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Content

INTRODUCTION

The purpose of studying the discipline "Biochemistry" is to form students knowledge about the structure and properties of the main biomolecules and their transformation in the process of vital activity of the organism.

Biochemistry is combining the core tenets of biology and chemistry, the field plays a huge role in the development of novel new scientific approaches.

As a result of studying the discipline student has ***to know***:

Understanding of the fundamentals of chemistry and biology and the key principles of biochemistry and molecular biology.

Ability to dissect a problem into its key features.

The structure and properties of cell

The structure and properties of the main biomolecules (proteins, carbohydrates, lipids, nucleic acids).

Structure, properties and classification of enzymes and vitamins.

Biochemical bases for preservation and transfer of genetic information and genetic engineering.

The student should ***be able to***:

Apply modern methods of biochemical research, conduct individual biochemical experiments and correctly interpret the obtained results.

Design experiments and understand the limitations of the experimental approach.

Work safely and effectively in a laboratory.

Aware of the available resources and how to use them.

Use computers as information and research tools.

Use oral, written and visual presentations to present their work to both a science literate and a science non-literate audience.

Think in an integrated manner and look at problems from different perspectives.

Aware of the ethical issues in the molecular life sciences.

Chapter №1. Cell structure and functions of organelles

Earth has an enormous diversity of organisms. The range of habitats, from hot springs to Arctic tundra, from animal intestines to college dormitories, is matched by a correspondingly wide range of specific biochemical adaptations, achieved within a common chemical framework.

Biochemistry describes in molecular terms the structures, mechanisms, and chemical processes shared by all organisms and provides organizing principles that underlie life in all its diverse forms. Although biochemistry provides important insights and practical applications in medicine, agriculture, nutrition, and industry, its ultimate concern is with the wonder of life itself.

All living things are made up of cells. This fact was first discovered by English scientist Robert Hooke, in 1665 (**Figure 1.1.**), when he used a microscope to look at a slice of cork and found that it seemed to be made up of tiny chambers that he named cells. Hooke had sliced thin sections of cork to view under a microscope of his own design. He was able to see the minute, boxlike units of which the cork was made up. Hooke called these structures cells because he thought the boxes looked like monastery cells. The first description of living cells was provided in 1674 by Dutch scientist Anthony van Leeuwenhoek (**Fig. 1.2.**), who observed bacteria and protozoa under his microscope. Ten years later Leeuwenhoek gave the first accurate description of red blood cells.

Figure 1.1. English scientist Robert Hooke

Figure 1.2. Dutch scientist Anthony van Leeuwenhoek

Improvements in microscopes by the 19th century allowed more detailed investigations. In the 1830s Scottish botanist Robert Brown discovered the cell nucleus, and two German scientists, Matthias J. Schleiden and Theodor Schwann (**Fig. 1.3.**), concluded independently that cells were the basis of all life, a view called the cell theory. Rudolf Virchow, another German scientist, stated in 1858 that all cells develop from previously existing cells. During the late 19th century, techniques of fixing and staining tissues to preserve cells opened the way for intensive research. Scientists use a variety of microscopes to study cells.

Figure 1.3. German scientists Matthias J. Schleiden and Theodor Schwann

Today, we know that organisms in all three domains of life – bacteria, archaea and eukaryotes (**Fig. 1.4.**), share this property – they are all made up of cells. For some, a single cell is the organism, while others are multicellular, like humans, wombats or weeping willows.

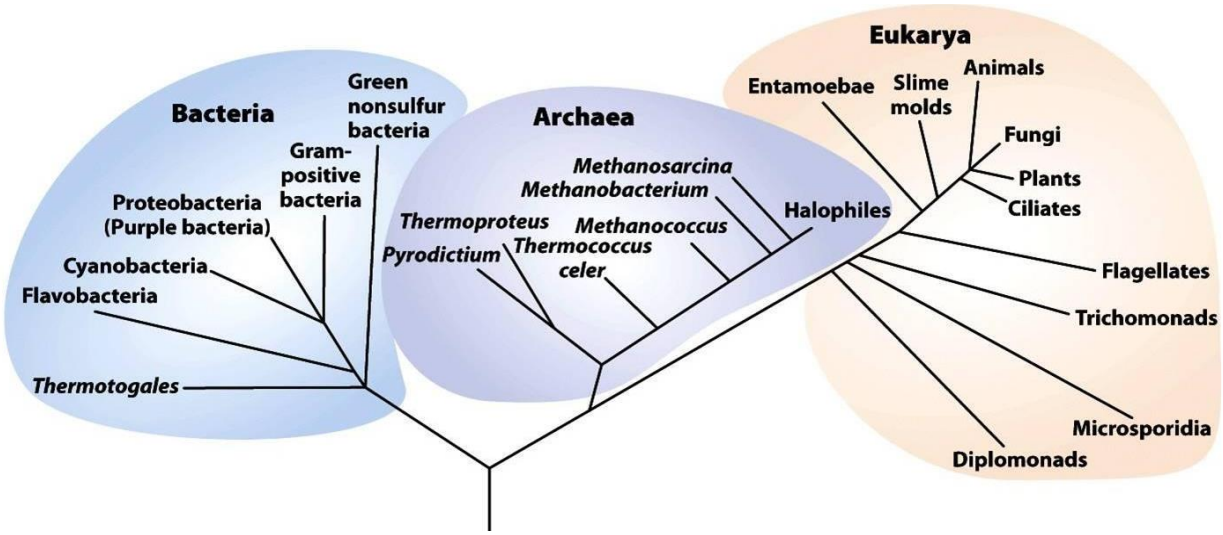

Figure 1.4. All three domains of life

Cells exist in a variety of shapes and sizes. Red blood cells are disk-shaped, while some skin cells resemble cubes. A single cell could be as large as a tennis ball or so small that thousands would fit on the period at the end of this sentence. Regardless of size, however, every cell contains the components needed to maintain life. Cells normally function with great efficiency, though they are vulnerable to disease. Cell size is usually measured in microns. A micron is equal to about one millionth of a meter, and about 25,000 microns equal 1 inch. The smallest bacteria are about 0.2 micron in diameter. The diameter of the average human cell is roughly 10 microns, making it barely visible without a microscope.

All cells have, for at least some part of their life, either a nucleoid or a nucleus, in which the genome—the complete set of genes, composed of DNA—is replicated and stored, with its associated proteins. The nucleoid, in bacteria and archaea, is not separated from the cytoplasm by a membrane; the nucleus, in eukaryotes, is enclosed within a double membrane, the nuclear envelope. Cells with nuclear envelopes make up the large domain Eukarya (Greek eu, “true,” and karyon, “nucleus”). Microorganisms

without nuclear membranes, formerly grouped together as prokaryotes (Greek pro, “before”). Based on fundamental differences in their cell structure, living organisms can be divided into two major groups—prokaryotes and eukaryotes. Bacteria and archaea are prokaryotes. Animals, plants, fungi, and protists are eukaryotes (**Fig. 1.5.**).

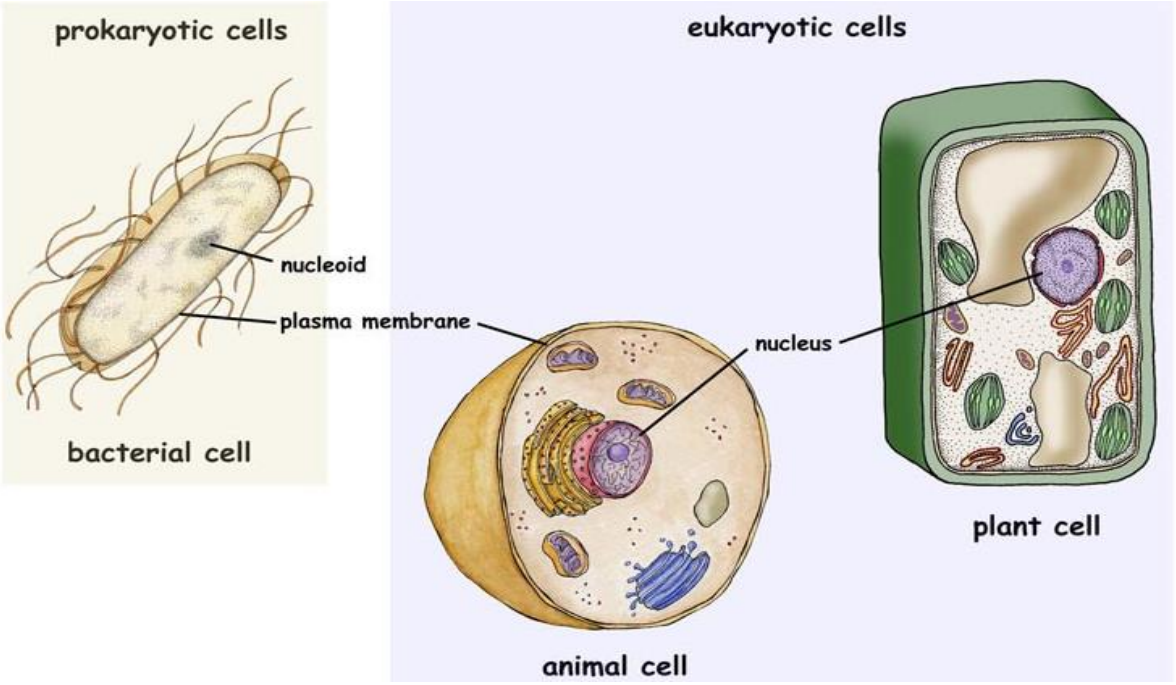

Figure 1.5. Prokaryotic and eukaryotic cells

Prokaryotic and eukaryotic cells are distinguished by several key characteristics. Both cell types contain DNA as their genetic material. However, prokaryotic DNA is single-stranded and circular and floats freely inside the cell. Eukaryotic DNA is double-stranded and linear and is enclosed in a membrane-bound structure called the nucleus. Eukaryotes also have other specialized membrane-bound structures called organelles that do much of the cell’s work. Prokaryotes lack organelles, though they must accomplish many similar vital tasks. This inability to “delegate” tasks makes prokaryotes less metabolically efficient than eukaryotes.

Among the eukaryotes, animal, plant, and fungal cells share many characteristics but can be distinguished by several key features (**Fig. 1.6.**). For example, plant cells and fungal cells have cell walls, but animal cells do not. However, plant and fungal cell walls can be distinguished by key materials they contain. Plant cell walls are composed mainly of cellulose, whereas fungal cell walls are rich in chitin. (Bacterial cells also have a cell wall, which is composed mainly of peptidoglycan.) All three eukaryote cell types contain a nucleus, organelles, and mitochondria. Plant cells also contain a central vacuole and chloroplasts.

All cells contain cytoplasm, a substance made up of water, proteins, and other molecules surrounded by a membrane. The cytoplasm of eukaryotic cells also contains numerous organelles. Much of the cell’s work takes place in the cytoplasm.

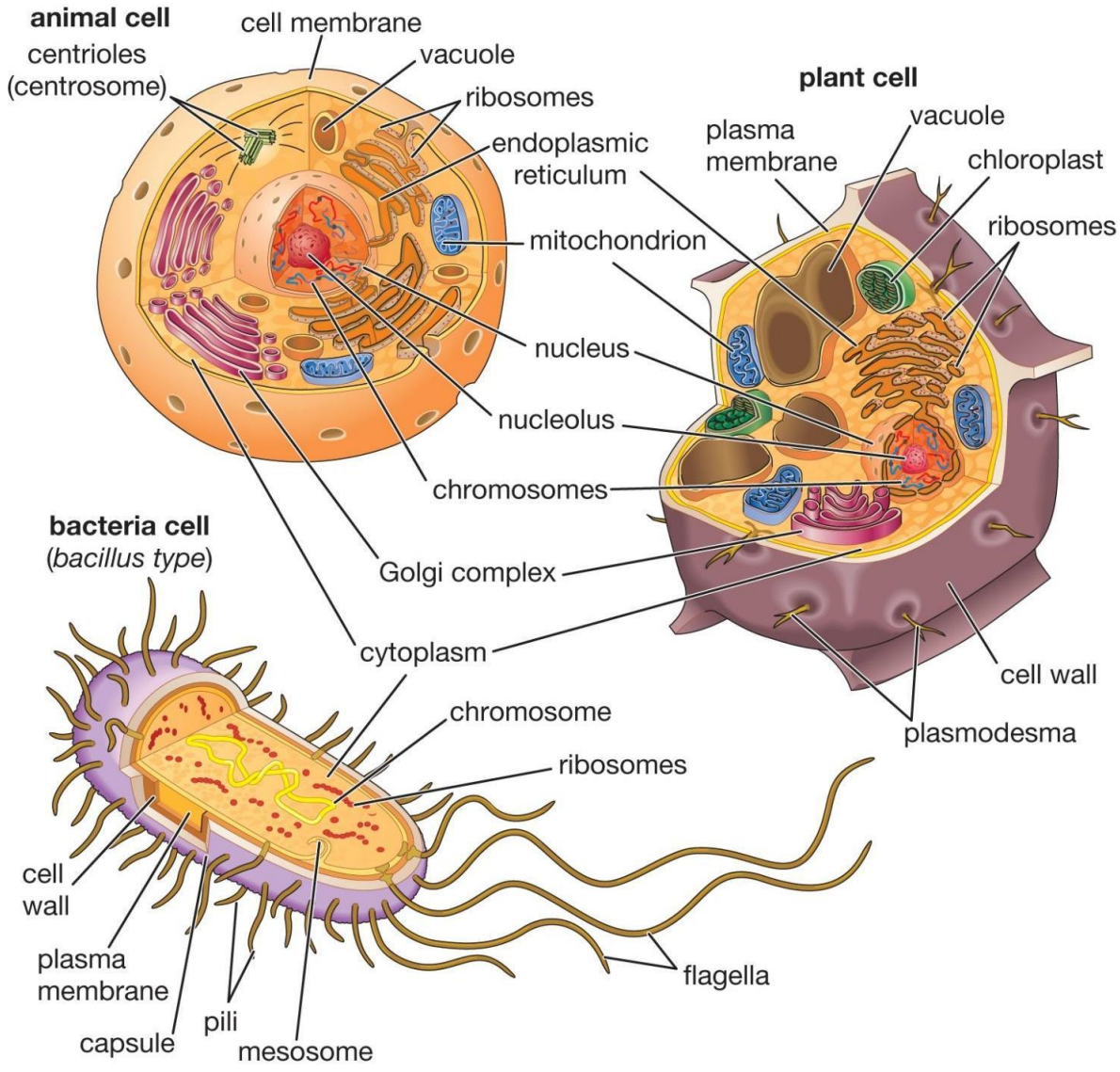

Figure 1.6. Cells structure of different organisms

Cell membrane

Cells can survive only in a liquid medium that brings in food and carries away waste. For unicellular (single-celled) organisms, such as bacteria, algae, and protozoa, this fluid can be an external body of water, such as a lake or stream. For multicellular (many-celled) organisms, however, the liquid medium is contained within the organism. In plants, for example, it is the sap; in animals, the blood and lymph.

The cell membrane is semipermeable—that is, some substances can pass through it but others cannot. This characteristic enables the cell to admit or block substances from the surrounding fluid and to excrete waste products into its environment. The cell membrane is composed of two thin layers of phospholipid molecules studded with large proteins. Phospholipids are chemically related to fats and oils. Some of the membrane proteins are structural; others form pores that function as gateways to allow or prevent the transport of substances across the membrane.

Substances pass through the cell membrane in several ways. Small uncharged molecules, such as water, pass freely down their concentration gradient (from the side of the membrane where they are in

The DNA strands, which are called chromatin because they readily stain with dyes, are usually too thin to be seen with an optical microscope. When a cell is ready to divide, the chromatin–protein strands coil repeatedly around themselves, condensing into chromosomes.

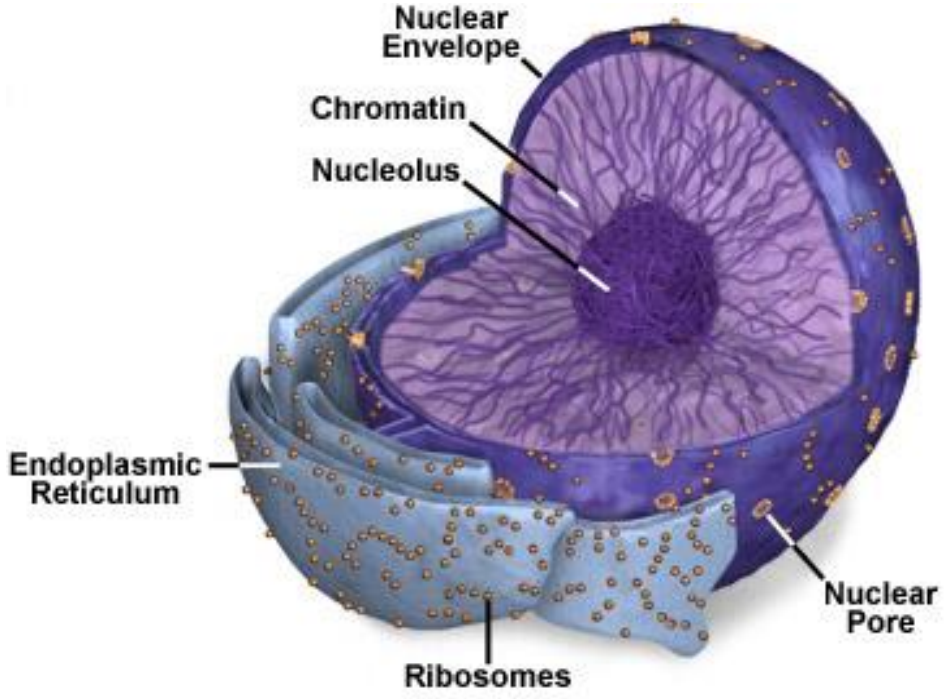

Figure 1.10. Nucleus structure

Vacuoles

Vacuoles (**Fig. 1.6**) are storage organelles that usually carry food molecules or wastes in solution. Plant cells have a large central vacuole that stores water and other materials. The central vacuole helps the cell maintain its volume and structure. The central vacuole in some cells plays a role in building stalks and stems. If a cambium cell is to become bark or wood, its membrane grows into the vacuole and deposits layers of cell wall to increase stiffness. In cells that become part of the vascular bundle that transmits sap, the vacuole becomes cylindrical and develops openings at each end that pass sap from cell to cell.

Some unicellular organisms, as well as the cells of certain simple animals such as sponges and hydra, have one or more contractile vacuoles that regulate the cell's water content. The vacuoles collect excess fluid and some wastes from the cell and then contract, releasing the liquid into the surrounding medium.

Cytoskeleton

The cytoskeleton helps the cell maintain its shape, aids in cellular movement, and helps with internal movement. Found only in eukaryotic cells, the cytoskeleton is a network of protein filaments and tubules that extends throughout the cytoplasm. Microtubules help form structures such as cilia and flagella, which help single-celled organisms move, and the spindle fibers that move chromosomes during cell division. Microfilaments in the cytoskeleton give the cell its shape and help it contract; intermediate filaments give it strength.

Self-assessment tasks

1. Most cell walls are composed of

   a) lipids; b) polysaccharides; c) proteins; d) amino acids.

2. Is it true that plastids and mitochondria have their own DNA?

   a) yes; b) no.

3. The portions of the endoplasmic reticulum that contain ribosomes are called

   a) rough endoplasmic reticulum (RER); b) smooth endoplasmic reticulum (SER);

   c) main endoplasmic reticulum (MER).

4. Who discovered the cell in 1665?

   a) Robert Hook; b) Robert Crook; c) David Thomson; d) Marie Francois.

5. Name the process in which the passage of water goes from a region of higher concentration to a region of lower concentration through a semipermeable membrane?

   a) diffusion; b) osmosis; c) both; a) and b) d) neither a) nor b).

6. Name the process in which the membrane of a vesicle can fuse with the plasma membrane and extrude its contents to the surrounding medium?

   a) exocytosis; b) endocytosis; c) osmosis; d) diffusion.

7. Chloroplast is found in

   a) plant cell only; b) animal cell only; c) both of these; d) none of these

8. The control unit of cell is

   a) nucleus; b) cell wall; c) cytoplasm; d) all of these.

9. What is passive and active transport?

10. Describe the difference between plant and animal cells.

11. Describe the difference between prokaryotic and eukaryotic cells.

12. Which organelles have DNA?

Chapter №2. Carbohydrates

Carbohydrates are the most abundant biomolecules on Earth. Each year, photosynthesis converts more than 100 billion metric tons of CO_2 and H_2O into cellulose and other plant products. Certain carbohydrates (sugar and starch) are a dietary staple in most parts of the world, and the oxidation of carbohydrates is the central energy-yielding pathway in most nonphotosynthetic cells. Carbohydrate polymers (also called glycans) serve as structural and protective elements in the cell walls of bacteria and plants and in the connective tissues of animals. Other carbohydrate polymers lubricate skeletal joints and participate in cell-cell recognition and adhesion.

Carbohydrates are polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis. Many, but not all, carbohydrates have the empirical formula $(CH_2O)n$; some also contain nitrogen, phosphorus, or sulfur. There are three major size classes of carbohydrates: monosaccharides, oligosaccharides, and polysaccharides (the word "saccharide" is derived from the Greek sakcharon, meaning "sugar").

Monosaccharides

Monosaccharides, or simple sugars, consist of a single polyhydroxy aldehyde or ketone unit. The most abundant monosaccharide in nature is the six-carbon sugar D-glucose, sometimes referred to as dextrose. Monosaccharides of four or more carbons tend to have cyclic structures.

Oligosaccharides consist of short chains of monosaccharide units, or residues, joined by characteristic linkages called glycosidic bonds. The most abundant are the disaccharides, with two monosaccharide units. Sucrose (cane sugar), for example, consists of the six-carbon sugars D-glucose and D-fructose.

All common monosaccharides and disaccharides have names ending with the suffix "-ose." In cells, most oligosaccharides consisting of three or more units do not occur as free entities but are joined to nonsugar molecules (lipids or proteins) in glycoconjugates.

The polysaccharides are sugar polymers containing more than 20 or so monosaccharide units; some have hundreds or thousands of units. Some polysaccharides, such as cellulose, are linear chains; others, such as glycogen, are branched. Both cellulose and glycogen consist of recurring units of D-glucose, but they differ in the type of glycosidic linkage and consequently have strikingly different properties and biological roles.

Monosaccharides are colorless, crystalline solids that are freely soluble in water but insoluble in nonpolar solvents. Most have a sweet taste. The backbones of common monosaccharides are unbranched carbon chains in which all the carbon atoms are linked by single bonds. In this open chain form, one of the carbon atoms is double-bonded to an oxygen atom to form a carbonyl group; each of the other carbon atoms has a hydroxyl group. If the carbonyl group is at an end of the carbon chain (that is, in an aldehyde group), the monosaccharide is an aldose; if the carbonyl group is at any other position (in a ketone group), the monosaccharide is a ketose **(Fig. 2.1.)**.

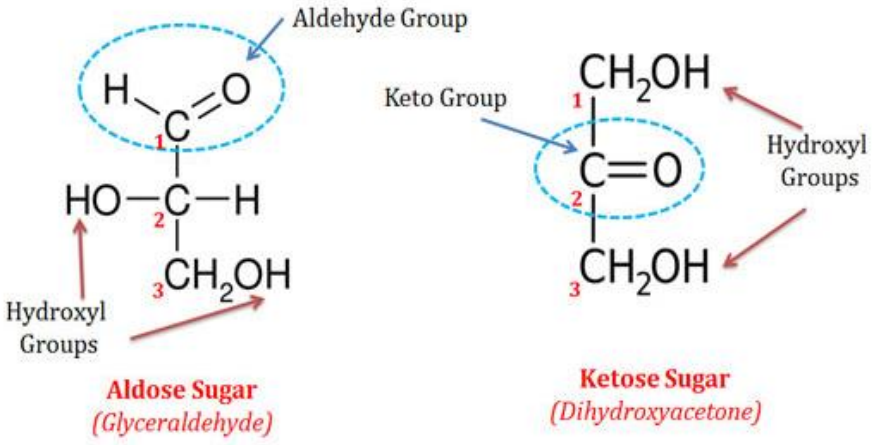

Figure 2.1. Difference between aldose and ketose sugars

Monosaccharides are classified as aldoses or ketoses. They are further classified according to the number of carbon atoms in the backbone, commonly designated with prefixes such as tri-(3), tetr-(4), pent-(5), hex-(6), etc. in the chemical name of the sugar. Glucose, e.g., with six carbons and ribose with five carbons are classified as hexose and pentose, respectively.

Monosaccharides are further classified stereochemically as D (dextro) and L (levo) **(Fig. 2.2.)** based on the configuration of the asymmetric carbon farthest away from the carbonyl group in straight chained compounds. If the farthest hydroxyl (–OH) group on the carbon atom next to the last CH_2OH is on the right as represented in the Fisher projection, it is classified as D and if on the left, classified as L.

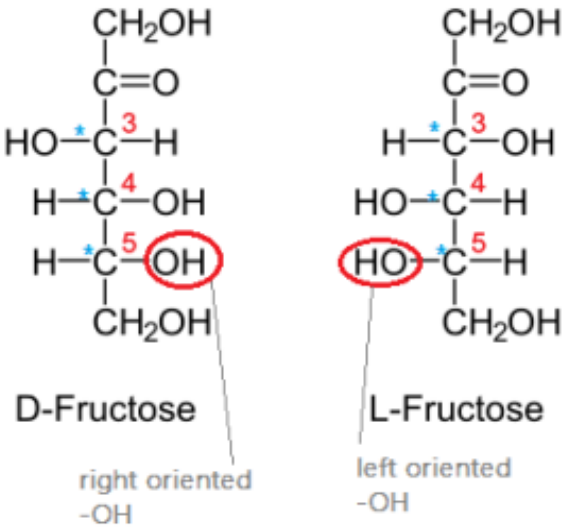

Figure 2.2. The stereochemical classification of monosaccharides

Aldoses such as ribose and glucose can exist in three structural forms: the open chain, the alpha (α) cyclic form, and the beta (β) cyclic form **(Fig. 2.3.)**. Ninety-nine percent (99%) of glucose molecules exist in the cyclic form (66%β and 33%α) and 1% in the open chain form. Upon cyclization, α-d-glucose is formed if the hydroxyl group on carbon-1 is pointed in the opposite direction to the CH_2OH group in

Haworth projection, while the β-d-glucose is formed if the hydroxyl group is pointed in the same direction as the CH_2OH group.

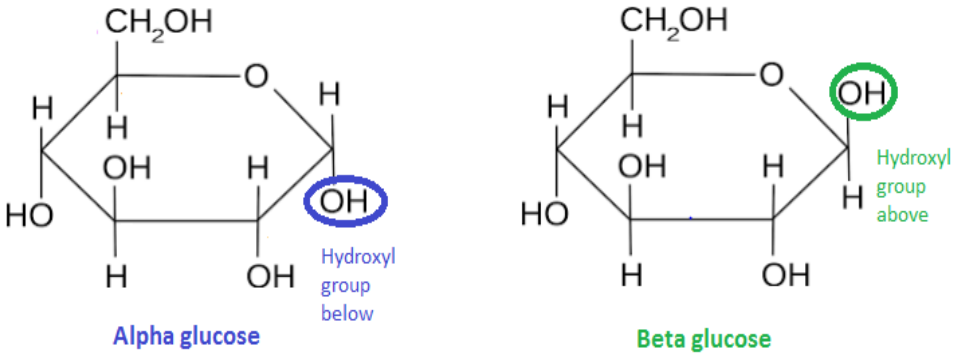

Figure. 2.3. Structural forms of glucose

Cyclic pentoses are referred to as furanoses, while hexoses are referred to as pyranoses. The cyclic formations are more thermodynamically stable than their open chain counterparts. Ketopentoses and ketohexoses such as ribulose and fructose can also exist in the open chain or cyclic forms. Fructose, the most common ketohexose, can cyclize to form either a furanose or pyranose ring depending on whether the C-2 keto group reacts with the hydroxyl group on C-6 or C-5.

The simplest monosaccharides are the two three-carbon trioses: glyceraldehyde, an aldotriose, and dihydroxyacetone, a ketotriose. Monosaccharides with four, five, six, and seven carbon atoms in their backbones are called, respectively, tetroses, pentoses, hexoses, and heptoses. The hexoses,
which include the aldohexose D-glucose and the ketohexose D-fructose, are the most common monosaccharides in nature—the products of photosynthesis and key intermediates in the central energy-yielding reaction sequence in most organisms. The aldopentoses D-ribose and 2-deoxy-D-ribose are components of nucleotides and nucleic acids.

All the monosaccharides except dihydroxyacetone contain one or more asymmetric (chiral) carbon atoms and thus occur in optically active isomeric forms **(Fig. 2.4.)**. The simplest aldose, glyceraldehyde, contains one chiral center (the middle carbon atom) and therefore has two different optical isomers, or enantiomers.

One of the two enantiomers of glyceraldehyde is, by convention, designated the D isomer; the other is the L isomer. To represent three-dimensional sugar structures on paper, often use Fischer projection formulas. In general, a molecule with n chiral centers can have 2**n** stereoisomers. Glyceraldehyde has **21 = 2**; the aldohexoses, with four chiral centers, have **24 = 16**. Those in which the configuration at this reference carbon is the same as that of D-glyceraldehyde are designated D isomers, and those with the same configuration as L-glyceraldehyde are L isomers. In other words, when the hydroxyl group on the reference carbon is on the right in a projection formula that has the carbonyl carbon at the top, the sugar is

the D isomer; when on the left, it is the L isomer. Of the 16 possible aldohexoses, eight are D forms and eight are L. Most of the hexoses of living organisms are D isomers.

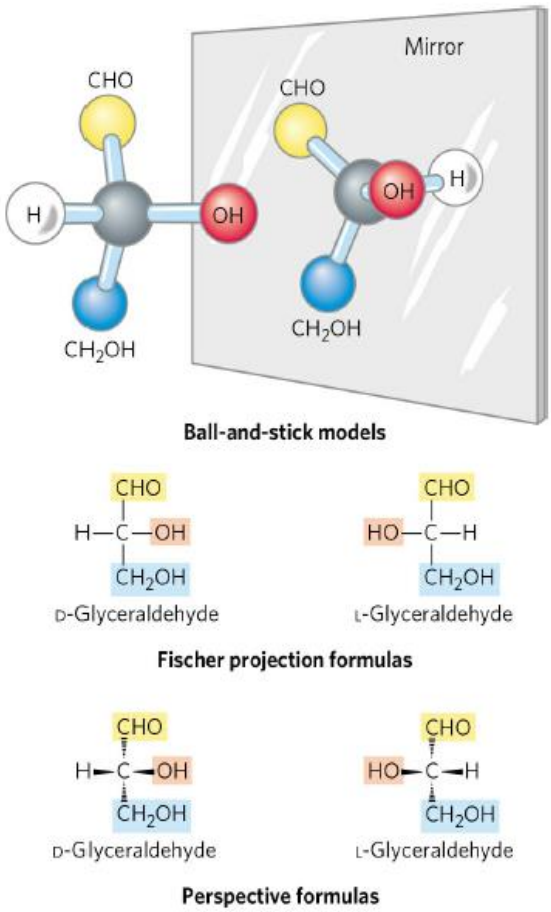

Figure. 2.4. Three ways to represent the two enantiomers of glyceraldehyde. The enantiomers are mirror images of each other. Ball-and stick models show the actual configuration of molecules

Figure 2.5. shows the structures of the D stereoisomers of all the aldoses and ketoses having three to six carbon atoms. The carbons of a sugar are numbered beginning at the end of the chain nearest the carbonyl group. Each of the eight D-aldohexoses, which differ in the stereochemistry at C-2, C-3, or C-4, has its own name: D-glucose, D-galactose, D-mannose, and so forth. The four- and five-carbon ketoses are designated by inserting “ul” into the name of a corresponding aldose; for example,
D-ribulose is the ketopentose corresponding to the aldopentose D-ribose. The ketohexoses are named otherwise: for example, fructose (from the Latin fructus, “fruit”; fruits are one source of this sugar) and sorbose (from Sorbus, the genus of mountain ash, which has berries rich in the related sugar alcohol sorbitol). Two sugars that differ only in the configuration around one carbon atom are called epimers; D-glucose and D mannose, which differ only in the stereochemistry at C-2, are epimers, as are D-glucose and D-galactose (which differ at C-4). Some sugars occur naturally in their L form; examples are L-arabinose and the L isomers of some sugar derivatives that are common components of glycoconjugates.

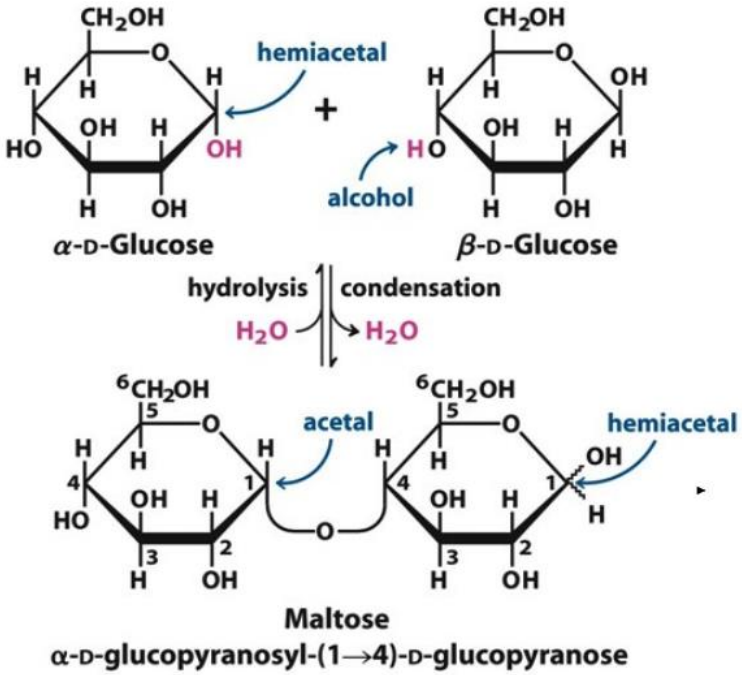

Figure 2.10. Formation of maltose. A disaccharide is formed from two monosaccharides (here, two molecules of D-glucose) when an —OH (alcohol) of one monosaccharide molecule (right) condenses with the intramolecular hemiacetal of the other (left), with elimination of H_2O and formation of a glycosidic bond. The reversal of this reaction is hydrolysis—attack by H_2O on the glycosidic bond. The maltose molecule, shown here, retains a reducing hemiacetal at the C-1 not involved in the glycosidic bond. Because mutarotation interconverts the α and β forms of the hemiacetal, the bonds at this position are sometimes depicted with wavy lines to indicate that the structure may be either α or β

The disaccharide lactose **(Fig. 2.11.)**, which yields D-galactose and Dglucose on hydrolysis, occurs naturally in milk. The anomeric carbon of the glucose residue is available for oxidation, and thus lactose is a reducing disaccharide. Its abbreviated name is Gal (β1→4)Glc. The enzyme lactase— absent in lactose-intolerant individuals—begins the digestive process in the small intestine by splitting the (β1→4) bond of lactose into monosaccharides, which can be absorbed from the small intestine. Lactose, like other disaccharides, is not absorbed from the small intestine, and in lactose intolerant individuals, the undigested lactose passes into the large intestine. Here, the increased osmolarity due to dissolved lactose opposes the absorption of water from the intestine into the bloodstream, causing watery, loose stools. In addition, fermentation of the lactose by intestinal bacteria produces large volumes of CO_2, which leads to the bloating, cramps, and gas associated with lactose intolerance.

Sucrose is a disaccharide of glucose and fructose. It is formed by plants but not by animals. In contrast to maltose and lactose, sucrose contains no free anomeric carbon atom; the anomeric carbons of both monosaccharide units are involved in the glycosidic bond **(Fig. 2.11.)**. Sucrose is therefore a nonreducing sugar, and its stability—its resistance to oxidation—makes it a suitable molecule for the storage and transport of energy in plants. Sucrose is a major intermediate product of photosynthesis; in

many plants it is the principal form in which sugar is transported from the leaves to other parts of the plant body.

Trehalose **(Fig. 2.11.)** —a disaccharide of D-glucose that, like sucrose, is a nonreducing sugar—is a major constituent of the circulating fluid (hemolymph) of insects. It serves as an energy-storage compound. Lactose gives milk its sweetness, and sucrose, of course, is table sugar. Trehalose is also used commercially as a sweetener.

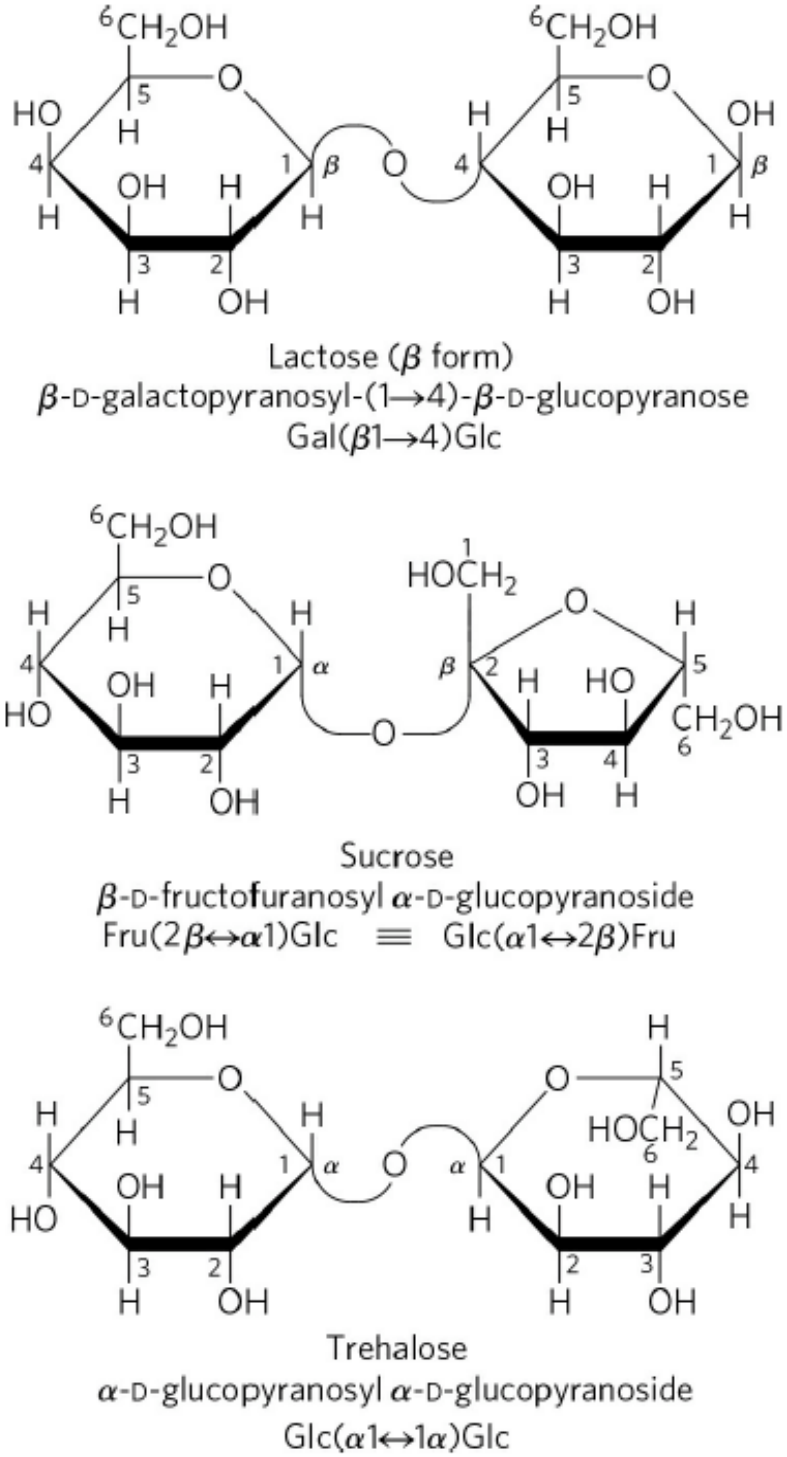

Figure 2.11. Common disaccharides

DID YOU KNOW?

Sweetness is one of the five basic flavors that humans can taste; the others are sour, bitter, salty, and umami. Sweet taste is detected by protein receptors in the plasma membranes of gustatory cells in the taste buds on the surface of the tongue. In humans, two closely related genes (T1R2 and T1R3) encode sweetness receptors. When a molecule with a compatible structure binds a gustatory receptor's extracellular domain, it triggers a series of events in the cell (including activation of a GTP-binding protein) that generate an electrical signal to the brain that is interpreted as "sweet." During evolution, there has probably been selection for the ability to taste compounds found in foods containing important nutrients, such as the carbohydrates that are major fuels for most organisms.

Several natural products are extraordinarily sweet. Stevioside, a sugar derivative isolated from the leaves of the stevia plant (Stevia rebaudiana Bertoni) **(Fig. 2.12.)**, is several hundred times sweeter than an equivalent amount of sucrose (table sugar).

The small (54 amino acids) protein brazzein, isolated from berries of the Oubli vine (Pentadiplandra brazzeana Baillon) **(Fig. 2.13.)** in Gabon and Cameroon, is 17,000 times sweeter than sucrose on a molar basis. Presumably, the sweet taste of the berries encourages their consumption by animals that then disperse the seeds so that new plants are established. There is great interest in the development of artificial sweeteners like aspartame as weight-reduction aids—compounds that give foods a sweet taste without adding the calories found in sugars.

Figure 2. 12. Stevia Rebaudiana Bertoni

Figure 2.13. Berries of the Oubli vine (Pentadiplandra brazzeana Baillon)

Polysaccharides

Most carbohydrates found in nature occur as polysaccharides, polymers of medium to high molecular weight. Polysaccharides, also called glycans, differ from each other in the identity of their recurring monosaccharide units, in the length of their chains, in the types of bonds linking the units, and in the

degree of branching. Homopolysaccharides contain only a single monomeric species; heteropolysaccharides contain two or more different kinds **(Fig. 2.14.)**.

Some homopolysaccharides serve as storage forms of monosaccharides that are used as fuels; starch and glycogen are homopolysaccharides of this type. Other homopolysaccharides (cellulose and chitin, for example) serve as structural elements in plant cell walls and animal exoskeletons. Heteropolysaccharides provide extracellular support for organisms of all kingdoms. For example, the rigid layer of the bacterial cell envelope (the peptidoglycan) is composed in part of a heteropolysaccharide built from two alternating monosaccharide units. In animal tissues, the extracellular space is occupied by several types of heteropolysaccharides, which form a matrix that holds individual cells together and provides protection, shape, and support to cells, tissues, and organs.

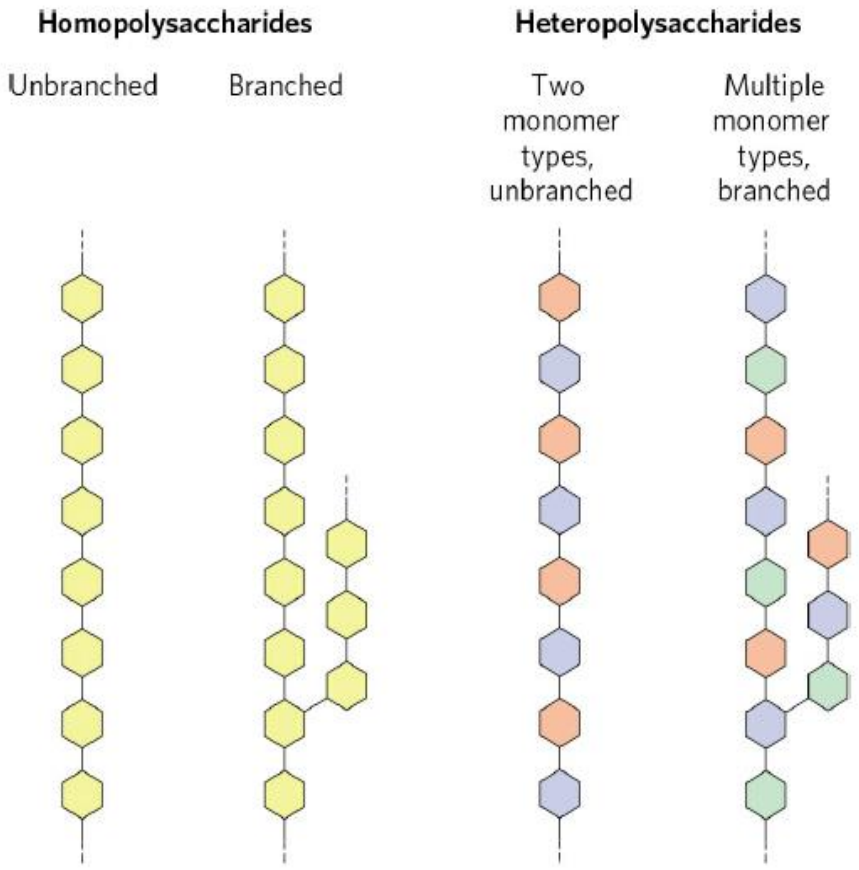

Figure 2.14. Homopolysaccharides and heteropolysaccharides. Polysaccharides may be composed of one, two, or several different monosaccharides, in straight or branched chains of varying length.

For example, the rigid layer of the bacterial cell envelope (the peptidoglycan) is composed in part of a heteropolysaccharide built from two alternating monosaccharide units. In animal tissues, the extracellular space is occupied by several types of heteropolysaccharides, which form a matrix that holds individual cells together and provides protection, shape, and support to cells, tissues, and organs.

The most important storage polysaccharides are starch in plant cells and glycogen in animal cells. Both polysaccharides occur intracellularly as large clusters or granules. Starch and glycogen molecules are heavily hydrated, because they have many exposed hydroxyl groups available to hydrogen bond with water. Most plant cells have the ability to form starch, and starch storage is especially abundant in tubers (underground stems), such as potatoes, and in seeds.

Starch contains two types of glucose polymer, amylose and amylopectin **(Fig. 2.15.)**. Amylose consists of long, unbranched chains of D-glucose residues. Such chains vary in molecular weight from a few thousand to more than a million. Amylopectin also has a high molecular weight (up to 200 million) but unlike amylose is highly branched.

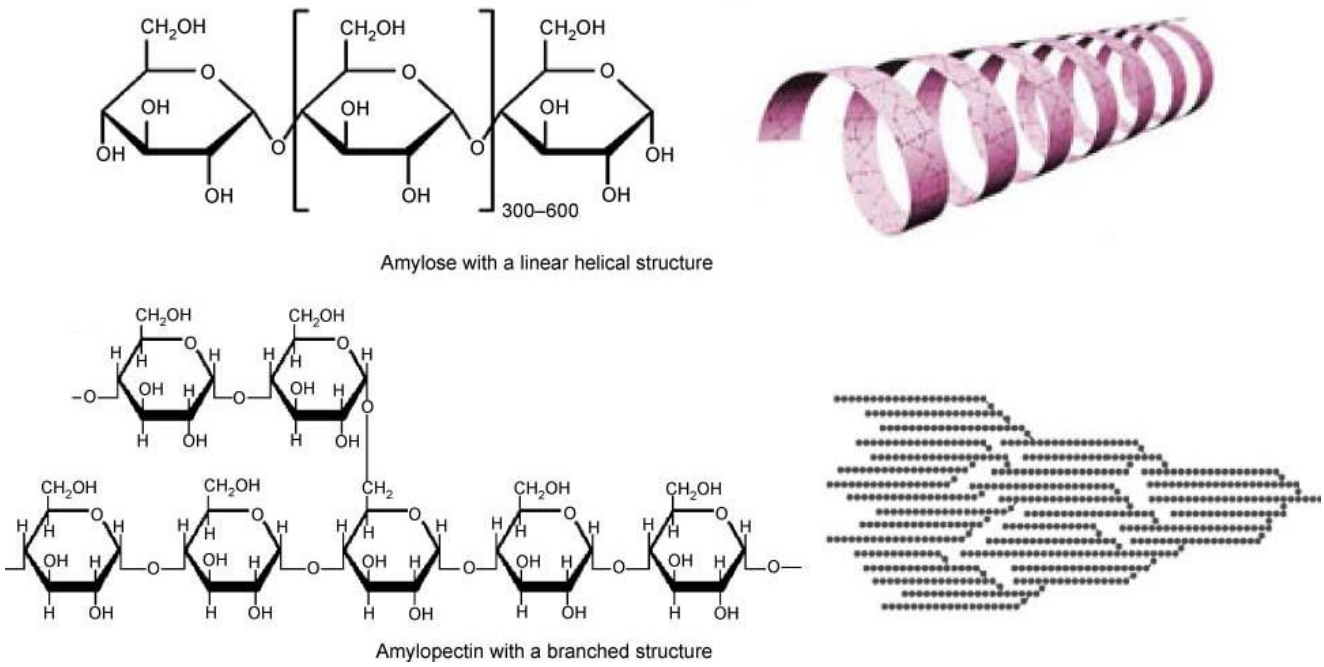

Figure 2.15. Amylose and amylopectin

Glycogen **(Fig.2.16.)** is the main storage polysaccharide of animal cells. Like amylopectin, glycogen is a polymer of (α1→4)-linked glucose subunits, with (α1→6)-linked branches, but glycogen is more extensively branched (on average, a branch every 8 to 12 residues) and more compact than starch. Glycogen is especially abundant in the liver, where it may constitute as much as 7% of the wet weight; it is also present in skeletal muscle. In hepatocytes glycogen is found in large granules, which are clusters of smaller granules composed of single, highly branched glycogen molecules with an average molecular weight of several million. The large glycogen granules also contain, in tightly bound form, the enzymes responsible for the synthesis and degradation of glycogen. Because each branch in glycogen ends with a nonreducing sugar unit, a glycogen molecule with n branches has n + 1 nonreducing ends, but only one reducing end.

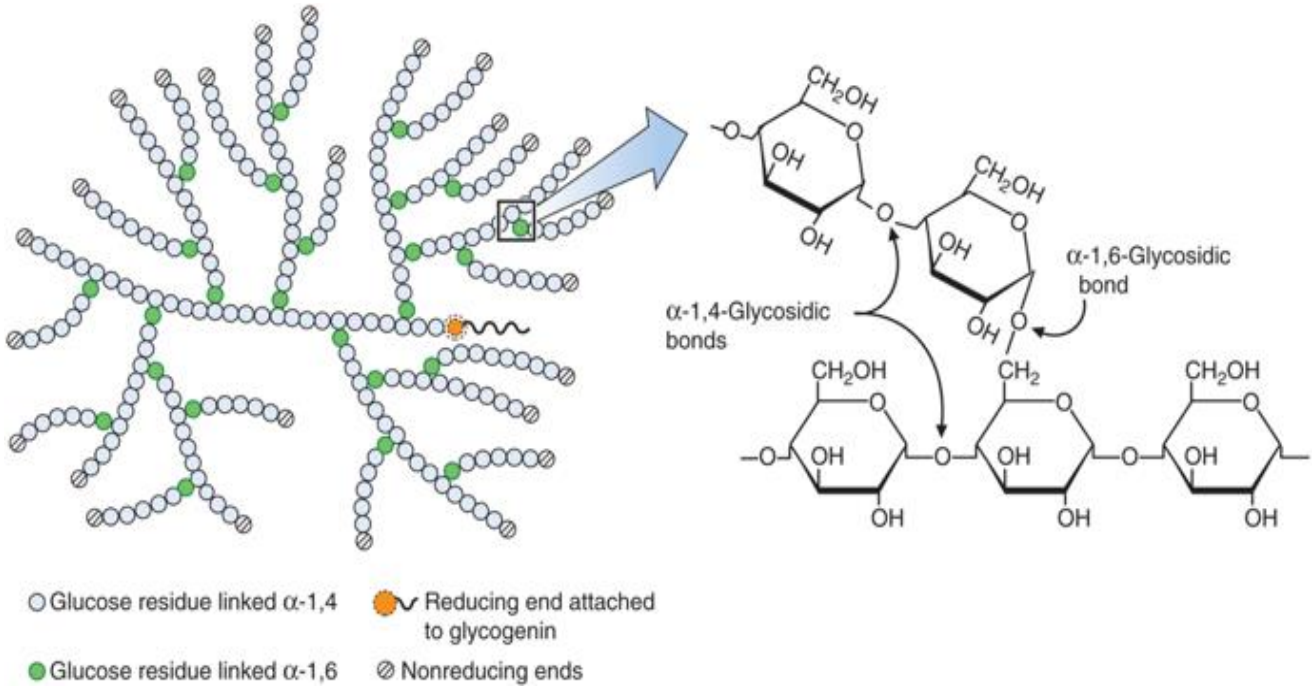

Figure 2.16. Glycogen is a polymer of (α1→4)-linked glucose subunits, with (α1→6)-linked branches

When glycogen is used as an energy source, glucose units are removed one at a time from the nonreducing ends. Degradative enzymes that act only at nonreducing ends can work simultaneously on the many branches, speeding the conversion of the polymer to monosaccharides.

Dextrans are bacterial and yeast polysaccharides. Dental plaque, formed by bacteria growing on the surface of teeth, is rich in dextrans, which are adhesive and allow the bacteria to stick to teeth and to each other. Dextrans also provide a source of glucose for bacterial metabolism. Synthetic dextrans are components of several commercial products (for example, Sephadex) used in the fractionation of proteins by size-exclusion chromatography. The dextrans in these products are chemically cross-linked to form insoluble materials of various sizes.

Cellulose **(Fig 2.17.)**, a tough, fibrous, water-insoluble substance, is found in the cell walls of plants, particularly in stalks, stems, trunks, and all the woody portions of the plant body. Cellulose constitutes much of the mass of wood, and cotton is almost pure cellulose. Like amylose, the cellulose molecule is a linear, unbranched homopolysaccharide, consisting of 10,000 to 15,000 D-glucose units. But there is a very important difference: in cellulose the glucose residues have the β configuration, whereas in amylose the glucose is in the α configuration.

The tough, fibrous nature of cellulose makes it useful in such commercial products as cardboard and insulation material, and it is a major constituent of cotton and linen fabrics. Cellulose is also the starting material for the commercial production of cellophane, rayon, and lyocell.

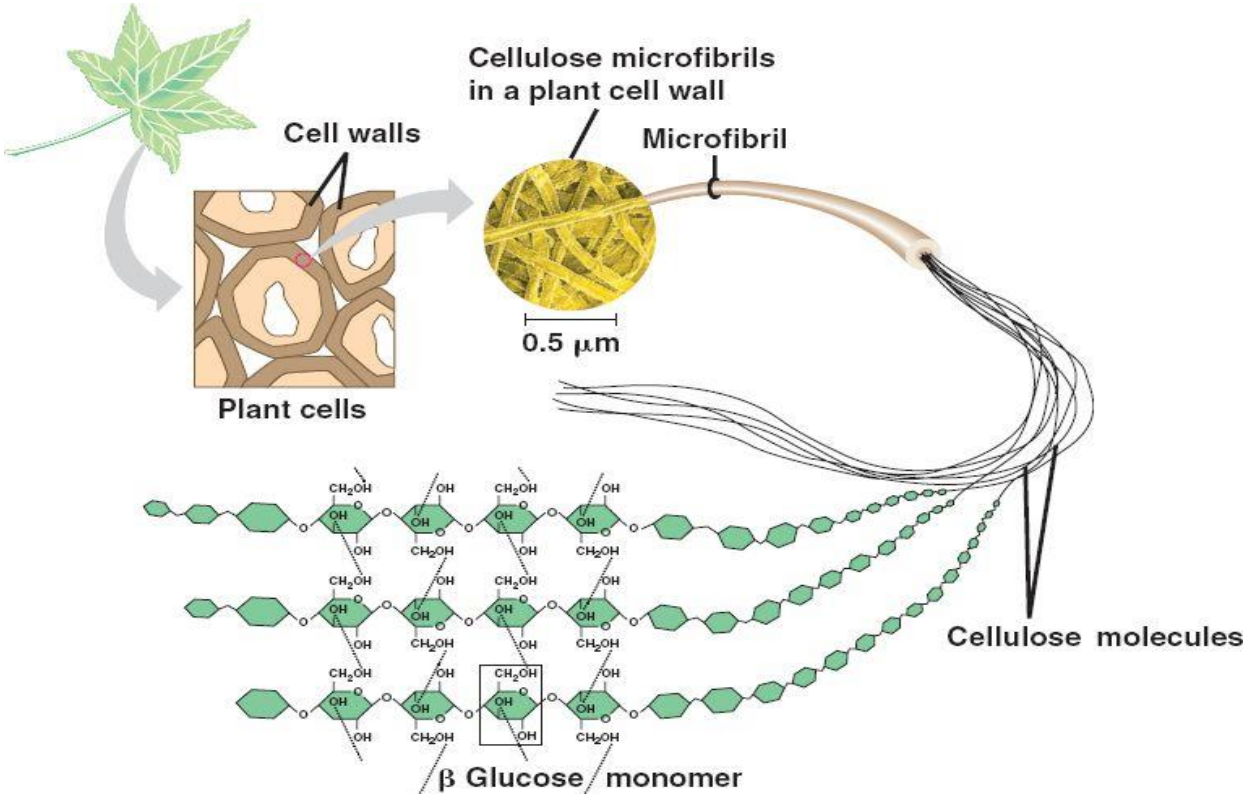

Figure 2.17. Cellulose

DID YOU KNOW?

Glycogen and starch ingested in the diet are hydrolyzed by α-amylases and glycosidases, enzymes in saliva and the small intestine that break glycosidic bonds between glucose units. Most vertebrate animals cannot use cellulose as a fuel source, because they lack an enzyme to hydrolyze the linkages. Termites (**Fig. 2.18.**) readily digest cellulose (and therefore wood), but only because their intestinal tract harbors a symbiotic microorganism, Trichonympha (**Fig. 2.19.)**, that secretes cellulase, which hydrolyzes the (β1→4) linkages.

Figure 2.18. Termites

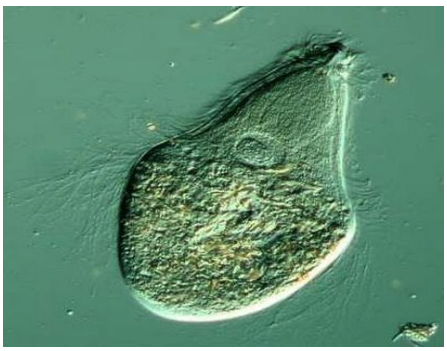

Figure 2.19. Trichonympha

Molecular genetic studies have revealed that genes encoding cellulose-degrading enzymes are present in the genomes of a wide range of invertebrate animals, including arthropods and nematodes. There is one important exception to the absence of cellulase in vertebrates: ruminant animals such as cattle, sheep, and goats harbor symbiotic microorganisms in the rumen (the first of their four stomach compartments) that can hydrolyze cellulose, allowing the animal to degrade dietary cellulose from soft grasses, but not from woody plants. Fermentation in the rumen yields acetate, propionate, and β-hydroxybutyrate, which the animal uses to synthesize the sugars in milk.

Biomass that is rich in cellulose can be used as starting material for the fermentation of carbohydrates to ethanol, to be used as a gasoline additive (switchgrass is a common biofuel crop) **(Fig. 2.20.)**. The annual production of biomass on Earth (accomplished primarily by photosynthetic organisms) is the energetic equivalent of nearly a trillion barrels of crude oil, when converted to ethanol by fermentation. Because of their potential use in biomass conversion to bioenergy, cellulose-degrading enzymes such as cellulase are under vigorous investigation.

Figure 2.20. Switchgrass is a common biofuel crop.

A major fraction of photosynthetic biomass is the woody portion of plants and trees, which consists of cellulose plus several other polymers derived from carbohydrates that are not easily digestible, either chemically or biologically. Lignins, for example, make up some 30% of the mass of wood. Synthesized from precursors that include phenylalanine and glucose, lignins are complex polymers with covalent cross-links to cellulose that complicate the digestion of cellulose by cellulase. If woody plants are to be used in the production of ethanol from biomass, better means of digesting wood components will need to be found.

Chitin is a linear homopolysaccharide composed of N-acetylglucosamine residues in (β1→4) linkage (**Fig. 2.21.**). The only chemical difference from cellulose is the replacement of the hydroxyl group at C-2 with an acetylated amino group.

presumably a defense against bacterial infections of the eye, and is also produced by certain bacterial viruses to ensure their release from the host bacterial cell, an essential step of the viral infection cycle.

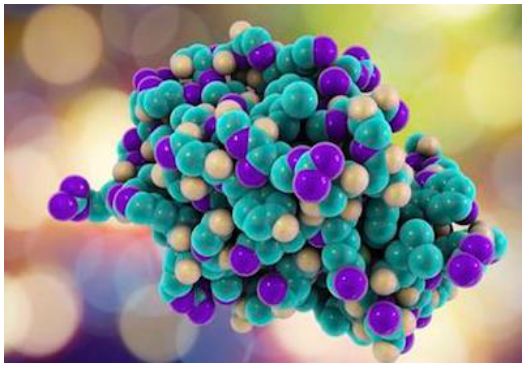

Figure 2.25. The enzyme lysozyme

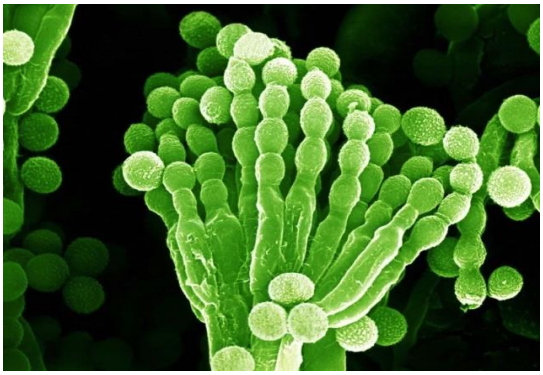

Figure 2.26. Penicillin

Penicillin (**Fig. 2.26.**) and related antibiotics kill bacteria by preventing synthesis of the peptidoglycan cross-links, leaving the cell wall too weak to resist osmotic lysis (p. 223). Certain marine red algae, including some of the seaweeds, have cell walls that contain agar, a mixture of sulfated heteropolysaccharides made up of D-galactose and an L-galactose derivative ether-linked between C-3 and C-6.

Agar **(Fig. 2.27.)** is a complex mixture of polysaccharides, all with the same backbone structure but substituted to varying degrees with sulfate and pyruvate.

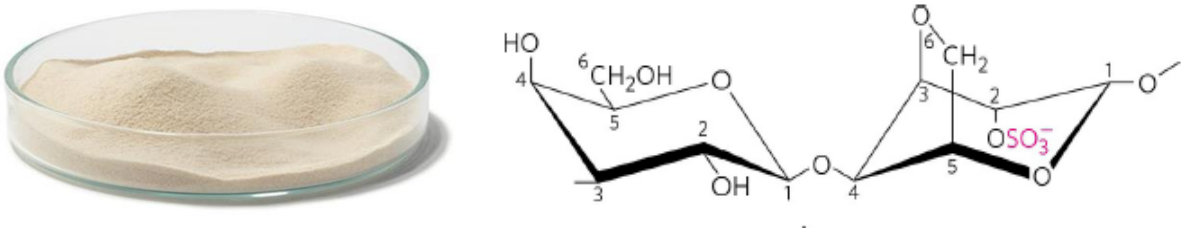

Figure 2.27. Agar and agar component - agarose

Agarose **(Fig. 2.27.)** is the agar component with the fewest charged groups (sulfates, pyruvates). The remarkable gel-forming property of agarose makes it useful in the biochemistry laboratory. When a suspension of agarose in water is heated and cooled, the agarose forms a double helix: two molecules in parallel orientation twist together with a helix repeat of three residues; water molecules are trapped in the central cavity. These structures in turn associate with each other to form a gel—a three dimensional matrix that traps large amounts of water. Agarose gels are used as inert supports for the electrophoretic separation of nucleic acids. Agar is also used to form a surface for the growth of bacterial colonies. Another commercial use of agar is for the capsules in which some vitamins and drugs are packaged; the dried agar material dissolves readily in the stomach and is metabolically inert.

The extracellular space in the tissues of multicellular animals is filled with a gel-like material, the extracellular matrix (ECM), also called ground substance, which holds the cells together and provides a porous pathway for the diffusion of nutrients and oxygen to individual cells. The ECM that surrounds fibroblasts and other connective tissue cells is composed of an interlocking meshwork of heteropolysaccharides and fibrous proteins such as fibrillar collagens, elastins, and fibronectins. Basement membrane is a specialized ECM that underlies epithelial cells; it consists of specialized collagens, laminins, and heteropolysaccharides. These heteropolysaccharides, the glycosaminoglycans, are a family of linear polymers composed of repeating disaccharide units **(Fig. 2.28)**. They are unique to animals and bacteria and are not found in plants. One of the two monosaccharides is always either N-acetylglucosamine or N-acetylgalactosamine; the other is in most cases a uronic acid, usually D-glucuronic or L-iduronic acid. Some glycosaminoglycans contain esterified sulfate groups. The sulfated glycosaminoglycans are attached to extracellular proteins to form proteoglycans.

With up to 50,000 repeats of the basic disaccharide unit, hyaluronan has a molecular weight of several million; it forms clear, highly viscous, noncompressible solutions that serve as lubricants in the synovial fluid of joints and give the vitreous humor of the vertebrate eye its jellylike consistency (the Greek hyalos means “glass”; hyaluronan can have a glassy or translucent appearance). Hyaluronan is also a component of the ECM of cartilage and tendons, to which it contributes tensile strength and elasticity as a result of its strong noncovalent interactions with other components of the matrix. Hyaluronidase, an enzyme secreted by some pathogenic bacteria, can hydrolyze the glycosidic linkages of hyaluronan, rendering tissues more susceptible to bacterial invasion. In many animal species, a similar enzyme in sperm hydrolyzes the outer glycosaminoglycan coat around an ovum, allowing sperm penetration. Other glycosaminoglycans differ from hyaluronan in three respects: they are generally much shorter polymers, they are covalently linked to specific proteins (proteoglycans), and one or both monomeric units differ from those of hyaluronan.

Chondroitin sulfate (Greek chondros, “cartilage”) contributes to the tensile strength of cartilage, tendons, ligaments, heart valves, and the walls of the aorta. Dermatan sulfate (Greek derma, “skin”) contributes to the pliability of skin and is also present in blood vessels and heart valves. In this polymer, many of the glucuronate residues present in chondroitin sulfate are replaced by their C-5 epimer, L-iduronate (IdoA). Keratan sulfates (Greek keras, “horn”) have no uronic acid, and their sulfate content is variable. They are present in cornea, cartilage, bone, and a variety of horny structures formed from dead cells: horn, hair, hoofs, nails, and claws. Heparan sulfate (Greek hēpar, “liver”; it was originally isolated from dog liver) is produced by all animal cells and contains variable arrangements of sulfated and nonsulfated sugars. The sulfated segments of the chain allow it to interact with a large number of proteins, including growth factors and ECM components, as well as various enzymes and factors present in plasma. Heparin is a highly sulfated, intracellular form of heparan sulfate produced primarily by mast cells (a type of leukocyte, or immune cell). Its physiological role is not yet clear, but purified heparin is

used as a therapeutic agent to inhibit coagulation of blood through its capacity to bind the protease inhibitor antithrombin.

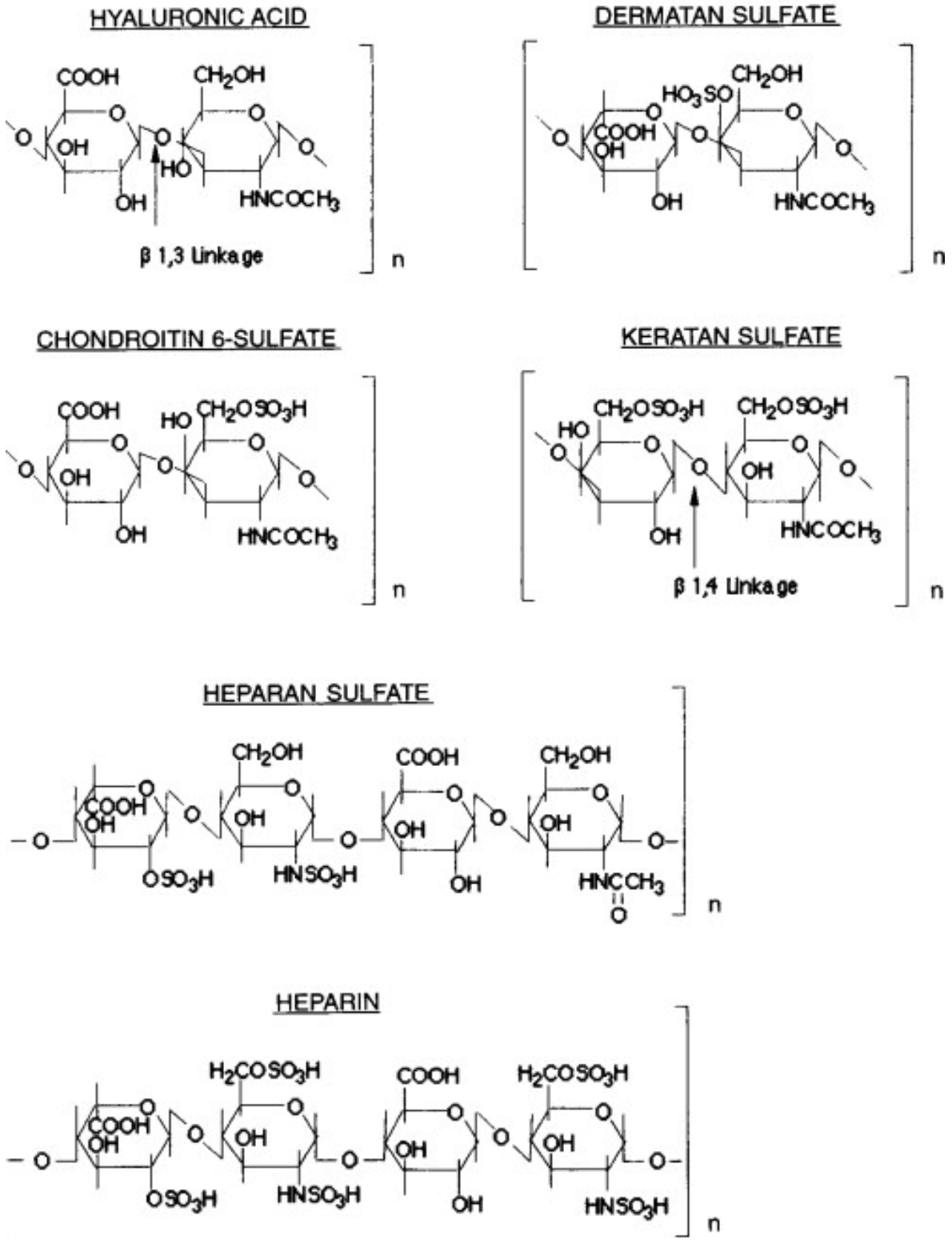

Figure 2.28. Repeating units of some common glycosaminoglycans of extracellular matrix

Self-assessment tasks

1. If the hydroxyl group is on the right in a projection formula, the sugar is
   a) D isomer; b) L isomer; c) J isomer; d) R isomer.
2. Pentoses are
   a) furanoses; b) pyranose; c) turanoses; d) muranoses.
3. There are two stereochemical classis of monosaccharides
   a) D and L; b) R and L; c) D and R; d) J and R.
4. Milk and other dairy products contain what specific type of sugar?
   a) glucose; b) sucrose; c) fructose; d) lactose.
5. Which one of the following is a monosaccharide?
   a) fructose; b) glycogen; c) lactose; d) sucrose.
6. Starch is a polymer of:
   a) amino acids; b) glucose; c) nucleic acids; d) sucrose.
7. Which of the following is a polysaccharide?
   a) cellulose; b) fructose; c) glucose; d) sucrose.
8. What kind of polysaccharide cannot be digested by human beings?
   a) cellulose; b) glucose; c) starch; d) sucrose.
9. Describe the monosaccharides.
10. What are isomers? What are the 2 structural isomers of glucose called?
11. What are the major functions of carbohydrates?
12. What is the function of the following carbohydrates in living things: glycogen, starch, cellulose, sucrose?

Chapter №3. Lipids. Biological membrane.

Biological lipids are a chemically diverse group of compounds, the common and defining feature of which is their insolubility in water. The biological functions of the lipids are as diverse as their chemistry.

According to the chemical structure lipids are divided into simple and complex. Simple include esters and their macromolecular components, insoluble in water. Depending on the nature of the alcohol, they are divided into fats (triacylglierols), waxes and steroids. Complex lipids have amphiphilic properties, because in addition to alcohol residues and fatty acids they also contain components of hydrophilic nature. By the chemical nature of the hydrophilic component, they are divided into phospholipids, glycolipids, sulfolipids.

Fats and oils are the principal stored forms of energy in many organisms. Phospholipids and sterols are major structural elements of biological membranes. Other lipids, although present in relatively small quantities, play crucial roles as enzyme cofactors, electron carriers, light-absorbing pigments, hydrophobic anchors for proteins, “chaperones” to help membrane proteins fold, emulsifying agents in the digestive tract, hormones, and intracellular messengers. The fats and oils used almost universally as stored forms of energy in living organisms are derivatives of fatty acids. The fatty acids are hydrocarbon derivatives, at about the same low oxidation state (that is, as highly reduced) as the hydrocarbons in fossil fuels. The cellular oxidation of fatty acids (to CO_2 and H_2O), like the controlled, rapid burning of fossil fuels in internal combustion engines, is highly exergonic.

Fatty acids

Fatty acids are carboxylic acids with hydrocarbon chains ranging from 4 to 36 carbons long (C4 to C36). In some fatty acids, this chain is unbranched and fully saturated (contains no double bonds); in unsaturated, the chain contains one or more double bonds **(Table 3.1.)**. A few contain three-carbon rings, hydroxyl groups, or methyl-group branches.

A simplified nomenclature for unbranched fatty acids specifies the chain length and number of double bonds, separated by a colon. For example, the 16-carbon saturated palmitic acid is abbreviated 16:0, and the 18-carbon oleic (octadecenoic) acid, with one double bond **(shown below Fig.3.1.)**, is 18:1. Each line segment of the zigzag in the structure represents a single bond between adjacent carbons. The carboxyl carbon is assigned the number 1 (C-1), and the carbon next to it is C-2. The positions of any double bonds, designated Δ (delta), are specified relative to C-1 by a superscript number indicating the lower-numbered carbon in the double bond. By this convention, oleic acid, with a double bond between C-9 and C-10, is designated 18:1(Δ9); a 20-carbon fatty acid with one double bond between C-9 and C-10 and another between C-12 and C-13 is designated 20:2(Δ9,12).

Table 3.1.

Common saturated and unsaturated fatty acids

Common Saturated Fatty Acids			
Number of Carbon Atoms	**Formula**	**Common Name**	**Source**
4	$CH_3(CH_2)_2COOH$	Butyric acid	Butter
6	$CH_3(CH_2)_4COOH$	Caproic acid	Butter
8	$CH_3(CH_2)_6COOH$	Caprylic acid	Coconut oil
10	$CH_3(CH_2)_8COOH$	Capric acid	Coconut oil
12	$CH_3(CH_2)_{10}COOH$	Lauric acid	Palm kernel oil
14	$CH_3(CH_2)_{12}COOH$	Myristic acid	Oil of nutmeg
16	$CH_3(CH_2)_{14}COOH$	Palmitic acid	Palm oil
18	$CH_3(CH_2)_{16}COOH$	Stearic acid	Beef tallow
18	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	Oleic acid	Olive oil
18	$CH_3(CH_2)_4CH{=}CHCH_2CH(CH_2)_7COOH$	Linoleic acid	Soybean oil
18	$CH_3CH_2(CH{=}CHCH_2)_3(CH_2)_6COOH$	Linolenic acid	Fish oils
20	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COOH$	Arachidonic	acid Liver
22	$CH_3(CH_2)_{20}COOH$	Beheric acid	Sesame oil
Common Unsaturated Fatty Acids			
Number of Carbon Atoms	**Formula**	**Common Name**	**Source**
16	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	Palmitoleic acid	Whale oil
18	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	Oleic acid	Olive oil
18	$CH_3(CH_2)_4CH{=}CHCH_2CH(CH_2)_7COOH$	Linoleic acid	Soybean oil, safflower oil
18	$CH_3CH_2(CH{=}CHCH_2)_3(CH_2)_6COOH$	Linolenic acid	Fish oils, linseed oil
20	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COOH$	Arachidonic	acid Liver

The most commonly occurring fatty acids have even numbers of carbon atoms in an unbranched chain of 12 to 24 carbons. There is also a common pattern in the location of double bonds; in most monounsaturated fatty acids the double bond is between C-9 and C-10 (Δ9), and the other double bonds of polyunsaturated fatty acids are generally Δ12 and Δ15. (Arachidonic acid is an exception to this generalization) The double bonds of polyunsaturated fatty acids are almost never conjugated (alternating single and double bonds, as in —CH=CH—CH=CH —), but are separated by a methylene group: —CH=CH—CH_2—CH=CH—. In nearly all naturally occurring unsaturated fatty acids, the double bonds are in the cis configuration. Trans fatty acids are produced by fermentation in the rumen of dairy animals and are obtained from dairy products and meat.

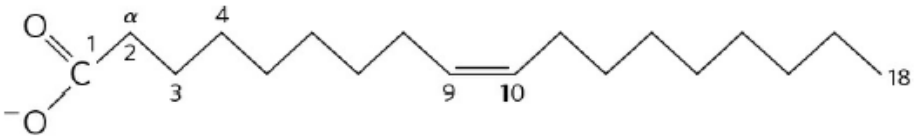

Figure 3.1. The 18-carbon oleic (octadecenoic) acid

The family of polyunsaturated fatty acids (PUFAs) with a double bond between the third and fourth carbon from the methyl end of the chain are of special importance in human nutrition. Because the physiological role of PUFAs is related more to the position of the first double bond near the methyl end of the chain than to that near the carboxyl end, an alternative nomenclature is sometimes used for these fatty acids. The carbon of the methyl group—that is, the carbon most distant from the carboxyl group —is called the ω (omega; the last letter in the Greek alphabet) carbon and is given the number 1 (C-1); the carboxyl carbon in this convention has the highest number. The positions of the double bonds are indicated relative to the ω carbon. In this convention, PUFAs with a double bond between C-3 and C-4 are called omega-3 (ω-3) fatty acids, and those with a double bond between C-6 and C-7 are omega-6 (ω-6) fatty acids. Shown below is eicosapentaenoic acid (**Fig. 3.2.)**, which can be designated as 20:5(Δ5,8,11,14,17) by the standard nomenclature but is also referred to as an omega-3 fatty acid, emphasizing the biologically important double bond in the omega-3 position.

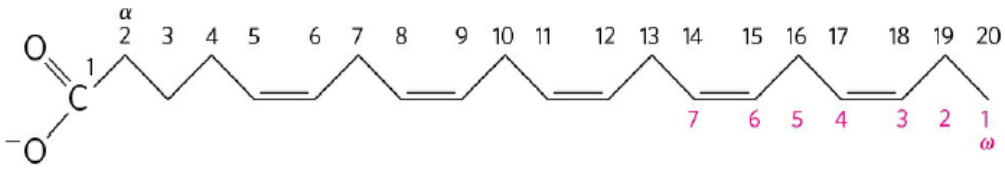

Figure 3.2. Eicosapentaenoic acid

Humans require the omega-3 PUFA Δ-linolenic acid (ALA; 18:3(Δ9,12,15), in the standard convention), but do not have the enzymatic capacity to synthesize it and must therefore obtain it in the diet **(Fig. 3.3.)**. From ALA, humans can synthesize two other omega-3 PUFAs important in cellular

human body can store less than a day's energy supply in the form of glycogen. Carbohydrates such as glucose do offer certain advantages as quick sources of metabolic energy, one of which is their ready solubility in water. In some animals, triacylglycerols stored under the skin serve not only as energy stores but as insulation against low temperatures. Seals, walruses, penguins, and other warm-blooded polar animals are amply padded with triacylglycerols. In hibernating animals (bears, for example), the huge fat reserves accumulated before hibernation serve the dual purposes of insulation and energy storage.

Many animals depend on fat stores for energy during hibernation, during migratory periods, and in other situations involving radical metabolic adjustments. One of the most pronounced adjustments of fat metabolism occurs in hibernating grizzly bears **(Fig.3.7.)**.

Figure 3.7. Grizzly bear

The winter sleep of bears is sometimes called torpor. These animals remain in a continuous state of dormancy for as long as seven months. Unlike most hibernating species, the bear maintains a body temperature of about 31 °C, close to the normal (nonhibernating) level (~40 °C). Although expending about 25,000 kJ/day while hibernating, the bear does not eat, drink, urinate, or defecate for months at a time. Its heart rate drops from 90 to 8 beats per minute, and its respiration (breathing) drops from 6 to 10 breaths to approximately 1 breath per minute. Mitochondrial electron transfer can be uncoupled from ATP production so that all of the energy of fuel oxidation is dissipated as heat, to maintain a body temperature near normal in the face of much lower ambient temperatures. Fat oxidation yields sufficient energy to maintain body temperature, synthesize amino acids and proteins, and carry out other energy-

requiring activities, such as membrane transport. Fat oxidation also releases large amounts of water, as described in the text, which replenishes water lost in breathing. The glycerol released by degradation of triacylglycerols is converted into blood glucose by gluconeogenesis. Urea formed during breakdown of amino acids is reabsorbed in the kidneys and recycled, with the amino groups reused to make new amino acids for maintaining body proteins.

Bears store an enormous amount of body fat in preparation for their long sleep. Large amounts of triacylglycerols are formed from the huge intake of carbohydrates during the fattening-up period. The bear will emerge from hibernation having lost 15% to 40% of its maximum body weight. Studies of hibernation mechanisms may yield insight into several problems in human medicine; for example, slowing the metabolism of organs donated for transplantation might extend the period of their viability. And if humans are to make long trips into space, inducing a torpor like state might relieve the monotony of long missions and conserve on-board resources such as food and oxygen.

Most natural fats, such as those in vegetable oils, dairy products, and animal fat, are complex mixtures of simple and mixed triacylglycerols.

These contain a variety of fatty acids differing in chain length and degree of saturation. Vegetable oils such as corn (maize) oil and olive oil are composed largely of triacylglycerols with unsaturated fatty acids and thus are liquids at room temperature **(Fig 3.7.)**. Triacylglycerols containing only saturated fatty acids, such as tristearin, the major component of beef fat, are white, greasy solids at room temperature. When lipid-rich foods are exposed too long to the oxygen in air, they may spoil and become rancid.

Vegetable oils such as corn (maize) oil and olive oil are composed largely of triacylglycerols with unsaturated fatty acids and thus are liquids at room temperature. Triacylglycerols containing only saturated fatty acids, such as tristearin, the major component of beef fat, are white, greasy solids at room temperature. When lipid-rich foods are exposed too long to the oxygen in air, they may spoil and become rancid.

The unpleasant taste and smell associated with rancidity result from the oxidative cleavage of double bonds in unsaturated fatty acids, which produces aldehydes and carboxylic acids of shorter chain length and therefore higher volatility. Throughout the twentieth century, to improve the shelf life of vegetable oils used in cooking, and to increase their stability at the high temperatures used in deep-frying, commercial vegetable oils were prepared by partial hydrogenation. Unsaturated fatty acids may be converted to saturated fatty acids by the relatively simple hydrogenation reaction **(Fig. 3.8.)**. This process converts many of the cis double bonds in the fatty acids to single bonds and increases the melting temperature of the oils so that they are more nearly solid at room temperature (margarine is produced from vegetable oil in this way).

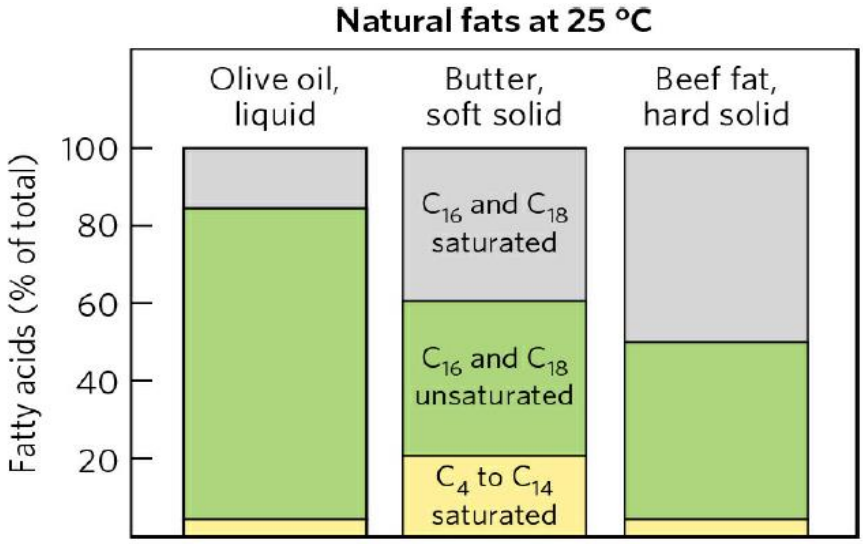

Figure 3.7. Fatty acid composition of three food fats. Olive oil, butter, and beef fat consist of mixtures of triacylglycerols differing in their fatty acid composition. The melting points of these fats—and hence their physical state at room temperature (25 °C)—are a direct function of their fatty acid composition. Olive oil has a high proportion of long-chain (C16 and C18) unsaturated fatty acids, which accounts for its liquid state at 25 °C. The higher proportion of long-chain (C16 and C18) saturated fatty acids in butter increases its melting point, so butter is a soft solid at room temperature. Beef fat, with an even higher proportion of long-chain saturated fatty acids, is a hard solid.

Partial hydrogenation, however, has another, undesirable, effect: some cis double bonds are converted to trans double bonds **(Fig.3.9.)**. There is now strong evidence that dietary intake of trans fatty acids (often referred to simply as “trans fats”) leads to a higher incidence of cardiovascular disease, and that avoiding these fats in the diet substantially reduces the risk of coronary heart disease.

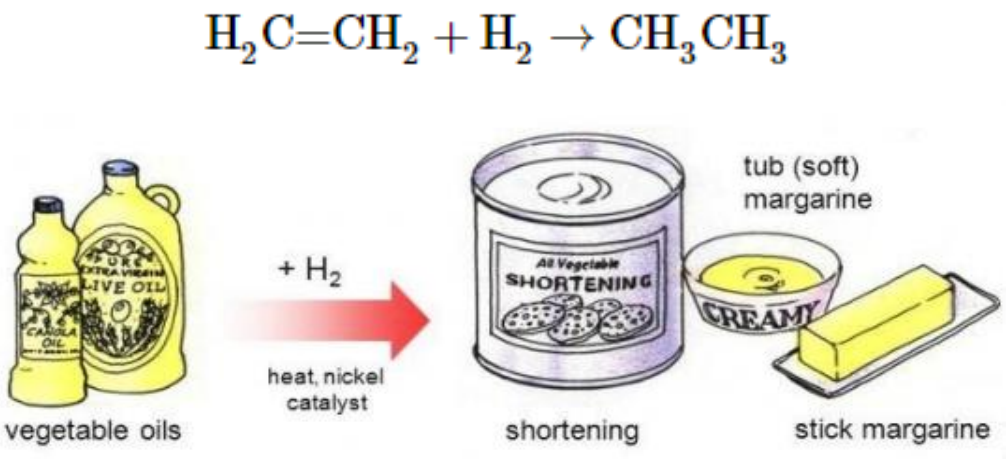

Figure. 3.8. Hydrogenation reaction. Hydrogenation of a double bond.

Dietary trans fatty acids raise the level of triacylglycerols and of LDL (“bad”) cholesterol in the blood, and lower the level of HDL (“good”) cholesterol, and these changes alone are enough to increase

the risk of coronary heart disease. But trans fatty acids may have further adverse effects. They seem, for example, to increase the body's inflammatory response, which is another risk factor for heart disease. Regulatory agencies around the world now limit or ban the use of trans fatty acids in prepared and packaged foods.

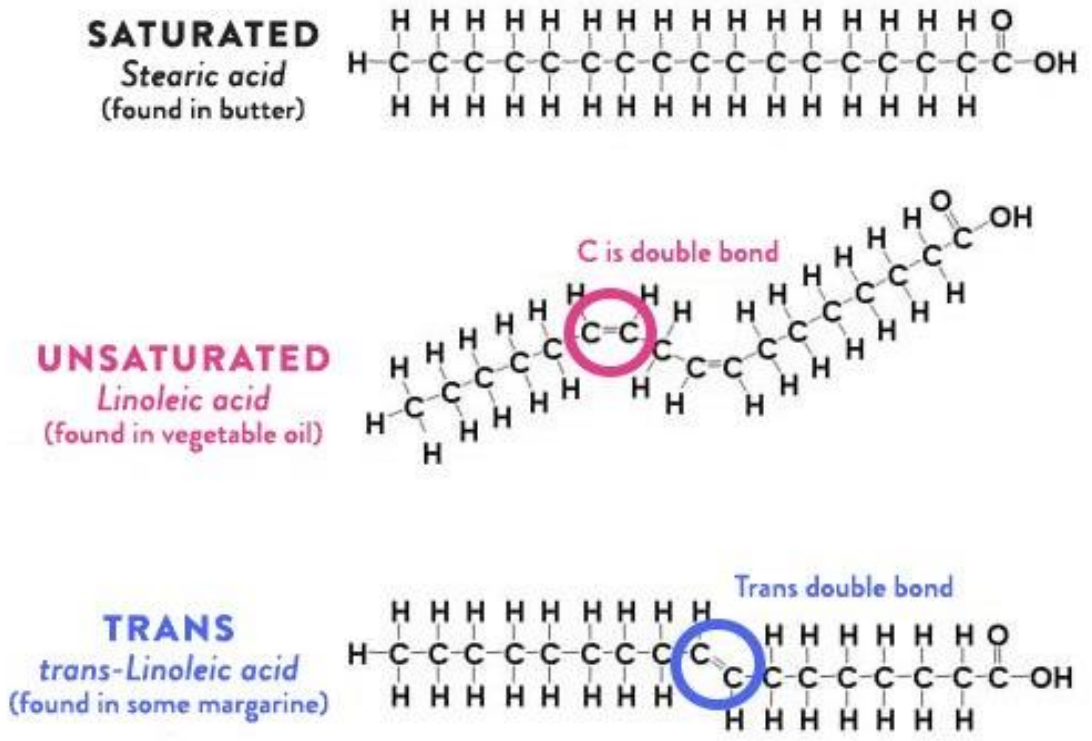

Figure 3.9. Converting cis double bonds to trans double bonds

Biological waxes

Biological waxes are esters of long-chain (C14 to C36) saturated and unsaturated fatty acids with long-chain (C16 to C30) alcohols. Their melting points (60 to 100 °C) are generally higher than those of triacylglycerols. In plankton, the free-floating microorganisms at the bottom of the food chain for marine animals, waxes are the chief storage form of metabolic fuel. Waxes also serve a diversity of other functions related to their water repellent properties and their firm consistency. Certain skin glands of vertebrates secrete waxes to protect hair and skin and keep it pliable, lubricated, and waterproof. Birds, particularly waterfowl, secrete waxes from their preen glands to keep their feathers water-repellent. The shiny leaves of holly, rhododendrons, poison ivy, and many tropical plants are coated with a thick layer of waxes, which prevents excessive evaporation of water and protects against parasites.

Biological waxes find a variety of applications in the pharmaceutical, cosmetic, and other industries. Lanolin (from lamb's wool), beeswax, carnauba wax (from a Brazilian palm tree) **(Fig. 3.10.)**, and wax extracted from the seeds of the jojoba bush are widely used in the manufacture of lotions, ointments, and polishes.

Figure 3.10. Lanolin, beeswax, carnauba and jojoba waxes

Burning longer, cleaner, and brighter, the spermaceti candle was the height of candle-making technology for Americans in the 18th and 19th centuries.

Demand for spermaceti dramatically impacted the American East Coast whaling industry, as spermaceti can only be found in one species of whale: the sperm whale. The perils associated with retrieving spermaceti from sperm whales, as well as the lengthy production process, kept the cost of the spermaceti candle high and allowed only the richest of Americans to fully enjoy the benefits of this type of candle. Numerous primary sources allow for an even greater understanding of early Americans fascination with the whaling industry, most notably, Herman Melville's Moby Dick.

The sperm whale's head is very large, accounting for over one-third of its total body weight. About 90% of the weight of the head is made up of the spermaceti organ **(Figure 3.11.)**, a blubbery mass that contains up to 3,600 kg (about 4 tons) of spermaceti oil, a mixture of triacylglycerols and waxes containing an abundance of unsaturated fatty acids. This mixture is liquid at the normal resting body temperature of the whale, about 37 °C, but it begins to crystallize at about 31°C and becomes solid when the temperature drops several more degrees.

These mammals feed almost exclusively on squid in very deep water. In their feeding dives they descend 1,000 m or more; the record dive is 3,000 m (almost 2 miles). For a marine animal to remain at a given depth, without a constant swimming effort, it must have the same density as the surrounding water.

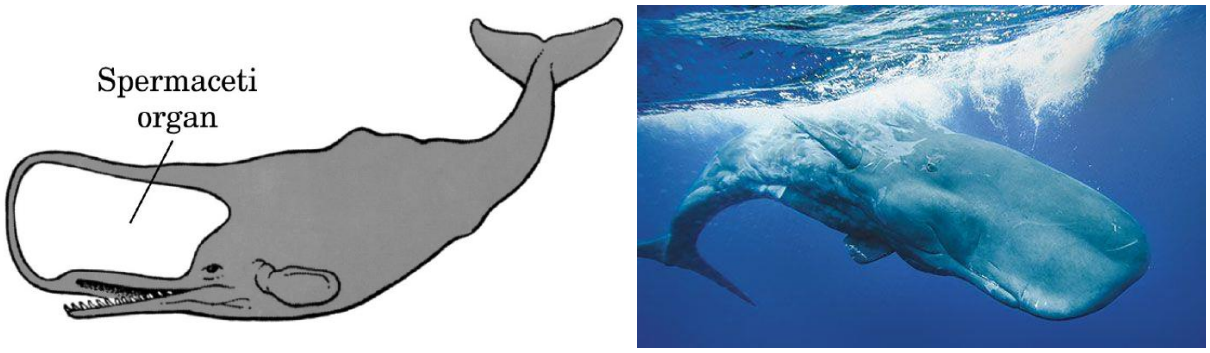

Figure 3.11. The spermaceti organ of whale

The key to the sperm whale's ability to change its buoyancy is the freezing point of spermaceti oil. When the temperature of liquid spermaceti oil is lowered several degrees during a deep dive, it congeals or crystallizes and becomes more dense, thus changing the buoyancy of the whale to match the density of seawater. Various physiological mechanisms promote rapid cooling of the oil during a dive. During the return to the surface, the congealed spermaceti oil is warmed again and melted, decreasing its density to match that of the surface water. Thus we see in the sperm whale a remarkable anatomical and biochemical adaptation, perfected by evolution. The triacylglycerols synthesized by the sperm whale contain fatty acids of the necessary chain length and degree of unsaturation to give the spermaceti oil the proper melting point for the animal's diving habits. Unfortunately for the sperm whale population, spermaceti oil is commercially valuable as a lubricant. Several centuries of intensive hunting of these mammals have depleted the world's population of sperm whales.

Eicosanoids

There are also group of lipids, present in much smaller amounts, includes those with active roles in the metabolic traffic as metabolites and messengers. Some serve as potent signals—as hormones, carried in the blood from one tissue to another, or as intracellular messengers generated in response to an extracellular signal (hormone or growth factor). Others function as enzyme cofactors in electron-transfer reactions in chloroplasts and mitochondria, or in the transfer of sugar moieties in a variety of glycosylation reactions. A third group consists of lipids with a system of conjugated double bonds: pigment molecules that absorb visible light. Some of these act as light-capturing pigments in vision and photosynthesis; others produce natural colorations, such as the orange of pumpkins and carrots and the yellow of canary feathers. Finally, a very large group of volatile lipids produced in plants consists of signaling molecules that pass through the air, allowing plants to communicate with each other and to invite animal friends and deter foes.

Eicosanoids are paracrine hormones, substances that act only on cells near the point of hormone synthesis instead of being transported in the blood to act on cells in other tissues or organs. These fatty acid derivatives have a variety of dramatic effects on vertebrate tissues. They are involved in reproductive function; in the inflammation, fever, and pain associated with injury or disease; in the formation of blood

clots and the regulation of blood pressure; in gastric acid secretion; and in various other processes important in human health or disease. Eicosanoids are derived from arachidonate (arachidonic acid; 20:4(Δ5,8,11,14)) and eicosapentaenoic acid (EPA; 20:5(Δ5,8,11,14,17)), from which they take their general name (Greek eikosi, "twenty"). There are four major classes of eicosanoids: prostaglandins, thromboxanes, leukotrienes, and lipoxins **(Fig. 3.12.)**. Eicosanoid names include letter designations for the functional groups on the ring and numbers indicating the number of double bonds in the hydrocarbon chain.

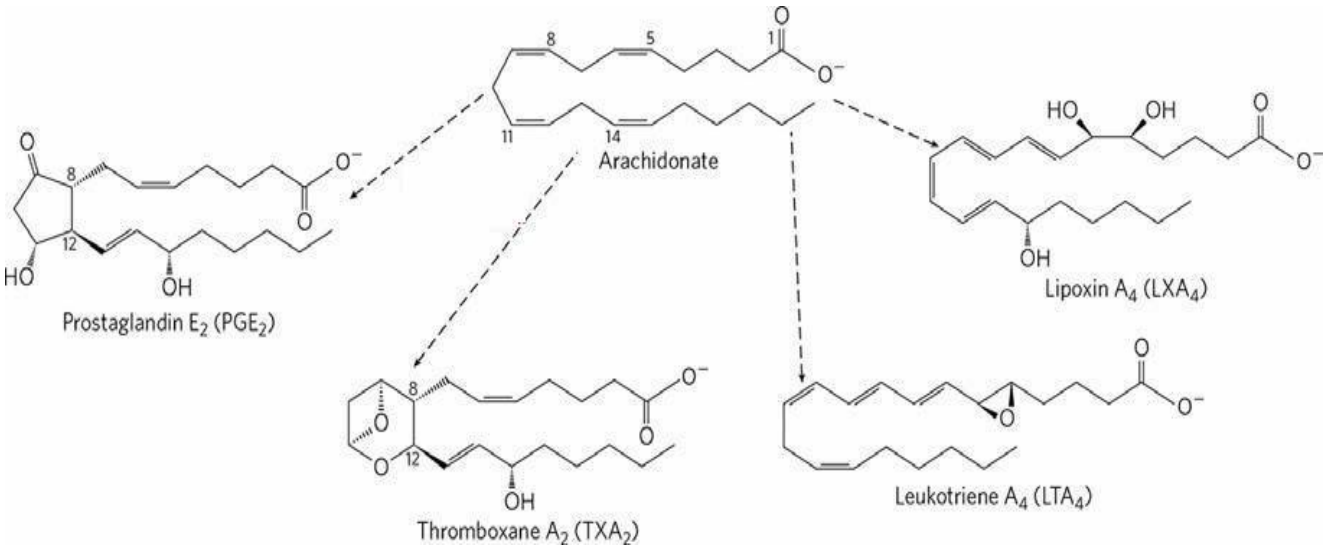

Figure. 3.12. Four major classes of eicosanoids

Arachidonic acid (arachidonate at pH 7) is the precursor of eicosanoids, including the prostaglandins, thromboxanes, leukotrienes, and lipoxins. In prostaglandin E2, C-8 and C-12 of arachidonate are joined to form the characteristic five-membered ring. In thromboxane A2, the C-8 and C-12 are joined and an oxygen atom is added to form the six-membered ring. Nonsteroidal antiinflammatory drugs (NSAIDs) such as aspirin and ibuprofen block the formation of prostaglandins and thromboxanes from arachidonate by inhibiting the enzyme cyclooxygenase (prostaglandin H_2 synthase). Leukotriene A4 has a series of three conjugated double bonds, and no cyclic moiety. Lipoxins are also noncyclic derivatives of arachidonate, with several hydroxyl groups.

Prostaglandins (PG) contain a five-carbon ring. Their name derives from the prostate gland, the tissue from which they were first isolated by Bengt Samuelsson and Sune Bergström. Prostaglandins have an array of functions. Some stimulate contraction of the smooth muscle of the uterus during menstruation and labor. Others affect blood flow to specific organs, the wake-sleep cycle, and the responsiveness of certain tissues to hormones such as epinephrine and glucagon. Prostaglandins in a third group elevate body temperature (producing fever) and cause inflammation and pain.

The thromboxanes (TX) have a six-membered ring containing an ether. They are produced by platelets (also called thrombocytes) and act in the formation of blood clots and reduction of blood flow to the site of a clot. As shown by John Vane, the nonsteroidal antiinflammatory drugs (NSAIDs)— aspirin,

ibuprofen, and meclofenamate, for example—inhibit the enzyme prostaglandin H_2 synthase (also called cyclooxygenase2, or COX-2), which catalyzes an early step in the pathway from arachidonate to series 2 prostaglandins and thromboxanes and from EPA to series 3 prostaglandins and thromboxanes.

Leukotrienes (LT), first found in leukocytes, contain three conjugated double bonds. They are powerful biological signals. For example, leukotriene D4, derived from leukotriene A4, induces contraction of the smooth muscle lining the airways to the lung. Overproduction of leukotrienes causes asthmatic attacks, and leukotriene synthesis is one target of antiasthmatic drugs such as prednisone. The strong contraction of the smooth muscle of the lungs that occurs during anaphylactic shock is part of the potentially fatal allergic reaction in individuals hypersensitive to bee stings, penicillin, or other agents.

Lipoxins (LX), like leukotrienes, are linear eicosanoids. Their distinguishing feature is the presence of several hydroxyl groups along the chain. These compounds are potent antiinflammatory agents. Because their synthesis is stimulated by low doses (81 mg) of aspirin taken daily, this low dose is commonly prescribed for individuals with cardiovascular disease.

Biological membrane

Biological membranes surround ceils and organelles, divide the interior of eukaryotic cells into distinct compartments, and provide surfaces for the localization of metabolic enzymes, transport proteins, receptors, and various substrates. In addition, membranes are semipermeable barriers which regulate the transport of water, ions, and other metabolites, thereby providing a means of controlling the internal environment. Our basic understanding of membrane structure has changed little since Singer and Nicholson (1972) first proposed the fluid-mosaic model **(Fig. 3.13.)** over 20 years ago.

Biological membranes are fluid (liquid<crystalline) lipid bilayers about 7 nm thick, into which proteins can insert or associate at the surface. Until recently, membrane research has focused primarily on the protein components, with the lipid portion viewed as a convenient barrier and environment for enzymes. However, biological membranes contain a wide diversity of lipids, far more than are needed to perform structural functions, and these lipids require elaborate metabolic pathways for their synthesis and transport.

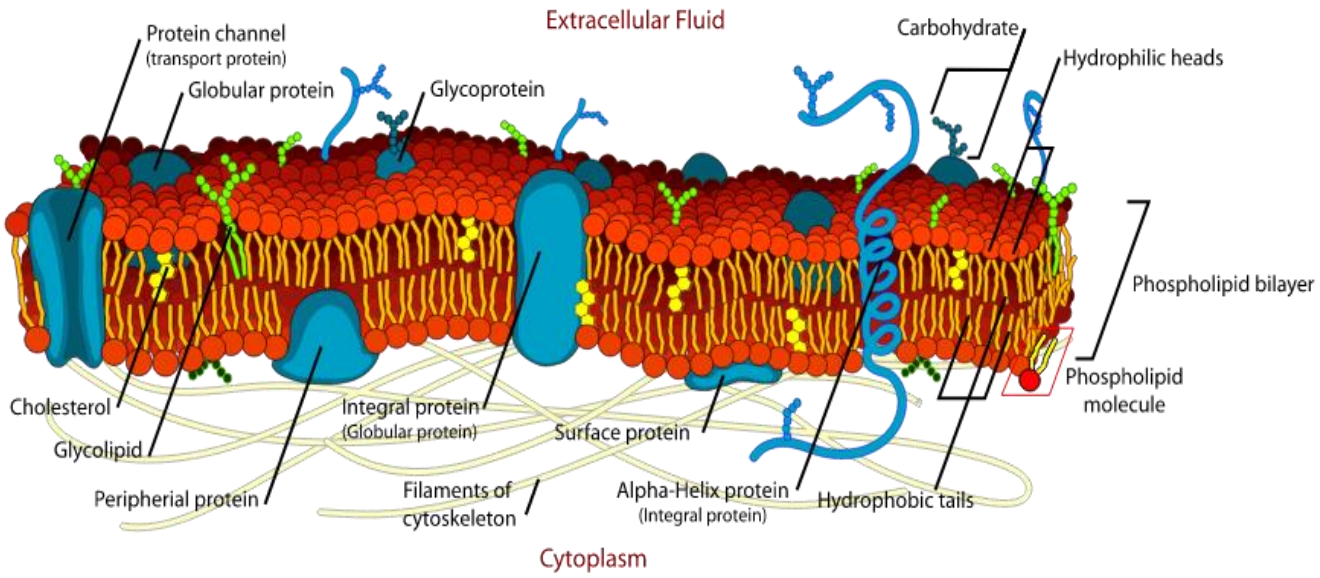

Figure. 3.13. The fluid-mosaic model of membrane (Singer and Nicholson (1972))

The chemical composition of the hydrophobic layer of the membrane determines such a property as permeability. When replacing unsaturated radicals in bacterial membranes with saturated ones, the diffusion of substances slows down 20 times. Water easily penetrates through the membranes, but they are impermeable to ions of the type Na^+, Cl^-, H^+ and to polar molecules of the glucose type. Such substances, unlike hydrophobic ones, require special transport systems or carriers to penetrate the membrane.

The structural organization and properties of cell membranes are determined by their molecular components (lipids, proteins and carbohydrates). Lipids are essential for membranes because they spatially organize in lipid bilayers, i.e., cellular membranes, and can modulate membrane properties since there are more than 1000 different lipid species in eukaryote membranes. Lipids are in charge of physical features of membranes. Length and saturation of their fatty acid chains regulate fluidity and thickness of membranes. The uneven distribution between the two lipid mono-layers generates membrane asymmetry. Electrical charges located in the hydrophilic lipid heads influence the electrical gradient that membranes have between the external and internal membrane surfaces, modulating the membrane potential. Through lateral interactions, lipids can modulate the activity of the membrane proteins. Furthermore, lipids may work as second messengers, leaving membranes and diffusing to intracellular compartments to trigger cellular responses. The lateral heterogeneity of cell membranes are thought to be caused by lateral lipid-lipid interactions, forming small and plastic domains of higher densely packed lipids that contain higher proportions of certain type of lipids and proteins. These regions are known as lipid rafts or membrane domains.

Membrane lipids show a hydrophobic domain toward the inner part of the membrane and a hydrophilic domain toward the aqueous environment. That is why they are known as amphiphilic **(Fig. 3.14.)**.

glycosphingolipids occur largely in the outer face of the plasma membrane. Cerebrosides have a single sugar linked to ceramide; those with galactose are characteristically found in the plasma membranes of cells in neural tissue, and those with glucose, in the plasma membranes of cells in nonneural tissues.

Gangliosides, the most complex sphingolipids, contain very large polar heads made up of several sugar units. One or more of the terminal sugar units of gangliosides is N-acetylneuraminic acid, also called sialic acid, which has a negative charge at pH 7. Gangliosides make up about 6% of the membrane lipids in the gray matter of the human brain, and they are present in lesser amounts in the membranes of most nonneural animal tissues.

When the sphingolipids were discovered a century ago by the physician-chemist Johann Thudicum, their biological role seemed as enigmatic as the Sphinx, for which he named them. Sphingolipids are now known to be involved in various recognition events at the cell surface. For example, glycosphingolipids are the determinants of the human blood groups A, B, and O. The ganglioside GM1, which doubtless plays some role of value to the animal cell that contains it, is the point of attachment of cholera toxin as it attacks an animal cell, a case of coevolution of a host cell and its pathogenic parasite. The membranes of the human nervous system contain at least 15 different gangliosides for which no function is yet known. However, it is clearly important that the synthesis and breakdown of these compounds be tightly regulated; derangements in the metabolism of cerebrosides and gangliosides underlie the devastating effects of several human genetic diseases, including Tay-Sachs and Niemann-Pick diseases. For example, in Niemann-Pick disease, sphingomyelin accumulates in the brain, spleen, and liver. The disease first becomes evident in infants, causing mental retardation and early death. Niemann-Pick disease is caused by a rare genetic defect in the hydrolytic enzyme sphingomyelinase, which cleaves phosphocholine from sphingomyelin. Much more common is Tay-Sachs disease, in which a specific ganglioside accumulates in the brain and spleen owing to the lack of the lysosomal enzyme hexosaminidase A, a degradative enzyme that normally hydrolyzes a specific bond between an N-acetyl-n-galactosamine and a n-galactose residue in the polar head of the ganglioside. As a result, the partially degraded gangliosides accumulate, causing degeneration of the nervous system. The symptoms of Tay-Sachs disease are progressive retardation in development, paralysis, blindness, and death by the age of 3 or 4 yr.

Sterols

Sterols are structural lipids present in the membranes of most eukaryotic cells. Their characteristic structure is the steroid nucleus consisting of four fused rings, three with six carbons and one with five. The steroid nucleus is almost planar, and relatively rigid; the fused rings do not allow rotation about C-C bonds. Cholesterol, the major sterol in animal tissues, is amphipathic, with a polar head group (the hydroxyl group at C-3) and a nonpolar hydrocarbon body (the steroid nucleus and the hydrocarbon side chain at C-17) about as long as a 16-carbon fatty acid in its extended form. Similar sterols are found in other eukaryotes: stigmasterol in plants and ergosterol in fungi, for example. With rare exceptions,

bacteria lack sterols. The sterols of all species are synthesized from simple five-carbon isoprene subunits (as are the fat-soluble vitamins, quinones, and dolichols described below).

Cholesterol **(Fig.3.17.)** is the most important sterol in animal cell membranes and the third most abundant type of lipid in plasma membrane (up to 25 % of the total lipids) of animal cells, whereas it is scarce in membranes of organelles like endoplasmic reticulum (about 1 % of the total lipids), mitochondria and chloroplasts.

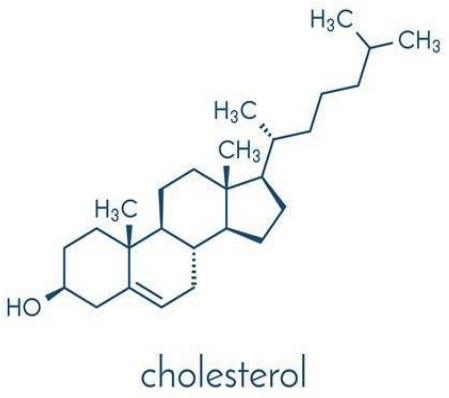

Figure 3.17. Cholesterol is the most important sterol in animal cell membranes

Cholesterol is located among the fatty acid chains of other lipids. Cholesterol is not present in plant cell membranes, in some unicellular eukaryotes, nor in bacteria. However, these cells bear other types of sterols in their membranes. Sterols are essential for the integrity and functions of eukaryotic plasma membranes. Cholesterol influences fluidity, stiffness and permeability of membranes. Moreover, it may modulate the activity of GPCR (G protein-coupled receptors), signal transduction and vesicular traffic. It is important for the organization of membranes, particularly the plasma membrane, because, together with sphingolipids, contributes to create lateral membrane domains. It also participates in metabolic processes such us the synthesis of steroid hormones and bile salts.

Although the lipid bilayer structure itself is stable, the individual phospholipid and sterol molecules have great freedom of motion within the plane of the membrane. They diffuse laterally so fast that an individual lipid molecule can circumnavigate an erythrocyte in a few seconds. The interior of the bilayer is also fluid; individual hydrocarbon chains of fatty acids are in constant motion produced by rotation about the carbon-carbon bonds of the long acyl side chains.

DID YOU KNOW?

The degree of fluidity depends on lipid composition and temperature. At low temperature, relatively little lipid motion occurs and the bilayer exists as a nearly crystalline (paracrystalline) array. Above a temperature that is characteristic for each membrane, lipids can undergo rapid motion. The temperature of the transition from paracrystalline solid to fluid depends upon the lipid composition of the

membrane. Saturated fatty acids pack well into a paracrystalline array, but the kinks in unsaturated fatty acids interfere with this packing, preventing the formation of a paracrystalline solid state. The higher the proportion of saturated fatty acids, the higher is the solid-to-fluid transition temperature of the membrane. When the temperature of the environment decreases, the membranes of bacteria and plants are enriched with unsaturated fatty acids, which ensures the maintenance of the liquid state. Organisms of warm-blooded animals counteract the hardening of membranes, maintaining a constant body temperature through metabolism. In the penguin's limbs **(Fig. 3.18.)** in the coldest parts of the body, the content of unsaturated fatty acids in the membranes is higher than in warm areas.

Figure 3.18. Organisms of penguins counteract the hardening of membranes, maintaining a constant body temperature through metabolism.

When individual protein molecules and multiprotein complexes in freeze-fractured biological membranes are visualized with the electron microscope, some proteins appear on only one face of the membrane; most span the full thickness of the bilayer, and protrude from both inner and outer membrane surfaces. Among the latter are some proteins that conduct solutes or signals across the membrane.

Membrane proteins may be divided operationally into two groups: integral (intrinsic) proteins, which are very firmly bound to the membrane, and peripheral (extrinsic) proteins, which are bound more loosely, or reversibly. Peripheral membrane proteins can be released from membranes by relatively mild treatments, and once released from the membrane they are generally water soluble. In contrast, the release of integral proteins from membranes requires the action of agents (detergents, organic solvents, or denaturants) that interfere with hydrophobic interactions. The insolubility of integral membrane proteins results from the presence of domains rich in hydrophobic amino acids; hydrophobic interactions between the protein and the lipids of the membrane account for the firm attachment of the protein.

The protein has four subunits, three of which contain α-helical segments that span the membrane. These segments are rich in nonpolar amino acids, and their hydrophobic side chains are oriented toward the outside of the protein, interacting with the hydrocarbon side chains of membrane lipids. The architecture of the reaction center protein is therefore the inverse of that seen in most water-soluble

proteins, which have their hydrophobic residues buried within the protein core and their hydrophilic residues on the surface available for polar interactions with water

Many peripheral proteins are held to the membrane by electrostatic interactions and hydrogen bonding with the hydrophilic domains of integral membrane proteins, and perhaps with the polar head groups of membrane lipids. Lipids attached covalently to certain membrane proteins anchor these proteins to the lipid bilayer by hydrophobic interactions.

On the outer surface of cell membranes, proteins and lipids form complexes with carbohydrates **(Fig. 3.19.)** - glycoproteins and glycolipids, which cause adhesive interactions, in particular contacts between cells of the same type, tissue compatibility. Branched oligosaccharide chains are located as "antennas" on the outer side of cell membranes, forming a shell of about 10 nm, which is 10 times thicker than the double lipid layer.

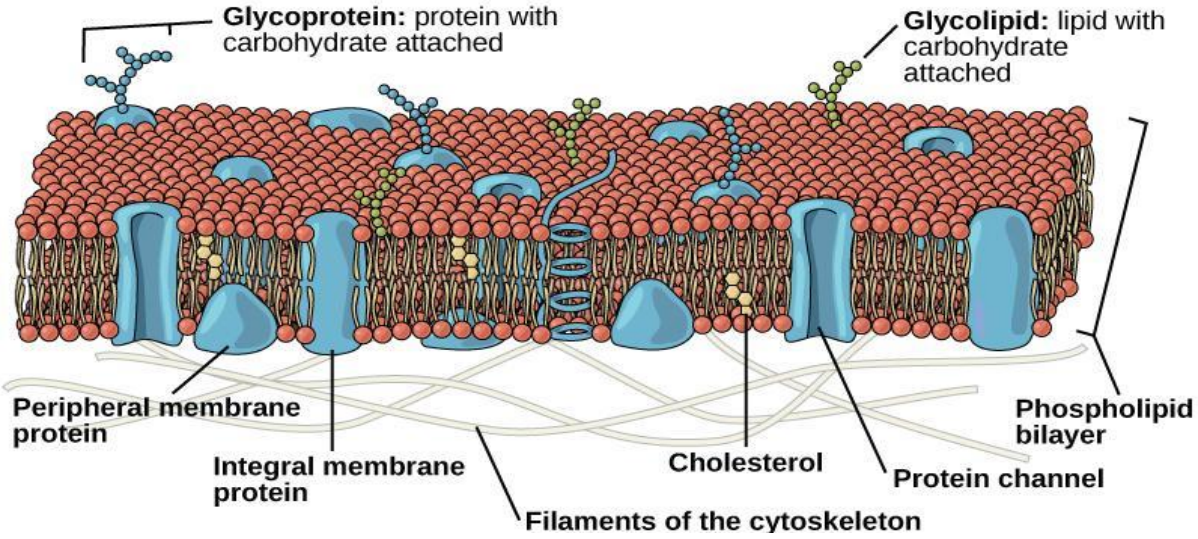

Figure 3.19. Proteins and lipids form complexes with carbohydrates

Self-assessment tasks

1. Which lipids are major structural elements of biological membranes?

a) phospholipids and sterols; b) oils and sterols; c) phospholipids and waxes; d) oils and waxes.

2.How do people get omega-3 PUFA Δ-linolenic acid?

a) they synthesize; b) obtain it in the diet; c) create by DNA; d) by hydrolyzing.

3.In which configuration the double bonds of unsaturated fatty acids are in nature?

a) wans; b) trans; c) cis; d) sis.

4.What is the name of the carbon most distant from the carboxyl group in PUFAs?

a) y; b) b; c) a; d) ω.

5.Where large amounts of triacylglycerols in vertebrates are stored?

a) nephrons; b) erythrocytes; c) adipocytes; d) platelets.

6.The most abundant steroid in the human body is

a) progesterone; b) testosterone; c) estradiol; d) cholesterol.

7.What are the monomers of lipids?

a) amino acids; b) simple sugars; c) fatty acids and glycerol; d) nucleic acids.

8.Fats that have fatty acids with only single covalent bonds in their carbon skeletons are

a) saturated; b) unsaturated; c) found in plants instead of animals; d) liquid at room temperature.

9. Describe lipids.

10. What kinds of lipids are there?

11. Explain the difference between saturated and unsaturated fats.

12. Describe biological membrane.

Chapter №4. Amino Acids, Peptides, and Proteins. The structure of proteins.

Proteins, whose name derives from the Greek protos, meaning "first" or "foremost." Proteins mediate virtually every process that takes place in a cell, exhibiting an almost endless diversity of functions. The proteins of every organism, from the simplest of bacteria to human beings, are constructed from the set of 20 amino acids. Relatively simple monomeric subunits provide the key to the structure of the thousands of different proteins. All proteins, whether from the most ancient lines of bacteria or from the most complex forms of life, are constructed from the same ubiquitous set of 20 amino acids, covalently linked in characteristic linear sequences. Because each of these amino acids has a distinctive side chain that determines its chemical properties, this group of 20 precursor molecules may be regarded as the alphabet in which the language of protein structure is written. To generate a particular protein, amino acids are covalently linked in a characteristic linear sequence. What is most remarkable is that cells can produce proteins with strikingly different properties and activities by joining the same 20 amino acids in many different combinations and sequences. From these building blocks, different organisms can make such widely diverse products as enzymes, hormones, antibodies, transporters, muscle fibers, the lens protein of the eye, feathers, spider webs, rhinoceros horn, milk proteins, antibiotics, mushroom poisons, and myriad other substances having distinct biological activities.

Proteins are polymers of amino acids, with each amino acid residue joined to its neighbor by a specific type of covalent bond. (The term “residue” reflects the loss of the elements of water when one amino acid is joined to another.) Proteins can be broken down (hydrolyzed) to their constituent amino acids by a variety of methods, and the earliest studies of proteins naturally focused on the free amino acids derived from them. Twenty different amino acids are commonly found in proteins. The first to be discovered was asparagine, in 1806. The last of the 20 to be found, threonine, was not identified until 1938. All the amino acids have trivial or common names, in some cases derived from the source from which they were first isolated. Asparagine was first found in asparagus, and glutamate in wheat gluten; tyrosine was first isolated from cheese (its name is derived from the Greek tyros,“cheese”); and glycine (Greek glykos, “sweet”) was so named because of its sweet taste.

One of the most important, technically and historically, is the ninhydrin reaction, which has been used for many years to detect and quantify microgram amounts of amino acids. When amino acids are heated with excess ninhydrin, all those having a free α-amino group yield a purple product. Proline, in which the a-amino group is substituted (forming an imino group), yields a yellow product. For determining the aromatic groups use the interaction with nitric acid (xanthoproteic reaction), and to determine cysteine - the interaction of sulfide formed after alkaline hydrolysis of the radical on the thiol group, with lead ions - (Fol's reaction). Individual amino acids are determined by the products of the reaction of substitution of their α-amino groups with 1-fluoro-2,4-dinitrobenzene (DNFB, Sanger's reagent) in a slightly alkaline medium. This reaction is used to determine the primary structure of proteins.

For all the common amino acids except glycine, the α carbon is bonded to four different groups: a carboxyl group, an amino group, an R group (they differ from each other in their side chains, or R groups, which vary in structure, size, and electric charge, and which influence the solubility of the amino acids in water), and a hydrogen atom (in glycine, the R group is another hydrogen atom). The α-carbon atom is thus a chiral center **(Fig. 4.1.)**.

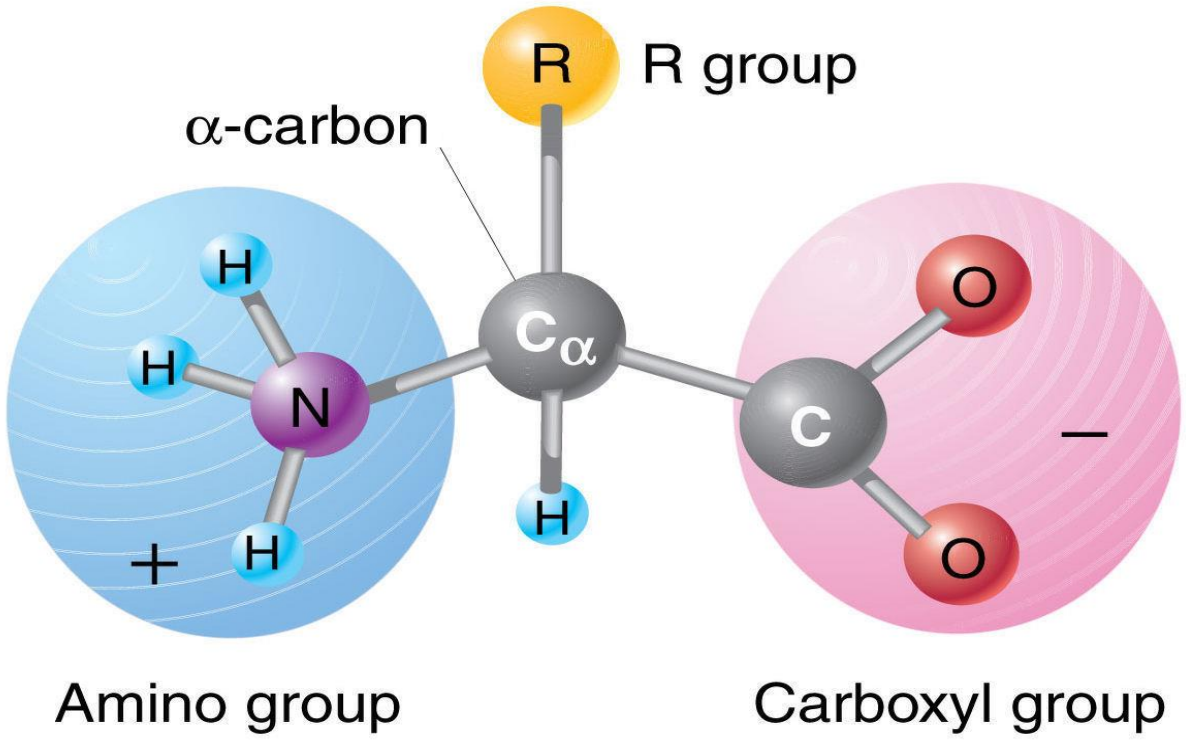

Figure 4.1. Amino acid structure

The common amino acids of proteins have been assigned three letter abbreviations and one-letter symbols, which are used as shorthand to indicate the composition and sequence of amino acids polymerized in proteins.

The three-letter code is transparent, the abbreviations generally consisting of the first three letters of the amino acid name. The one letter code was devised by Margaret Oakley Dayhoff, considered by many to be the founder of the field of bioinformatics.

There are 5 classes of amino acids which are based on the properties of their R groups **(Fig 4.2.)**, particularly their polarity, or tendency to interact with water at biological pH (near pH 7.0). The polarity of the R groups varies widely, from nonpolar and hydrophobic (water-insoluble) to highly polar and hydrophilic (water-soluble). A few amino acids are somewhat difficult to characterize or do not fit perfectly in any one group, particularly glycine, histidine, and cysteine. Their assignments to particular groupings are the results of considered judgments rather than absolutes.

The spatial arrangement of atoms in a protein is called a conformation. The term conformation refers to a structural state that can, without breaking any covalent bonds, interconvert with other structural states. A change in conformation could occur, for example, by rotation about single bonds. Of the innumerable conformations that are theoretically possible in a protein containing hundreds of single bonds, one generally predominates. This is usually the conformation that is thermodynamically the most stable, having the lowest Gibbs' free energy (G). Proteins in their functional conformation are called native proteins.

Levels of protein structure

The term, structure, when used in relation to proteins, takes on a much more complex meaning than it does for small molecules. Proteins are macromolecules and have four different levels of structure – primary, secondary, tertiary and quaternary **(Fig. 4.7.)**.

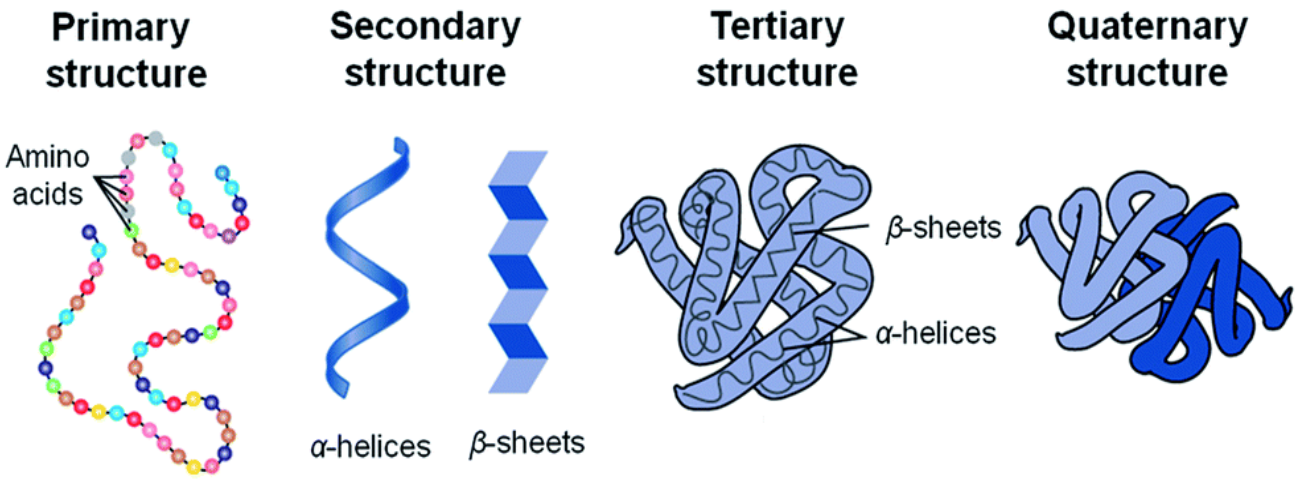

Figure 4.7. Summary of the Four Levels of Protein Structure

Primary structure refers to the amino acid sequence and the location of disulfide bonds. Secondary structure refers to regular, recurring arrangements in space of adjacent amino acid residues in a polypeptide chain. There are a few common types of secondary structure, the most prominent being the α-helix and the β conformation. Tertiary structure is the three-dimensional conformation of an entire polypeptide chain. Quaternary structure involves the spatial relationship of multiple polypeptide chains (e.g., enzyme subunits) that are tightly associated.

The primary structure

The simplest level of protein structure, primary structure, is simply the sequence of amino acids in a polypeptide chain and the location of disulfide bonds **(Fig. 4.8.)**. Each chain has its own set of amino acids, assembled in a particular order.

The sequence of a protein is determined by the DNA of the gene that encodes the protein (or that encodes a portion of the protein, for multi-subunit proteins). A change in the gene's DNA sequence may lead to a change in the amino acid sequence of the protein. Even changing just one amino acid in a protein’s sequence can affect the protein’s overall structure and function.

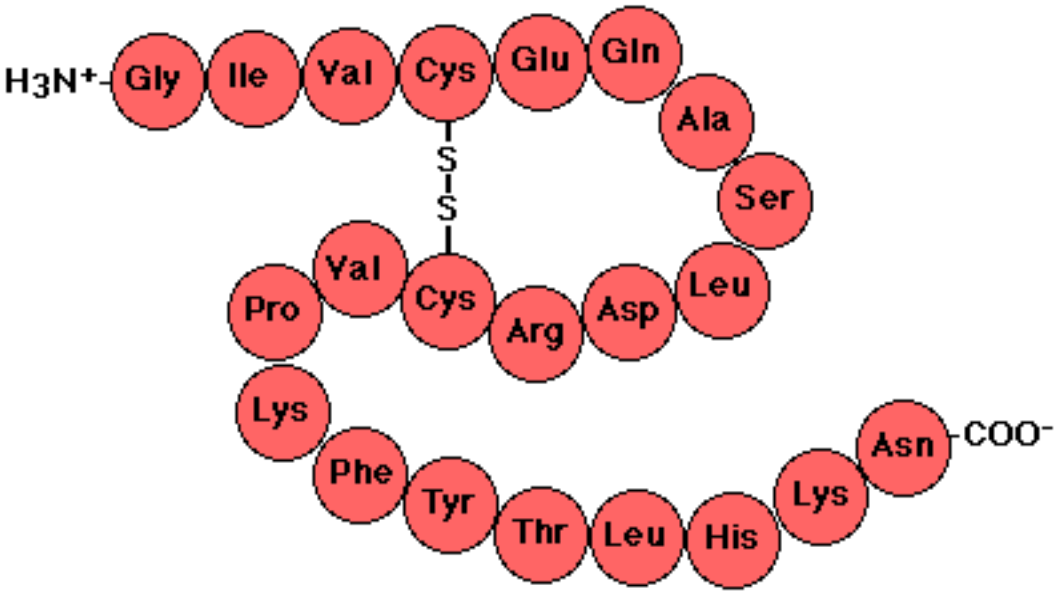

Figure 4.8. Amino acid sequence with disulfide bonds

A single amino acid change is associated with sickle cell anemia **(Fig. 4.9.)**, an inherited disease that affects red blood cells. In sickle cell anemia, one of the polypeptide chains that make up hemoglobin, the protein that carries oxygen in the blood, has a slight sequence change. The glutamic acid that is normally the sixth amino acid of the hemoglobin β chain (one of two types of protein chains that make up hemoglobin) is replaced by a valine.

A person whose body makes only sickle cell hemoglobin will suffer symptoms of sickle cell anemia. These occur because the glutamic acid-to-valine amino acid change makes the hemoglobin molecules assemble into long fibers. The fibers distort disc-shaped red blood cells into crescent shapes. Examples of "sickled" cells can be seen mixed with normal, disc-like cells in the blood sample below.

The sickled cells get stuck as they try to pass through blood vessels. The stuck cells impair blood flow and can cause serious health problems for people with sickle cell anemia, including breathlessness, dizziness, headaches, and abdominal pain.

Sickle cell anemia is an inherited red blood cell disorder in which there aren't enough healthy red blood cells to carry oxygen throughout your body. Normally, the flexible, round red blood cells move easily through blood vessels.

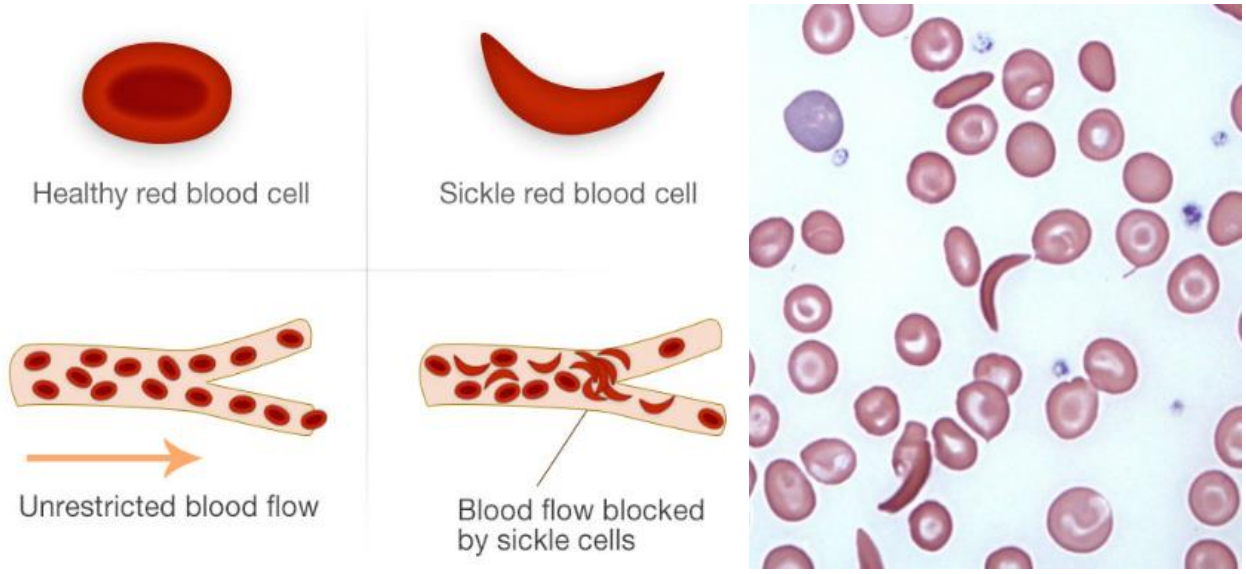

Figure 4.9. Sickle Cell Disease

Secondary structure

Stretches or strands of proteins or peptides have distinct, characteristic local structural conformations, or secondary structure, dependent on hydrogen bonding.

Several types of secondary structure are particularly stable and occur widely in proteins. The two main types of secondary structure are the α-helix and the ß-sheet **(Fig. 4.10.)**. Using fundamental chemical principles and a few experimental observations, Linus Pauling and Robert Corey predicted the existence of these secondary structures in 1951, several years before the first complete protein structure was elucidated.

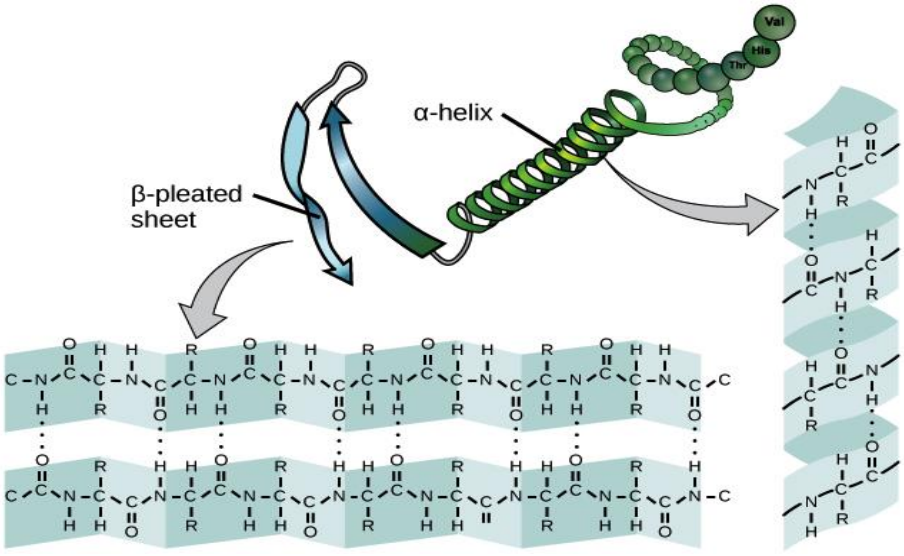

Figure 4.10. The two main types of secondary structure - the α-helix and the ß-sheet

In considering secondary structure, it is useful to classify proteins into two major groups: fibrous proteins, having polypeptide chains arranged in long strands or sheets, and globular proteins, with polypeptide chains folded into a spherical or globular shape **(Fig 4.11.)**.

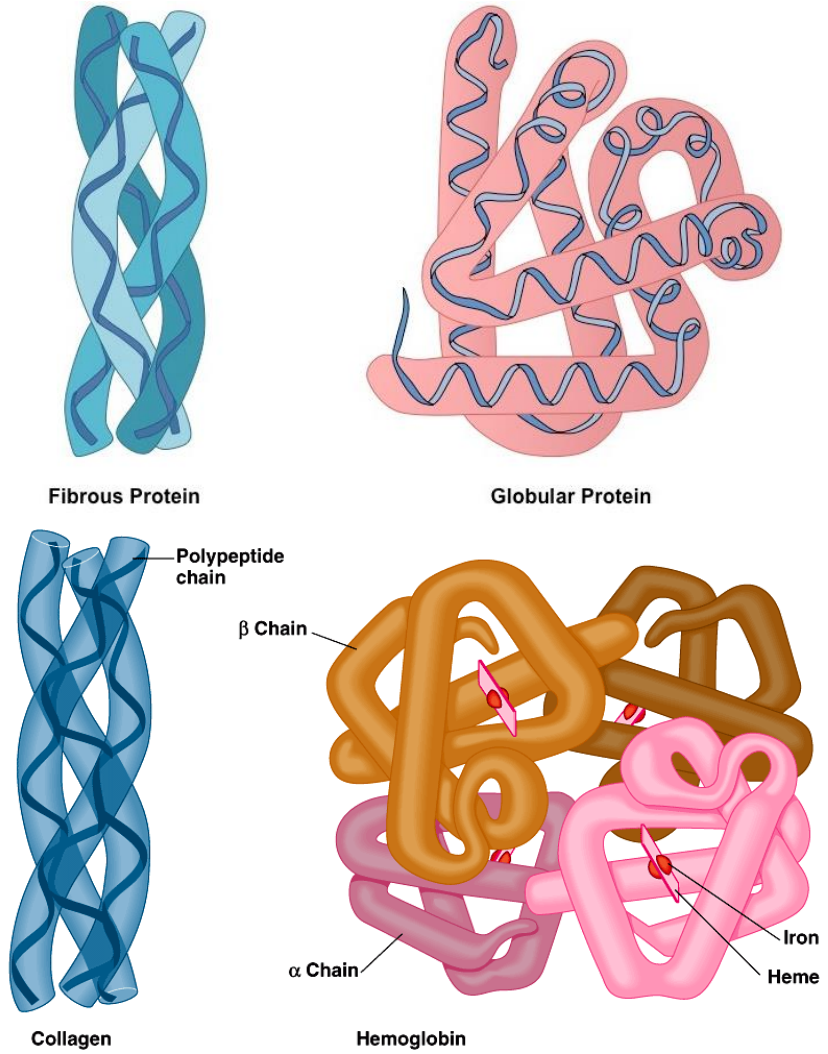

Figure 4.11. Two major groups fibrous and globular proteins: Collagen is fibrous and hemoglobin is globular protein.

Fibrous proteins play important structural roles in the anatomy and physiology of vertebrates, providing external protection, support, shape, and form. They may constitute one-half or more of the total body protein in larger animals. Most enzymes and peptide hormones are globular proteins. Globular proteins tend to be structurally complex, often containing several types of secondary structure; fibrous proteins usually consist largely of a single type of secondary structure. Because of this structural simplicity, certain fibrous proteins played a key role in the development of the modern understanding of protein structure and provide particularly clear examples of the relationship between structure and function; they are considered in some detail after the general discussion of secondary structure.

The simplest arrangement the polypeptide chain could assume with its rigid peptide bonds (but with the other single bonds free to rotate) is a helical structure, which Pauling and Corey called the α helix. In this structure the polypeptide backbone is tightly wound around the long axis of the molecule, and the R groups of the amino acid residues protrude outward from the helical backbone. The repeating unit is a single turn of the helix, which extends about 0.56 nm along the long axis, corresponding closely to the

periodicity. The twisting of the helix has a right-handed sense in the most common form of the α helix, although a very few left-handed variants have been observed.

The α-helix is a right-handed coiled strand. The side-chain substituents of the amino acid groups in an α-helix extend to the outside. Hydrogen bonds form between the oxygen of each C=O bond in the strand and the hydrogen of each N-H group four amino acids below it in the helix. The hydrogen bonds make this structure especially stable. The side-chain substituents of the amino acids fit in beside the N-H groups.

The α helix is one of two prominent types of secondary structure in proteins. It is the predominant structure in α-keratins. In globular proteins, about one-fourth of all amino acid residues are found in α helices, the fraction varying greatly from one protein to the next.

Why does such a helix form more readily than many other possible conformations? The answer is, in part, that it makes optimal use of internal hydrogen bonds. The structure is stabilized by a hydrogen bond between the hydrogen atom attached to the electronegative nitrogen atom of each peptide linkage and the electronegative carbonyl oxygen atom of the fourth amino acid on the amino-terminal side of it in the helix. Every peptide bond of the chain participates in such hydrogen bonding. Each successive coil of the α helix is held to the adjacent coils by several hydrogen bonds, which in summation give the entire structure considerable stability.

The α helix and the β conformation are the major repetitive secondary structures easily recognized in a wide variety of proteins. Other repetitive structures exist, often in only one or a few specialized proteins. An example is the collagen helix. One other type of secondary structure is common enough to deserve special mention. This is a β bend or β turn, often found where a polypeptide chain abruptly reverses direction. (These turns often connect the ends of two adjacent segments of an antiparallel β pleated sheet, hence the name.)

The hydrogen bonding in a ß-sheet is between strands (inter-strand) rather than within strands (intra-strand). The sheet conformation consists of pairs of strands lying side-by-side. The carbonyl oxygens in one strand bonds with the amino hydrogens of the adjacent strand. The two strands can be either parallel or anti-parallel depending on whether the strand directions (N-terminus to C-terminus) are the same or opposite. The anti-parallel ß-sheet is more stable due to the more well-aligned hydrogen bonds.

α-Keratin, collagen, and elastin provide clear examples of the relationship between protein structure and biological function. These proteins share properties that give strength and/or elasticity to structures in which they occur. They have relatively simple structures, and all are insoluble in water, a property conferred by a high concentration of hydrophobic amino acids both in the interior of the protein and on the surface. These proteins represent an exception to the rule that hydrophobic groups must be buried. The hydrophobic core of the molecule therefore contributes less to structural stability, and covalent bonds assume an especially important role. α-Keratin and collagen **(Fig. 4.12.)** have evolved for strength. In

vertebrates, α-keratins constitute almost the entire dry weight of hair, wool, feathers, nails, claws, quills, scales, horns, hooves, tortoise shell, and much of the outer layer of skin. Collagen is found in connective tissue such as tendons, cartilage, the organic matrix of bones, and the cornea of the eye. The food product gelatin is derived from collagen. Although it is protein, it has little nutritional value because collagen lacks significant amounts of many amino acids that are essential in the human diet.

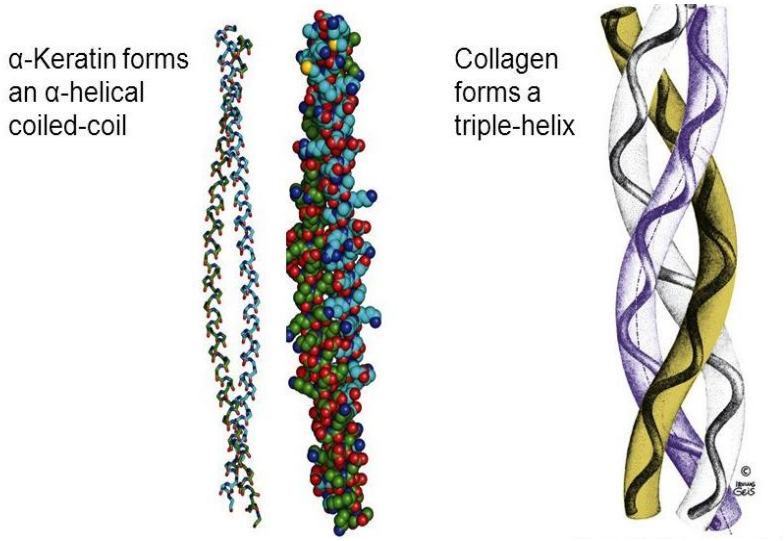

Figure 4.12. The secondary structure α-Keratin and collagen

In both α-keratin and collagen, strength is amplified by wrapping multiple helical strands together in a superhelix, much the way strings are twisted to make a strong rope.

The tight wrapping of the collagen triple helix provides great tensile strength with no capacity to stretch: Collagen fibers can support up to 10,000 times their own weight and are said to have greater tensile strength than a steel wire of equal cross section.

The strength of these structures is also enhanced by covalent cross-links between polypeptide chains within the multi-helical "ropes" and between adjacent ones. In α-keratin, the cross-links are contributed by disulfide bonds. In the hardest and toughest α-keratins, such as those of tortoise shells and rhinoceros horns, up to 18% of the residues are cysteines involved in disulfide bonds. The arrangement of α-keratin to form a hair fiber is shown in. In collagen, the cross-links are contributed by an unusual type of covalent link between two Lys residues that creates a nonstandard amino acid residue called lysin or leucine, found only in certain fibrous proteins.

Collagen fibrils consist of recurring three-stranded polypeptide units called tropocollagen, arranged head to tail in parallel bundles. The rigid, brittle character of the connective tissue in older people is the result of an accumulation of covalent cross-links in collagen as we age.

Human genetic defects involving collagen illustrate the close relationship between amino acid sequence and three-dimensional structure in this protein. Osteogenesis imperfecta results in abnormal bone formation in human babies. Ehlers-Danlos syndrome is characterized by loose joints. Both can be lethal

and both result from the substitution of a Cys or Ser residue, respectively, for a Gly (a different Gly residue in each case) in the amino acid sequence of collagen.

Although fibrous proteins typically have only one type of secondary structure, globular proteins may include several types of secondary structure in the same molecule. Globular proteins, including enzymes, transport proteins, some peptide hormones, and immunoglobulins, are folded structures that are much more compact than α or β conformations.

The three-dimensional arrangement of all atoms in a protein is called the tertiary structure, and this is now becoming our focus. While the secondary structure of polypeptide chains is determined by the short-term structural relationships of amino acid residues, the tertiary structure is provided by aspects of the amino acid sequence with a longer range of action. Amino acids that are far apart in the polypeptide sequence and contain different types of secondary structure can interact when the protein is formed. The formation of bends in the polypeptide chain during folding and the direction and angle of these bends are determined by the number and location of certain amino acids that produce bends, such as residues Pro, Thr, Ser and Gly. Moreover, the loops of a strongly composed polypeptide chain are held in their characteristic tertiary positions by various types of interactions of weak bonds (and sometimes covalent bonds, such as disulfide cross-links) between R groups of adjacent loops.

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Secondary structures promote tertiary folding of a polypeptide chain in a globular protein, and this structure is stabilized by weak interactions, including hydrophobic interactions involving nonpolar amino acid side chains in a tightly packed protein nucleus.

Tertiary structure

The overall three-dimensional shape of a protein molecule is the tertiary structure. The protein molecule will bend and twist in such a way as to achieve maximum stability or lowest energy state. Although the three-dimensional shape of a protein may seem irregular and random, it is fashioned by many stabilizing forces due to bonding interactions between the side-chain groups of the amino acids.

Under physiologic conditions, the hydrophobic side-chains of neutral, non-polar amino acids such as phenylalanine or isoleucine tend to be buried on the interior of the protein molecule, thereby shielding them from the aqueous medium. The alkyl groups of alanine, valine, leucine and isoleucine often form

hydrophobic interactions between one another, while aromatic groups such as those of phenylalanine and tyrosine often stack together. Acidic or basic amino acid side-chains will generally be exposed on the surface of the protein as they are hydrophilic.

The formation of disulfide bridges by oxidation of the sulfhydryl groups on cysteine is an important aspect of the stabilization of protein tertiary structure, allowing different parts of the protein chain to be held together covalently. Additionally, hydrogen bonds may form between different side-chain groups. As with disulfide bridges, these hydrogen bonds can bring together two parts of a chain that are some distance away in terms of sequence. Salt bridges, ionic inter- actions between positively and negatively charged sites on amino acid side chains, also help to stabilize the tertiary structure of a protein **(Fig. 4.13.)**.

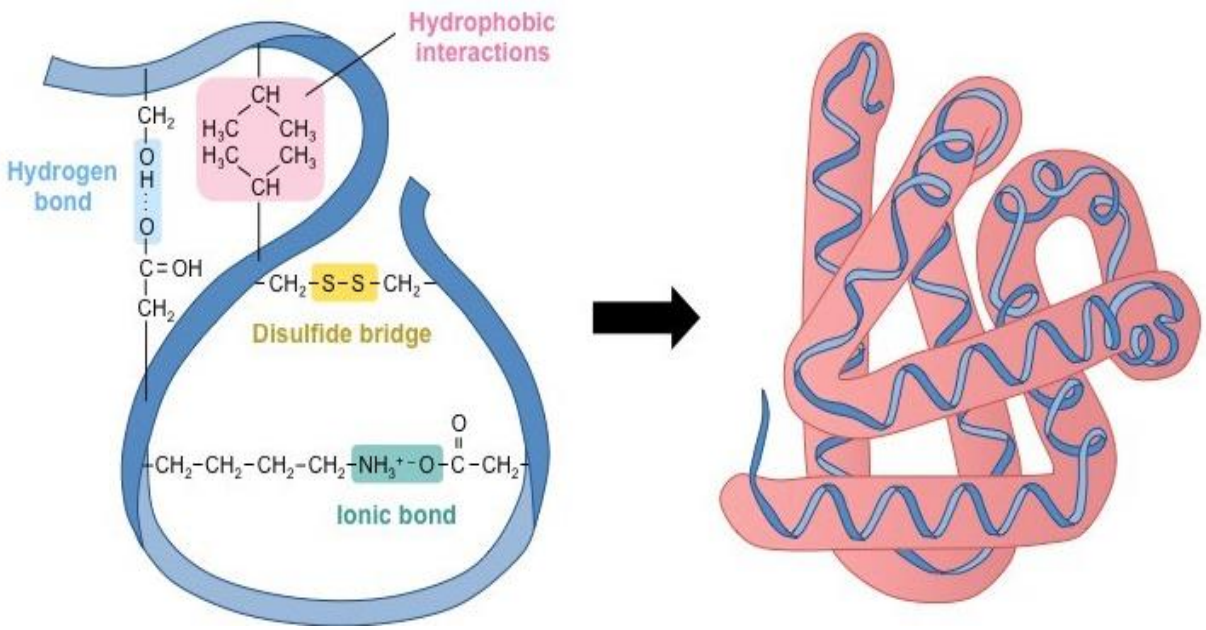

Figure 4.13. Tertiary structure: types of interaction

The breakthrough in understanding globular protein structure came from x-ray diffraction studies of the protein myoglobin **(Fig 4.14.)** carried out by John Kendrew and his colleagues in the 1950s. Myoglobin is a relatively small (Mr 16,700), oxygen-binding protein of muscle cells that functions in the storage and transport of oxygen for mitochondrial oxidation of cell nutrients. Myoglobin contains a single polypeptide chain of 153 amino acid residues of known sequence and a single iron porphyrin, or heme, group, identical to that of hemoglobin, the oxygen-binding protein of erythrocytes. The heme group is responsible for the deep red-brown color of both myoglobin and hemoglobin. Myoglobin is particularly abundant in the muscles of diving mammals such as the whale, seal, and porpoise, whose muscles are so rich in this protein that they are brown. Storage of oxygen by muscle myoglobin permits these animals to remain submerged for long periods of time.

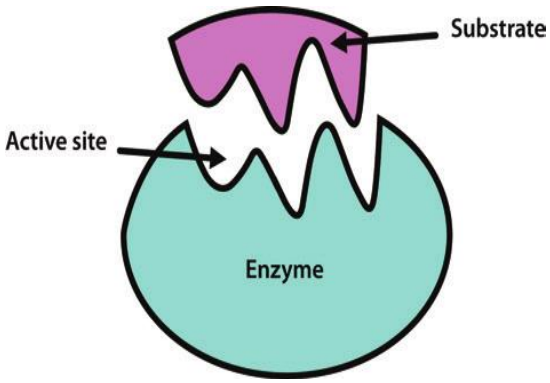

Figure 5.2. Enzyme and the active site

Studies on enzyme specificity carried out by Emil Fischer led him to propose, in 1894, that enzymes were structurally complementary to their substrates, so that they fit together like a "lock and key"**(Fig. 5.3.)**. However, the "lock and key" hypothesis can be misleading when applied to the question of enzymatic catalysis. An enzyme completely complementary to its substrate would be a very poor enzyme. The modern notion of enzymatic catalysis was first proposed by Haldane in 1930, and elaborated by Linus Pauling in 1946. In order to catalyze reactions, an enzyme must be complementary to the reaction transition state. This means that the optimal interactions (through weak bonding) between substrate and enzyme can occur only in the transition state.

Enzyme-catalyzed reactions occur in at least two steps. In the first step, an enzyme molecule (E) and the substrate molecule or molecules (S) collide and react to form an intermediate compound called the enzyme-substrate (E–S) complex. (This step is reversible because the complex can break apart into the original substrate or substrates and the free enzyme.)

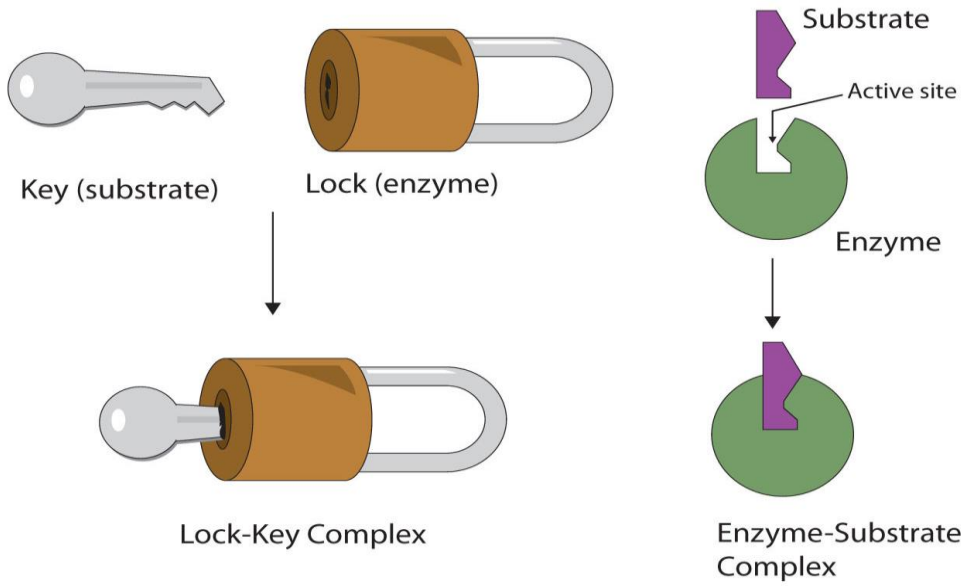

Figure 5.3. The lock and key model of enzyme activity

Once the E–S complex forms, the enzyme is able to catalyze the formation of product (P), which is then released from the enzyme surface:

$$S + E \rightarrow E–S$$

$$E–S \rightarrow P + E$$

The structural changes that occur when an enzyme and a substrate join together bring specific parts of a substrate into alignment with specific parts of the enzyme's active site. Amino acid side chains in or near the binding site can then act as acid or base catalysts, provide binding sites for the transfer of functional groups from one substrate to another or aid in the rearrangement of a substrate. The participating amino acids, which are usually widely separated in the primary sequence of the protein, are brought close together in the active site as a result of the folding and bending of the polypeptide chain or chains when the protein acquires its tertiary and quaternary structure. Binding to enzymes brings reactants close to each other and aligns them properly, which has the same effect as increasing the concentration of the reacting compounds.

To ensure that the body's systems work correctly, sometimes enzymes need to be slowed down. For instance, if an enzyme is making too much of a product, there needs to be a way to reduce or stop production.

Enzymes' activity can be inhibited in a number of ways:

- Competitive inhibitors – a molecule blocks the active site so that the substrate has to compete with the inhibitor to attach to the enzyme.
- Non-competitive inhibitors – a molecule binds to an enzyme somewhere other than the active site and reduces how effectively it works.
- Uncompetitive inhibitors – the inhibitor binds to the enzyme and substrate after they have bound to each other. The products leave the active site less easily, and the reaction is slowed down.
- Irreversible inhibitors – an irreversible inhibitor binds to an enzyme and permanently inactivates it.

There are thousands of enzymes in the human body, here are just a few examples:

- ❖ Lipases – a group of enzymes that help digest fats in the gut.
- ❖ Amylase – helps change starches into sugars. Amylase is found in saliva.
- ❖ Maltase – also found in saliva; breaks the sugar maltose into glucose. Maltose is found in foods such as potatoes, pasta, and beer.
- ❖ Trypsin – found in the small intestine, breaks proteins down into amino acids.
- ❖ Lactase – also found in the small intestine, breaks lactose, the sugar in milk, into glucose and galactose.
- ❖ Acetylcholinesterase – breaks down the neurotransmitter acetylcholine in nerves and muscles.
- ❖ Helicase – unravels DNA.

❖ DNA polymerase – synthesize DNA from deoxyribonucleotides.

In the absence of an enzyme, biochemical reactions hardly proceed at all, whereas in its presence the rate can be increased up to 107-fold. Thus, they are crucial for normal metabolism of living systems. Besides in the body, extracted and purified enzymes have many applications.

Medical applications of enzymes include:

To treat enzyme related disorders. To assist in metabolism. To assist in drug delivery. To diagnose & detect diseases. In manufacture of medicines.

Industrial applications of enzymes include:

Amylase, lactases, cellulases are enzymes used to break complex sugars into simple sugars. Pectinase like enzymes which act on hard pectin is used in fruit juice manufacture. Lipase enzymes act on lipids to break them in fatty acids and glycerol. Lipases are used to remove stains of grease, oils, butter.

Enzymes are used in detergents and washing soaps. Protease enzymes are used to remove stains of protein nature like blood, sweat etc.

Hundreds of enzymes have been purified and studied in an effort to understand how they work so effectively and with such specificity. The resulting knowledge has been used to design drugs that inhibit or activate particular enzymes. An example is the intensive research to improve the treatment of or find a cure for acquired immunodeficiency syndrome (AIDS). AIDS is caused by the human immunodeficiency virus (HIV). Researchers are studying the enzymes produced by this virus and are developing drugs intended to block the action of those enzymes without interfering with enzymes produced by the human body. Several of these drugs have now been approved for use by AIDS patients.

The first enzymes to be discovered were named according to their source or method of discovery. The enzyme pepsin, which aids in the hydrolysis of proteins, is found in the digestive juices of the stomach (Greek pepsis, meaning “digestion”). Papain, another enzyme that hydrolyzes protein (in fact, it is used in meat tenderizers), is isolated from papayas. As more enzymes were discovered, chemists recognized the need for a more systematic and chemically informative identification scheme.

Penicillin **(Fig 5.5.)**, one of the most widely used antibiotics in the world, was fortuitously discovered by Alexander Fleming **(Fig. 5.4.)** in 1928, when he noticed antibacterial properties in a mold growing on a bacterial culture plate. In 1938, Ernst Chain and Howard Florey began an intensive effort to isolate penicillin from the mold and study its properties. The large quantities of penicillin needed for this research became available through development of a corn-based nutrient medium that the mold loved and through the discovery of a higher-yielding strain of mold at a United States Department of Agriculture

research center near Peoria, Illinois. Even so, it was not until 1944 that large quantities of penicillin were being produced and made available for the treatment of bacterial infections.

Penicillin functions by interfering with the synthesis of cell walls of reproducing bacteria. It does so by inhibiting an enzyme—transpeptidase—that catalyzes the last step in bacterial cell-wall biosynthesis. The defective walls cause bacterial cells to burst. Human cells are not affected because they have cell membranes, not cell walls.

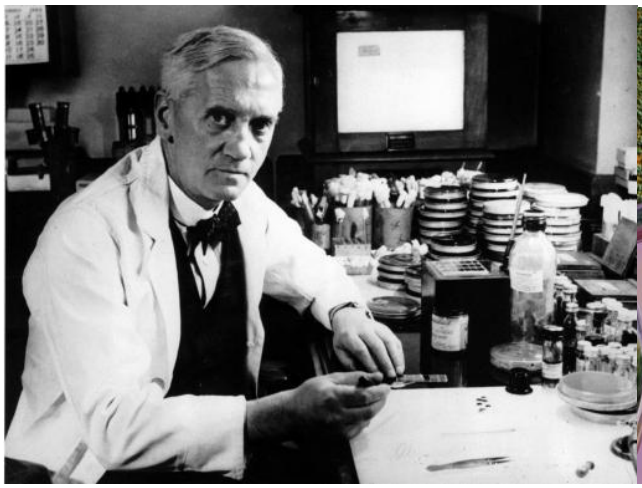

Figure 5.4. Alexander Fleming

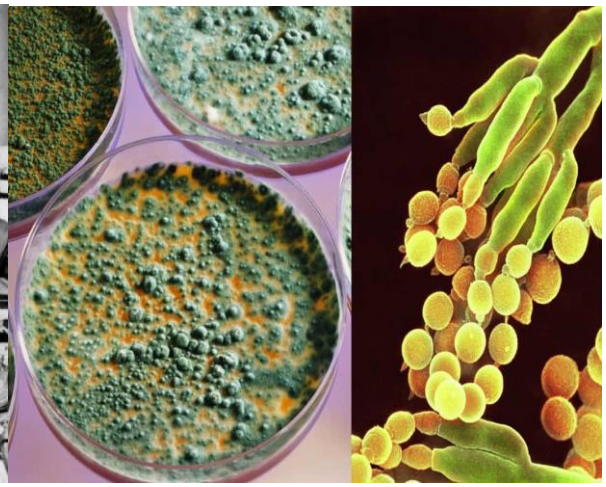

Figure 5.5. Penicillin

Self-assessment tasks

1. A prosthetic group is

a) holoenzyme that is covalently bound to the enzyme protein; b) a coenzyme or metal ion that is covalently bound to the enzyme protein; c) a coenzyme or metal ion that is covalently bound to the lipid; d) lipid that is covalently bound to the enzyme protein.

2. Holoenzyme is

a) active enzyme together with its coenzyme; b) passive enzyme together with its coenzyme;

c) active lipid together with its coenzyme; d) active passive together with its coenzyme.

3. The active site is

a) the place where cycling hormones; b) the place where cycling enzymes; c) the place where hydrolyzation of nucleic acid occurs; d) the place where enzyme-catalyzed reaction occurs.

4. What is the function of enzymes within living systems?

a) structural elements; b) neurotransmitters; c) catalysts; d) hormones.

5. Which of these do not affect enzyme activity?

a) temperature; b) competitive inhibitors; c) pH; d) carbohydrates.

6. An enzyme is primarily made of:

a) carbohydrate; b) protein; c) nucleic acid; d) lipid.

7. Coenzyme is

a) Fe2+, Mg2+, Mn2+; b) Cu2+, K+, Mn2+; c) a complex organic or metallo organic molecule; d) amino acid.

8. Who firstly studied that enzymes were structurally complementary to their substrates, so that they fit together like a key and lock?

a) Emil Fischer; b) Rosalind Elsie Franklin ; c) Ivan Horbachevsky; d) James Watson

9. Describe what an enzyme is made of?

10. What is the "active site" of an enzyme, and how is it critical in how an enzyme functions?

11. What is a coenzyme and what is the difference between a coenzyme and a cofactor?

12. What is an inhibitor?

Chapter № 6. Vitamins.

Vitamins may be defined as organic compounds occur-ring in small quantities in different natural foods and necessary for growth and maintenance of good health in human beings. Vitamins are essential food factors, which are required for the proper utilization of the proximate principles of food like carbohydrates, lipids and proteins. “A vitamin is a substance that makes you ill if you don’t eat it” (Albert Szent-Györgyi, Nobel Prize winner, 1937) Discovery of vitamins started from observation of deficiency manifestations, e.g. scurvy, rickets, beriberi, etc. The term “vitamine” was coined from the words vital + amine, since the earlier identified ones had amino groups. Later work showed that most of them did not contain amino groups, so the last letter ‘e' was dropped in the modern term of vitamin. The vitamins are mainly classified into two:

1. The fat soluble vitamins are A, D, E and K
2. Water soluble vitamins are named as B complex and C.

Vitamins are required to perform specific cellular functions, for example, many of the water-soluble vitamins are precursors of coenzymes for the enzymes of intermediary metabolism. In contrast to the water-soluble vitamins, only one fat soluble vitamin (vitamin K) has a coenzyme function. These vitamins are released, absorbed, and transported with the fat of the diet. They are not readily excreted in the urine, and significant quantities are stored in the liver and adipose tissue.

Vitamin A

McCollum, Simmonds and Kennedy isolated vitamin A in 1913. Richard Kuhn (Nobel Prize, 1938) identified arotenes. Paul Karrer in 1931 elucidated the structure of vitamin A1 (Nobel Prize, 1937).

Vitamin A (retinol) is a pigment essential to vision. It was first recognized as an essential nutritional factor for laboratory animals, and was later isolated from fish liver oils.

The active form is present only in animal tissues. The provitamin, beta-carotene is present in plant tissues. Vitamin A itself does not occur in plants, but many plants contain carotenoids, light absorbing pigments that can be enzymatically converted into vitamin A by most animals. **Fig. 6.1.** shows, for example, how vitamin A can be formed by cleavage of, β-carotene, the pigment that gives carrots, sweet potatoes, and other yellow vegetables their characteristic color. Deficiency of vitamin A leads to a variety of symptoms in humans and experimental animals, which include dry skin, xerophthalmia (dry eyes), dry mucous membranes, retarded development and growth, sterility in male animals, and night blindness, an early symptom commonly used in the medical diagnosis of vitamin A deficiency.

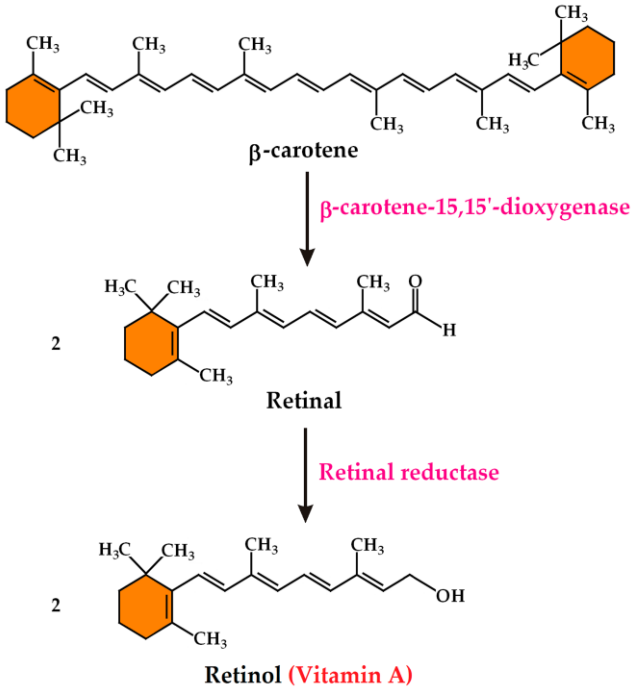

Figure 6.1. Structure of vitamin A

Beta-carotene has two beta ionone rings **(Fig. 6.1.)**. All the compounds with vitamin A activity are referred to as retinoids. They have a beta-ionone (cyclohexyl) ring system. Three different compounds with vitamin A activity are retinol (vitamin A alcohol), retinal (vitamin A aldehyde) and retinoic acid (vitamin A acid). The retinal may be reduced to retinol by retinal reductase. This reaction is readily reversible. Retinal is oxidized to retinoic acid, which cannot be converted back to the other forms **(Fig. 6.2.)**. Biologically important compound is 11-cis-retinal. Beta carotene is cleaved by a di-oxygenase, to form retinal. The retinal is reduced to retinol by retinal reductase present in the intestinal mucosa. Intestine is the major site of absorption. The absorption is along with other fats and requires bile salts. Vitamin is incorporated into chylomicrons and transported to the liver. In the liver, vitamin is stored as retinol palmitate.

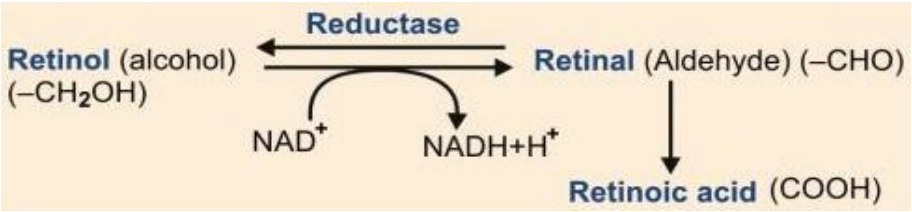

Figure 6.2. Interconversion of vitamin A molecules

The vitamin A from the liver is transported to peripheral tis-sues as trans-retinol by the retinol binding protein or RBP. In the case of vitamin A deficiency, that RBP level in blood falls The retinol-RBP complex binds to specific receptors on the retina, skin, gonads and other tissues. Vitamin binds to cellular

preterm infants be given prophy-lactic doses of vitamin K (1 mg Menadione). In children and adults, Vitamin K deficiency may be manifested as bruising tendency, ecchymotic patches, mucous membrane hemorrhage, post-traumatic bleeding and internal bleeding. Prolongation of prothrombin time and delayed clotting time are characteristic of vita-min K deficiency.

The vitamin acts in the formation of prothrombin, a blood plasma protein essential in blood-clot formation. Prothrombin is a proteolytic enzyme that splits specific peptide bonds in the blood protein fibrinogen, converting it to fibrin, the insoluble, fibrous protein that holds blood clots together. Deficiency of vitamin K results in slowed blood clotting, which can be fatal to a wounded animal. Henrik Dam and Edward A. Doisy are given credit for having independently discovered the antihemorrhagic action of vitamin K. Warfarin is a synthetic analog of vitamin K, which acts as a competitive inhibitor of prothrombin formation.

Sources of vitamin K are green leafy vegetables are good dietary sources. Even if the diet does not contain the vitamin, intestinal bacterial synthesis will meet the daily requirements, as long as absorption is normal. Hemolysis, hyperbilirubinemia, kernicterus and brain damage are the manifestations of hypervitaminosis.

Next vitamins are classified as water-soluble.

Vitamin C

A description of scurvy was found in the Ebers papyrus written in 1500 B.C. in Egypt. During the voyage of Vasco da Gama, around the Cape of Good Hope to India in 1498, he lost two-thirds of the crew due to scurvy. The French explorer, Jacques Cartier, in 1536, during the voyages to discover eastern parts of Canada, was laid up with scurvy. A friendly native gave an extract from the leaves of the spruce tree, which produced a remarkable cure for scurvy **(Fig. 6.10.)**. James Lind published ‘Treatise on Scurvy', in 1753. These observations led to compulsory rationing of lime or lemon juice to all the crew of the British Royal Navy from 1795 onwards. So the British sailors were nicknamed as “Limeys”. However, it helped to eliminate scurvy from the British Navy, while opponents continued to suffer. No wonder, in course of time, Britain had the colonies in which the sun never set. Zilva and associates, in 1928, showed that the antiscorbutic factor present in lemon juice is a reducing substance. The factor was isolated in 1930 and named as “Hexuronic acid” by Albert Szent-Gyorgi (Nobel prize, 1937). In 1933, Haworth established the molecular structure. He renamed it as ascorbic acid (Nobel prize, 1937).

Figure 6.10. The history of scurvy

Vitamin C (ascorbic acid) is a crystalline solid which is soluble in water and is easily destroyed by heat, alkali and storage. In the process of cooking, 70% of vitamin C is lost. The structural formula of ascorbic acid closely resembles that of carbohydrates. The strong reducing property of vitamin C depends on the double-bonded (enediol) carbons. Only L-ascorbic acid and dehydroascorbic acid have antiscorbutic activity. The D-ascorbic acid has no activity.

Most animals and plants synthesize ascorbic acid from glucose; however, primates and humans cannot synthesize ascorbic acid lack gluconolactone oxidase, a key enzyme in the final step of ascorbate synthesis. They lack the genes responsible for the synthesis of this enzyme. Thus, in these species, the daily requirements of vitamin C should come from the diet. Oranges, lemons, grapefruit, leafy green vegetables and beef liver are the best sources of vitamin C.

The active form of vitamin C is ascorbic acid. Vitamin C is needed to form collagen that gives strength to the connective tissues and required for wound healing and normal immune function. Vitamin C defciency causes changes in the connective, leading to the development of scurvy, a disease in which the synthesized collagen is unstable. The symptoms of scurvy include muscle pain, joint swelling, and bleeding. Vitamin C acts as an antioxidant and free oxygen radical scavenger and can be used topically in skin disorders, including those caused by photo-aging. The hyperpigmentation of the skin can be treated with vitamin C, since it inhibits the activity of melanocytes, i.e. the cells involved in the synthesis of melanin.

Ascorbic acid is readily absorbed from the gastrointestinal tract. The vitamin is excreted in the urine. Oxidation of ascorbic acid yields dehydroascorbic acid, which is oxidized further to oxalic acid through dike to-Lgulonic acid **(Fig. 6.11)**.

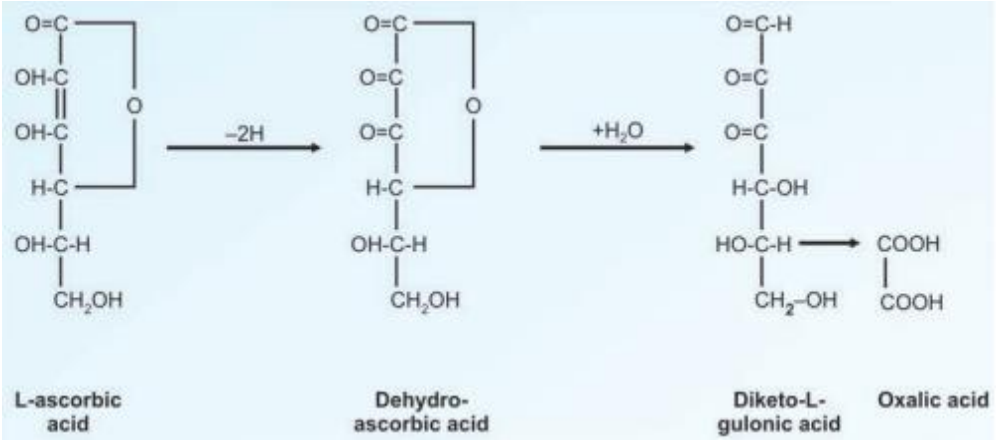

Figure 6.11. Vitamin C: Structure and catabolism

Ascorbic acid is partly excreted unchanged and partly as oxalic acid. Most of the oxalates in urine are derived from ascorbic acid and the rest from glycine metabolism.

A very high concentration of vitamin C is observed locally in healing wounds. Vitamin C is essential for wound healing. Ascorbic acid stimulates phagocytic action of leukocytes and helps in the formation of antibodies.

As an anti-oxidant, it may prevent cancer formation. Aniline dyes are known to induce bladder cancer in factory workers. Daily intake of vitamin C reduces this risk for cancer. Cataract Vitamin C is concentrated in the lens of eye. Regular intake of ascorbic acid reduces the risk of cataract formation.

Deficiency Manifestations of Vitamin C

1. Scurvy.

2. Infantile scurvy (Barlow's disease) In infants between 6 to 12 months of age, (the period in which weaning from breast milk), the diet should be supplemented with vitamin C sources.

3. Hemorrhagic tendency In ascorbic acid deficiency, collagen is abnormal and the intercellular cement substance is brittle. So capillaries are fragile, leading to the tendency to bleed even under minor pressure.

4. Internal hemorrhage In severe cases, hemorrhage may occur in the conjunctiva and retina.

5. Oral cavity In severe cases of scurvy, the gum becomes painful, swollen, and spongy **(Fig. 6.12.)**. The pulp is separated from the dentine and finally, teeth are lost.

6. Bones In the bones, the deficiency results in the failure of the osteoblasts to form the intercellular substance, osteoid.

7. Anemia.

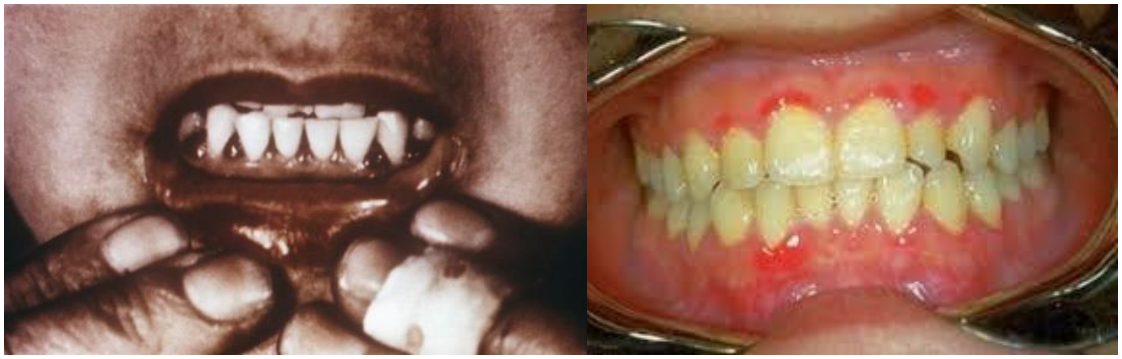

Figure 6.12. Hemorrhage and swollen gums of a patient with scurvy

Thiamine

Thiamine is also called as vitamin B1. In old literature, it is designated as Aneurine (it can relieve neuritis) or antiberiberi factor. In 1900, Christian Eijkman produced beriberi in chicken by feeding polished rice (Nobel Prize, 1929). Adolf Windaus (Nobel prize, 1928) elucidated the structure of the vitamin.

Aleurone layer of cereals (food grains) is a rich source of thiamine. Therefore whole wheat flour and unpolished hand-pound rice have better nutritive value than completely polished refined foods. When the grains are polished, aleurone layer is usually removed. Yeast is also a very good source. Thiamine is partially destroyed by heat.

Thiamine contains a substituted pyrimidine ring connected to a substituted thiazole ring by means of methylene bridge. The vitamin is then converted to its active coenzyme form by addition of two phosphate groups, with the help of ATP **(Fig. 6.13.)**. It is catalyzed by thiamine pyrophosphotransferase.

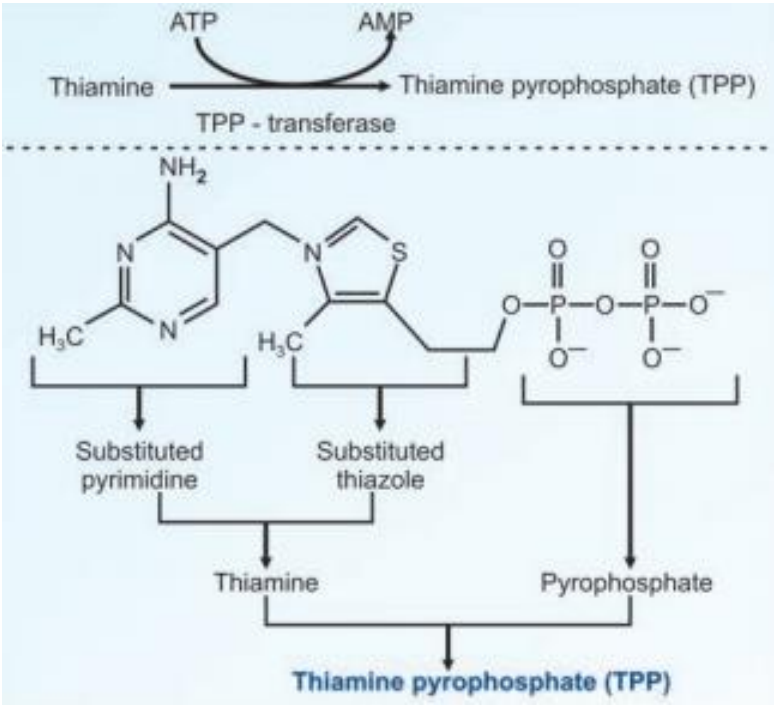

Figure 6.13. Structure of thiamine pyrophosphate

Thiamine contains a substituted pyrimidine ring connected to a substituted thiazole ring by means of methylene bridge. The vitamin is then converted to its active co-enzyme form by addition of two phosphate groups, with the help of ATP. It is catalyzed by thiamine pyrophosphotransferase.

The main role of thiamine (TPP) is in carbohydrate metabolism. So, the requirement of thiamine is increased along with higher intake of carbohydrates.

Deficiency Manifestations of Thiamine

Beriberi: Deficiency of thiamine leads to beriberi. It is a Singhalese word, meaning "weakness". The early symptoms are anorexia, dyspepsia, heaviness and weakness. Subjects feel weak and get easily exhausted.

Wet beriberi: Here cardiovascular manifestations are prominent. Edema of legs, face, trunk and serous cavities are the main features. Palpitation, breathlessness and distended neck veins are observed. Death occurs due to heart failure.

Dry beriberi: In this condition, CNS manifestations are the major features. Walking becomes difficult. Peripheral neuritis with sensory disturbance leads to complete paralysis.

Infantile beriberi: It occurs in infants born to mothers suffering from thiamine deficiency. Restlessness and sleeplessness are observed.

Wernicke-Korsakoff syndrome: It is also called as cerebral beriberi. Carl Wernicke in 1894 and Sergiei Sergievich Korsakoff in 1887 described the condition. Clinical features are those of encephalopathy (ophthalmoplegia, nystagmus, cerebellar ataxia) plus psychosis. It is seen only when the nutritional status is severely affected.

Polyneuritis: It is common in chronic alcoholics.

Alcohol utilization needs large doses of thiamine. Alcohol inhibits intestinal absorption of thiamine, leading to thiamine deficiency.

Polyneuritis may also be associated with pregnancy and old age.

Such thiamine deficiency in alcoholism may cause impairment of conversion of pyruvate to acetyl CoA. This results in increased plasma concentration of pyruvate and lactate, leading to lactic acidosis.

Riboflavin

Lactoflavin (milk), hepatoflavin (liver) and ovoflavin (eggs) are chemically identical to riboflavin. Riboflavin was the first B complex component to be isolated in a pure state. This vitamin is synthesized by green plants and micro-organisms. Warburg (Nobel prize 1931), isolated the “yellow enzyme” of cellular respiration. Later Axel Theorell (Nobel prize, 1955) isolated riboflavin. In 1935, Paul Karrer (Nobel prize, 1937) determined the structure.

Riboflavin has a dimethyl isoalloxazine ring to which a ribitol is attached **(Fig. 6.14.)**. Ribitol is the alcohol of ribose sugar. Riboflavin is converted to its active coenzyme forms (FMN and FAD) with the help of ATP. Riboflavin is heat stable.

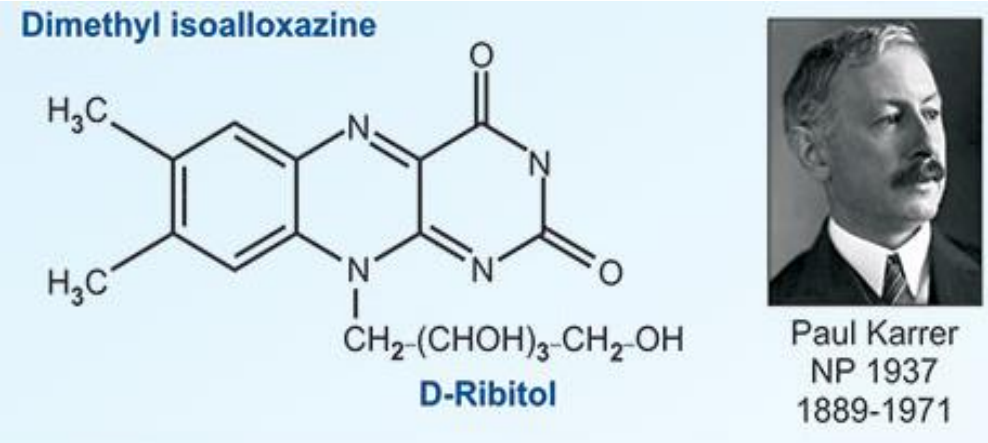

Figure 6.14. Riboflavin structure

Riboflavin (or lactoflavin) is a yellow crystalline substance. Riboflavin is found in grain, milk, eggs, liver, oats, and green verdant vegetables. It is involved in tissue respiration.

The two biologically active forms are flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), formed by the transfer of an adenosine monophosphate moiety from ATP to FMN. FMN and FAD are each capable of reversibly accepting two hydrogen atoms, forming $FMNH_2$ or $FADH_2$. FMN and FAD are bound tightly—sometimes covalently—to flavoenzymes that catalyze the oxidation or reduction of a substrate. Riboflavin deficiency is not associated with a major human disease, although it frequently accompanies other vitamin deficiencies. Its deficiency results in a condition known as ariboflavinosis. Ariboflavinosis is characterized by symptoms include dermatitis, cheilosis (fissuring at the corners of the mouth), and glossitis (the tongue appearing smooth and purplish).

Niacin (Nicotinic acid)

Niacin and Nicotinic acid are synonyms. It is a water-soluble vitamin essential to the human diet, but can be synthesized in the body from tryptophan. The term nicotinic acid should not be confused with nicotine. Nicotinic acid is a vitamin; but, nicotine is the potent poison from tobacco. Niacinamide is the active form of the vitamin, present in tissues. Warburg (Nobel prize, 1931) elucidated the structure of NAD+, and Alexander Todd (Nobel prize, 1957) demonstrated its function. It was originally named as co-enzyme-I, which was later designated as DPN (diphosphopyridine nucleotide), and finally in 1965 as NAD+.

Niacin is pyridine-3-carboxylic acid. Niacinamide is the acid amide **(Fig. 6.15.)**. In NAD+ or NADP+, the reactive site is the carbon atom 4 and the nitrogen atom of the nicotinamide ring.

Niacin, or nicotinic acid, is a substituted pyridine derivative. The biologically active coenzyme forms are nicotinamide adenine dinucleotide (NAD+) and its phosphorylated derivative, nicotinamide adenine dinucleotide phosphate (NADP+). Nicotinamide, a derivative of nicotinic acid that contains an amide instead of a carboxyl group, also occurs in the diet. Nicotinamide is readily deaminated in the body and, therefore, is nutritionally equivalent to nicotinic acid. NAD+ and NADP+ serve as coenzymes in

oxidation-reduction reactions in which the coenzyme undergoes reduction of the pyridine ring by accepting a hydride ion.

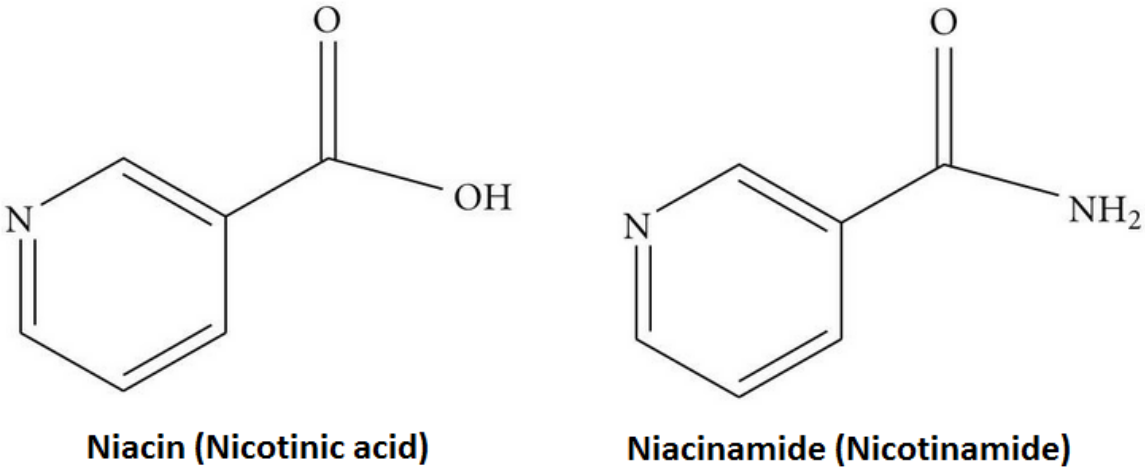

Figure 6.15. Structure of niacin

Niacin has lipid-lowering effects and can be used in the treatment of diabetes mellitus.

Deficiency of niacin leads to the clinical condition called pellagra **(Fig. 6.16.)**. Pellagra is an Italian word, meaning "rough skin". Pellagra is caused by the deficiency of Tryptophan as well as Niacin. Pellagra is seen more in women; this may be because tryptophan metabolism is inhibited by estrogen metabolites. The symptoms of pellagra are:

1) Dermatitis: In early stages, bright red erythema occurs, especially in the feet, ankles and face. Increased pigmentation around the neck is known as Casal's necklace **(Fig. 6.16.)**. The dermatitis is precipitated by exposure to sunlight.

2) Diarrhea: The diarrhea may be mild or severe with blood and mucus. This may lead to weight loss. Nausea and vomiting may also be present.

3) Dementia: It is frequently seen in chronic cases. Delerium is common in acute pellagra. Irritability, inability to concentrate and poor memory are more common in mild cases.

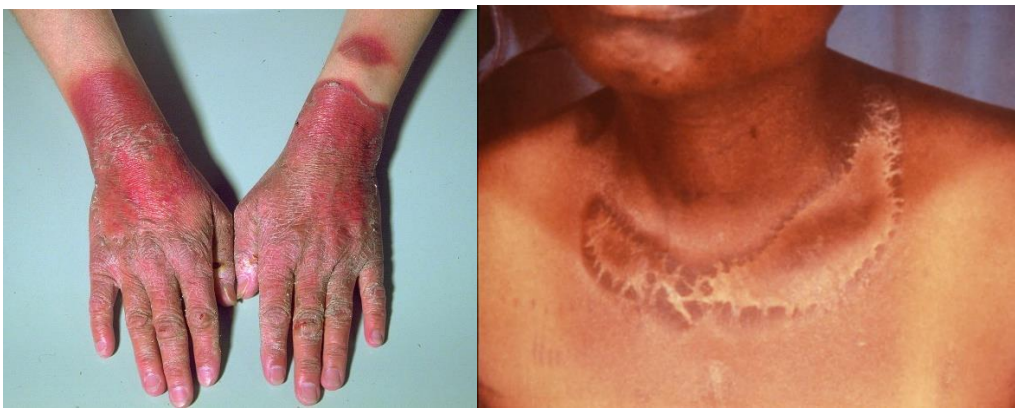

Figure 6.16. Pellagra. Casal's necklace

Pellagra is seen among people whose staple diet is maize (South and Central America). In maize, niacin is present; but it is in a bound form, and is unavailable. Pellagra is also seen when staple diet is sorghum (jowar or guinea corn) as in Central and Western India. Sorghum, contains leucine in high quantities. Leucine inhibits the QPRT enzyme, and so niacin cannot be converted to NAD+ (Leucine pellagra).

The richest natural sources of niacin are dried yeast, rice polishing, liver, peanut, whole cereals, legumes, meat and fish. About half of the requirement is met by the conversion of tryptophan to niacin.

Vitamin B6

Vitamin B6 is a collective term for pyridoxine, pyridoxal, and pyridox - amine, all derivatives of pyridine. They differ only in the nature of the functional group attached to the ring **(Figure 6.17.)**. Pyridoxine occurs primarily in plants, whereas pyridoxal and pyridoxamine are found in foods obtained from animals. All three compounds can serve as precursors of the biologically active coenzyme, pyridoxal phosphate. Active form of pyridoxine is pyridoxal phosphate (PLP). Pyridoxal phosphate functions as a coenzyme for a large number of enzymes, particularly those that catalyze reactions involving amino acids. It is synthesized by pyridoxal kinase, utilizing ATP. Main supply of B6 compounds in food is in the form of pyridoxine which can be readily converted to pyridoxal and pyridoxamine in the body. Richard Kuhn (Nobel prize 1938) did the isolation and structural analysis.

Pyridoxine is the only water-soluble vitamin with significant toxicity.

Neurologic symptoms (sensory neuropathy) occur at intakes above 200 mg/day.

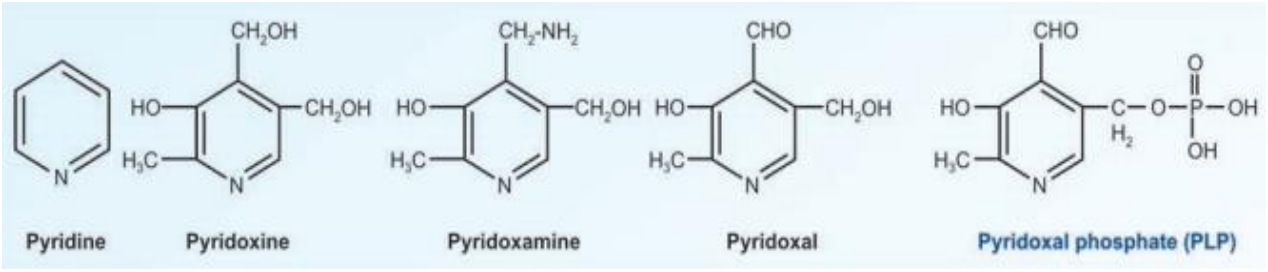

Figure 6.17. Structure of B6 related compounds

All decarboxylation reactions of amino acids require PLP as coenzyme. In vitamin B6 deficiency, PLP dependent enzymes function poorly. So, serotonin, epinephrine, noradrenalin and gamma amino butyric acid (GABA) are not produced properly. Neurological symptoms are therefore quite common in B6 deficiency. In children, B6 deficiency leads to convulsions due to decreased formation of GABA. PLP is involved in the synthesis of sphingolipids; so B6 deficiency leads to demyelination of nerves and consequent peripheral neuritis. Deficiency of B6 will also affect tryptophan metabolism. Since niacin is produced from tryptophan, B6 deficiency in turn leads to niacin deficiency which is manifested as pellagra. In adults hypochromic microcytic anemia may occur due to the inhibition of heme biosynthesis.

DID YOU KNOW?

In 1947, Dorothy Hodgkin was the head of research on X-ray diffraction analysis at Margaret Thatcher (Prime Minister of England in 1979-1990) when she was studying at Oxford University **(Fig. 6.24.)**.

Figure 6.24. Prime Minister Margaret Thatcher greets her former tutor, 78-year-old Professor Dorothy Crowfoot Hodgkin, before a luncheon for Nobel Laureates at Downing Street, London, 14 April 1989

Vitamin B_{12} is water soluble, heat stable and red in color. It contains 4.35% cobalt by weight. It contains one cobalt atom. Four pyrrole rings coordinated with a cobalt atom is called a Corrin ring. The 5th valency of the cobalt is covalently linked to a substituted benzimidazole ring. This is then called cobalamin. The 6th valency of the cobalt is satisfied by any of the following groups: cyanide, hydroxyl, adenosyl or methyl **(Fig. 6.25.)**.

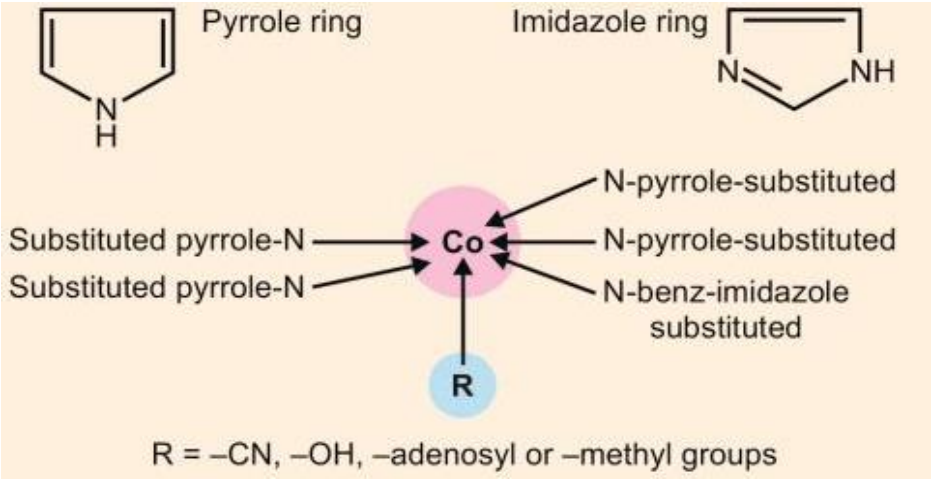

Figure 6.25. Simplified structure of vitamin B_{12}

When hydroxyl group is attached at the R position, it is called hydroxy cobalamin or vitamin B_{12}a. Injectable preparations are in this form. When taken up by the cells, these groups are removed and deoxy adenosyl cobalamin or Ado-B_{12} is formed **(Fig. 6.26.)**. This is the major storage form, seen in liver.

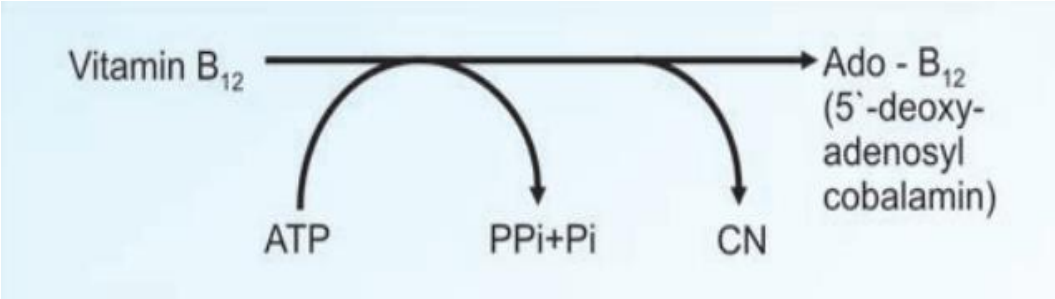

Figure 6.26. Storage form of vitamin B_{12}

When the methyl group replaces adenosyl group, it is known as methyl cobalamin. This is the major form seen in blood circulation as well as in cytoplasm of cells. The Ado-B_{12} and methyl B_{12} are the functional coenzymes in the body.

Absorption of vitamin B_{12} requires two binding proteins. First is the intrinsic factor (IF) of Castle **(Fig. 6.27.)**. William B Castle described it in 1929. The B_{12} is otherwise known as extrinsic factor (EF), that is, the factor derived from external sources. Intrinsic factor is secreted by the gastric parietal cells. It is a glycoprotein with a molecular weight of 50,000.

The second factor is cobalophilin, secreted in the saliva. Gastric pepsin release the vitamin from proteins of the food, and then B_{12} binds with cobalophilin. In duodenum, cobalophilin is hydrolyzed by trypsin of pancreatic juice; vitamin is released, and then vitamin binds to intrinsic factor. In pancreatic insufficiency (absence of trypsin), the vitamin may not be released. Then vitamin cobalophilin complex is excreted, resulting in vitamin deficiency.

One molecule of IF can combine with 2 molecules of B_{12}. This IF-B_{12} complex is attached with specific receptors on mucosal cells. The whole IF-B_{12} complex is internalized. It may be noted that B_{12} is absorbed from ileum, while folic acid is from jejunum.

In the blood, methyl B_{12} form is predominant. Transcobalamin, a glycoprotein, is the specific carrier. It is stored in the liver cells, as ado-B_{12} form, in combination with transcorrin. Generally, B complex vitamins are not stored in the body, B_{12} is an exception. Whole liver contains about 2 mg of B_{12}, which is sufficient for the requirement for 2-3 years. So, B_{12} deficiency is seen only years after gastrectomy.

Nutritional vitamin B_{12} deficiency is very common in India, especially among vegetarians of low socioeconomic group. The only source for B_{12} in vegetarian diet is curd/milk, and lower income group may not be able to afford it.

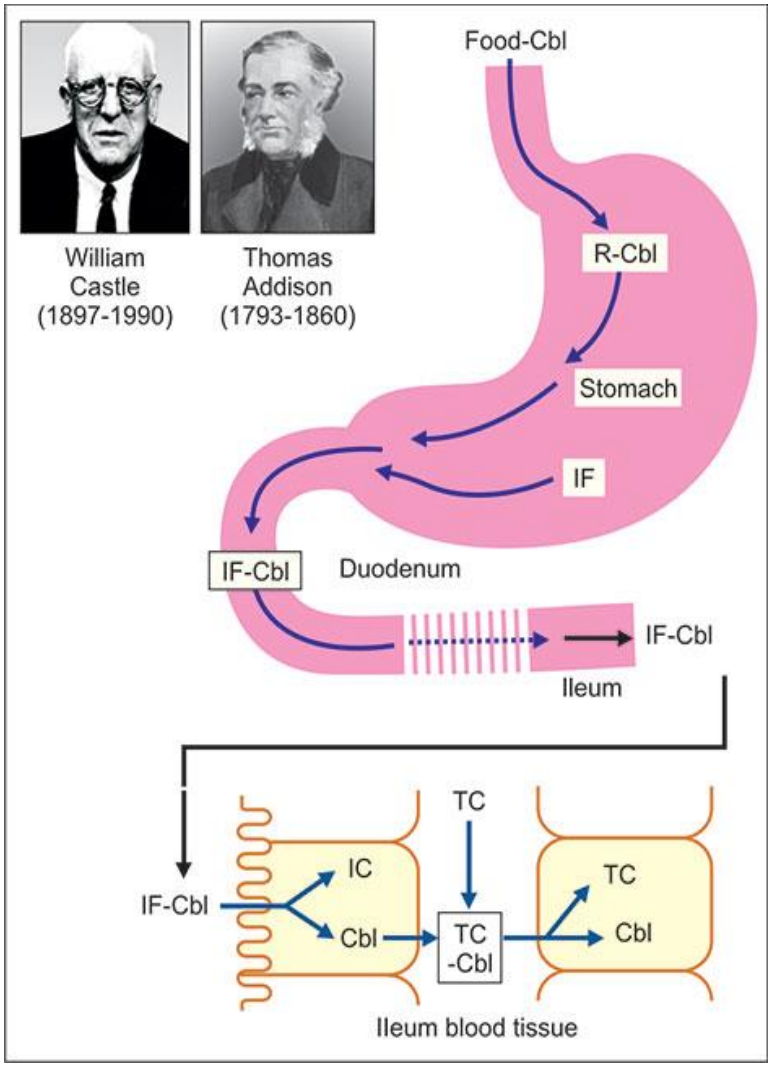

Figure 6.27. Absorption and storage of vitamin B_{12}. R = cobalophilin; Cbl = cobalamin; IF = intrinsic factor; TC = trans cobalamin

Addisonian pernicious anemia is also causes of B_{12} deficiance.

When it was described in 1849 by Thomas Addison, it was pernicious (fatal), without any known remedy. It is manifested usually in persons over 40 years. It is an autoimmune disease with a strong familial background. Antibodies are generated against IF. So, IF becomes deficient, leading to defective absorption of B_{12}.

Patients with cobalamin deficiency are usually anemic, but later in the development of the disease they show neuropsychiatric symptoms. However, central nervous system (CNS) symptoms may occur in the absence of anemia. The disease is treated by giving high-dose B_{12} orally, or intramuscular (IM) injection of cyanocobalamin. Therapy must be continued throughout the lives of patients with pernicious anemia.

Vitamin B_{12} deficiency causes simultaneous folate deficiency due to the folate trap. Therefore all the manifestations of folate deficiency are also seen. In vitamin B_{12} deficiency, step No. 2 is blocked, so that homocysteine is accumulated, leading to homocystinuria. Homocysteine level in blood has a positive

correlation with myocardial infarction. So, B_{12} and folic acid are protective against ischemic heart disease.

Also, Fish tapeworm although not seen in India, the fish tapeworm, diphillobothrium latum infection is common in Scandinavian countries where eating live fish is a delicacy. This tapeworm has a special affinity to B_{12} causing reduction in available vitamin.

Vitamin B_{12} is synthesized only by microorganisms; it is not present in plants. Animals obtain the vitamin preformed from their natural bacterial flora or by eating foods derived from other animals. Cobalamin is present in appreciable amounts in liver, whole milk, eggs, oysters, fresh shrimp, pork, and chicken.

Self-assessment tasks

1. Which vitamins belong to fat-soluble vitamins?

   a) A, D, E, K; b) A, B12, E, K; c) A, D, E, B6; d) B12, D, E, K.

2. What the earliest symptoms of vitamin A deficiency are?

   a) night blindness; b) scurvy; c) rickets; d) blood failure.

3. An early sign of retinol deficiencies in man is

   a) night blindness; b) keratinization; c) xerophthalmia; d) none of these.

4. Results from a dietary vitamin C deficiency is

   a) scurvy; b) night blindness; c) rickets; d) blood failure.

5. A deficiency of thiamin produces the disease known as

   a) beri-beri; b) scurvy; c) cataract; d) night blindness.

6. Vitamin-D deficiency can cause

   a) rickets; b) pernicious anemia; c) cataract; d) beri-beri.

7. Vitamins are essential because the organism

   a) can't synthesize these compounds at all; b) can synthesize these compounds partially;

   c) can't synthesize these compounds in the adequate amounts; d) none of the above.

8. What metal ion is specifically bound by vitamin B12?

   a) Cobalt; b) Copper; c) Zinc; d) Iron.

9. Why did Eskimos tell European explorers not to eat the liver of a polar bear?

10. Why is it important to eat whole grains?

11. Why is it important to get enough vitamin C?

12. What are the functions of vitamins?

Chapter № 7. Nucleotides and nucleic acids.

Nucleotides themselves participate in a plethora of crucial supporting roles in cell metabolism, and polymers of nucleotides, the nucleic acids, provide the script for everything that occurs in a cell. Nucleotides are energy-rich compounds that drive metabolic processes (primarily biosyntheses) in all cells. They also serve as chemical signals, key links in cellular systems that respond to hormones and other extracellular stimuli, and are structural components of a number of enzyme cofactors and metabolic intermediates. The nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), are the molecular repositories for genetic information. The structure of every protein, and ultimately of every cell constituent, is a product of information programmed into the nucleotide sequence of a cell's nucleic acids.

The amino acid sequence of every protein and the nucleotide sequence of every RNA molecule in a cell are specified by that cell's DNA. The necessary protein or RNA sequence information is found in corresponding nucleotide sequences in the DNA. A segment of DNA that contains the information required for the synthesis of a functional biological product (protein or RNA) is referred to as a gene. A cell typically has many thousands of genes, and DNA molecules, not surprisingly, tend to be very large. The storage of biological information is the only known function of DNA.

Several classes of RNAs **(Fig.7.1.)** are found in cells, each with a distinct function.

Ribosomal RNAs (rRNA) are structural components of ribosomes, the large complexes that carry out the synthesis of proteins.

Messenger RNAs (mRNA) are nucleic acids that carry the information from one or a few genes to the ribosome, where the corresponding proteins can be synthesized.

Transfer RNAs (tRNA) are adapter molecules that faithfully translate the information in mRNA into a specific sequence of amino acids.

In addition to these major classes there are a wide variety of special-function RNAs.

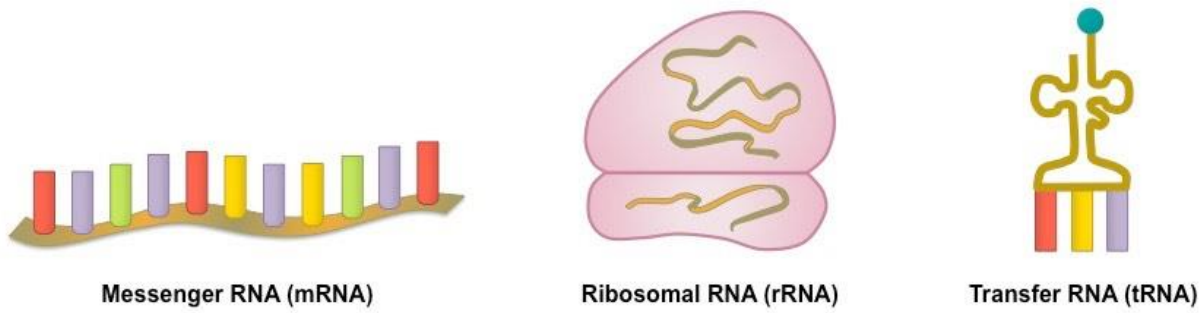

Figure 7.1. Types of RNA: mRNA, rRNA and tRNA

Nucleic acids are polymers whose monomers are nucleotides.

Nucleotides have three characteristic components: (1) a nitrogenous base, (2) the pentose sugar (ribose in RNA and deoxyribose in DNA), and (3) a phosphate **(Fig. 7.2.)**. The nitrogenous bases are derivatives of two parent compounds, pyrimidine and purine ((**Fig. 7.2.)**.

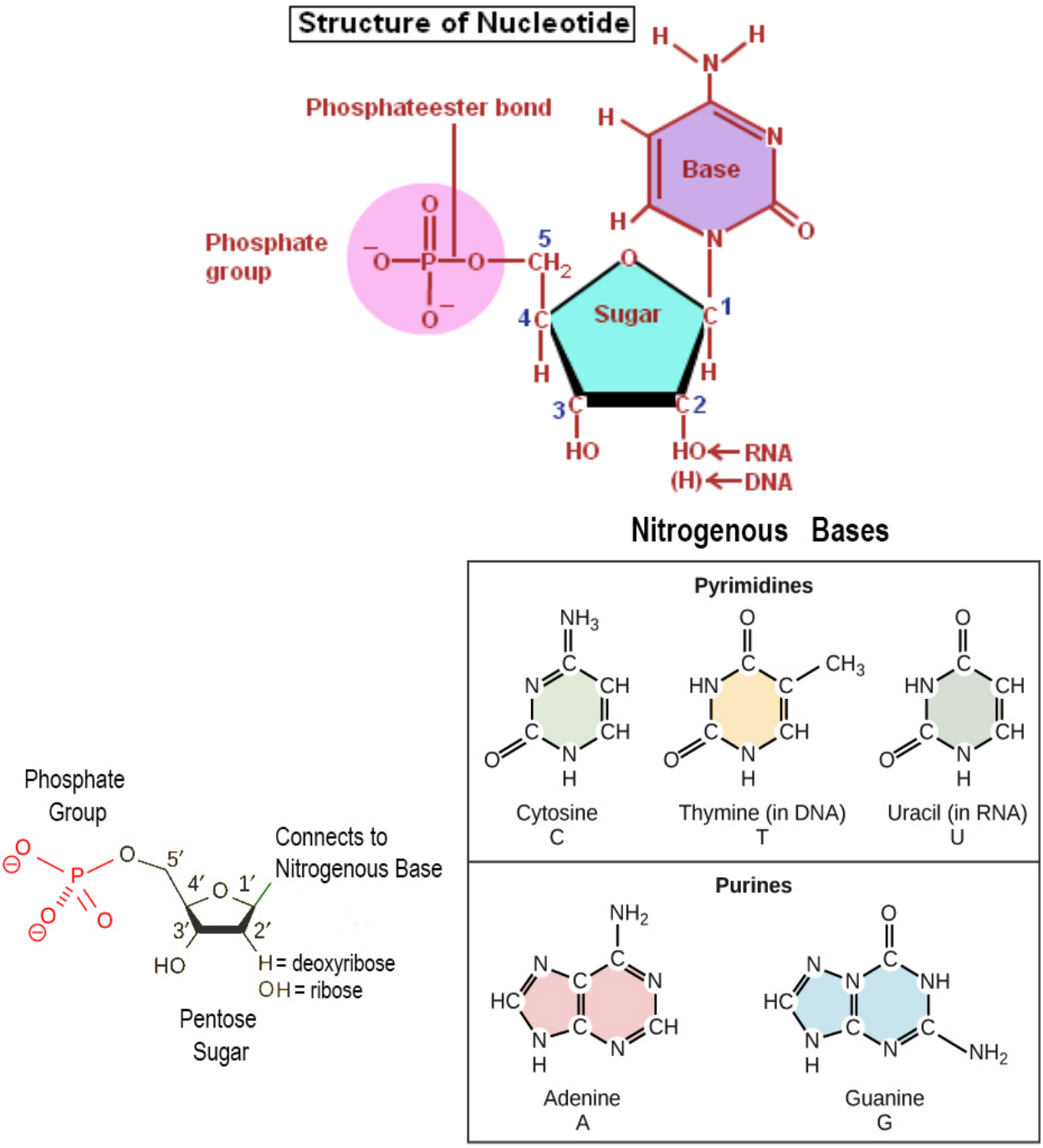

Figure 7.2. Structure of nucleotide and nitrogenous bases

The carbon and nitrogen atoms in the parent structures are conventionally numbered to facilitate naming and identification of the many derivative compounds. The base is joined covalently (at N-1 of pyrimidines and N-9 of purines) in an N-glycosidic linkage to the 1' carbon of the pentose, and the phosphate is esterified to the 5' carbon. The N-glycosidic bond is formed by removal of the elements of

Figure 7.5. James Watson and Francis Crick

The biochemical investigation of DNA began with Friedrich Miescher, who carried out the first systematic chemical studies of cell nuclei. In 1868 Miescher isolated a phosphorus-containing substance, which he called "nuclein," from the nuclei of pus cells (leukocytes) obtained from discarded surgical bandages. He found nuclein to consist of an acidic portion, which we know today as DNA, and a basic portion, protein. Miescher later found a similar acidic substance in the heads of salmon sperm cells. Although he partially purified the nucleic acid and studied its properties, the covalent (primary) structure of DNA did not become known with certainty until the late 1940s.

Miescher and many others suspected that nuclein or nucleic acid was associated in some way with cell inheritance, but the first direct evidence that DNA is the bearer of genetic information came in 1944 through a discovery made by Oswald T. Avery, Colin MacLeod, and Maclyn McCarty. These investigators found that DNA extracted from a virulent (disease-causing) strain of the bacterium Streptococcus pneumoniae, also known as pneumococcus, genetically transformed a nonvirulent strain of this organism into a virulent form. Avery and his colleagues concluded that the DNA extracted from the virulent strain carried the inheritable genetic message for virulence. Not everyone accepted these conclusions, because traces of protein impurities present in the DNA could have been the actual carrier of the genetic information. This possibility was soon eliminated by the finding that treatment of the DNA with proteolytic enzymes did not destroy the transforming activity, but treatment with deoxyribonucleases (DNA hydrolyzing enzymes) did.

A second important experiment provided independent evidence that DNA carries genetic information. In 1952 Alfred D. Hershey and Martha Chase used radioactive phosphorus (32P) and radioactive sulfur (35S) tracers to show that when the bacterial virus (bacteriophage) T2 infects its host cell, E. coli, it is the phosphorus-containing DNA of the viral particle, not the sulfur-containing protein of the viral coat, that actually enters the host cell and furnishes the genetic information for viral replication. These important early experiments and many other lines of evidence have shown that DNA is definitely the exclusive chromosomal component bearing the genetic information of living cells.

A most important clue to the structure of DNA came from the work of Erwin Chargaff and his colleagues in the late 1940s. They found that the four nucleotide bases in DNA occur in different ratios in the DNAs of different organisms and that the amounts of certain bases are closely related. These data, collected from DNAs of a great many different species, led Chargaff to the following conclusions:

1. The base composition of DNA generally varies from one species to another.
2. DNA specimens isolated from different tissues of the same species have the same base composition.
3. The base composition of DNA in a given species does not change with the organism's age, nutritional state, or changing environment.
4. In all DNAs, regardless of the species, the number of adenine residues is equal to the number of thymine residues (that is, A = T), and the number of guanine residues is equal to the number of cytosine residues (G = C). From these relationships it follows that the sum of the purine residues equals the sum of the pyrimidine residues; that is, A + G = T + C.

These quantitative relationships, sometimes called "Chargaff s rules," were confirmed by many subsequent researchers. They were a key to establishing the three-dimensional structure of DNA and yielded clues to how genetic information is encoded in DNA and passed from one generation to the next.

To shed more light on the structure of DNA, Rosalind Franklin and Maurice Wilkins **(Fig. 7.6.)** used the powerful method of x-ray diffraction to analyze DNA crystals. They showed in the early 1950s that DNA produces a characteristic x-ray diffraction pattern. From this pattern it was deduced that DNA polymers are helical with two periodicities along their long axis, a primary one of 0.34 nm and a secondary one of 3.4 nm. The pattern also indicated that the molecule contains two strands, a clue that was crucial to determining the structure. The problem then was to formulate a three-dimensional model of the DNA molecule that could account not only for the x-ray diffraction data but also for the specific A = T and G = C base equivalences discovered by Chargaff and for the other chemical properties of DNA.

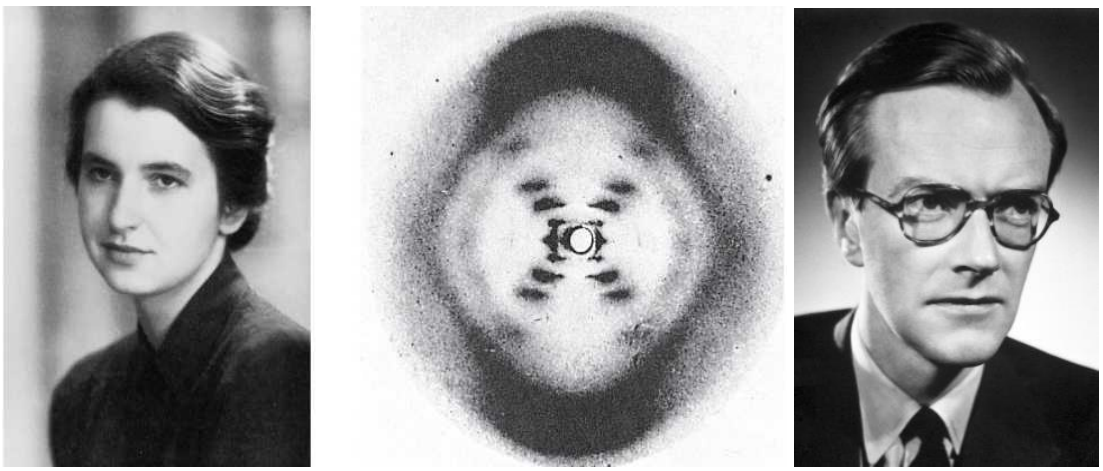

Figure 7.6. Rosalind Franklin and Maurice Wilkins and the x-ray diffraction pattern of DNA. The spots forming a cross in the center denote a helical structure. The heavy bands at the top and bottom correspond to the recurring bases.

In 1953 Watson and Crick postulated a three-dimensional model of DNA structure that accounted for all of the available data **(Fig. 7.7.)**. It consists of two helical DNA chains coiled around the same axis to form a right-handed double helix. The hydrophilic backbones of alternating deoxyribose and negatively charged phosphate groups are on the outside of the double helix, facing the surrounding water. The purine and pyrimidine bases of both strands are stacked inside the double helix, with their hydrophobic and nearly planar ring structures very close together and perpendicular to the long axis of the helix. The spatial relationship between these strands creates a major groove and minor groove between the two strands. Each base of one strand is paired in the same plane with a base of the other strand. Watson and Crick found that the hydrogen-bonded base pairs illustrated in **Figure 7.4.** are those that fit best within the structure, providing a rationale for Chargaff's rules. It is important to note that three hydrogen bonds can form between G and C, symbolized G≡C, but only two can form between A and T, symbolized A=T.

In the Watson-Crick structure, the two chains or strands of the helix are antiparallel; their 5',3'-phosphodiester bonds run in opposite directions. Later work with DNA polymerases provided experimental evidence, confirmed by x-ray crystallography, that the strands are indeed antiparallel.

To account for the periodicities observed in the x-ray diffraction pattern, Watson and Crick used molecular models to show that the vertically stacked bases inside the double helix would be 0.34 nm apart and that the secondary repeat distance of about 3.4 nm could be accounted for by the presence of 10 (now 10.5) nucleotide residues in each complete turn of the double helix. The two antiparallel polynucleotide chains of double-helical DNA are not identical in either base sequence or composition. Instead they are complementary to each other. Wherever adenine appears in one chain, thymine is found in the other; similarly, wherever guanine is found in one chain, cytosine is found in the other.

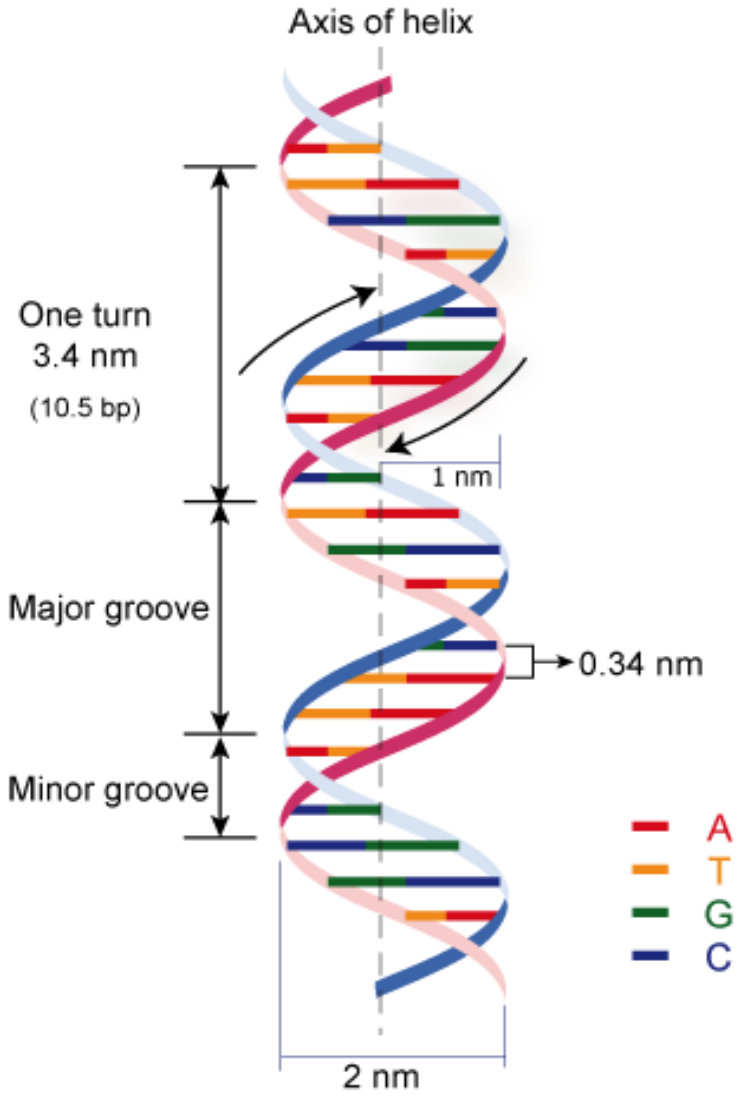

Figure 7.7. The Watson-Crick model for the structure of DNA. The original model proposed that there are 10 base pairs or 3.4 nm per turn of the helix. Subsequent measurements have shown that there are 10.5 base pairs or 3.6 nm per turn.

The DNA double helix or duplex is held together by two sets of forces, as described earlier: hydrogen bonding between complementary base pairs and base-stacking interactions. The specificity that maintains a given base sequence in each DNA strand is contributed entirely by the hydrogen bonding between base pairs. The basestacking interactions, which are largely nonspecific with respect to the identity of the stacked bases, make the major contribution to the stability of the double helix.

The essential feature of the model is the complementarity of the two DNA strands. Making a copy of this structure (replication) could logically proceed by (1) separating the two strands and (2) synthesizing a complementary strand for each by joining nucleotides in a sequence specified by the base-paring rules stated above. Each preexisting strand could function as a template to guide the synthesis of the complementary strand **(Fig. 7.8.)**.

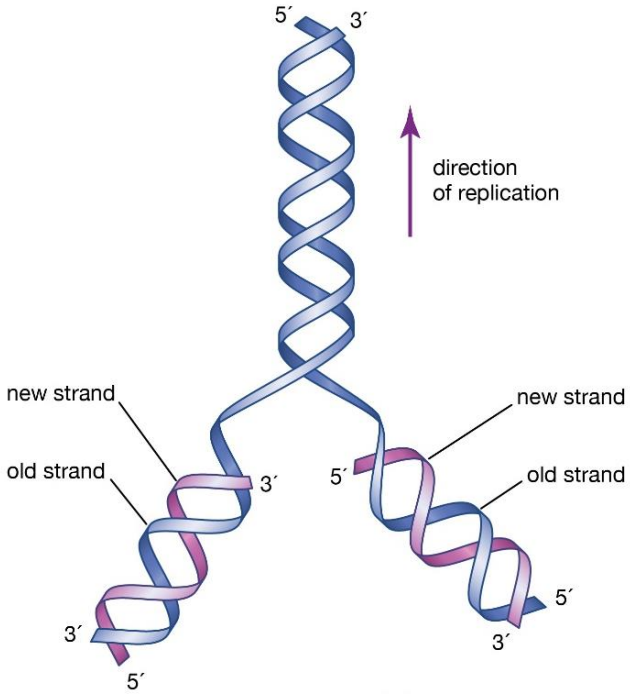

Figure 7.8. Replication of DNA as suggested by Watson and Crick. The parent strands become separated, and each forms the template for biosynthesis of a complementary daughter strand.

The Watson-Crick structure is also referred to as B-form DNA. The B form is the most stable structure for a random-sequence DNA molecule under physiological conditions, and is therefore the standard point of reference in any study of the properties of DNA.

Iwo DNA structural variants that have been well characterized in crystal structures are the A and Z forms **(Fig. 7.9.)**. For a given DNA molecule, the A form will be shorter and have a greater diameter than the B form.

The reagents used to promote crystallization of DNA tend to dehydrate it, and this leads to a tendency for many DNAs to crystallize in the A form. Z-form DNA is a more radical departure from the B structure; the most obvious distinction is the left-handed helical rotation.

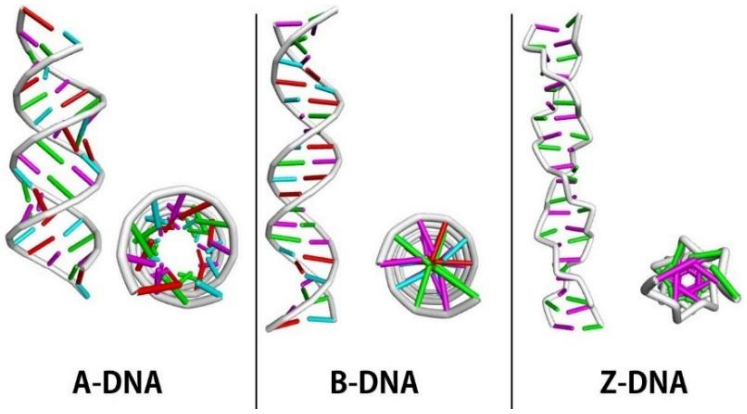

Figure 7.9. Comparison of the A, B and Z forms of DNA

A number of other sequence-dependent structural variations have been detected that may serve locally important functions in DNA metabolism. For example, some sequences cause bends in the DNA helix. Bends are produced whenever four or more adenine residues appear sequentially in one of the two strands. Six adenines in a row produce a bend of about 180 The bending observed with this and other sequences may be important in the binding of some proteins to DNA.

A rather common type of sequence found in DNA is a palindrome. A palindrome is a word, phrase, or sentence that is spelled identically reading forward or backward; two examples are ROTATOR and NURSES RUN. The term is applied to regions of DNA in which there are inverted repetitions of base sequence with twofold symmetry occurring over two strands of DNA. Such sequences are self complementary within each of the strands and therefore have the potential to form hairpin or cruciform (cross-shaped) structures **(Fig. 7.10.)**..

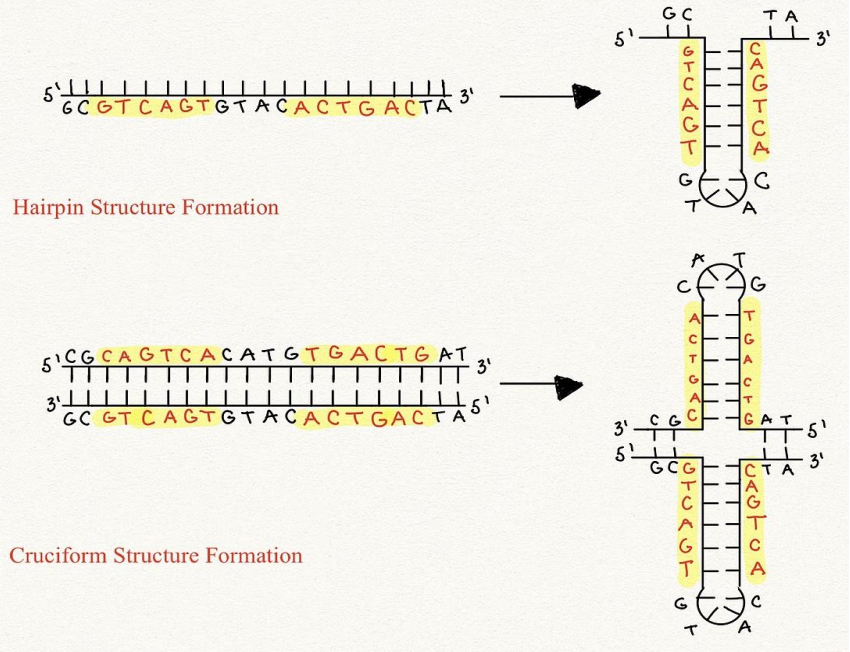

Figure 7.10. Hairpins and cruciforms. Palindromic DNA (or RNA) sequences can form alternative structures with intrastrand base pairing. When only a single DNA (or RNA) strand is involved it is called a hairpin. When both strands of a duplex DNA are involved, the structure is called a cruciform.

RNA, the second major form of nucleic acid in cells, plays the role of intermediary in converting this information into a functional protein.

In eukaryotes DNA is largely confined to the nucleus, whereas protein synthesis occurs on ribosomes in the cytoplasm. Therefore some molecule other than DNA must carry the genetic message for protein synthesis from the nucleus to the cytoplasm. As early as the 1950s, RNA was considered the logical candidate: RNA is found in both the nucleus and cytoplasm, and the onset of protein synthesis is accompanied by an increase in the amount of RNA in the cytoplasm and an increase in its rate of

turnover. These and other observations led several researchers to suggest that RNA carries genetic information from DNA to the protein biosynthetic machinery of the ribosome. In 1961, Francois Jacob and Jacques Monod presented a unified (and essentially correct) picture of many aspects of this process. They proposed the name messenger RNA (mRNA) for that portion of the total cell RNA carrying the genetic information from DNA to the ribosomes, where the messengers provide the templates for specifying amino acid sequences in polypeptide chains. Although mRNAs from different genes can vary greatly in length, the mRNAs from a particular gene will generally have a defined size. The process of forming mRNA on a DNA template is known as transcription.

In prokaryotes a single mRNA molecule may code for one or several polypeptide chains. If it carries the code for only one polypeptide, the mRNA is monocistronic; if it codes for two or more different polypeptides, the mRNA is polycistronic. In eukaryotes, most mRNAs are monocistronic. The minimum length of an mRNA is set by the length of the polypeptide chain for which it codes. For example, a polypeptide chain of 100 amino acid residues requires an RNA coding sequence of at least 300 nucleotides, because each amino acid is coded by a nucleotide triplet. However, mRNAs transcribed from DNA are always somewhat longer than needed simply to specify the code for the polypeptide sequence(s).

Messenger RNA is only one of several classes of cellular RNA. Transfer RNAs serve as adapter molecules in protein synthesis; covalently linked to an amino acid at one end, they pair with the mRNA in such a way that the amino acids are joined in the correct sequence. Ribosomal RNAs are structural components of ribosomes.

Regardless of the class of RNA being synthesized, the product of transcription is always a single strand of RNA. The single-stranded nature of these molecules does not mean their structure is random. The single strands tend to take up a right-handed helical conformation that is dominated by base-stacking interactions. The stacking interactions are stronger between two purines than between a purine and a pyrimidine or between two pyrimidines. The purine interaction is so strong that a pyrimidine separating two purines will often be displaced from the stacking pattern so that the purines can interact. Any self complementary sequences in the molecule will lead to more complex and specific structures. RNA can base-pair with complementary strands of either RNA or DNA. The standard base-pairing rules are identical to those for DNA: guanine pairs with cytosine and adenine pairs with uracil (or thymine). One difference is that one unusual base pairing-between guanine and uracil-is fairly common between two strands of RNA.

Solutions of carefully isolated, native DNA are highly viscous at pH 7.0 and room temperature (20 to 25°C). When such a solution is subjected to extremes of pH or to temperatures above 80 to 90°C, its viscosity decreases sharply, indicating that the DNA has undergone a physical change. Just as heat and extremes of pH cause denaturation of globular proteins, so too will they cause denaturation or melting of

double helical DNA. This involves disruption of the hydrogen bonds between the paired bases and the hydrophobic interactions between the stacked bases. As a result, the double helix unwinds to form two single strands, completely separate from each other along the entire length, or part of the length (partial denaturation), of the molecule. No covalent bonds in the DNA are broken.

Renaturation of DNA is a rapid one-step process as long as a double-helical segment of a dozen or more residues still unites the two strands. When the temperature or pH is returned to the biological range, the unwound segments of the two strands spontaneously rewind or anneal to yield the intact duplex. However, if the two strands are completely separated, renaturation occurs in two steps. The first step is relatively slow, because the two strands must first "find" each other by random collisions and form a short segment of complementary double helix. The second step is much faster: the remaining unpaired bases successively come into register as base pairs, and the two strands "zipper" themselves together to form the double helix.

Each species of DNA has a characteristic denaturation temperature or melting point: the higher its content of G≡C base pairs, the higher the melting point of the DNA. This is because G≡C base pairs, with three hydrogen bonds, are more stable and require more heat energy to dissociate than A=T base pairs.

Self-assessment tasks

1. Which of the following is not considered a pyrimidine?

   a) C; b) T; c) U; d) G.

2. What type of sugar is found in the nucleotides of DNA?

   a) deoxyribose; b) ribose; c) glucose; d) none of the above.

3. What is the role of hydrogen bonds in the structure of DNA?

   a) to code for proteins; b) to synthesize proteins; c) to separate the strands; d) to connect the base pairs.

4. Which of the following is found on RNA but not DNA?

   a) uracil; b) deoxyribose; c) phosphate; d) adenine.

5. Which is true about the pairing of bases in the DNA molecule?

   a) purines always pair with pyrimidines b) a single ring base pairs with another single ring base c) a double ring base pairs with another double ring base d) purines pair with purines and pyrimidines with pyrimidines

6. The synthesis of DNA by DNA polymerase occurs in the

   a) 3' to 5' direction; b) 5' to 5' direction; c) 5' to 3' direction; d) 3' to 3' direction.

7. What are the 4 nitrogen bases in DNA?

   a) adenine, thymine, cytokinin, guanine; b) adetamin, thymine, cytosine, guanine; c) adenine, thymine, cytosine, glikogen; d) adenine, thymine, cytosine, guanine.

8. DNA codes for

   a) cholesterol; b) fatty acids; c) proteins; d) carbohydrates

9. What is the basic unit of DNA and how are these units arranged?

10. What are the four possible nitrogen bases found in DNA and what are their abbreviations?

11. Describe the Watson-Crick model for the structure of DNA?

12. How are the nitrogen bases connected to the backbone and how are they connected to each other?

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