid. The name of a salt starts with the name of the cation (ammonium, magnesium, etc.) followed by the name of the anion (chloride, sulfate, etc.). For example:

$$NH_3 + HCl \rightarrow NH_4Cl \text{ (ammonium chloride)}$$

$$Mg + H_2SO_4 \rightarrow MgSO_4 + H_2$$

Sometimes salts are referred to less specifically by the name of the cation (e.g., sodium salt, ammonium salt) or by the name of the anion (e.g., chloride, acetate). Common salt-forming cations and anions are shown in Table 2.4.

Salts are usually solid crystals but can exist as a liquid at room temperature and are called *ionic liquids.* Different salts can stimulate sensations of all five basic tastes: salty (sodium chloride), sweet (lead diacetate), sour (potassium bitartrate), bitter (magnesium sulfate), and umami (monosodium glutamate). Pure salts are odorless whereas impure salts may smell acidic (acetates) or basic (ammonium salts). Salts can be clear and transparent (sodium chloride), opaque (titanium dioxide), or metallic (iron disulfate). They also exist in different colors (Table 2.5). Most mineral, inorganic pigments and many synthetic organic dyes are salts.

**TABLE 2.4 Common Salt-forming Anions and Cations**

| Salt-forming Anions* | | Salt-forming Cations | |
|---|---|---|---|
| **Name** | **Formula** | **Name** | **Formula** |
| Acetate (acetic acid) | $CH_3COO^-$ | Ammonium | $NH_4^+$ |
| Carbonate (carbonic acid) | $CO_3^{2-}$ | Calcium | $Ca^{2+}$ |
| Chloride (hydrochloric acid) | $Cl^-$ | Iron | $Fe^{2+}$ and $Fe^{3+}$ |
| Hydroxide | $OH^-$ | Magnesium | $Mg^{2+}$ |
| Nitrate (nitric acid) | $NO_3^-$ | Potassium | $K^+$ |
| Oxide | $O_2^-$ | Pyridinium | $C_5H_5NH^+$ |
| Phosphate (phosphoric acid) | $PO_4^{3-}$ | Quaternary ammonium | $NR_4^{+\dagger}$ |
| Sulfate (sulfuric acid) | $SO_4^{2-}$ | Sodium | $Na^+$ |

*Parent acid in parentheses.
†A side chain/group.



**TABLE 2.5 Colors of Salts**

| Name of Salt | Formula | Color |
|---|---|---|
| Sodium chloride | $NaCl$ | Clear, transparent |
| Titanium dioxide | $TiO_2$ | Opaque, white |
| Iron disulfide | $FeS_2$ | Metallic |
| Sodium chromate | $Na_2Cr_2O_4{\cdot}2H_2O$ | Yellow |
| Sodium dichromate | $Na_2Cr_2O_7{\cdot}2H_2O$ | Orange |
| Mercury sulfide | $HgS$ | Red |
| Cobalt dichloride hexahydrate | $CoCl_2{\cdot}6H_2O$ | Mauve |
| Copper sulfate pentahydrate | $CuSO_4{\cdot}5H_2O$ | Blue |
| Ferric hexacyanoferrate | $Fe_4[Fe(CN)_6]_3$ | Blue |
| Nickel oxide | $Ni_2O_3$ | Green |
| Magnesium sulfate | $MgSO_4$ | Colorless |
| Manganese dioxide | $MnO_2$ | Black |

## Water

Life on earth most likely exists because of the abundance of liquid water. Water is unique because it can exist in three different temperature-dependent states: gas (steam), liquid, and solid (ice). Water is a molecule that consists of hydrogen and oxygen at a ratio of 2 to 1 and is absolutely necessary for all life forms. The bond between the oxygen atom and the hydrogen atom is a polar covalent bond, resulting in the molecule having a slightly positive side and a slightly negative side. Water molecules are held together by hydrogen bonding. Water molecules can quickly break down and reform their hydrogen bonds, which results in the property of cohesion. Water's high level of cohesion, or "stickiness," allows things to float easily on its surface at the air-water interface, and also causes water to form beads when dispersed. It is the most abundant molecule in the human body and in microorganisms, which contain at least 70% water, and the earth's surface is 71% covered by water as well.

**LIFE APPLICATION**

### Water Insects

Many insects can skate across the surface of water because of the water's high surface tension. One of the most common examples is the water strider, an insect that never breaks the surface as it skates on the water of streams, rivers, ponds, and even the ocean. Long-distance water movement is essential to the survival of land plants. On a dry, warm, sunny day a leaf may lose almost 100% of its water in a very short time. Therefore the water evaporated from the leaves must be replaced by the uptake of water from the soil. This water transport can be explained by the *cohesion-tension theory,* which states that the driving force of this transport is the evaporation (transpiration) of water from the leaf surfaces. The water molecules stick together (cohere) and are pulled up the plant by capillary action (tension) exerted by the evaporation at the leaf surface.

Water's freezing and boiling points at sea level are the baseline from which temperature is measured. Zero degrees Celsius (0° C) is water's freezing point and 100° C is its boiling point. The solid form of water (ice) is less dense than the liquid form and for that reason ice floats on water. Whereas most liquids contract as they become colder, water expands until it is solid.

Water is a contributor in most chemical reactions of cells and is essential to break down polymers into monomers by the process of hydrolysis. The amount of water needed for metabolic activities varies among different microorganisms. The availability of water influences microbial growth rates (see Chapter 4). Some of the properties of water are shown in Box 2.3.

Water is a **solvent** and can dissolve many different substances, and is therefore often called the "universal solvent." The substances dissolved in water or another solvent are **solutes,** and the combination of a solvent and its solutes is referred to as a **solution** (Figure 2.10). The solubility of molecules is determined by their molecular structure. Molecules that exhibit local differences in electrical properties, or polar areas, are water soluble and those that do not are insoluble in water. The survival of living cells depends on the appropriate concentration of solutes in a solution. Most organisms do not tolerate environments where the concentration of solutes is much higher than that in their intracellular environment. Depending on the amount of solutes within or outside a cell, the environment can be isotonic, hypertonic, or hypotonic.

- **Isotonic:** The solute concentration, and hence the osmotic pressure within the cell (intracellular), is the same as it is outside of the cell (extracellular). A cell placed in an isotonic solution will not change its cell volume.
- **Hypertonic:** The solute concentration in the cell is less than in the extracellular environment, which causes a net loss of water from the cell, resulting in cell shrinkage. The cell shape becomes notched or crenated.
- **Hypotonic:** The solute concentration in the extracellular environment is less than that inside the cell (intracellular), causing the uptake of water into the cell, resulting in the bursting of the cell (Figure 2.11).

**BOX 2.3 Properties of Water**

- Water is colorless, tasteless, and odorless.
- Water feels wet.
- Water makes a distinctive sound when dripping or crashing as a wave.
- Water exists in three forms: liquid, solid, and gas.
- Water can absorb a large amount of heat.
- Water has cohesive properties and "sticks" together into drops.
- Water has surface tension (the tendency of water molecules to stick together at the surface due to cohesion) and capillary action (the tendency of water molecules to stick to another surface and to move along with it due to adhesion).
- Water is part of all living organisms.

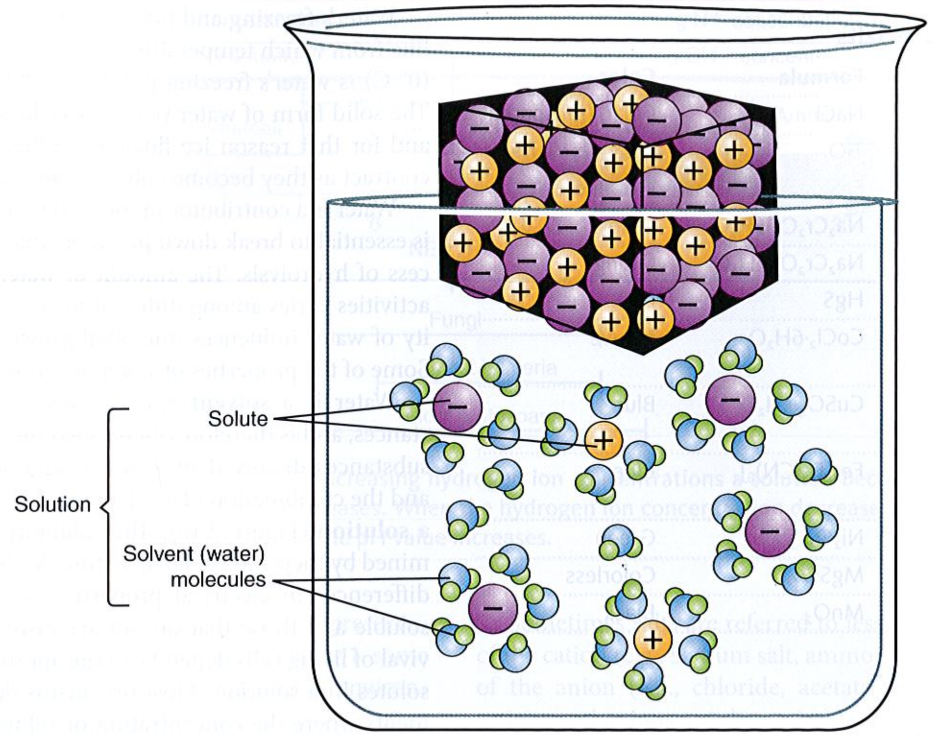



**FIGURE 2.10 Solvent, solutes, and solution.** In this diagram water acts as the solvent, sodium and chloride ions are the solutes, and when solutes are dissolved (dissociated) in a solvent, a solution is formed.

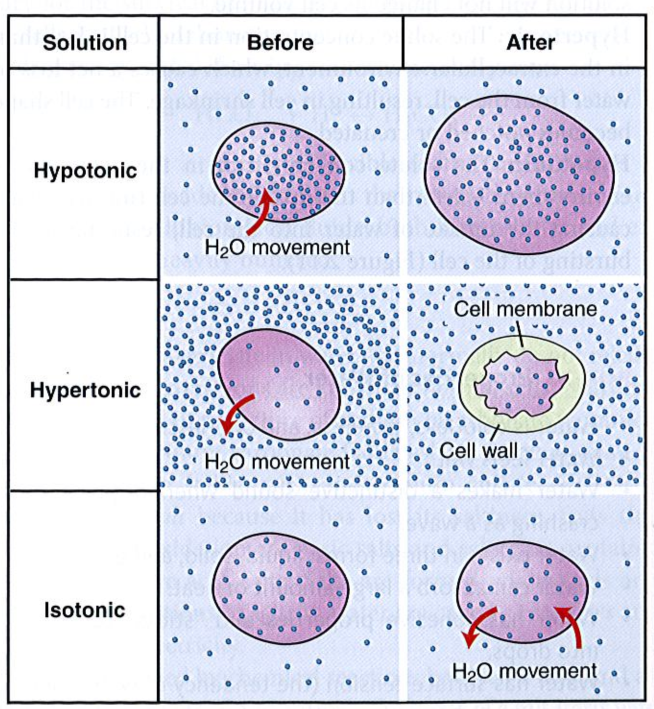



**FIGURE 2.11 Tonicity.** Cells placed into a hypotonic environment will take up water, swell, and may eventually burst. Cells placed into a hypertonic environment will lose water and shrink. Cells placed in an isotonic environment will remain unchanged.

Because of their polarity, ions attract the polar water molecules that surround the ions, which in turn attract other water molecules to form hydration spheres around each ion (Figure 2.12). Formations of hydration spheres are responsible for the solubility of ions in water. Organic molecules such as glucose, amino acids, and others are water soluble if the covalent bonding pattern permits the formation of hydration spheres around their atoms of oxygen, nitrogen, and phosphorus. These molecules are **hydrophilic** (water loving) water-soluble compounds. Molecules held together by nonpolar covalent bonds are **hydrophobic** (water repelling) and insoluble in water because of their inability to form hydration spheres. Parts of drug molecules may be hydrophilic, conferring water solubility properties on them, and vice versa for hydrophobic parts of drug molecules (see Chapter 10).

# Organic Molecules

All organic molecules contain atoms of carbon and hydrogen. Organic molecules have a backbone of chains or rings formed by the carbon and hydrogen atoms, referred to as a hydrocarbon backbone. Carbons commonly form covalent bonds not only with hydrogen, but also with oxygen, nitrogen, sulfur, and phosphorus. It is this covalent bonding of carbons with other carbons that yields the immense number and variety of organic molecules and allows different arrangements of chains or rings (Figure 2.13). The major organic molecules in living organisms



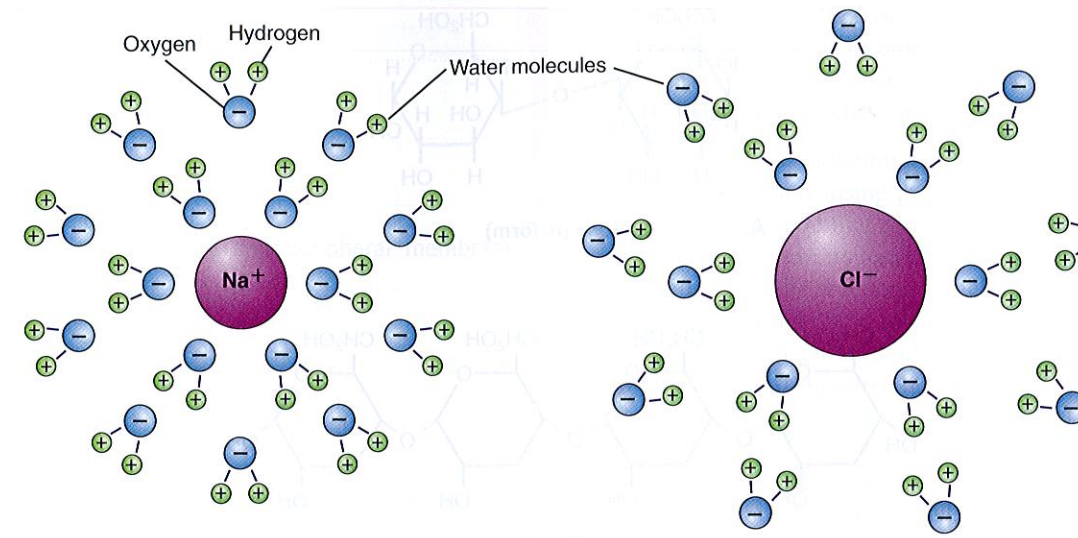



**FIGURE 2.12 Hydration spheres.** Water molecules form hydration spheres around ions.

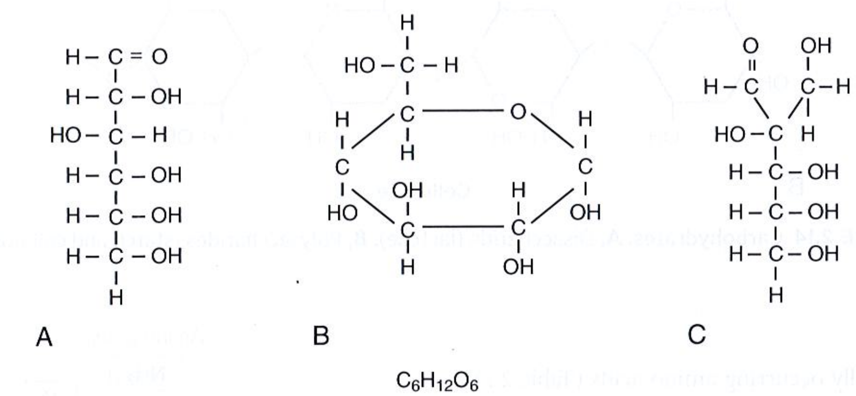



**FIGURE 2.13 Carbon backbone of organic molecules.** Carbon atoms can be arranged in **(A)** chains, **(B)** rings, or **(C)** branched chains.

are carbohydrates, proteins, lipids, and nucleic acids. Each of these compounds is composed of specific unit molecules or monomers (Table 2.6).

## Carbohydrates

Carbohydrates (sugars) include monosaccharides (monomer), disaccharides (two monosaccharides), and polysaccharides (many monosaccharides—polymer), all of which have a characteristic ratio (2:1:2) of carbon, hydrogen, and oxygen atoms. The name "carbohydrate" (hydrates [water] of carbon) is derived from this ratio. Sugars store carbon as well as large amounts of energy that are extracted during catabolism. Many microorganisms prefer sugars, when they are available, as their source of energy. Carbohydrates are also present in a large variety of cellular structures.

**TABLE 2.6 Organic Molecules and Their Monomers**

| Organic Molecule | Monomer |
|---|---|
| Carbohydrates | Monosaccharides |
| Proteins | Amino acids |
| Lipids | Glycerol and fatty acids |
| Nucleic acids | Nucleotides |

**Monosaccharides** are simple sugars that contain three to seven carbon atoms and an aldehyde group or a keto group. Monosaccharides represent the unit molecules (monomers) of carbohydrates. Monosaccharides include glucose ($C_6H_{12}O_6$), fructose, galactose, ribose ($C_5H_{10}O_5$), and deoxyribose ($C_5H_{10}O_4$).

**Disaccharides** (Figure 2.14, *A*) are compounds formed when two monosaccharides combine with the loss of a water molecule. Disaccharides include the following:

- Sucrose, composed of glucose and fructose
- Lactose, composed of glucose and galactose
- Maltose, composed of two glucose molecules

**Polysaccharides** (Figure 2.14, *B*) are formed when many monosaccharides combine to form a larger compound. Starch in plants and glycogen in animals are polysaccharide storage forms of glucose. The most abundant polysaccharide is cellulose, a major component of the cell walls of plants, fungi, and most algae.

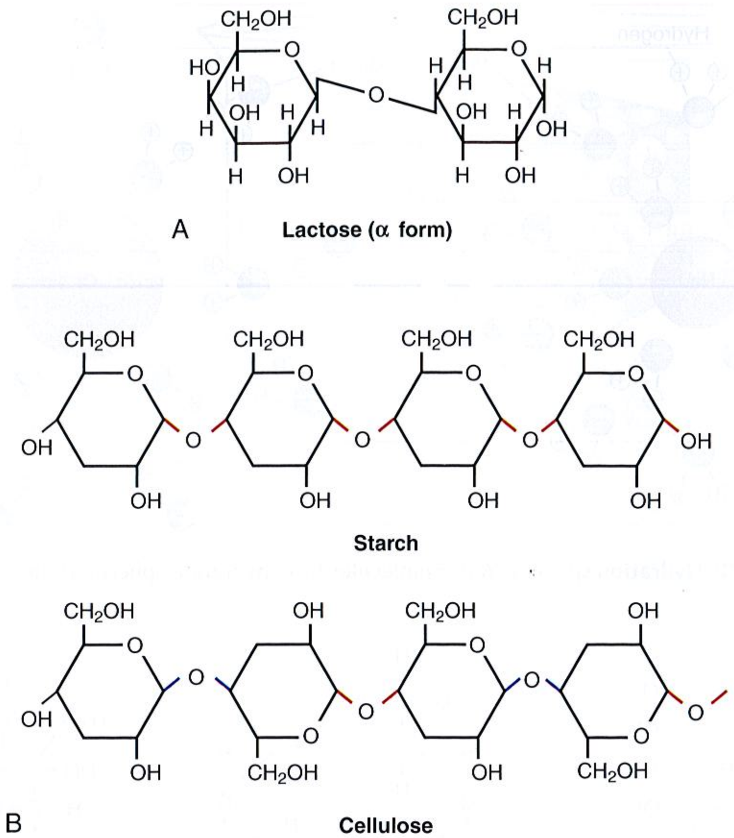



**FIGURE 2.14 Carbohydrates. A,** Disaccharide (lactose). **B,** Polysaccharides (starch and cellulose).

## Proteins

There are 20 known naturally occurring amino acids (Table 2.7), and they are the monomers of proteins. All amino acids consist of an amino group, a carboxyl group, and a variable side chain designated chemically as R (the R group; Figure 2.15). Two amino acids joined together form a dipeptide; 3 amino acids form a tripeptide; and a chain of 10 or more amino acids form a polypeptide.

Recalling Crick's central dogma that deoxyribonucleic acid (DNA) makes messenger ribonucleic acid (mRNA) and mRNA makes protein, the sequence of amino acids in a polypeptide is determined in the codons of mRNA, which translates codons (three-letter genetic words) into a polypeptide chain. The mRNA codons are transcribed from codons in DNA. All living things are dependent on proteins for structure and function (Box 2.4). Proteins can contain up to 10,000 amino acids. The sequence and folding of the amino acid chains determine the shape, which in turn determines the function and specificity of a protein. Different combinations of amino acids yield an infinite variety of polypeptides, which provides the chemical basis for the incredible biological diversity in structure and function among living organisms.

**TABLE 2.7 Naturally Occurring Amino Acids**

| Amino Acid | Abbreviation | Amino Acid | Abbreviation |
|---|---|---|---|
| Alanine | Ala | Leucine | Leu |
| Arginine | Arg | Lysine | Lys |
| Asparagine | Asn | Methionine | Met |
| Aspartic acid | Asp | Phenylalanine | Phe |
| Cysteine | Cys | Proline | Pro |
| Glutamic acid | Glu | Serine | Ser |
| Glutamine | Gln | Threonine | Thr |
| Glycine | Gly | Tryptophan | Trp |
| Histidine | His | Tyrosine | Tyr |
| Isoleucine | Ile | Valine | Val |

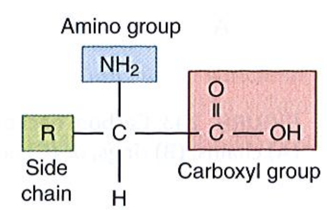



**FIGURE 2.15 General structure of amino acids.** Amino acids contain amino and carboxyl groups, as well as the variable R group side chain.

Proteins occur in four different structural arrangements (Figure 2.16):

- A *primary structure* is represented by a single chain of amino acids. Examples of primary structure proteins are small hypothalamic hormones such as gonadotropin-releasing hormone and thyrotropin-releasing hormone.



**BOX 2.4 Functions of Proteins**

- Structural support
- Contractile elements in muscles
- Receptors
- Enzymes
- Hormones
- Integral (transmembrane) and peripheral membrane proteins
- Transcription factors
- Antigens (some but not all)
- Antibodies
- Histocompatibility molecules

- A *secondary structure* is made of polypeptide chains that are either folded into a β sheet (β conformation) or form an α helix. The right-handed helix makes a complete turn in a clockwise direction every 3.6 amino acids. The α helix is held together by hydrogen bonds. The β sheets consist of two or more polypeptide chains lying side by side and are also stabilized by hydrogen bonds. Examples of such helical proteins are keratin, myosin, and collagen; silk represents a β-pleated sheet.
- A *tertiary structure* has a globular shape because of the additional coiling of secondary structure proteins. This structure is stabilized by the formation of additional hydrogen, ionic, and disulfide bonds. Properties of solubility are determined by hydrophobic nonpolar chains, which are generally positioned on the inside of the protein, and by hydrophilic polar chains, which are positioned on the outside of protein molecules. Examples of tertiary structure proteins are enzymes and some peptide hormones such as insulin.
- A *quaternary structure* contains several polypeptides that form a functional unit. Such complexes can consist of several copies of the same polypeptide or different polypeptides. An example of a quaternary structure protein is the hemoglobin molecule.

Heat, pH, salts, radiation, and heavy metals can change the shape of a protein, causing protein denaturation. This process results in nonfunctioning protein compounds. Denatured enzymes (proteins) can no longer function as biological catalysts, and their metabolic reactions come to a stop. Denatured antibodies can no longer bind to an antigen and fail to produce the all-important antigen-antibody complex in immune reactions (see Chapter 13). Denatured hormones are no longer able to act on their target cells, and the denaturing of bacterial or viral proteins often will eliminate the microbe (see Chapter 11).

When proteins are combined with inorganic or organic nonprotein compounds they are called *conjugated proteins.* These compounds are named accordingly as glycoproteins, lipoproteins, nucleoproteins, and phosphoproteins.

## Lipids

Lipids are molecules that vary markedly in their chemical structures. With the exception of phospholipids they are hydrophobic and are soluble in organic solvents such as ether, acetone, chloroform, benzene, and alcohols. They consist of hydrocarbon chains and rings, as triglycerides, phospholipids, steroids, cholesterol, prostaglandins, or leukotrienes.

**Triglycerides** (fats and oils) consist of glycerol and fatty acid chains (neutral fats). At room temperature fats are solid whereas oils are liquid. Structurally a fatty acid has a tail portion, which is a long hydrocarbon chain, and a head portion that consists of a carboxyl group (COOH). The tails of the fatty

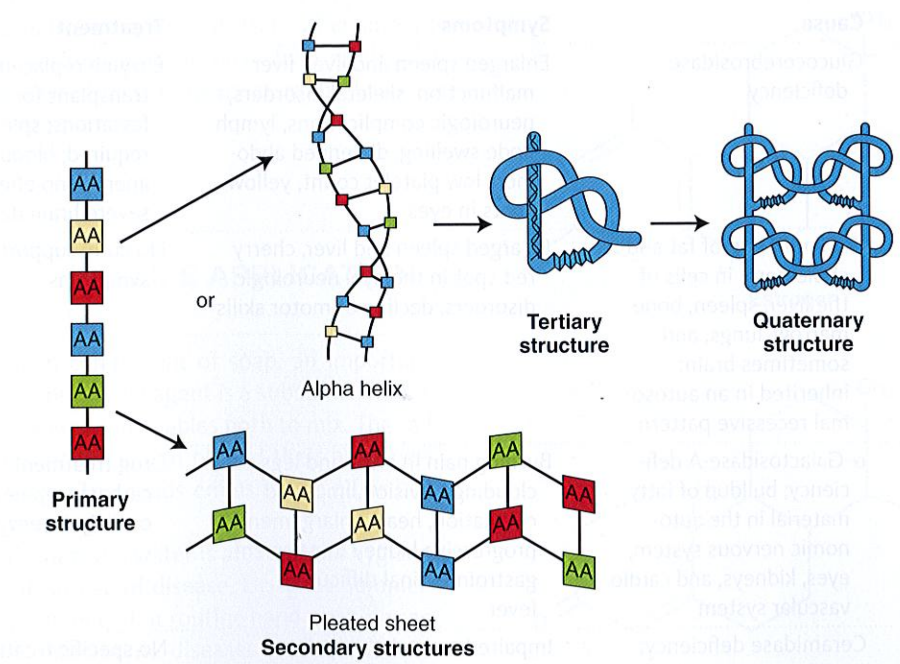



**FIGURE 2.16 Structural arrangements of proteins.** The primary structure consists of a chain of amino acids (AA), the secondary structure can be either an α helix or a β sheet, the tertiary structure has a globular shape, and the quaternary structure contains several different polypeptides forming a functional unit.

acids are hydrophobic and the heads are hydrophilic. When the head portion of the fatty acid is attached to a glycerol molecule to form fat, the entire molecule becomes hydrophobic and therefore insoluble in water (Figure 2.17).

Depending on the absence or presence of double bonds between the carbon atoms of the fatty acid chains, the fats are called *saturated, monounsaturated,* or *polyunsaturated* (Figure 2.18). In animal fats the carbons of the fatty acid chains are all bonded by single covalent bonds, meaning that all carbons are bonded to the maximal number of hydrogens. Therefore animal fats are saturated, closely packed together, and solid at room temperature. On the other hand, plant lipids are oils. They have some double bonds between the carbons, causing bends in the shape of the molecule. These oils are unsaturated fats and are liquid at room temperature.

Fats and oils are essential forms of energy storage. Animals convert excess sugars into fats if the glycogen storage capacity is reached. Although some seeds and fruits store energy as oil, most plants store excess sugars as starch. Fats can store over twice the amount of energy (9.3 kcal/g) than do carbohydrates (3.79 kcal/g).

**Phospholipids** consist of glycerol, two fatty acid chains, and a phosphate group at one end (Figure 2.19). These molecules are composed of polar (hydrophilic) heads and nonpolar (hydrophobic) tails. The fatty acid groups are hydrophobic whereas

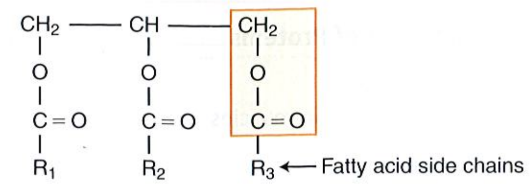



**FIGURE 2.17 Triglycerides.** The triglyceride molecule consists of glycerol and fatty acid side chains.

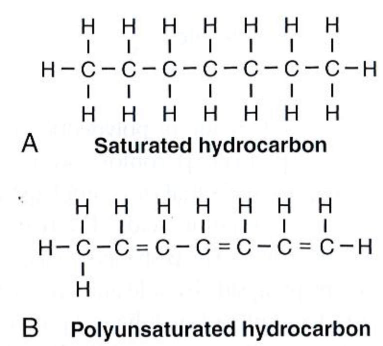



**FIGURE 2.18 Saturated and unsaturated fats. A,** Saturated fat with no double bonds within the carbon chain. **B,** Polyunsaturated fat with several double bonds within the carbon chain.

## HEALTHCARE APPLICATION

### Selected Lipid Storage Diseases

| Disease | Cause | Symptoms | Treatment |
|---|---|---|---|
| Gaucher disease (three common clinical subtypes) | Glucocerebrosidase deficiency | Enlarged spleen and liver, liver malfunction, skeletal disorders, neurologic complications, lymph node swelling, distended abdomen, low platelet count, yellow spots in eyes | Enzyme replacement; bone marrow transplant for nonneurologic manifestations; splenectomy may be required; blood transfusion for anemia; no effective treatment for severe brain damage |
| Niemann-Pick disease (four categories) | Accumulation of fat and cholesterol in cells of the liver, spleen, bone marrow, lungs, and sometimes brain; inherited in an autosomal recessive pattern | Enlarged spleen and liver, cherry red spot in the eye, neurologic disorders, decline of motor skills | No cure, supportive treatment of symptoms |
| Fabry disease | α-Galactosidase-A deficiency; buildup of fatty material in the autonomic nervous system, eyes, kidneys, and cardiovascular system | Burning pain in arms and legs, clouding of vision, impaired circulation, heart enlargement, progressive kidney impairment, gastrointestinal difficulties, fever | Drug treatment for pain (phenytoin, carbamazepine); kidney transplant or dialysis; enzyme replacement |
| Farber's disease | Ceramidase deficiency; accumulation of fatty material in joints, tissues, and central nervous system | Impaired mental ability; liver, heart, and kidneys may be affected; vomiting, arthritis, swollen lymph nodes, swollen joints, joint contractures | No specific treatment; corticosteroids to relieve pain |



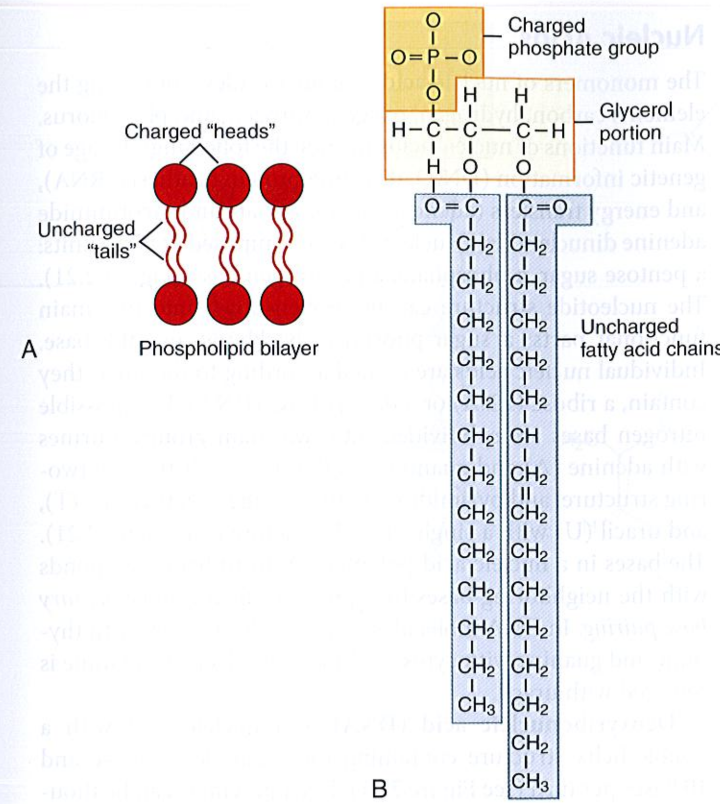



**FIGURE 2.19 Phospholipid. A,** Phospholipid bilayer as seen in plasma membranes. **B,** The phospholipid molecule is composed of a polar, hydrophilic head and a nonpolar, hydrophobic tail.

the phosphate group is hydrophilic. Phospholipids therefore have a water-soluble polar head and a nonpolar hydrophobic tail. Cell membranes, for example, are composed of a phospholipid bilayer with the tails facing toward each other and the heads facing outward, making their surfaces hydrophilic and their interior hydrophobic. This arrangement is the basis for their biological barrier properties.

## LIFE APPLICATION

### Hand Washing

Fatty acids are the main component of soap, an important emulsifying agent. An emulsifying agent is a substance that is soluble in both oil and water and enables both to mix. The tail portions of fatty acids are soluble in oil and their head portions are soluble in water. The heads emulsify the oil or oily dirt and wash it away.

Hand washing with soap and water is an essential hygiene practice to prevent the spread of disease. Dr. Ignaz Semmelweis first demonstrated in 1847 that routine hand washing can prevent the spread of hand-borne diseases (see Chapter 1). This was a landmark achievement in healthcare settings and for public health in general. Healthcare specialists now state that hand washing is the single most effective way to prevent the transmission of disease.

**Steroids** (Figure 2.20) have a carbon skeleton consisting of four fused rings with various functional groups attached. Hundreds of different steroids have been found in plants, animals, and fungi. Categories are as follows:

- *Anabolic steroids* interact with androgen receptors to increase muscle and bone mass. Besides the naturally occurring anabolic steroids, synthetic ones exist that are used (sometimes illegally) by athletes in an attempt to enhance their performance.
- *Sex steroids* are responsible for the secondary sex characteristics of males and females. They include androgens, estrogens, and progesterones.
- *Mineralocorticoids* help maintain blood volume, electrolyte balance, and osmolarity by controlling the renal secretion of electrolytes.
- *Glucocorticoids* play a role in many aspects of metabolism and in immune function. They are often prescribed to reduce inflammatory conditions.

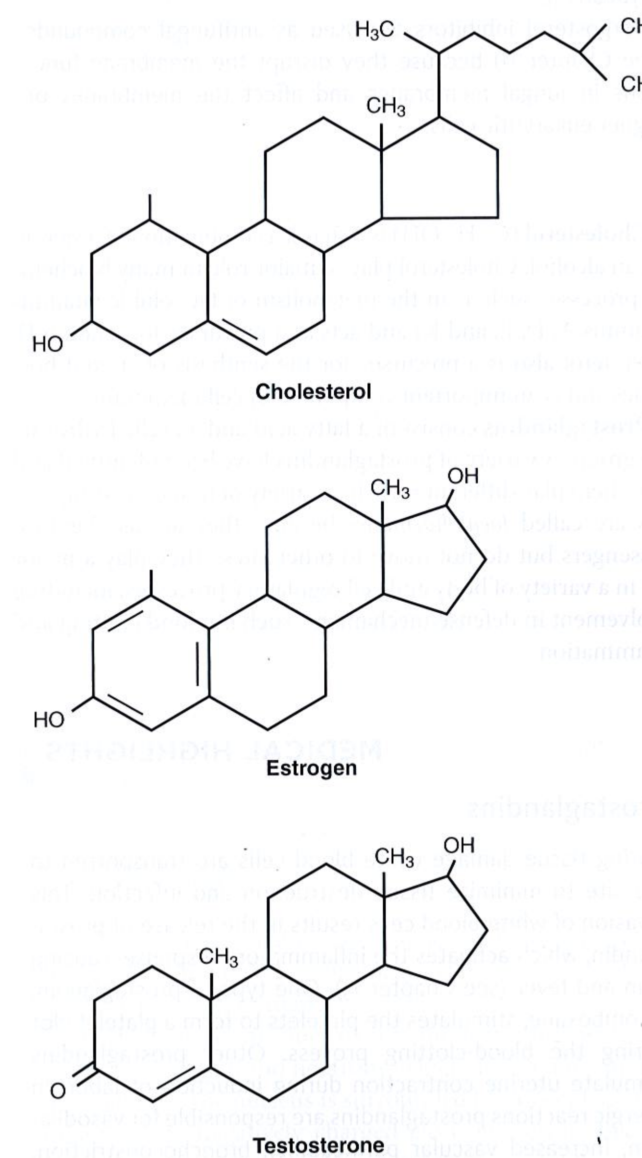



**FIGURE 2.20 Steroids.** Various steroid molecules are shown, all with four fused carbon rings but differing in their functional groups.

- Phytosterols are a group of steroid alcohols that naturally occur in plants such as yeasts and fungi. Plants contain a wide range of phytosterols that are a structural component in their cell membrane and serve the same function as cholesterol does in animal cells. Ergosterol, a phytosterol, is also referred to as provitamin $D_2$. It is a biological precursor that is converted by ultraviolet irradiation into ergocalciferol, or vitamin $D_2$.

**MEDICAL HIGHLIGHTS**

## Phytosterol and Ergosterol

Phytosterol is used as a food additive because it lowers cholesterol by reducing cholesterol absorption in the intestines. It may act in cancer prevention as well. Small amounts of phytosterols naturally occur in vegetable oils, especially in soybean oil.

Ergosterol inhibitors are used as antifungal compounds (see Chapter 11) because they disrupt the membrane functions in fungal membranes and affect the membranes of higher eukaryotic cells.

**Cholesterol** ($C_{27}H_{45}OH$) is a sterol, a combination of a steroid and an alcohol. Cholesterol plays a major role in many biochemical processes such as in the metabolism of fat-soluble vitamins (vitamins A, D, E, and K) and acts as a precursor for vitamin D. Cholesterol also is a precursor for the synthesis of steroid hormones and is an important component of cell membranes.

**Prostaglandins** consist of a fatty acid and a cyclic hydrocarbon group. A variety of prostaglandins have been identified and all of them play different roles in a variety of tissues. Prostaglandins are called *local hormones* because they act as chemical messengers but do not move to other sites. They play a major role in a variety of body and cell regulatory processes, including involvement in defense mechanisms such as blood clotting and inflammation.

**MEDICAL HIGHLIGHTS**

## Prostaglandins

During tissue damage white blood cells are transported to the site to minimize tissue destruction and infection. This invasion of white blood cells results in the release of prostaglandin, which activates the inflammatory response, causing pain and fever (see Chapter 13). One type of prostaglandin, thromboxane, stimulates the platelets to form a platelet clot during the blood-clotting process. Other prostaglandins stimulate uterine contraction during induction of labor. In allergic reactions prostaglandins are responsible for vasodilation, increased vascular permeability, bronchoconstriction, and increased sensitivity to pain. Antiinflammatory drugs such as aspirin and corticosteroids act by preventing the actions of prostaglandins (see Chapters 10 and 11).

## Nucleic Acids

The monomers of nucleic acids are nucleotides, containing the elements carbon, hydrogen, oxygen, nitrogen, and phosphorus. Main functions of nucleic acids include the following: storage of genetic information (DNA), directing protein synthesis (RNA), and energy transfers (adenosine triphosphate and nicotinimide adenine dinucleotide). Nucleotides are composed of three units: a pentose sugar, a phosphate, and nitrogen base (Figure 2.21). The nucleotide structure can be broken down into two main functional parts: a sugar-phosphate backbone and the base. Individual nucleic acids are named according to the sugar they contain, a ribose (RNA) or a deoxyribose (DNA). Five possible nitrogen bases are subdivided into two main groups: purines with adenine (A) and guanine (G) that have a distinctive two-ring structure, and pyrimidines with cytosine (C), thymine (T), and uracil (U) with a single-ringed structure (see Figure 2.21). The bases in a nucleic acid polymer can form hydrogen bonds with the neighboring bases by a process called *complementary base pairing*. In DNA molecules, adenine always pairs with thymine and guanine with cytosine. In RNA molecules, thymine is replaced with uracil.

**Deoxyribonucleic acid (DNA)** is a nucleic acid with a double-helix structure containing the sugar deoxyribose and 10 bases per turn (see Figure 2.21). DNA polymers can be thousands of bases long. DNA contains the genetic code and therefore serves for information storage. DNA is responsible for inherited characteristics, growth, and cell reproduction. It is present in both prokaryotic and eukaryotic cells, as well as in a group of viruses. DNA contains the information necessary for protein synthesis. The language of DNA, the genetic code, consists of four letters that represent the nitrogen bases, C, G, A, and T, used in three-letter "words" called *codons* to indicate the 20 naturally occurring amino acids. The combination of codons can create an infinite variety of "sentences." Each codon represents a specific amino acid and the combination of amino acids results in different polypeptides (see Table 3.6 in Chapter 3).

Chromosomes are the microscopic structures that carry DNA within the nucleus of cells. One chromosome represents a single molecule of DNA. In bacteria chromosomes with two types of DNA are present: the chromosomal DNA located in the nucleoid area and *plasmids*, which are simple circles of DNA floating freely in the organism. Plasmids are capable of autonomous replication and therefore can replicate independently of the chromosomal DNA. In eukaryotes the chromosomes are highly complex structures and DNA molecules are linear rather than circular.

**Ribonucleic acid (RNA)** is similar to DNA but is a single-stranded molecule, its sugar is ribose, and uracil replaces thymine. RNA is specialized for the synthesis of proteins. Three different types of RNA are necessary for the process of protein synthesis: ribosomal RNA (rRNA), messenger RNA (mRNA), and transfer RNA (tRNA). Ribosomes composed of rRNA are made in the nucleus and transported into the cytoplasm, where they attach to rough endoplasmic reticulum (rER) or remain free in the cytoplasm as polyribosomes. Both polyribosomes and ribosomes on the rER (see Chapter 3) are sites of protein synthesis. Messenger RNA contains genetic information that encodes the



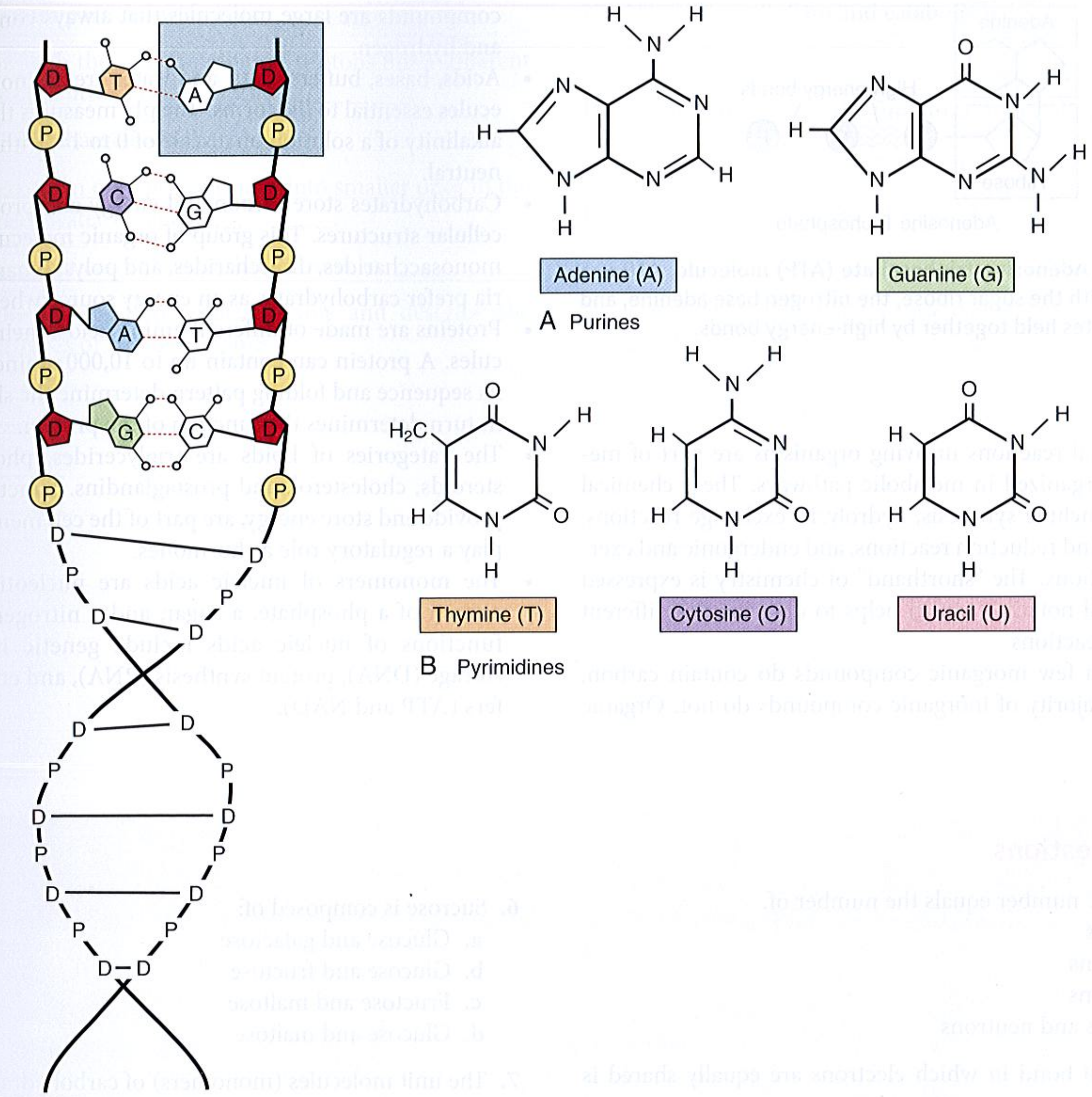



**FIGURE 2.21 DNA molecule.** Deoxyribonucleic acid (DNA) is composed of nucleotides, each of which consists of a phosphate, a pentose sugar (deoxyribose), and a nitrogen base *(shaded area)*. The nitrogen bases of nucleic acids are either **(A)** purines or **(B)** pyrimidines. The pyrimidine uracil is only found in RNA.

sequence of amino acids in proteins. Base triplets, referred to as codons, direct the amino acid sequence in a polypeptide chain. The third type of RNA, tRNA, contains a triplet of nitrogen bases called the *anticodon*. Anticodons contain complementary bases to the codons on mRNA and are necessary for the synthesis of polypeptides (see Protein Synthesis in Chapter 3).

**Adenosine triphosphate (ATP)** is the energy molecule of cells. When energy is released during catabolism it is captured in the high-energy bonds of ATP (Figure 2.22). In transferring energy to other molecules, ATP loses one or two of its phosphate groups, resulting in adenosine diphosphate (ADP) or adenosine monophosphate (AMP). This is an exergonic reaction. Both ADP and AMP can be converted back to ATP by photosynthesis or through chemical energy during anabolism. Photosynthetic microorganisms use sunlight as an energy source during their anabolism. Microorganisms that need nutrient molecules for ATP production are called *chemotrophs* and the ones that use sunlight for energy are called *phototrophs* (see Chapter 4).

# Summary

- Matter is anything that occupies space and has mass. It can be in solid, liquid, or gaseous form. All living and nonliving matter consists of elements.
- Atoms have an atomic nucleus containing protons, which are positively charged, and neutrons, which do not have an electrical charge. The nucleus is surrounded by concentric shells in which the negatively charged electrons reside. Because atoms have the same number of protons and electrons they are electrically neutral.
- Molecules are formed when two or more atoms combine through covalent, ionic, or hydrogen bonding.

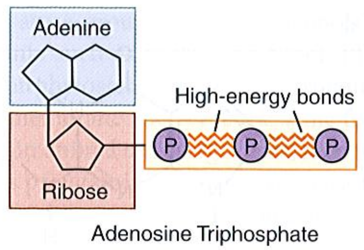



**FIGURE 2.22 Adenosine triphosphate (ATP) molecule.** ATP is a nucleic acid with the sugar ribose, the nitrogen base adenine, and three phosphates held together by high-energy bonds.

- All chemical reactions in living organisms are part of metabolism organized in metabolic pathways. These chemical reactions include synthesis, hydrolysis, exchange reactions, oxidation and reduction reactions, and endergonic and exergonic reactions. The "shorthand" of chemistry is expressed as chemical notation, which helps to describe the different chemical reactions.
- Although a few inorganic compounds do contain carbon, the vast majority of inorganic compounds do not. Organic compounds are large molecules that always contain carbon and hydrogen.
- Acids, bases, buffers, salts, and water are all inorganic molecules essential to life forms. The pH measures the acidity or alkalinity of a solution on a scale of 0 to 14, with pH 7 set as neutral.
- Carbohydrates store carbon and energy and provide part of cellular structures. This group of organic molecules includes monosaccharides, disaccharides, and polysaccharides. Bacteria prefer carbohydrates as an energy source when available.
- Proteins are made of different amino acids, their unit molecules. A protein can contain up to 10,000 amino acids, and its sequence and folding pattern determine the shape, which in turn determines the function of the protein.
- The categories of lipids are triglycerides, phospholipids, steroids, cholesterol, and prostaglandins. Functionally, fats provide and store energy, are part of the cell membrane, and play a regulatory role as hormones.
- The monomers of nucleic acids are nucleotides, which consist of a phosphate, a sugar, and a nitrogen base. The functions of nucleic acids include genetic information storage (DNA), protein synthesis (RNA), and energy transfers (ATP and NAD).

## Review Questions

1. The atomic number equals the number of:
   a. Protons
   b. Neutrons
   c. Electrons
   d. Protons and neutrons
2. A chemical bond in which electrons are equally shared is a(n):
   a. Ionic bond
   b. Polar covalent bond
   c. Nonpolar covalent bond
   d. Hydrogen bond
3. The bond between water molecules is a(n):
   a. Ionic bond
   b. Polar covalent bond
   c. Nonpolar covalent bond
   d. Hydrogen bond
4. The outermost shell of an atom can hold up to __________ electrons.
   a. 2
   b. 6
   c. 8
   d. 10
5. The bond between sodium and chlorine atoms in sodium chloride is a(n):
   a. Hydrogen bond
   b. Ionic bond
   c. Polar covalent bond
   d. Nonpolar covalent bond
6. Sucrose is composed of:
   a. Glucose and galactose
   b. Glucose and fructose
   c. Fructose and maltose
   d. Glucose and maltose
7. The unit molecules (monomers) of carbohydrates are:
   a. Monosaccharides
   b. Amino acids
   c. Nucleic acids
   d. Fatty acids
8. The bond between amino acids is a(n):
   a. Ionic bond
   b. Peptide bond
   c. Hydrogen bond
   d. Covalent bond
9. The RNA nucleotide base that pairs with adenine of DNA is:
   a. Cytosine
   b. Guanine
   c. Thymine
   d. Uracil
10. Glucose and fructose are examples of:
    a. Monosaccharides
    b. Disaccharides
    c. Polysaccharides
    d. Lipids



11. Neutrons are particles with a(n) __________ charge.
12. An atom with the same number of protons but a different number of neutrons is called a(n) __________.
13. A positively charged ion is a(n) __________.
14. The breakdown of large molecules into smaller ones in the presence of water is called __________.
15. Molecules that can absorb hydrogen ions are __________.
16. From the strongest to weakest, name and describe the different types of chemical bonds.
17. Describe anabolism and catabolism.
18. Name and describe the functions of proteins.
19. Compare and contrast saturated and unsaturated fats.
20. Describe complementary base pairing and compare DNA and RNA.