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21 ENVIRONMENTAL BIOCHEMISTRY

21.1. BIOCHEMISTRY

The effects of pollutants and potentially harmful chemicals on living organisms are of particular importance in environmental chemistry. These effects are addressed under the topic of "Toxicological Chemistry" in Chapter 22, and for specific substances in Chapter 23. The current chapter, "Environmental Biochemistry," is designed to provide the fundamental background in biochemistry required to understand toxicological chemistry.

Most people have had the experience of looking through a microscope at a single cell. It may have been an ameba, alive and oozing about like a blob of jelly on the microscope slide, or a cell of bacteria, stained with a dye to make it show up more plainly. Or, it may have been a beautiful cell of algae with its bright green chlorophyll. Even the simplest of these cells is capable of carrying out a thousand or more chemical reactions. These life processes fall under the heading of **biochemistry**, that branch of chemistry that deals with the chemical properties, composition, and biologically-mediated processes of complex substances in living systems.

Biochemical phenomena that occur in living organisms are extremely sophisticated. In the human body complex metabolic processes break down a variety of food materials to simpler chemicals, yielding energy and the raw materials to build body constituents such as muscle, blood, and brain tissue. Impressive as this may be, consider a humble microscopic cell of photosynthetic cyanobacteria only about a micrometer in size, which requires only a few simple inorganic chemicals and sunlight for its existence. This cell uses sunlight energy to convert carbon from CO_2, hydrogen and oxygen from H_2O, nitrogen from NO_3^-, sulfur from SO_4^{2-}, and phosphorus from inorganic phosphate into all the proteins, nucleic acids, carbohydrates, and other materials that it requires to exist and reproduce. Such a simple cell accomplishes what could not be done by humans in even a vast chemical factory costing billions of dollars.

Ultimately, most environmental pollutants and hazardous substances are of concern because of their effects upon living organisms. The study of the adverse

effects of substances on life processes requires some basic knowledge of biochemistry. Biochemistry is discussed in this chapter, with emphasis upon aspects that are especially pertinent to environmentally hazardous and toxic substances, including cell membranes, DNA, and enzymes.

Biochemical processes not only are profoundly influenced by chemical species in the environment, they largely determine the nature of these species, their degradation, and even their syntheses, particularly in the aquatic and soil environments. The study of such phenomena forms the basis of **environmental biochemistry**.[1]

Biomolecules

The biomolecules that constitute matter in living organisms are often polymers with molecular masses of the order of a million or even larger. As discussed later in this chapter, these biomolecules may be divided into the categories of carbohydrates, proteins, lipids, and nucleic acids. Proteins and nucleic acids consist of macromolecules, lipids are usually relatively small molecules, carbohydrates range from relatively small sugar molecules to high molar mass macromolecules such as those in cellulose.

The behavior of a substance in a biological system depends to a large extent upon whether the substance is hydrophilic ("water-loving") or hydrophobic ("water-hating"). Some important toxic substances are hydrophobic, a characteristic that enables them to traverse cell membranes readily. Part of the detoxification process carried on by living organisms is to render such molecules hydrophilic, therefore water-soluble and readily eliminated from the body.

21.2. BIOCHEMISTRY AND THE CELL

The focal point of biochemistry and biochemical aspects of toxicants is the **cell**, the basic building block of living systems where most life processes are carried out. Bacteria, yeasts, and some algae consist of single cells. However, most living things are made up of many cells. In a more complicated organism the cells have different functions. Liver cells, muscle cells, brain cells, and skin cells in the human body are quite different from each other and do different things. Cells are divided into two major categories depending upon whether or not they have a nucleus: **eukaryotic** cells have a nucleus and **prokaryotic** cells do not. Prokaryotic cells are found predominately in single-celled organisms such as bacteria.[2] Eukaryotic cells occur in multicellular plants and animals—higher life forms.

Major Cell Features

Figure 21.1 shows the major features of the **eukaryotic cell**, which is the basic structure in which biochemical processes occur in multicellular organisms. These features are the following:

- **Cell membrane**, which encloses the cell and regulates the passage of ions, nutrients, lipid-soluble ("fat-soluble") substances, metabolic products, toxicants, and toxicant metabolites into and out of the cell interior because of its varying **permeability** for different substances. The cell membrane

protects the contents of the cell from undesirable outside influences. Cell membranes are composed in part of phospholipids that are arranged with their hydrophilic ("water-seeking") heads on the cell membrane surfaces and their hydrophobic ("water-repelling") tails inside the membrane. Cell membranes contain bodies of proteins that are involved in the transport of some substances through the membrane. One reason the cell membrane is very important in toxicology and environmental biochemistry is because it regulates the passage of toxicants and their products into and out of the cell interior. Furthermore, when its membrane is damaged by toxic sustances, a cell may not function properly and the organism may be harmed.

- **Cell nucleus**, which acts as a sort of "control center" of the cell. It contains the genetic directions the cell needs to reproduce itself. The key substance in the nucleus is **deoxyribonucleic** acid (**DNA**). **Chromosomes** in the cell nucleus are made up of combinations of DNA and proteins. Each chromosome stores a separate quantity of genetic information. Human cells contain 46 chromosomes. When DNA in the nucleus is damaged by foreign substances, various toxic effects, including mutations, cancer, birth defects, and defective immune system function may occur.
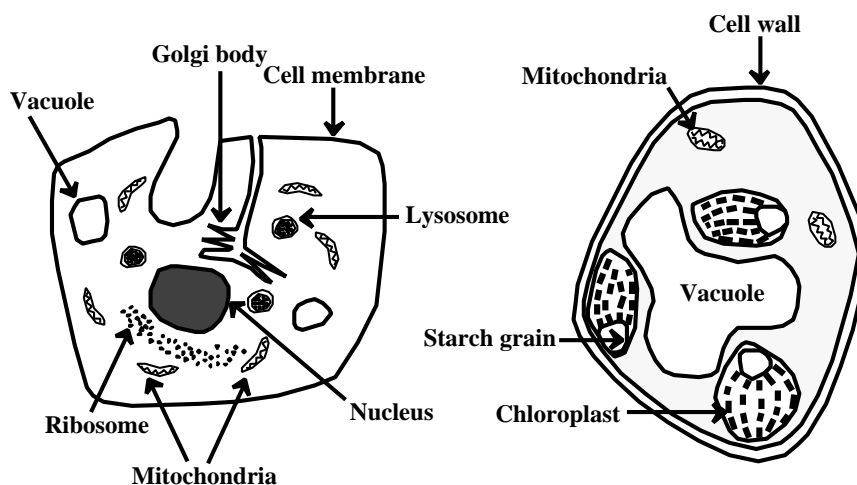

Figure 21.1. Some major features of the eukaryotic cell in animals (left) and plants (right).

- **Cytoplasm**, which fills the interior of the cell not occupied by the nucleus. Cytoplasm is further divided into a water-soluble proteinaceous filler called **cytosol**, in which are suspended bodies called **cellular organelles**, such as mitochondria or, in photosynthetic organisms, chloroplasts.

- **Mitochondria**, "powerhouses" which mediate energy conversion and utilization in the cell. Mitochondria are sites in which food materials—carbohydrates, proteins, and fats—are broken down to yield carbon dioxide, water, and energy, which is then used by the cell. The best example of this is the oxidation of the sugar glucose, $C_6H_{12}O_6$:
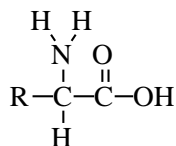
$$C_6H_{12}O_6 \ + \ 6O_2 \qquad 6CO_2 \ + \ 6H_2O \ + \ energy$$

This kind of process is called **cellular respiration.**

- **Ribosomes**, which participate in protein synthesis.

- **Endoplasmic reticulum**, which is involved in the metabolism of some toxicants by enzymatic processes.

- **Lysosome**, a type of organelle that contains potent substances capable of digesting liquid food material. Such material enters the cell through a "dent" in the cell wall, which eventually becomes surrounded by cell material. This surrounded material is called a **food vacuole**. The vacuole merges with a lysosome, and the substances in the lysosome bring about digestion of the food material. The digestion process consists largely of **hydrolysis reactions** in which large, complicated food molecules are broken down into smaller units by the addition of water.

- **Golgi bodies**, that occur in some types of cells. These are flattened bodies of material that serve to hold and release substances produced by the cells.

- **Cell walls** of plant cells. These are strong structures that provide stiffness and strength. Cell walls are composed mostly of cellulose, which will be discussed later in this chapter.

- **Vacuoles** inside plant cells that often contain materials dissolved in water.

- **Chloroplasts** in plant cells that are involved in photosynthesis (the chemical process which uses energy from sunlight to convert carbon dioxide and water to organic matter). Photosynthesis occurs in these bodies. Food produced by photosynthesis is stored in the chloroplasts in the form of **starch grains**.
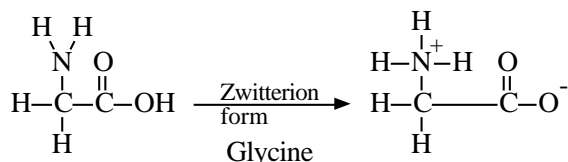
21.3. PROTEINS

Proteins are nitrogen-containing organic compounds which are the basic units of live systems. Cytoplasm, the jelly-like liquid filling the interior of cells, consists largely of protein. Enzymes, which act as catalysts of life reactions, are proteins; they are discussed later in the chapter. Proteins are made up of **amino acids** joined together in huge chains. Amino acids are organic compounds which contain the carboxylic acid group, $-CO_2H$, and the amino group, $-NH_2$. They are sort of a hybrid of carboxylic acids and amines (See Chapter 29 if these terms are unfamiliar). Proteins are polymers or **macromolecules** of amino acids containing from approximately 40 to several thousand amino acid groups joined by peptide linkages. Smaller molecule amino acid polymers, containing only about 10 to about 40 amino acids per molecule, are called **polypeptides**. A portion of the amino acid left after the elimination of H_2O during polymerization is called a **residue**. The amino acid sequence of these residues is designated by a series of three-letter abbreviations for the amino acid.

Natural amino acids all have the following chemical group:

$$\begin{array}{c} \overset{\displaystyle H}{\underset{\displaystyle}{N}} \overset{\displaystyle H}{} \overset{\displaystyle O}{} \\ R-C-C-OH \\ \overset{|}{H} \end{array}$$

In this structure the $-NH_2$ group is always bonded to the carbon next to the $-CO_2H$ group. This is called the "alpha" location, so natural amino acids are alpha-amino acids. Other groups, designated as "R," are attached to the basic alpha-amino acid structure. The R groups may be as simple as an atom of H found in glycine,

Glycine → Zwitterion form

or, they may be as complicated as the structure,

R group found in tryptophan
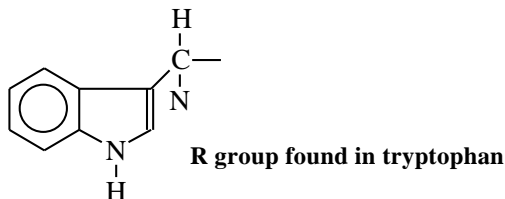
found in tryptophan. There are 20 common amino acids in proteins, examples of which are shown in Figure 21.2. The amino acids are shown with uncharged $-NH_2$ and $-CO_2H$ groups. Actually, these functional groups exist in the charged **zwitterion** form as shown for glycine, above.

Valine (val)   Tyrosine (tyr)   Isoleucine (ile)

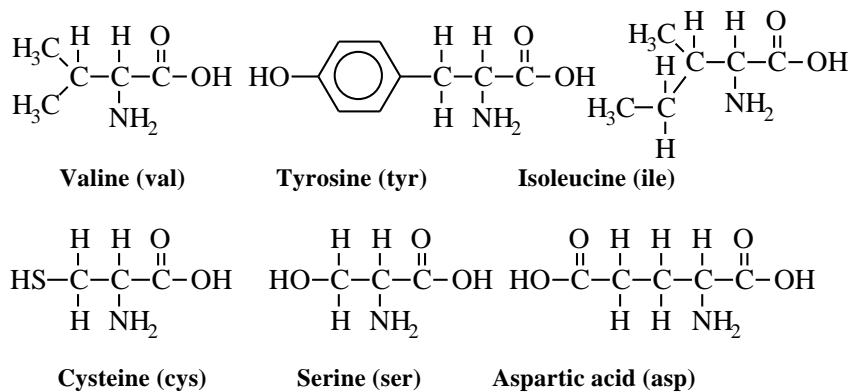Cysteine (cys)   Serine (ser)   Aspartic acid (asp)

Figure 21.2. Examples of naturally occurring amino acids.

Amino acids in proteins are joined together in a specific way. These bonds are called the **peptide linkage**. The formation of peptide linkages is a condensation process involving the loss of water. Consider as an example the condensation of

alanine, leucine, and tyrosine shown in Figure 21.3. When these three amino acids join together, two water molecules are eliminated. The product is a *tri*peptide since there are three amino acids involved. The amino acids in proteins are linked as shown for this tripeptide, except that many more monomeric amino acid groups are involved.
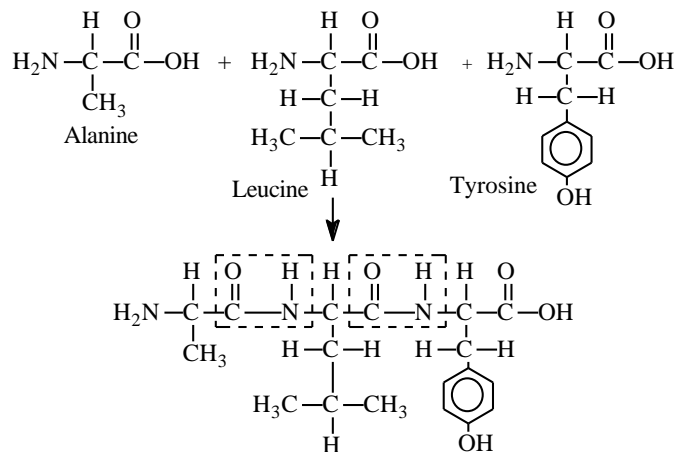

Figure 21.3. Condensation of alanine, leucine, and tyrosine to form a tripeptide consisting of three amino acids joined by peptide linkages (outlined by dashed lines).

Proteins may be divided into several major types that have widely varying functions. These are given in Table 21.1.

Protein Structure

The order of amino acids in protein molecules, and the resulting three-dimensional structures that form, provide an enormous variety of possibilites for **protein structure**. This is what makes life so diverse. Proteins have primary, secondary, tertiary, and quaternary structures. The structures of protein molecules determine the behavior of proteins in crucial areas such as the processes by which the body's immune system recognizes substances that are foreign to the body. Proteinaceous enzymes depend upon their structures for the very specific functions of the enzymes.

The order of amino acids in the protein molecule determines its primary structure. **Secondary protein structures** result from the folding of polypeptide protein chains to produce a maximum number of hydrogen bonds between peptide linkages:

Illustration of hydrogen bonds between N and O atoms in peptide linkages, which constitute protein secondary structures
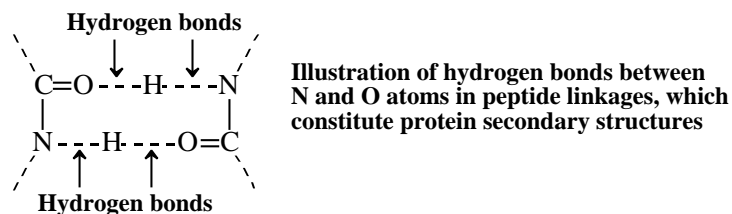
The nature of the R groups on the amino acids determines the secondary structure. Small R groups enable protein molecules to be hydrogen-bonded together in a parallel arrangement. With larger R groups the molecules tend to take a spiral form. Such a spiral is known as an **alpha-helix**.

Table 21.1. Major Types of Proteins

Type of protein	Example	Function and characteristics
Nutrient	Casein (milk protein)	Food source. People must have an adequate supply of nutrient protein with the right balance of amino acids for adequate nutrition.
Storage	Ferritin	Storage of iron in animal tissues
Structural	Collagen (tendons) keratin (hair)	Structural and protective components in organisms
Contractile	Actin, myosin in muscle tissue	Strong, fibrous proteins that can contract and cause movement to occur
Transport	Hemoglobin	Transport inorganic and organic species across cell membranes, in blood, between organs
Defense	- - -	Antibodies against foreign agents such as viruses produced by the immune system
Regulatory	Insulin, human growth hormone	Regulate biochemical processes such as sugar metabolism or growth by binding to sites inside cells or on cell membranes
Enzymes	Acetylcholin-esterase	Catalysts of biochemical reactions (see Section 21.6.)

Tertiary structures are formed by the twisting of alpha-helices into specific shapes. They are produced and held in place by the interactions of amino side chains on the amino acid residues constituting the protein macromolecules. Tertiary protein structure is very important in the processes by which enzymes identify specific proteins and other molecules upon which they act. It is also involved with the action of antibodies in blood which recognize foreign proteins by their shape and react to them. This is basically what happens in the case of immunity to a disease where antibodies in blood recognize specific proteins from viruses or bacteria and reject them.

Two or more protein molecules consisting of separate polypeptide chains may be further attracted to each other to produce a **quaternary structure**.

Some proteins are **fibrous proteins**, which occur in skin, hair, wool, feathers, silk, and tendons. The molecules in these proteins are long and threadlike and are laid out parallel in bundles. Fibrous proteins are quite tough and they do not dissolve in water.

Aside from fibrous protein, the other major type of protein form is the **globular**

protein. These proteins are in the shape of balls and oblongs. Globular proteins are relatively soluble in water. A typical globular protein is hemoglobin, the oxygen-carrying protein in red blood cells. Enzymes are generally globular proteins.

Denaturation of Proteins

Secondary, tertiary, and quaternary protein structures are easily changed by a process called **denaturation**. These changes can be quite damaging. Heating, exposure to acids or bases, and even violent physical action can cause denaturation to occur. The albumin protein in egg white is denatured by heating so that it forms a semisolid mass. Almost the same thing is accomplished by the violent physical action of an egg beater in the preparation of meringue. Heavy metal poisons such as lead and cadmium change the structures of proteins by binding to functional groups on the protein surface.

21.4. CARBOHYDRATES

Carbohydrates have the approximate simple formula CH_2O and include a diverse range of substances composed of simple sugars such as glucose:


Glucose molecule
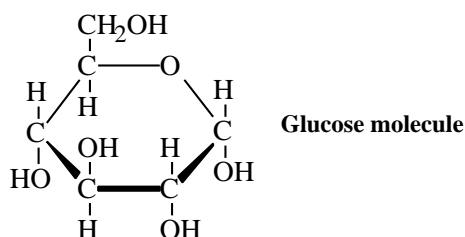
High-molar-mass **polysaccharides**, such as starch and glycogen ("animal starch"), are biopolymers of simple sugars.

When photosynthesis occurs in a plant cell, the energy from sunlight is converted to chemical energy in a carbohydrate. This carbohydrate may be transferred to some other part of the plant for use as an energy source. It may be converted to a water-insoluble carbohydrate for storage until it is needed for energy. Or it may be transformed to cell wall material and become part of the structure of the plant. If the plant is eaten by an animal, the carbohydrate is used for energy by the animal.

The simplest carbohydrates are the **monosaccharides**, also called **simple sugars**. Because they have six carbon atoms, simple sugars are sometimes called *hex*oses. Glucose (formula shown above) is the most common simple sugar involved in cell processes. Other simple sugars with the same formula but somewhat different structures are fructose, mannose, and galactose. These must be changed to glucose before they can be used in a cell. Because of its use for energy in body processes, glucose is found in the blood. Normal levels are from 65 to 110 mg glucose per 100 ml of blood. Higher levels may indicate diabetes.

Units of two monosaccharides make up several very important sugars known as **disaccharides**. When two molecules of monosaccharides join together to form a disaccharide,

$$C_6H_{12}O_6 \ + \ C_6H_{12}O_6 \qquad C_{12}H_{22}O_{11} \ + \ H_2O \qquad\qquad (21.4.1)$$
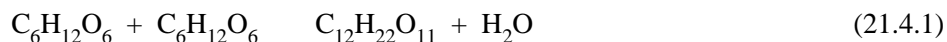
a molecule of water is lost. Recall that proteins are also formed from smaller amino acid molecules by condensation reactions involving the loss of water molecules. Disaccharides include sucrose (cane sugar used as a sweetener), lactose (milk sugar), and maltose (a product of the breakdown of starch).

Polysaccharides consist of many simple sugar units hooked together. One of the most important polysaccharides is **starch**, which is produced by plants for food storage. Animals produce a related material called **glycogen**. The chemical formula of starch is $(C_6H_{10}O_5)_n$, where n may represent a number as high as several hundreds. What this means is that the very large starch molecule consists of many units of $C_6H_{10}O_5$ joined together. For example, if n is 100, there are 6 times 100 carbon atoms, 10 times 100 hydrogen atoms, and 5 times 100 oxygen atoms in the molecule. Its chemical formula is $C_{600}H_{1000}O_{500}$. The atoms in a starch molecule are actually present as linked rings represented by the structure shown in Figure 21.4. Starch occurs in many foods, such as bread and cereals. It is readily digested by animals, including humans.
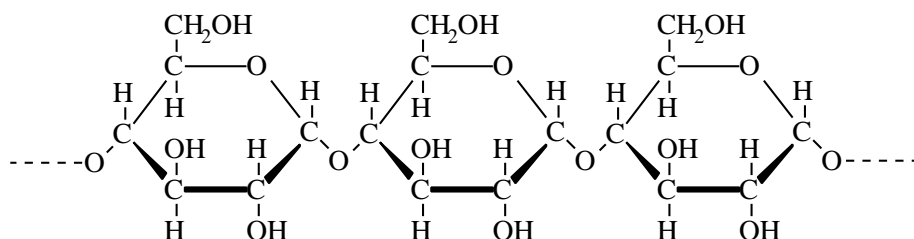

Figure 21.4. Part of a starch molecule showing units of $C_6H_{10}O_5$ condensed together.

Cellulose is a polysaccharide which is also made up of $C_6H_{10}O_5$ units. Molecules of cellulose are huge, with molecular weights of around 400,000. The cellulose structure (Figure 21.5) is similar to that of starch. Cellulose is produced by plants and forms the structural material of plant cell walls. Wood is about 60% cellulose, and cotton contains over 90% of this material. Fibers of cellulose are extracted from wood and pressed together to make paper.

Humans and most other animals cannot digest cellulose because they lack the enzyme needed to hydrolyze the oxygen linkages between the glucose molecules. Ruminant animals (cattle, sheep, goats, moose) have bacteria in their stomachs that break down cellulose into products which can be used by the animal. Chemical processes are available to convert cellulose to simple sugars by the reaction

$$(C_6H_{10}O_5)_n \ + \ nH_2O \qquad nC_6H_{12}O_6 \qquad\qquad (21.4.2)$$
$$\text{cellulose} \qquad\qquad\qquad \text{glucose}$$
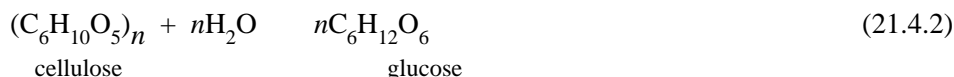
where n may be 2000-3000. This involves breaking the linkages between units of $C_6H_{10}O_5$ by adding a molecule of H_2O at each linkage, a hydrolysis reaction. Large amounts of cellulose from wood, sugar cane, and agricultural products go to waste each year. The hydrolysis of cellulose enables these products to be converted to sugars, which can be fed to animals.
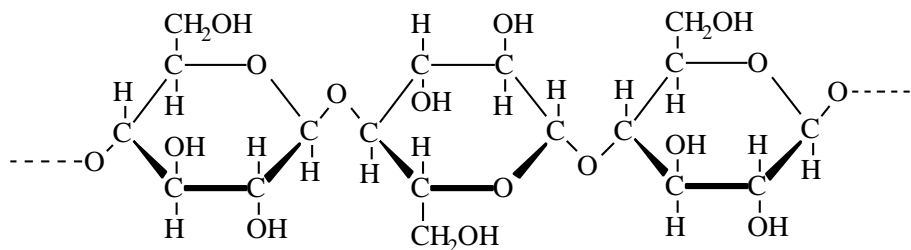
Figure 21.5. Part of the structure of cellulose.

Carbohydrate groups are attached to protein molecules in a special class of materials called **glycoproteins**. Collagen is a crucial glycoprotein that provides structural integrity to body parts. It is a major constituent of skin, bones, tendons, and cartilage.

21.5. LIPIDS

Lipids are substances that can be extracted from plant or animal matter by organic solvents, such as chloroform, diethyl ether, or toluene (Figure 21.6). Whereas carbohydrates and proteins are characterized predominately by the mono-mers (monosaccharides and amino acids) from which they are composed, lipids are defined essentially by their physical characteristic of organophilicity. The most common lipids are fats and oils composed of **triglycerides** formed from the alcohol glycerol, $CH_2(OH)CH(OH)CH_2OH$, and a long-chain fatty acid such as stearic acid, $CH_3(CH_2)_{16}COOH$ as shown in Figure 21.7. Numerous other biological materials, including waxes, cholesterol, and some vitamins and hormones, are classified as lipids. Common foods such as butter and salad oils are lipids. The longer chain fatty acids such as stearic acid are also organic-soluble and are classified as lipids.

Lipids are toxicologically important for several reasons. Some toxic substances interfere with lipid metabolism, leading to detrimental accumulation of lipids. Many toxic organic compounds are poorly soluble in water but are lipid-soluble, so that bodies of lipids in organisms serve to dissolve and store toxicants.

An important class of lipids consists of **phosphoglycerides** (glycerophosphat-ides), which may be regarded as triglyderides in which one of the acids bonded to glycerol is orthophosphoric acid. These lipids are especially important because they are essential constituents of cell membranes. These membranes consist of bilayers in which the hydrophilic phosphate ends of the molecules are on the outside of the membrane and the hydrophobic "tails" of the molecules are on the inside.

Waxes are also esters of fatty acids. However, the alcohol in a wax is not glycerol, but is often a very long chain alcohol. For example, one of the main com-pounds in beeswax is myricyl palmitate,
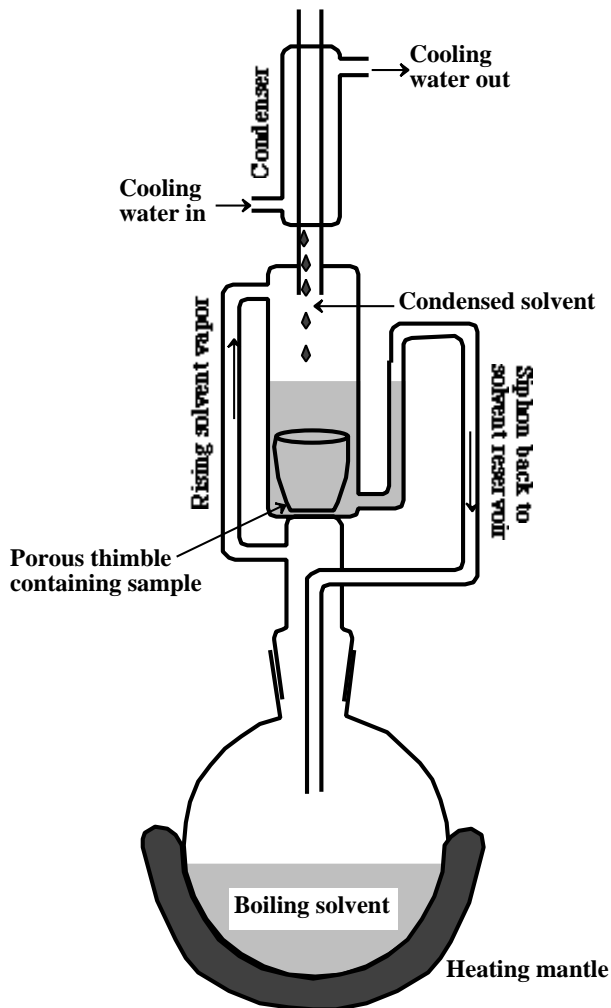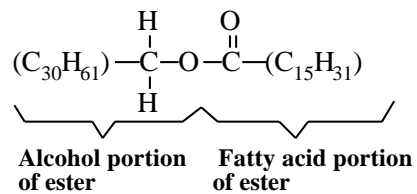
Figure 21.6. Lipids are extracted from some biological materials with a soxhlet extractor (above). The solvent is vaporized in the distillation flask by the heating mantle, rises through one of the exterior tubes to the condenser, and is cooled to form a liquid. The liquid drops onto the porous thimble containing the sample. Siphon action periodically drains the solvent back into the distillation flask. The extracted lipid collects as a solution in the solvent in the flask.
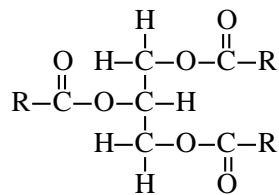
Figure 21.7. General formula of triglycerides, which make up fats and oils. The R group is from a fatty acid and is a hydrocarbon chain, such as $-(CH_2)_{16}CH_3$.

in which the alcohol portion of the ester has a very large hydrocarbon chain. Waxes are produced by both plants and animals, largely as protective coatings. Waxes are found in a number of common products. Lanolin is one of these. It is the "grease" in sheep's wool. When mixed with oils and water, it forms stable colloidal emulsions consisting of extremely small oil droplets suspended in water. This makes lanolin useful for skin creams and pharmaceutical ointments. Carnauba wax occurs as a coating on the leaves of some Brazilian palm trees. Spermaceti wax is composed largely of cetyl palmitate,

Cetyl palmitate
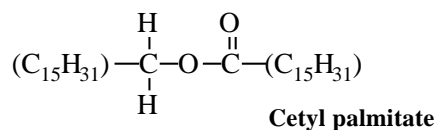
extracted from the blubber of the sperm whale. It is very useful in some cosmetics and pharmaceutical preparations.

Steroids are lipids found in living systems which all have the ring system shown in Figure 21.8 for cholesterol. Steroids occur in bile salts, which are produced by the liver and then secreted into the intestines. Their breakdown products give feces its characteristic color. Bile salts act upon fats in the intestine. They suspend very tiny fat droplets in the form of colloidal emulsions. This enables the fats to be broken down chemically and digested.

Cholesterol, a typical steroid
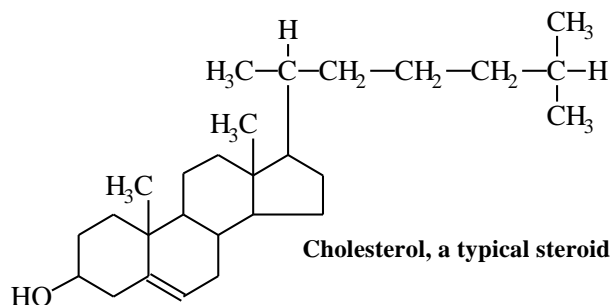
Figure 21.8. Steroids are characterized by the ring structure shown above for cholesterol.

Some steroids are **hormones**. Hormones act as "messengers" from one part of the body to another. As such, they start and stop a number of body functions. Male and female sex hormones (estrogens) are examples of steroid hormones. Hormones are given off by glands in the body called **endocrine glands**. The locations of important endocrine glands are shown in Figure 21.9.

21.6. ENZYMES

Catalysts are substances that speed up a chemical reaction without themselves being consumed in the reaction. The most sophisticated catalysts of all are those found in living systems. They bring about reactions that could not be performed at all, or only with great difficulty, outside a living organism. These catalysts are called **enzymes**. In addition to speeding up reactions by as much as ten- to a hundred million-fold, enzymes are extremely selective in the reactions they promote.
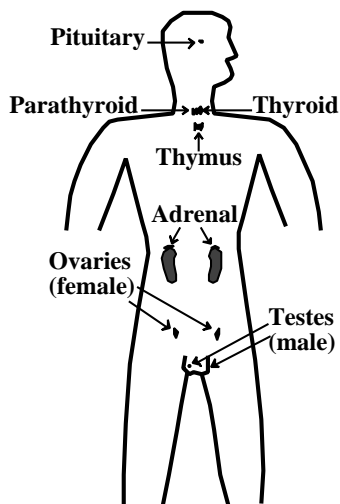

Figure 21.9. Locations of important endocrine glands.

Enzymes are proteinaceous substances with highly specific structures that inter-act with particular substances or classes of substances called **substrates**. Enzymes act as catalysts to enable biochemical reactions to occur, after which they are regenerated intact to take part in additional reactions. The extremely high specificity with which enzymes interact with substrates results from their "lock and key" action based upon the unique shapes of enzymes as illustrated in Figure 21.10. This illustration shows that an enzyme "recognizes" a particular substrate by its molecular structure and binds to it to produce an **enzyme-substrate complex**. This complex then breaks apart to form one or more products different from the original substrate, regenerating the unchanged enzyme, which is then available to catalyze additional reactions. The basic process for an enzyme reaction is, therefore,

enzyme + substrate   enzyme-substrate complex
                                                           enzyme + product   (21.6.1)
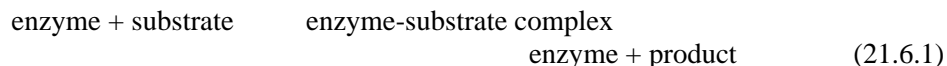
Several important things should be noted about this reaction. As shown in Figure 21.10, an enzyme acts on a specific substrate to form an enzyme-substrate complex because of the fit between their structures. As a result, something happens to the

substrate molecule. For example, it might be split in two at a particular location. Then the enzyme-substrate complex comes apart, yielding the enzyme and products. The enzyme is not changed in the reaction and is now free to react again. Note that the arrows in the formula for enzyme reaction point both ways. This means that the reaction is **reversible**. An enzyme-substrate complex can simply go back to the enzyme and the substrate. The products of an enzymatic reaction can react with the enzyme to form the enzyme-substrate complex again. It, in turn, may again form the enzyme and the substrate. Therefore, the same enzyme may act to cause a reaction to go either way.
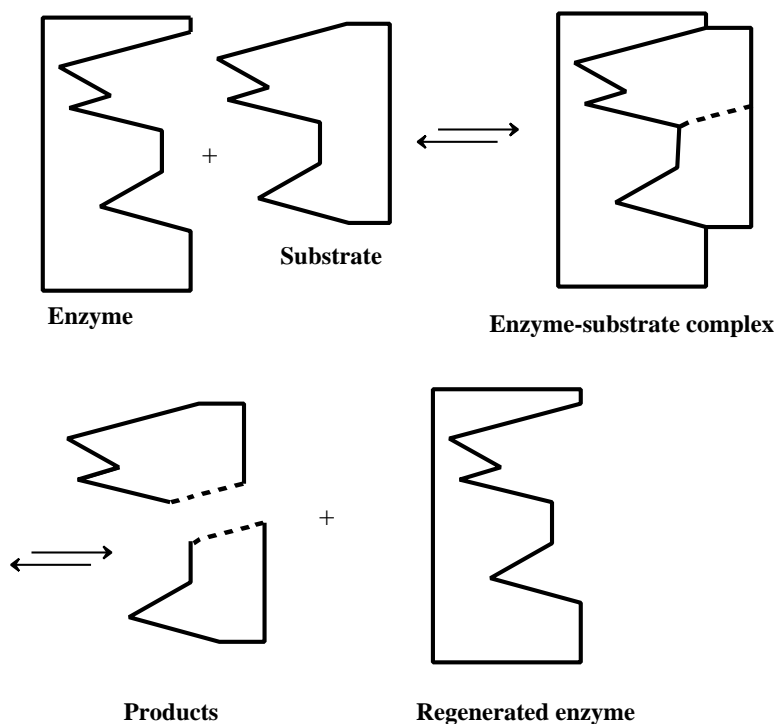

Figure 21.10. Representation of the "lock-and-key" mode of enzyme action which enables the very high specificity of enzyme-catalyzed reactions.

Some enzymes cannot function by themselves. In order to work, they must first be attached to **coenzymes**. Coenzymes normally are not protein materials. Some of the vitamins are important coenzymes.

Enzymes are named for what they do. For example, the enzyme given off by the stomach, which splits proteins as part of the digestion process, is called *gastric proteinase*. The "gastric" part of the name refers to the enzyme's origin in the stomach. The "proteinase" denotes that it splits up protein molecules. The common name for this enzyme is pepsin. Similarly, the enzyme produced by the pancreas that breaks down fats (lipids) is called *pancreatic lipase*. Its common name is steapsin. In general, lipase enzymes cause lipid triglycerides to dissociate and form glycerol and fatty acids.

The enzymes mentioned above are **hydrolyzing enzymes**, which bring about the breakdown of high-molecular-weight biological compounds by the addition of water. This is one of the most important types of the reactions involved in digestion. The three main classes of energy-yielding foods that animals eat are carbohydrates, proteins, and fats. Recall that the higher carbohydrates humans eat are largely disaccharides (sucrose, or table sugar) and polysaccharides (starch). These are formed by the joining together of units of simple sugars, $C_6H_{12}O_6$, with the elimination of an H_2O molecule at the linkage where they join. Proteins are formed by the condensation of amino acids, again with the elimination of a water molecule at each linkage. Fats are esters which are produced when glycerol and fatty acids link together. A water molecule is lost for each of these linkages when a protein, fat, or carbohydrate is synthesized. In order for these substances to be used as a food source, the reverse process must occur to break down large, complicated molecules of protein, fat, or carbohydrate to simple, soluble substances which can penetrate a cell membrane and take part in chemical processes in the cell. This reverse process is accomplished by hydrolyzing enzymes.

Biological compounds with long chains of carbon atoms are broken down into molecules with shorter chains by the breaking of carbon-carbon bonds. This commonly occurs by the elimination of CO_2 from carboxylic acids. For example, *pyruvate decarboxylase* enzyme acts upon pyruvic acid,

$$
\underset{\textbf{Pyruvic acid}}{H-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{\overset{\displaystyle O}{\|}}{C}-\overset{\overset{\displaystyle O}{\|}}{C}-OH} \;\xrightarrow[\text{decarboxylase}]{\text{Pyruvate}}\; \underset{\textbf{Acetaldehyde}}{H-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{\overset{\displaystyle O}{\|}}{C}-H} \;+\; CO_2
\tag{21.6.2}
$$
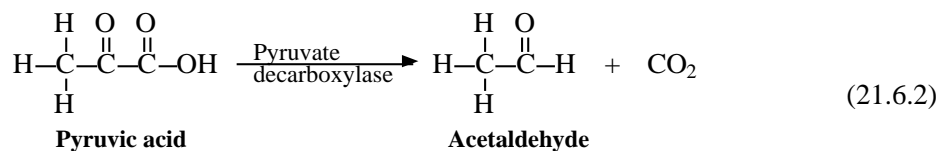
to split off CO_2 and produce a compound with one less carbon. It is by such carbon-by-carbon breakdown reactions that long chain compounds are eventually degraded to CO_2 in the body, or that long-chain hydrocarbons undergo biodegradation by the action of microorganisms in the water and soil environments.

Oxidation and reduction are the major reactions for the exchange of energy in living systems. Cellular respiration is an oxidation reaction in which a carbohydrate, $C_6H_{12}O_6$, is broken down to carbon dioxide and water with the release of energy.

$$
C_6H_{12}O_6 + 6O_2 \qquad 6CO_2 + 6H_2O + \text{energy}
\tag{21.6.3}
$$
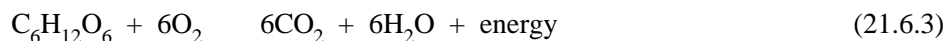
Actually, such an overall reaction occurs in living systems by a complicated series of individual steps. Some of these steps involve oxidation. The enzymes that bring about oxidation in the presence of free O_2 are called **oxidases**. In general, biological oxidation-reduction reactions are catalyzed by **oxidoreductase enzymes**.

In addition to the types of enzymes discussed above, there are many enzymes that perform miscellaneous duties in living systems. Typical of these are **isomerases**, which form isomers of particular compounds. For example, of the several simple sugars with the formula $C_6H_{12}O_6$, only glucose can be used directly for cell processes. The other isomers are converted to glucose by the action of isomerases. **Transferase enzymes** move chemical groups from one molecule to another, **lyase enzymes** remove chemical groups without hydrolysis and participate in the formation of C=C bonds or addition of species to such bonds, and **ligase**

enzymes work in conjunction with ATP (adenosine triphosphate, a high-energy molecule that plays a crucial role in energy-yielding, glucose-oxidizing metabolic processes) to link molecules together with the formation of bonds such as carbon-carbon or carbon-sulfur bonds.

Enzyme action may be affected by many different things. Enzymes require a certain hydrogen ion concentration (pH) to function best. For example, gastric proteinase requires the acid environment of the stomach to work well. When it passes into the much less acidic intestines, it stops working. This prevents damage to the intestine walls, which would occur if the enzyme tried to digest them. Temperature is critical. Not surprisingly, the enzymes in the human body work best at around 98.6°F (37°C), which is the normal body temperature. Heating these enzymes to around 140°F permanently destroys them. Some bacteria that thrive in hot springs have enzymes that work best at temperatures as high as that of boiling water. Other "cold-seeking" bacteria have enzymes adapted to near the freezing point of water.

One of the greatest concerns regarding the effects of surroundings upon enzymes is the influence of toxic substances. A major mechanism of toxicity is the alteration or destruction of enzymes by toxic agents—cyanide, heavy metals, or organic compounds such as insecticidal parathion. An enzyme that has been destroyed obviously cannot perform its designated function, whereas one that has been altered may either not function at all or may act improperly. The detrimental effects of toxicants on enzymes are discussed in more detail in Chapter 22.

21.7. NUCLEIC ACIDS

The "essence of life" is contained in **deoxyribonucleic acid** (**DNA**, which stays in the cell nucleus) and **ribonucleic acid** (**RNA**, which functions in the cell cytoplasm). These substances, which are known collectively as **nucleic acids**, store and pass on essential genetic information that controls reproduction and protein synthesis.

The structural formulas of the monomeric constituents of nucleic acids are given in Figure 21.11. These are pyrimidine or purine nitrogen-containing bases, two sugars, and phosphate. DNA molecules are made up of the nitrogen-containing bases adenine, guanine, cytosine, and thymine; phosphoric acid (H_3PO_4); and the simple sugar 2-deoxy- -D-ribofuranose (commonly called deoxyribose). RNA molecules are composed of the nitrogen-containing bases adenine, guanine, uracil, and cytosine; phosphoric acid (H_3PO_4) and the simple sugar -D-ribofuranose (commonly called ribose).

The formation of nucleic acid polymers from their monomeric constituents may be viewed as the following steps.

- Monosaccharide (simple sugar) + cyclic nitrogenous base yields **nucleoside**:

Deoxyctidine formed by the dimerization of cytosine and deoxyribose with the elimination of a molecule of H$_2$O.

Thymine (T)

Occur only in DNA

2-Deoxy- β-D-ribofuranose

Cytosine (C)

Adenine (A)

Guanine(G)

Phosphate

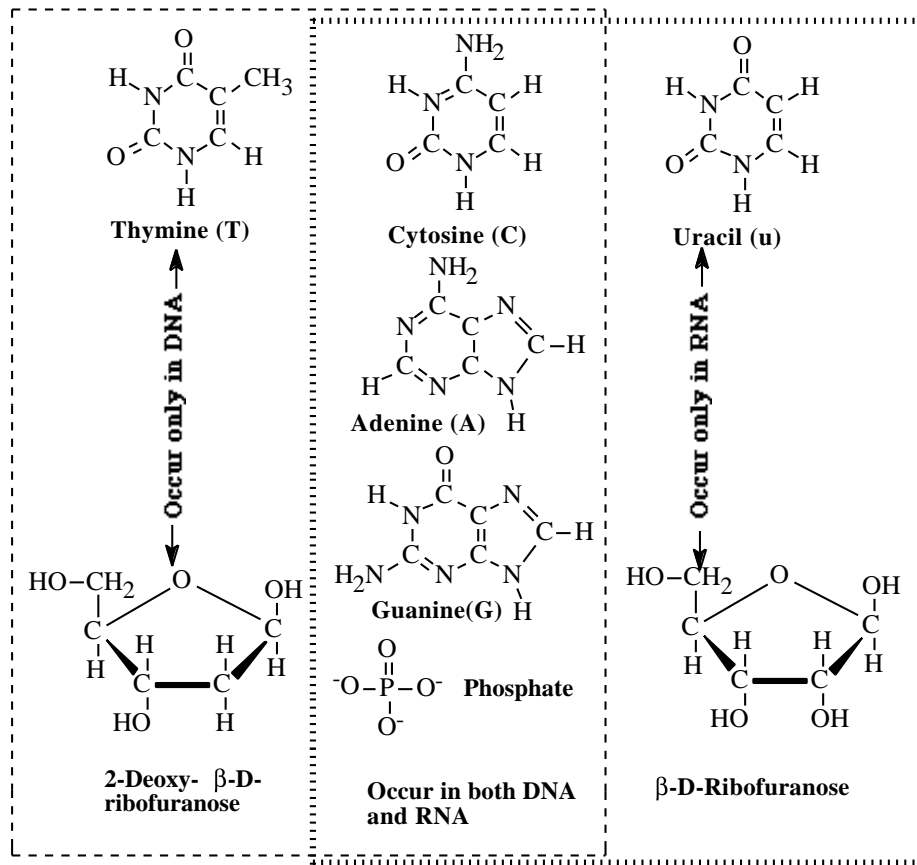Occur in both DNA and RNA

Uracil (u)

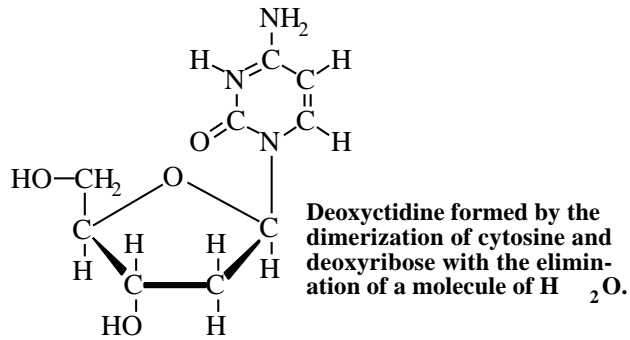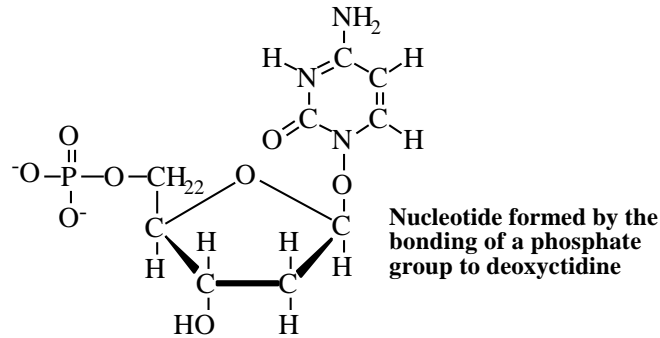Occur only in RNA

β-D-Ribofuranose

Figure 21.11. Constituents of DNA (enclosed by ----) and of RNA (enclosed by ⁞⁞⁞⁞).

• Nucleoside + phosphate yields **phosphate ester nucleotide**.

Nucleotide formed by the bonding of a phosphate group to deoxyctidine

- Polymerized nucleotide yields **nucleic acid**. In the nucleic acid the phosphate negative charges are neutralized by metal cations (such as Mg^{2+}) or positively-charged proteins (histones).

Segment of the DNA polymer showing linkage of two nucleotides
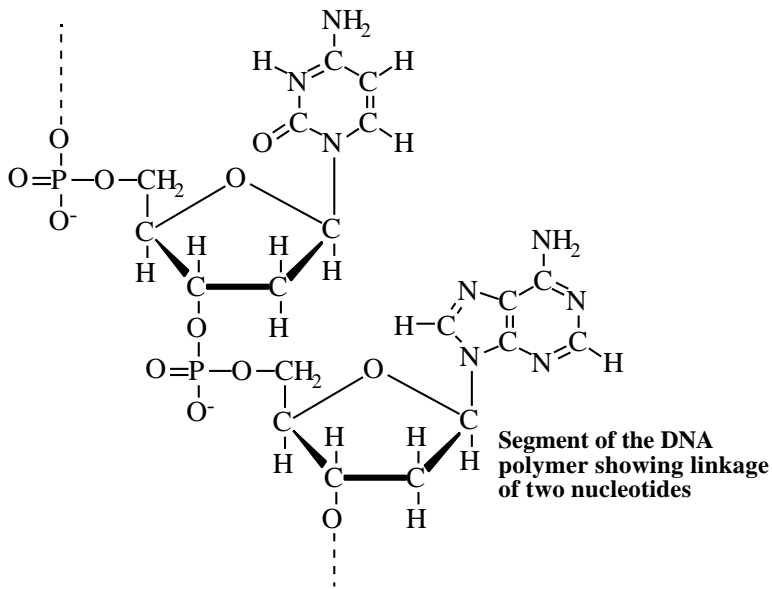
Molecules of DNA are huge, with molecular weights greater than one billion. Molecules of RNA are also quite large. The structure of DNA is that of the famed "double helix." It was figured out in 1953 by an American scientist, James D. Watson, and Francis Crick, a British scientist. They received the Nobel prize for this scientific milestone in 1962. This model visualizes DNA as a so-called double - helix structure of oppositely-wound polymeric strands held together by hydrogen bonds between opposing pyrimidine and purine groups. As a result, DNA has both a primary and a secondary structure; the former is due to the sequence of nucleotides in the individual strands of DNA, and the latter results from the -helix interaction of the two strands. In the secondary structure of DNA, only cytosine can be opposite guanine and only thymine can be opposite adenine and vice versa. Basically, the structure of DNA is that of two spiral ribbons "counter-wound" around each other (Fig. 21.12). The two strands of DNA are **complementary**. This means that a particular portion of one strand fits like a key in a lock with the corresponding

portion of another strand. If the two strands are pulled apart, each manufactures a new complementary strand, so that two copies of the original double helix result. This occurs during cell reproduction.

The molecule of DNA is sort of like a coded message. This "message," the genetic information contained in and transmitted by nucleic acids, depends upon the sequence of bases from which they are composed. It is somewhat like the message sent by telegraph which consists only of dots, dashes, and spaces in between. The key aspect of DNA structure that enables storage and replication of this information is its double helix structure mentioned above.

Nucleic Acids in Protein Synthesis

Whenever a new cell is formed, the DNA in its nucleus must be accurately reproduced from the parent cell. Life processes are absolutely dependent upon accurate protein synthesis as regulated by cell DNA. The DNA in a single cell must
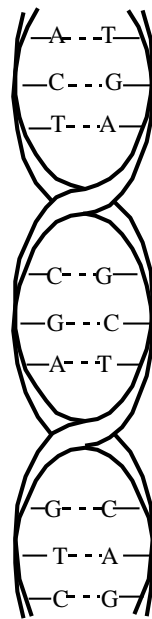

Figure 21.12. Representation of the double helix structure of DNA showing the allowed base pairs held together by hydrogen bonding between the phosphate/sugar polymer "backbones" of the two strands of DNA. The letters stand for adenine (A), cytosine (C), guanine (G), and thymine (T). The dashed lines, ---, represent hydrogen bonds.

be capable of directing the synthesis of up to 3000 or even more different proteins. The directions for the synthesis of a single protein are contained in a segment of DNA called a **gene**. The process of transmitting information from DNA to a newly-synthesized protein involves the following steps:

- The DNA undergoes **replication**. This process involves separation of a segment of the double helix into separate single strands which then

replicate such that guanine is opposite cytosine (and vice versa) and adenine is opposite thymine (and vice versa). This process continues until a complete copy of the DNA molecule has been produced.

- The newly replicated DNA produces **messenger RNA** (**m-RNA**), a complement of the single strand of DNA, by a process called **transcription**.

- A new protein is synthesized using m-RNA as a template to determine the order of amino acids in a process called **translation**.

Modified DNA

DNA molecules may be modified by the unintentional addition or deletion of nucleotides or by substituting one nucleotide for another. The result is a **mutation** that is transmittable to offspring. Mutations can be induced by chemical substances. This is a major concern from a toxicological viewpoint because of the detrimental effects of many mutations and because substances that cause mutations often cause cancer as well. DNA malfunction may also result in birth defects. The failure to control cell reproduction results in cancer. Radiation from X rays and radioactivity also disrupts DNA and may cause mutation.

21.8. RECOMBINANT DNA AND GENETIC ENGINEERING

As noted above, segments of DNA contain information for the specific syntheses of particular proteins. Within the last two decades it has become possible to transfer this information between organisms by means of **recombinant DNA technology**, which has resulted in a new industry based on **genetic engineering**. Most often the recipient organisms are bacteria, which can be reproduced (cloned) over many orders of magnitude from a cell that has acquired the desired qualities. Therefore, to synthesize a particular substance such as human insulin or growth hormone, the required genetic information can be transferred from a human source to bacterial cells, which then produce the substance as part of their metabolic processes.

The first step in recombinant DNA gene manipulation is to lyze, or "open up," a cell that has the genetic material needed and to remove this material from the cell. Through enzyme action the sought-after genes are cut from the donor DNA chain. These are next spliced into small DNA molecules. These molecules, called **cloning vehicles**, are capable of penetrating the host cell and becoming incorporated into its genetic material. The modified host cell is then reproduced many times and carries out the desired biosynthesis.

Early concerns about the potential of genetic engineering to produce "monster organisms" or new and horrible diseases have been largely allayed, although caution is still required with this technology. In the environmental area genetic engineering offers some hope for the production of bacteria engineered to safely destroy troublesome wastes and to produce biological substitutes for environmentally damaging synthetic pesticides.

Numerous possibilities exist for combining biology with chemistry to produce chemical feedstocks and products of various kinds. An example is production of polylactic acid using lactic acid produced enzymatically with corn and polymerized by standard chemical processes.[3] Much attention has been focused on the develop-

ment of enzymes to perform a variety of chemical conversions. Another important area that uses transgenic organisms is the breeding of plants that produce natural insecticides, specifically the insecticide from *Bacillus thuringiensis.*

21.9. METABOLIC PROCESSES

Biochemical processes that involve the alteration of biomolecules fall under the category of **metabolism**. Metabolic processes may be divided into the two major categories of **anabolism** (synthesis) and **catabolism** (degradation of substances). An organism may use metabolic processes to yield energy or to modify the constituents of biomolecules.

Energy-Yielding Processes

Organisms can gain energy by one of three major processes, which are listed as follows:

- **Respiration**, in which organic compounds undergo catabolism that requires molecular oxygen (**aerobic respiration**) or that occurs in the absence of molecular oxygen (**anaerobic repiration**). Aerobic respiration uses the **Krebs cycle** to obtain energy from the following reaction:

$$C_6H_{12}O_6 + 6O_2 \quad 6CO_2 + 6H_2O + \text{energy}$$

About half of the energy released is converted to short-term stored chemical energy, particularly through the synthesis of **adenosine triphosphate** (**ATP**) nucleotide. For longer-term energy storage, glycogen or starch polysaccharides are synthesized, and for still longer-term energy storage, lipids (fats) are generated and retained by the organism.

- **Fermentation**, which differs from respiration in not having an electron transport chain. Yeasts produce ethanol from sugars by fermentation:

$$C_6H_{12}O_6 \quad 2CO_2 + 2C_2H_5OH$$

- **Photosynthesis**, in which light energy captured by plant and algal chloroplasts is used to synthesize sugars from carbon dioxide and water:

$$6CO_2 + 6H_2O + h \quad C_6H_{12}O_6 + 6O_2$$
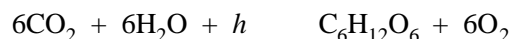
Plants cannot always get the energy that they need from sunlight. During the dark they must use stored food. Plant cells, like animal cells, contain mitochondria in which stored food is converted to energy by cellular respiration.

Plant cells, which use sunlight as a source of energy and CO_2 as a source of carbon, are said to be **autotrophic**. In contrast, animal cells must depend upon organic material manufactured by plants for their food. These are called **heterotrophic** cells. They act as "middlemen" in the chemical reaction between oxygen and food material using the energy from the reaction to carry out their life processes.

21.10. METABOLISM OF XENOBIOTIC COMPOUNDS

When toxicants or their metabolic precursors (**protoxicants**) enter a living organism they may undergo several processes, including those that may make them more toxic or that detoxify them. Chapter 22 discusses the metabolic processes that toxicants undergo and the mechanisms by which they may cause damage to an organism. Emphasis is placed on **xenobiotic compounds**, which are those that are normally foreign to living organisms; on chemical aspects; and on processes that lead to products that can be eliminated from the organism. Of particular importance is **intermediary xenobiotic metabolism** which results in the formation of somewhat transient species that are different from both those ingested and the ultimate product that is excreted. These species may have significant toxicological effects. Xenobiotic compounds in general are acted upon by enzymes that function on a material that is in the body naturally—an **endogenous substrate**. For example, flavin-containing monooxygenase enzyme acts upon endogenous cysteamine to convert it to cysta-mine, but also functions to oxidize xenobiotic nitrogen and sulfur compounds.

Biotransformation refers to changes in xenobiotic compounds as a result of enzyme action. Reactions that are not mediated by enzymes may also be important in some cases. As examples of nonenzymatic transformations, some xenobiotic compounds bond with endogenous biochemical species without an enzyme catalyst, undergo hydrolysis in body fluid media, or undergo oxidation/reduction processes. The metabolic Phase I and Phase II reactions of xenobiotics discussed here and in Chapter 22 are enzymatic, however.

The likelihood that a xenobiotic species will undergo enzymatic metabolism in the body depends upon the chemical nature of the species. Compounds with a high degree of polarity, such as relatively ionizable carboxylic acids, are less likely to enter the body system and, when they do, tend to be quickly excreted. Therefore, such compounds are unavailable, or only available for a short time, for enzymatic metabolism. Volatile compounds, such as dichloromethane or diethyl ether, are expelled so quickly from the lungs that enzymatic metabolism is less likely. This leaves as the most likely candidates for enzymatic metabolic reactions **nonpolar lipophilic compounds**, those that are relatively less soluble in aqueous biological fluids and more attracted to lipid species. Of these, the ones that are resistant to enzymatic attack (PCBs, for example) tend to bioaccumulate in lipid tissue.

Xenobiotic species may be metabolized in many body tissues and organs. As part of the body's defense against the entry of xenobiotic species, the most prominent sites of xenobiotic metabolism are those associated with entry into the body, such as the skin and lungs. The gut wall through which xenobiotic species enter the body from the gastrointestinal tract is also a site of significant xenobiotic compound metabolism. The liver is of particular significance because materials entering systemic circulation from the gastrointestinal tract must first traverse the liver.

Phase I and Phase II Reactions

The processes that most xenobiotics undergo in the body can be divided into the two categories of Phase I reactions and Phase II reactions. A **Phase I reaction** introduces reactive, polar functional groups into lipophilic ("fat-seeking") toxicant molecules. In their unmodified forms, such toxicant molecules tend to pass through lipid-containing cell membranes and may be bound to lipoproteins, in which form

they are transported through the body. Because of the functional group present, the product of a Phase I reaction is usually more water-soluble than the parent xenobiotic species, and more importantly, possesses a "chemical handle" to which a substrate material in the body may become attached so that the toxicant can be eliminated from the body. The binding of such a substrate is a **Phase II reaction**, and it produces a **conjugation product** that is amenable to excretion from the body.

In general, the changes in structure and properties of a compound that result from a Phase I reaction are relatively mild. Phase II processes, however, usually produce species that are much different from the parent compounds. It should be emphasized that not all xenobiotic compounds undergo both Phase I and Phase II reactions. Such a compound may undergo only a Phase I reaction and be excreted directly from the body. Or a compound that already possesses an appropriate functional group capable of conjugation may undergo a Phase II reaction without a preceding Phase I reaction. Phase I and Phase II reactions are discussed in more detail as they relate to toxicological chemistry in Chapter 22 and 23.

LITERATURE CITED

1. Stanley E. Manahan, *Toxicological Chemistry*, 2nd ed., Lewis Publishers/CRC Press, Inc., Boca Raton, FL, 1992.

2. White, David, *The Physiology and Biochemistry of Prokaryotes*, Oxford University Press, New York, 1999.

3. Thayer, Ann, "Best of All Industrial Worlds: Chemistry and Biology," *Chemical and Engineering News*, May 31, 1999, p. 18.

SUPPLEMENTARY REFERENCES

Bettelheim, Frederick A. and Jerry March, *Introduction to Organic and Biochemistry*, Saunders College Publishing, Fort Worth, TX, 1998.

Brownie, Alexander C. and John C. Kemohan, Illustrations by Jane Templeman and Chartwell Illustrators, *Biochemistry*, Churchill Livingstone, Edinburgh, Scotland, 1999.

Chesworth, J. M., T. Stuchbury, and J.R. Scaife, *An Introduction to Agricultural Biochemistry*, Chapman and Hall, London, 1998.

Garrett, Reginald H. and Charles M. Grisham, *Biochemistry*, Saunders College Publishing, Philadelphia, 1998.

Gilbert, Hiram F., Ed., *Basic Concepts in Biochemistry*, McGraw-Hill, Health Professions Division, New York, 2000.

Kuchel, Philip W., Ed., *Schaum's Outline of Theory and Problems of Biochemistry*, McGraw-Hill, New York, 1998.

Lea, Peter J. and Richard C. Leegood, Eds., *Plant Biochemistry and Molecular Biology*, 2nd ed., John Wiley & Sons, New York, 1999.

Marks, Dawn B., *Biochemistry*, Williams & Wilkins, Baltimore, 1999.

McKee, Trudy and James R. McKee, *Biochemistry: An Introduction*, WCB/McGraw-Hill, Boston, 1999.

Meisenberg,Gerhard and William H. Simmons, *Principles of Medical Biochemistry*, Mosby, St. Louis, 1998.

Switzer, Robert L. and Liam F. Garrity, *Experimental Biochemistry*, W. H. Freeman and Co., New York, 1999.

Voet, Donald, Judith G. Voet, and Charlotte Pratt, *Fundamentals of Biochemistry*, John Wiley & Sons, New York, 1998.

Vrana, Kent E., *Biochemistry*, Lippincott Williams & Wilkins, Philadelphia,, 1999.

Wilson, Keith and John M. Walker, *Principles and Techniques of Practical Biochemistry*, Cambridge University Press, New York, 1999.

QUESTIONS AND PROBLEMS

1. What is the toxicological importance of lipids? How are lipids related to hydrophobic pollutants and toxicants?

2. What is the function of a hydrolase enzyme?

3. Match the cell structure on the left with its function on the right, below:

   1. Mitochondria   (a) Toxicant metabolism
   2. Endoplasmic reticulum (b) Fills the cell
   3. Cell membrane   (c) Deoxyribonucleic acid
   4. Cytoplasm   (d) Mediate energy conversion and utilization
   5. Cell nucleus   (e) Encloses the cell and regulates the passage of materials into and out of the cell interior

4. The formula of simple sugars is $C_6H_{12}O_6$. The simple formula of higher carbohydrates is $C_6H_{10}O_5$. Of course, many of these units are required to make a molecule of starch or cellulose. If higher carbohydrates are formed by joining together molecules of simple sugars, why is there a difference in the ratios of C, H, and O atoms in the higher carbohydrates as compared to the simple sugars?

5. Why does wood contain so much cellulose?

6. What would be the chemical formula of a *tri*saccharide made by the bonding together of three simple sugar molecules?

7. The general formula of cellulose may be represented as $(C_6H_{10}O_5)_x$. If the molecular weight of a molecule of cellulose is 400,000, what is the estimated value of x?

8. During one month a factory for the production of simple sugars, $C_6H_{12}O_6$, by the hydrolysis of cellulose processes one million pounds of cellulose. The percentage of cellulose that undergoes the hydrolysis reaction is 40%. How many pounds of water are consumed in the hydrolysis of cellulose each month?

9. What is the structure of the largest group of atoms common to all amino acid molecules?

10. Glycine and phenylalanine can join together to form two different dipeptides. What are the structures of these two dipeptides?

11. One of the ways in which two parallel protein chains are joined together, or cross linked, is by way of an —S—S— link. What amino acid to you think might be most likely to be involved in such a link? Explain your choice.

12. Fungi, which break down wood, straw, and other plant material, have what are called "exoenzymes." Fungi have no teeth and cannot break up plant material physically by force. Knowing this, what do you suppose an exoenzyme is? Explain how you think it might operate in the process by which fungi break down something as tough as wood.

13. Many fatty acids of lower molecular weight have a bad odor. Speculate as to the reasons that rancid butter has a bad odor. What chemical compound is produced that has a bad odor? What sort of chemical reaction is involved in its production?

14. The long-chain alcohol with 10 carbons is called decanol. What do you think would be the formula of decyl stearate? To what class of compounds would it belong?

15. Write an equation for the chemical reaction between sodium hydroxide and cetyl stearate. What are the products?

16. What type of endocrine gland is found only in females? What type of these glands is found only in males?

17. The action of bile salts is a little like that of soap. What function do bile salts perform in the intestine? Look up the action of soaps in Chapter 5, and explain how you think bile salts may function somewhat like soap.

18. If the structure of an enzyme is illustrated as,
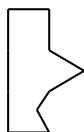
how should the structure of its substrate be represented?

19. Look up the structures of ribose and deoxyribose. Explain where the "deoxy" came from in the name deoxyribose.

20. In what respect is an enzyme and its substrate like two opposite strands of DNA?

21. For what discovery are Watson and Crick noted?

22. Why does an enzyme no longer function if it is denatured?