PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
DJILLALI LIABES UNIVERSITY
FACULTY OF MEDECIN
SIDI BEL ABBES
DEPARTMENT OF DENTISTRY





PEDAGOGICAL HANDOUT OF BIOCHEMISTRY DISTINGUISHED FOR STUDENTS OF MEDICAL SCIENCES

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Academic year: 2024-2025

LIST OF ABBREVIATIONS

AACC: American Association of Cereal Chemists

DNA: Nucleic acids, deoxyribonucleic

AMP : Adenosine monophosphate

ATP: Adenosine triphosphate

Ea: Activation energy

CoA: Coenzyme A

DA: Dalton

EOs : Essential Oils

GDP : Guanosine diphosphate

GDP : Geranyl DiPhosphate

GGDP: GeranylGeranyl Di Phosphate

HDL: high density lipoproteins

IDL: Intermediate density lipoproteins

LDL: low density lipoproteins

PUFA : Polyunsaturated fatty acids

SGLT: Sodium/GLucose co Transporter

TFA : Trans Fatty Acids

VLDL: very low density lipoproteins

Preface

Biochemistry is the language of biology. The tools for research in all the branches of medical science are based on principles of biochemistry. The study of biochemistry is essential to understand basic functions of the body. This will give information regarding the functioning of cells at the molecular level. How the food that we eat is digested, absorbed, and used to make ingredients of the body? How does the body derive energy for the normal day to day work? How are the various metabolic processes interrelated? What is the function of genes? What is the molecular basis for immunological resistance against invading organisms? Answer for such basic questions can only be derived by a systematic study of medical biochemistry. Modern day medical practice is highly dependent on the laboratory analysis of body fluids, especially the blood. The disease manifestations are reflected in the composition of blood and other tissues. Hence, the demarcation of abnormal from normal constituents of the body is another aim of the study of clinical biochemistry.

The ultimate goal of biochemistry is to explain all life processes in molecular detail. Because life processes are performed by organic molecules the discipline of biochemistry relies heavily on fundamental principles of organic chemistry and other basic sciences. It is of no surprise that the first "biochemists" actually were organic chemists who specialized in the chemistry of compounds derived from living organisms.

The purpose of studying the discipline "Biochemistry" is to form student's 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. By studying the discipline student has ***to know***:

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

- The structure and properties of the main bimolecular: 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.

It's my pleasure that our faculty members are mastering the art of scientific writing. There is vacume in the area of lucid and lucrative scientific documentation. If we want to propagate the scientific. knowledge we should present it in a more simple form, in a pictorial and diagrammatic manner. It should be more graphical and photographica1.

The present document content five chapters presented in simple manner to facilate understanding for the students. I hope that document will be appreciated by the students, teachers, researchers and professionals.

List of abbreviations

AACC: American Association of Cereal Chemists

DNA: Nucleic acids, deoxyribonucleic

AMP : Adenosine monophosphate

ATP: Adenosine triphosphate

Ea: Activation energy

CoA: Coenzyme A

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EOs : Essential Oils

GDP : Guanosine diphosphate

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PUFA : Polyunsaturated fatty acids

SGLT: Sodium/GLucose co Transporter

TFA : Trans Fatty Acids

VLDL: very low density lipoproteins

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General introduction

Biochemistry is the chemistry of living organisms. It bridges the gap between the conventional chemistry and biology. Living organisms have certain extraordinary properties. They can grow, respond to stimuli and replicate themselves with high fidelity. All these activities are ultimately interpretable in chemical terms. The lifeless organic molecules with appropriate complexity and properties make a living thing.

The basic phenomena of biochemistry is to understand how the collections of inanimate molecules that constitute living organisms interact with each other to maintain life. The basic life processes or chemistry remains broadly the same whether it is an unicellular microorganism or the higher organisms such as human or plants. Life is nothing but thousands of ordered chemical reactions. In other words, chemistry is the logic of all biological phenomena.

Biochemistry is rapidly progressing from a science performed almost entirely at the laboratory bench to one that may be explored through computers. The recently developed ability to determine entire genomic sequences has provided the data needed to accomplish massive comparisons of derived protein sequences, the results of which may be used to formulate and test hypotheses about biochemical function. The power of these new methods is explained by the impact of evolution: many molecules and biochemical pathways have been generated by duplicating and modifying existing ones.

Introduction to biochemistry

Biochemistry is the science of the chemical constituents of living cells and of the reactions and processes they undergo. The term "biochemistry" itself is derived from a combination of biology and chemistry. In 1877 German physician, chemist and physiologist Felix Hoppe-Seyler used the term as a synonym for physiological chemistry in the first issue of Journal of Physiological Chemistry.

Biochemistry is the chemistry of living cell. Life depends on various biochemical reactions, that is why biochemistry has become the basic language of all biological sciences. Controlling information flow through biochemical signalling and the flow of chemical energy through metabolism, biochemical processes give rise to the incredible complexity and diversity of life. Biochemistry mostly deals with the structures and functions of cellular components such as proteins, carbohydrates, lipids, nucleic acids and other biomolecules as well as with their conversions. Over the last 50 years biochemistry has become so successful at explaining living processes, that now almost all areas of the life sciences from botany to medicine are engaged in biochemical research.

Today the main focus of pure biochemistry lies in understanding how biological molecules give rise to the processes that occur within living cells which in turn relates greatly to the study and understanding of whole organisms. Biochemistry is a field of science concerned with the study of:

• chemical properties of the compounds constitutive of the living organism (**static biochemistry**);

• their conversions (**dynamic biochemistry**);

• relation of these conversions to the activity of organs and tissues (**functional biochemistry**).

. Biochemistry is concerned with the entire spectrum of life forms, from relatively simple viruses and bacteria to complex human beings. Depending on the **object of study** biochemistry is divided into:

• biochemistry of humans and animals

• biochemistry of plants

• biochemistry of microorganisms

The study of human biochemistry will open your eyes to how the body works as a chemical system. From a physician's point of view, biochemistry not only describes how the system works, but also provides a foundation for understanding how to improve its operation, how to diagnose problems and, where possible, how to remedy them. To understand links between nutrients, metabolism, health and disease, is one of the most important reasons to study biochemistry. Knowing biochemistry helps understand current therapies, which include recombinant proteins, such as human insulin or erythropoietin synthesized by bacteria. It helps understand the action of new drugs. In future, therapies will possibly involve gene rather than organ transplants. Pharmacogenomics and nutritional genomics will create a basis for designer treatments, customized to an individual's genetic makeup.

History of biochemistry

I period: ancient time – 15th century. In this period people used biochemical processes to make bread, cheese, wine, though the essence of these processes was unknown to them.

II period: 15th century– the first half of the 19th century. In this period German physician Paracelsus (1493-1541) put forward the concept of a close relationship between chemistry and medicine: chemical reactions formed the basis of vital activity and the cause of any disease is a disturbance of the natural course of chemical processes within the organism. The first controlled experiments in human metabolism were published by Santorio Santorio in 1614 in his book *"Ars de statica medicina"*. This book describes how he weighed himself before and after eating, sleeping, working, sex, fasting, drinking, and excreting.

He found that most of the food he took in was lost through what he called "insensible perspiration". Russian scientist Lomonosov (1711-1765) formulated the law of conservation of mass. French chemist Lavoisier (1743-1794) proposed that in respiration of living organism oxygen is consumed and carbon dioxide is evolved. Russian chemist Kirchhoff (1764-1833) described in 1814 enzymatic process of starch saccharization by the action of an extract from the germinated barleycorn. By the 1850s, other enzymes were discovered: salivary amylase, pepsin in gastric juice, trypsin of pancreatic juice.

III period: the second half of the 19th century – first half of the 20th century. In this period French scientist Pasteur (1821-1895) performed studies of fermentation with participation of living yeast cells. German chemist Buchner in 1897 provided an evidence for the ability of a

cell-free yeast juice to produce alcoholic fermentation. For his works on cell-free fermentation Buchner was awarded a Nobel Prize in chemistry in 1907.

In the second half of the 19th century special chairs of medical, or physiological, chemistry were instituted at the medical departments of many European universities.

IV period: 1950s – present time. Advent of biochemistry, development of new methodologic principles and techniques such as chromatography, X-ray diffraction, nuclear magnetic resonance spectroscopy, radioisotopic labelling, electron microscopy ,and molecular dynamics simulations. These techniques allowed for the discovery and detailed analysis of many molecules and metabolic pathways of the cell, such as glycolysis and the Krebs cycle (citric acid cycle). In 1960, the biochemist Robert K. Crane revealed his discovery of the sodium-glucose cotransport as the mechanism for intestinal glucose absorption. This was the very first proposal of a coupling between the fluxes of ions and a substrate that has been seen as sparking a revolution in biology.

Today, the findings of biochemistry are used in many areas, from genetics to molecular biology and from agriculture to medicine.

CHAPTER I : CARBOHYDRATES

I-1 Definition

Carbohydrates are the most abundant biomolecules on Earth. Each year, photosynthesis converts more than 100 billion metric tons of CO2 and H2O 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 (CH2O)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").

1-2 Classification

1- 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.

2-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.

3-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 non polar solvents. Most have a sweet taste. The backbones of common monosaccharides are un branched 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 **(Figure 1)**.

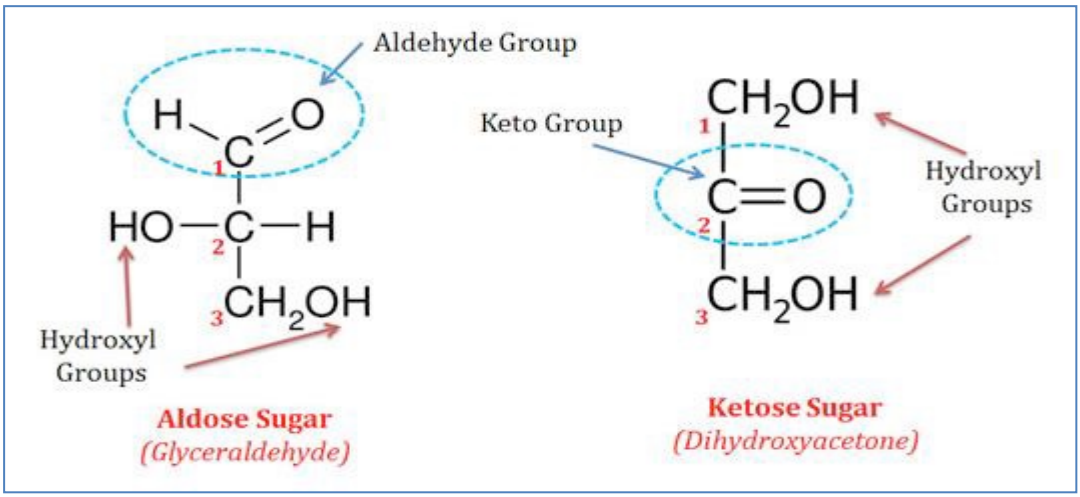


Figure 1: Difference between aldose and ketose sugars

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) **(Figure 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 CH2OH is on the right as represented in the Fisher projection, it is classified as D and if on the left, classified as L.

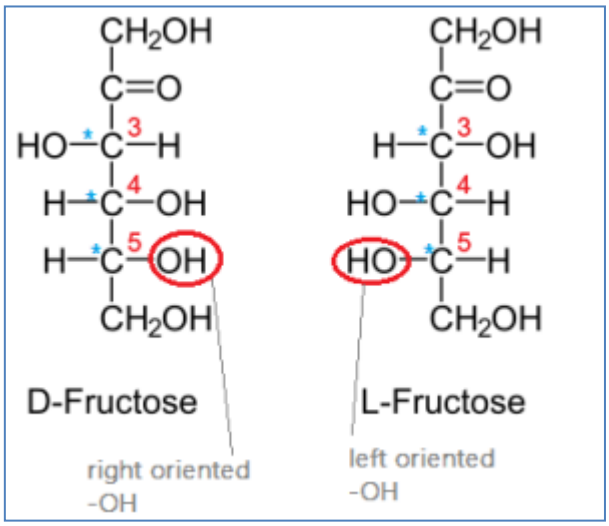


Figure 2: The stereochemical classification of monosaccharides

1-3 Cyclization of Monosaccharide

The predominant forms of ribose ,glucose ,fructose ,and many other sugars in solution are not open chains .Rather ,the open- chain forms of these sugars cyclize in to rings.For an aldohexose suchas glucose, Formation of ahemiacetal by reaction of the **C-1 aldehyde** group with **C5 hydroxyl group** to form an intra molecular hemiacetal.

The resulting cyclichemiacetal,a six member dring,is called **pyranose** because of its similarity to pyran.



Similarly,the **C-2** keto group in the open-chain form of a keto hexose,suchas fructose,can form an intra molecular hemiketal by reacting with either the **C-6**hydroxyl group to form a six-membered cyclichemiketalor the C-5hydroxyl group to form a five member cyclic hemiketal. The five-membered ringis called a furanose because of its similarity to furan

(Figure below)

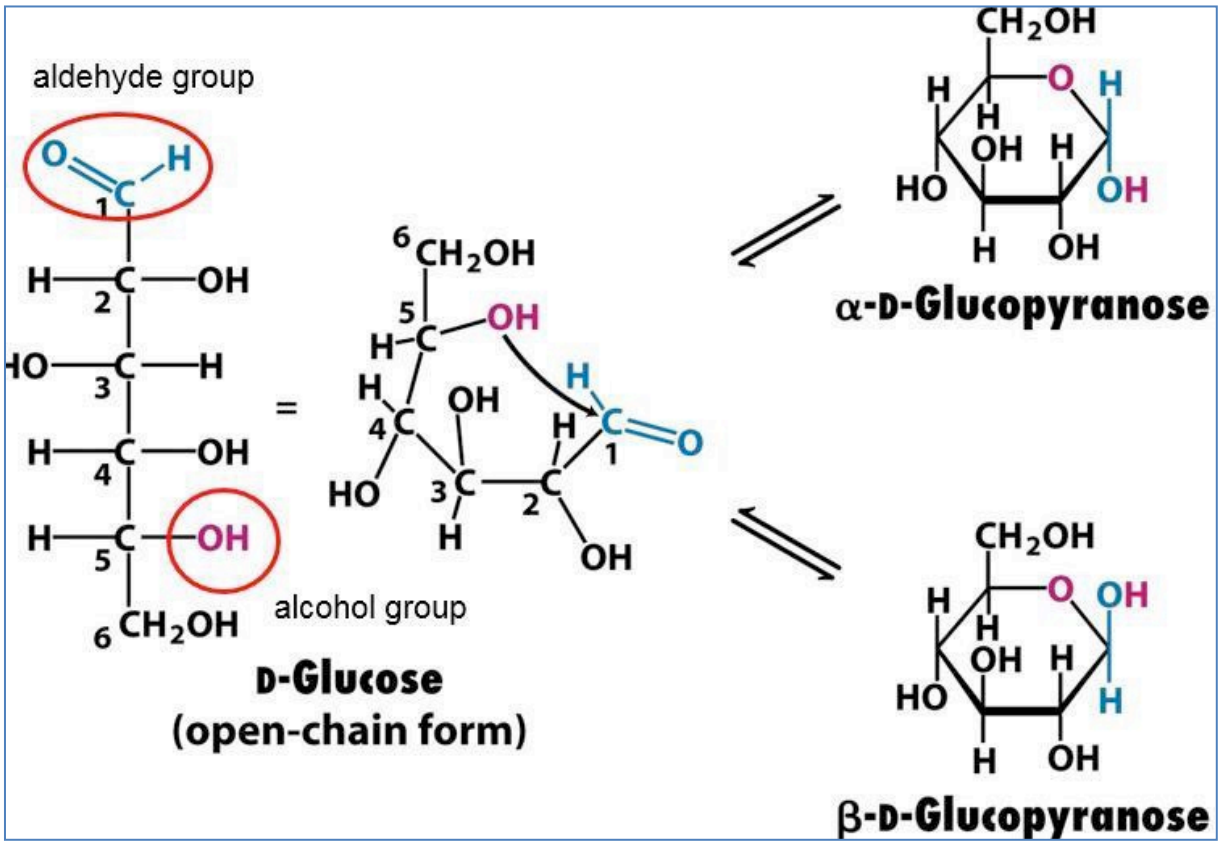


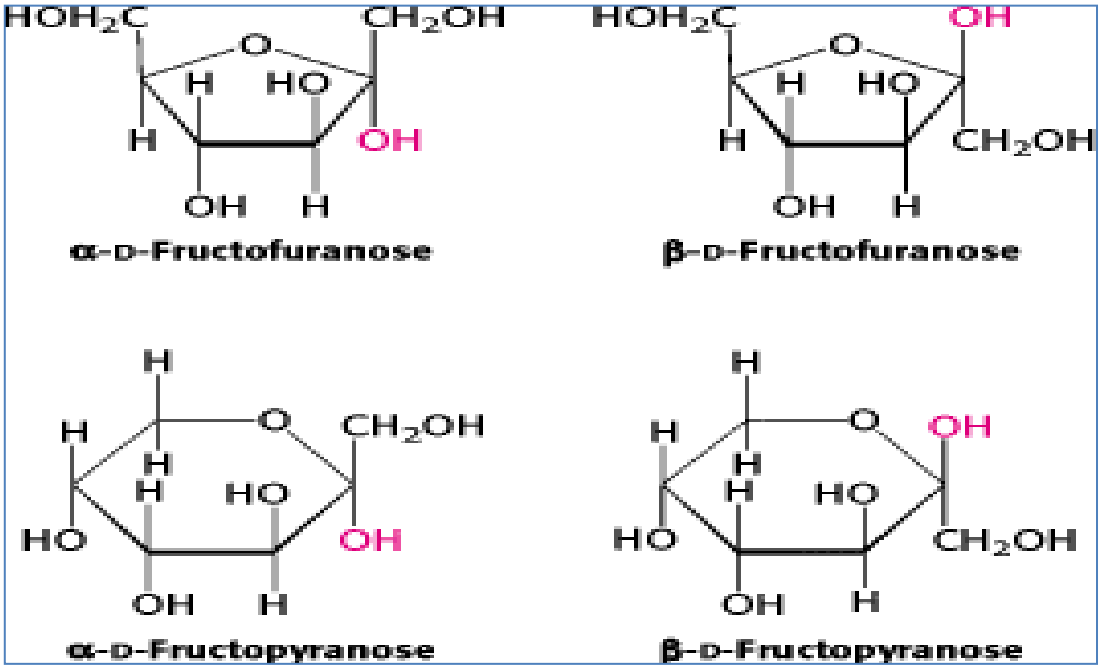
Aldoses such as ribose and glucose can exist in three structural forms: the open chain, the alpha (α) cyclic form, and the beta (β) cyclic form **(Figure3)**.

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 CH2OH group in Haworth projection, while the β-d-glucose is formed if the hydroxyl group is pointed in the same direction as the CH2OH group.

To convert between Haworth and Fischer projections is a little tricky. The Fischer projection curves away from you, with the OH and H groups sticking out like the plates on a stegosaur's back. If you turn the Fischer projection clockwise through 90° in your mind's eye, you will see that for carbons 2, 3, and 4, if the Fischer OH goes right, the Haworth OH goes down. Carbon 1 is the anomeric carbon, and doesn't exist in the open chain form, sowe're left with carbon 5 to understand. You need to realise there is free rotation of the H, OH and CH20H groups around carbon-5, hence you can twirl the three groups round so that the CH20H is pointing up as you curl the chain round into a ring.


aldehyde group
alcohol group
D-Glucose
(open-chain form)
α-D-Glucopyranose
β-D-Glucopyranose


α-D-Fructofuranose
β-D-Fructofuranose
α-D-Fructopyranose
β-D-Fructopyranose

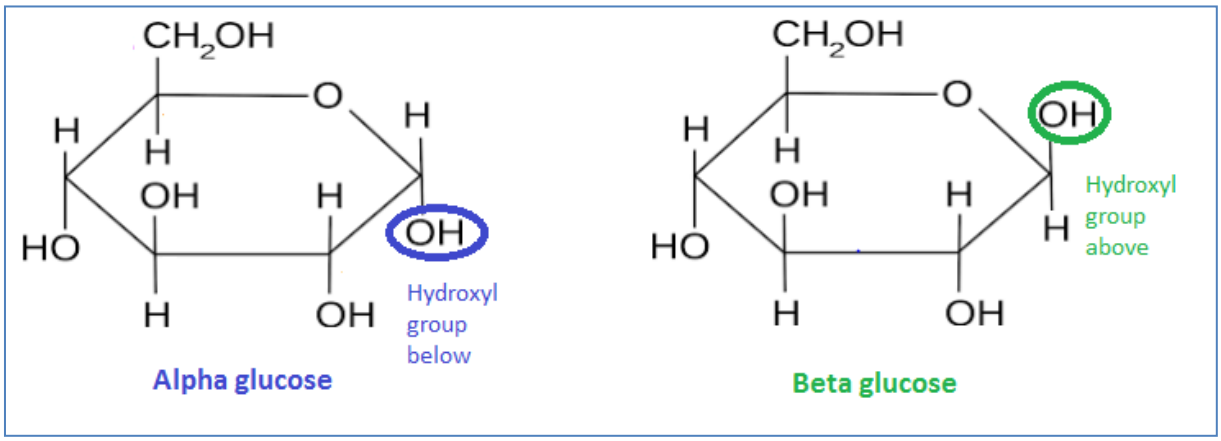


Figure 3: Structural forms of glucose

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.

1-4 Optical Activity

The presence of asymmetrical carbon atom causes optical activity. When a beam of plane-polarized light is passed through a solution of carbohydrates, it will rotate the light either to right or to left. Please note that the D- and L-notation has no bearing with the optical activity. Depending on the rotation, molecules are called dextrorotatory (+) (d) or levorotatory (-) (l).Thus **D-glucose is dextrorotatory but D-fructose is levorotatory**. Equimolecular mixture of optical isomers has no net rotation (**racemic mixture**). Heyrovsky was awarded Nobel prize in 1924 for polarographic analysis of sugars.

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.

- **D and L Isomerism of Glucose**

With reference to the penultimate carbon atom (i.e. C5 in the case of glucose), the configuration of H and OH groups is changed and two mirror images are produced (Fig. 6.3). It may be noted that in D and L varieties, the groups in 2nd, 3rd, 4th and 5th carbon atoms are totally reversed, so as to produce the mirror images. These two forms are also stereoisomers. **D sugars are naturally occurring** sugars and body can metabolise only D sugars **(Figure 4).**

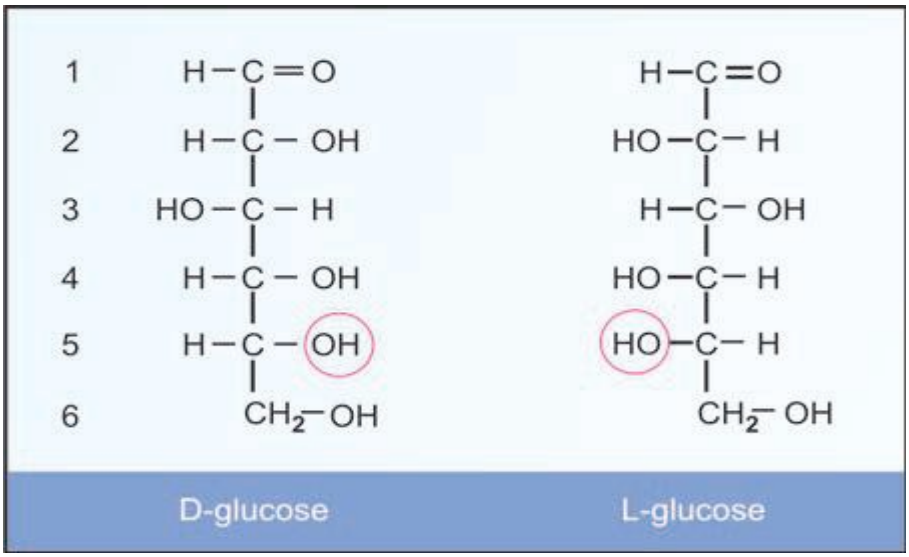


Figure 4: Penultimate carbon atom

❖ **Diastereo-isomers of Glucose**

Configurational changes with regard to C2, C3 and C4 will produce eight different monosaccharides. Out of these, only 3 are seen in human body. They are Glucose, Galactose and Mannose **(Figure 5)**.

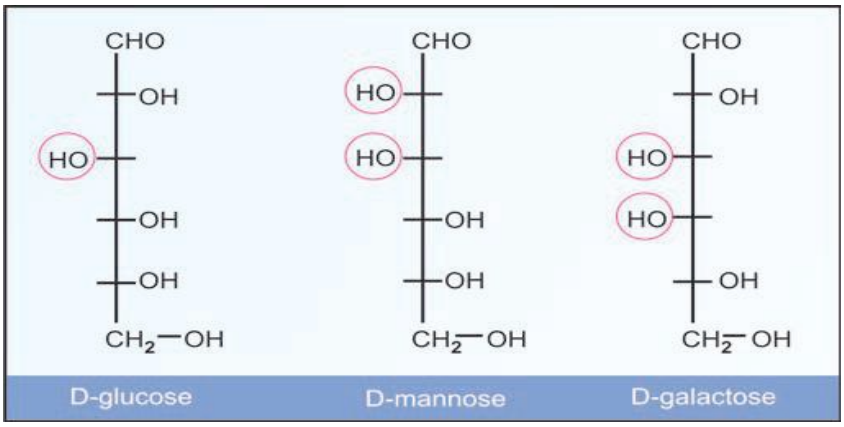


Figure 5: Epimers of D-glucose

There are 8 diastereoisomers for aldohexoses. With reference to C5, all of them will have D and L forms. Hence, the molecular formula of hexose ($C_6H_{12}O_6$) represents 16 different monosaccharides, due to spatial arrangement of constituent groups. Glucose is the most predominant sugar in human body. It is the major source of energy. It is present in blood D-glucose is dextrorotatory. In clinical practice, it is often called as dextrose (Box 6.5). Galactose is a constituent of lactose (milk sugar) and glycoproteins. Galactose is epimerised to glucose in liver and then utilized as a fuel. The term galactose is derived from Greek word gala, meaning milk. Mannose is a constituent of many glycoproteins. Mannose was isolated from plant mannans; hence the name.

1-5 Anomerism of Sugars

When D glucose is crystallized at room temperature, and a fresh solution is prepared, its specific rotation of polarized light is $+112^{o}$; but after 12-18 hours it changes to $+52.5^{o}$. If initial crystallization is taking place at $98^{o}C$ and then solubilized, the specific rotation is found to be $+19^{o}$, which also changes to$+52.5^{o}$ within a few hours. This change in rotation with time is called **mutarotation.** This is explained by the fact that D-glucose has two anomers, **alpha and beta varieties**.

These anomers are produced by the spatial configuration with reference to the first carbon atom in aldoses and second carbon atom in ketoses.Hence, these carbon atoms are known as **anomeric carbon atoms**. Thus α-D-glucose has specific rotation of $+112^{o}$ and β-D-glucose has $+19^{0}$. Both undergo mutarotation and at equilibrium one-third molecules are alpha type and 2/3rd are beta variety to get the specific rotation of $+52.5^{0}$. The hemiacetal structure of anomeric forms of glucose. The differences between α and β anomeric forms are dependent on the 1st carbon atom only. In the previous section 16 stereoisomers of glucose are described. Each of them will have 2 anomers; and hence there are a total of 32 isomers for glucose.

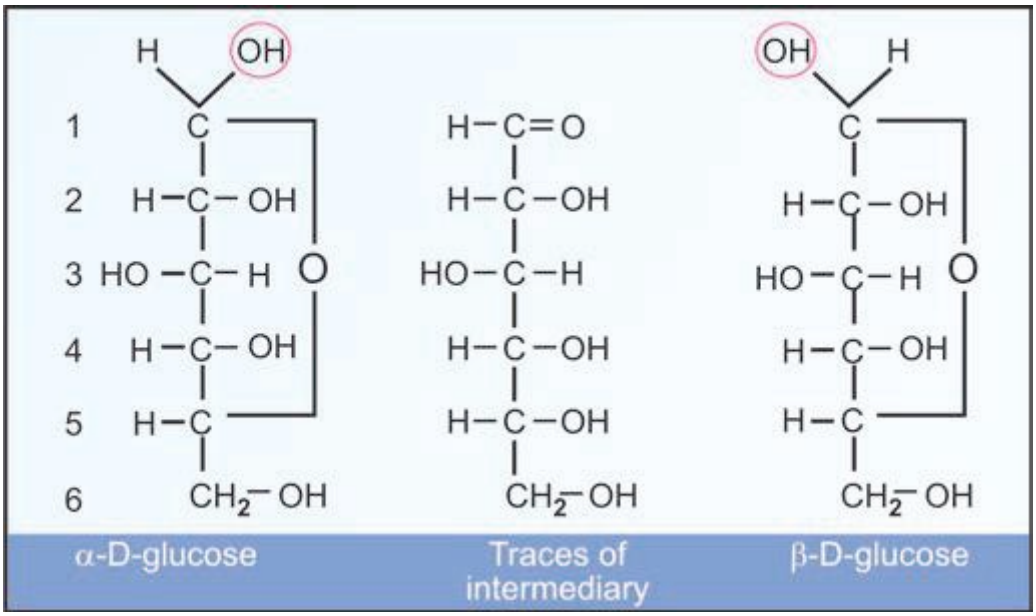


Figure 6: Anomers of D-glucose

All the monosaccharides except dihydroxyacetone contain one or more asymmetric (chiral) carbon atoms and thus occur in optically active isomeric forms **(Figure 6).** 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 2n 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 7)**.

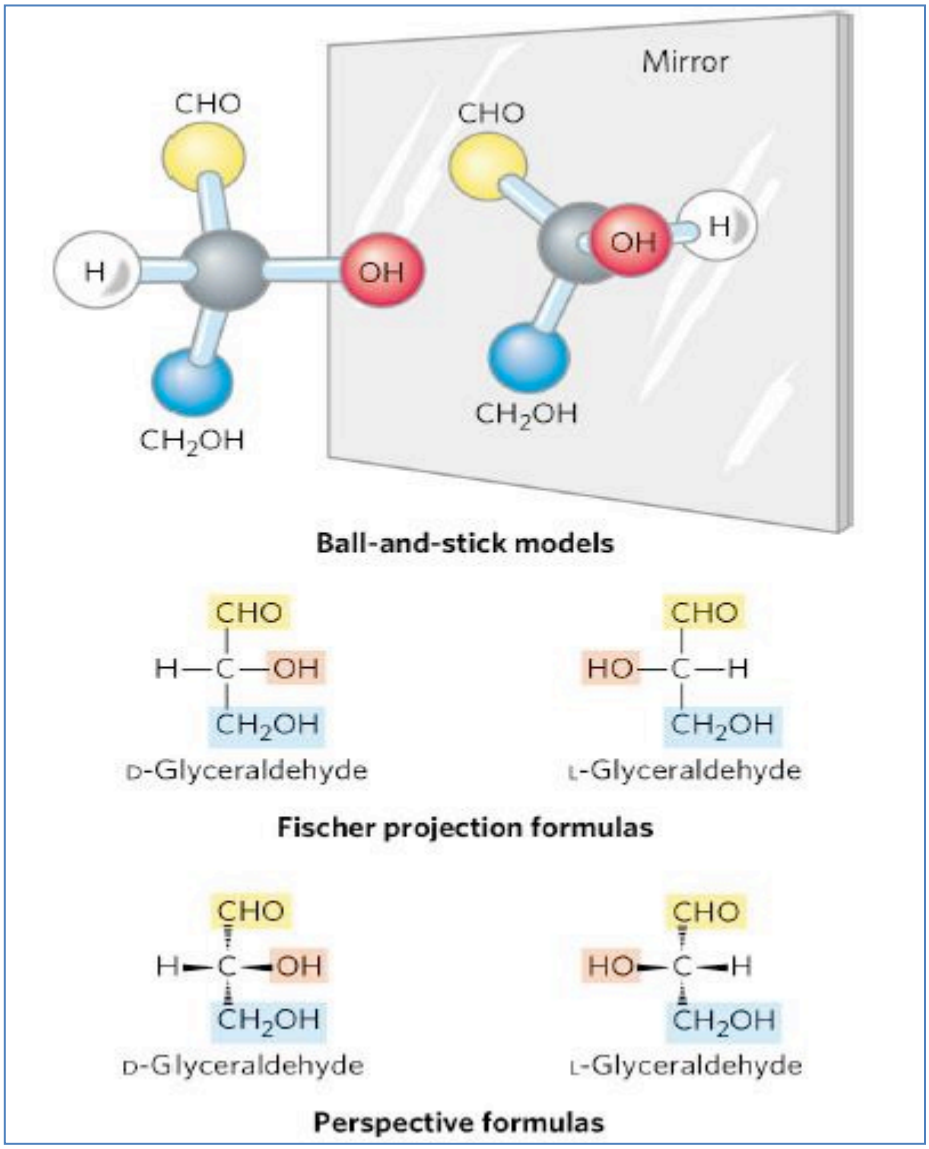


Figure 7: Three ways to represent the two enantiomers of glyceraldehyde.

The enantiomers are mirror images of each other. Ball-and stick models show the actual configuration of molecules.

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.

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 H2O and formation of a glycosidic bond. The reversal of this reaction is hydrolysis—attack by H2O 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*** **(Figure 8)**.

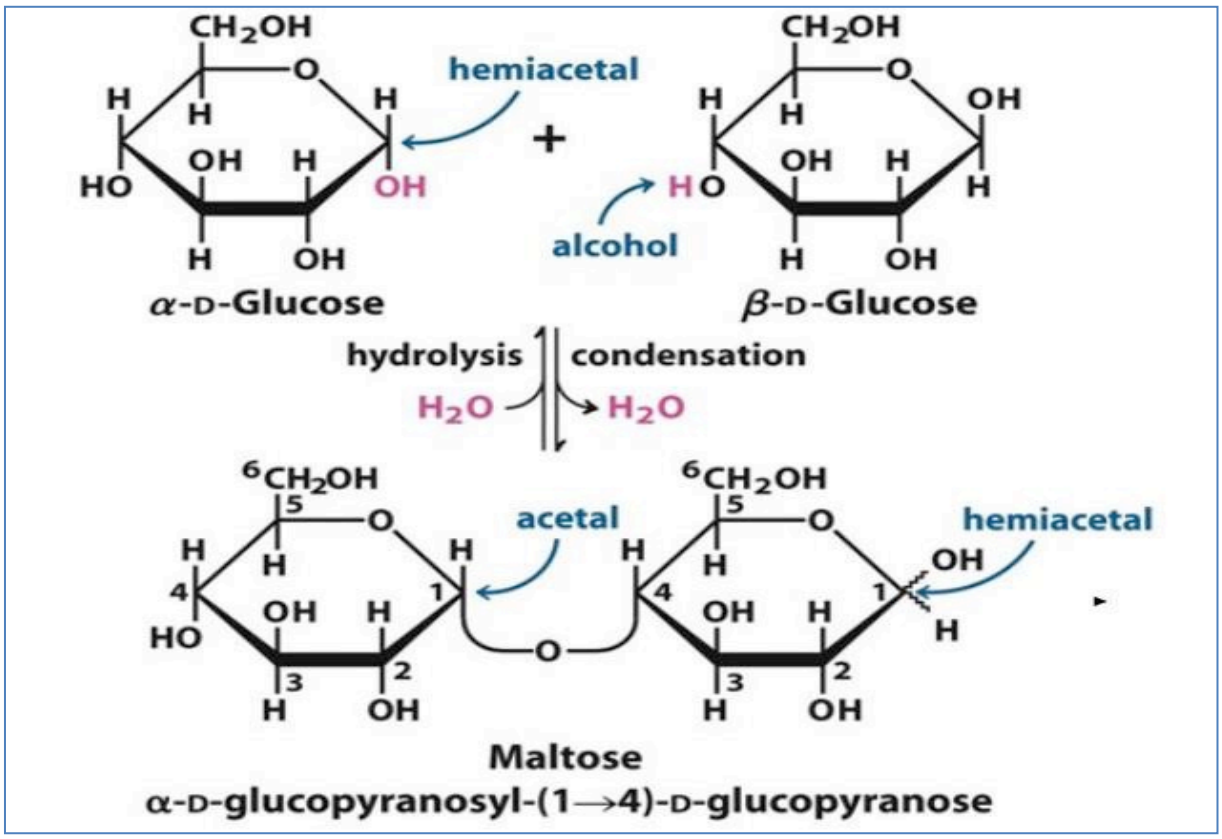


Figure 8: Formation of maltose

The disaccharide lactose , 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 CO2, 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.

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** 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. Trehalalso used commercially as a sweetener **(Figure 9)**.

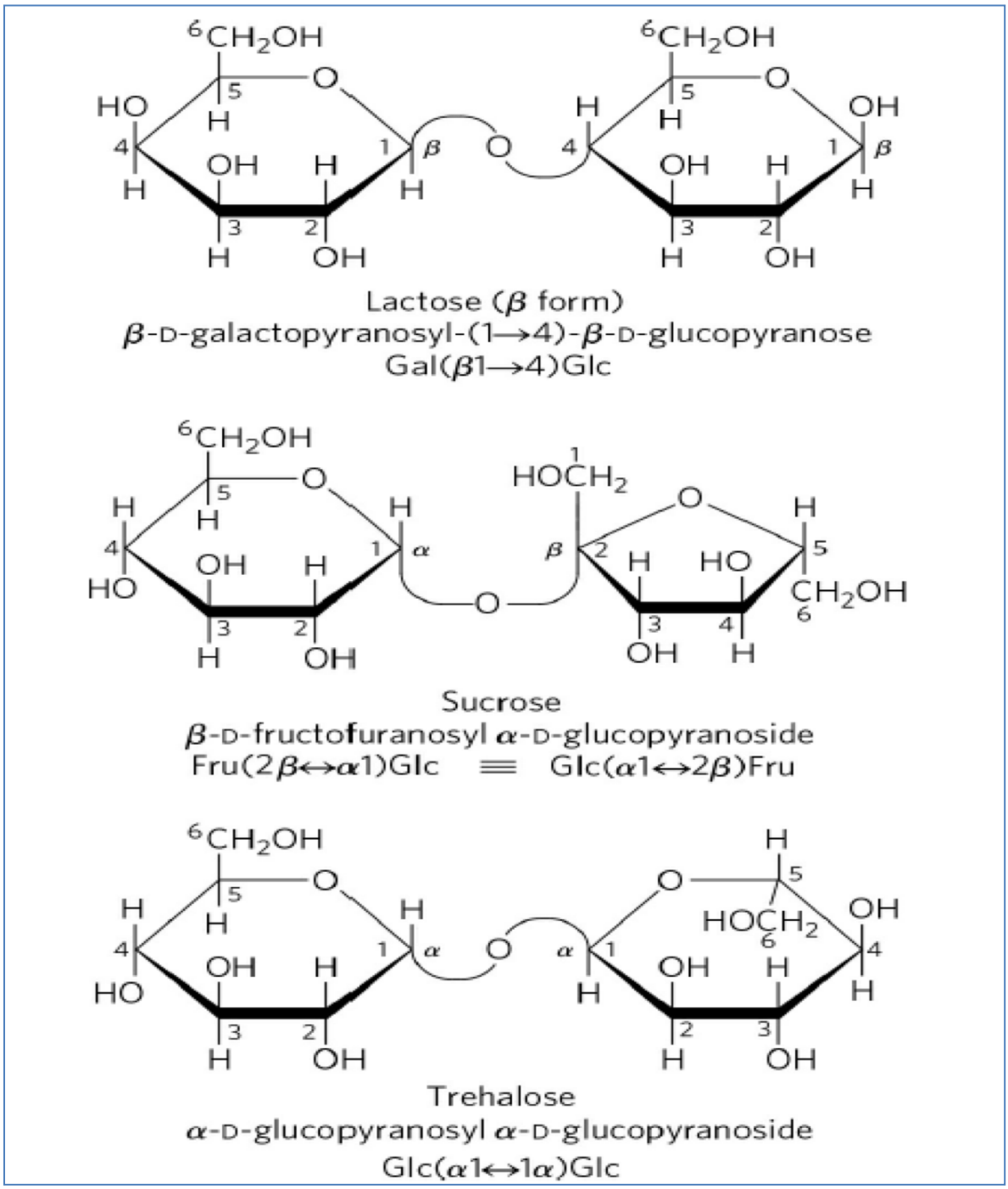


Figure 9: Common disaccharides

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) , is several hundred times sweeter than an equivalent amount of sucrose .

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.

1-6 Oligosaccharides

The general term oligosaccharide is often used for carbohydrates that contain from four to ten monosaccharide units. We will look at some of the common disaccharides, examine their bonding patterns and properties and then turn to some common polysaccharides. The oligosaccharides commonly encountered in nature belong to disaccharides. The physiologically important disaccharides are maltose, lactose, trehalose and sucrose. Disaccharides consist of two monosaccharides joined covalently by an O-glycosidic bond.

The hydroxyl group formed as a result of hemiacetal formation is highly reactive when compared to other hydroxyl groups. This hydroxyl group present in one monosaccharide reacts with anyone of the hydroxyl groups attached to C-l, C-2, C-3, C-4, or C-6 of another monosaccharide to produce 1 → 1 , 1 2, 1 3, 1 4, and 1 6 linked disaccharides. When only one anomeric carbon is involved in glycosidic bond formation, reducing disaccharides are formed. If both anomeric carbon atoms of monosaccharides are involved in glycosidic bond formation that results in the formation of a non-reducing disaccharides such as trehalose (aldosyl-aldosyl disaccharide) or sucrose (aldosyl-ketosyl disaccharide)'. In the case of reducing

disaccharides, one end of the molecule having free anomeric carbon is called reducing end and the other end, where the anomeric carbon is involved in glycosidic bond, is called as non-reducing end.

1-7 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 .

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 10)**.

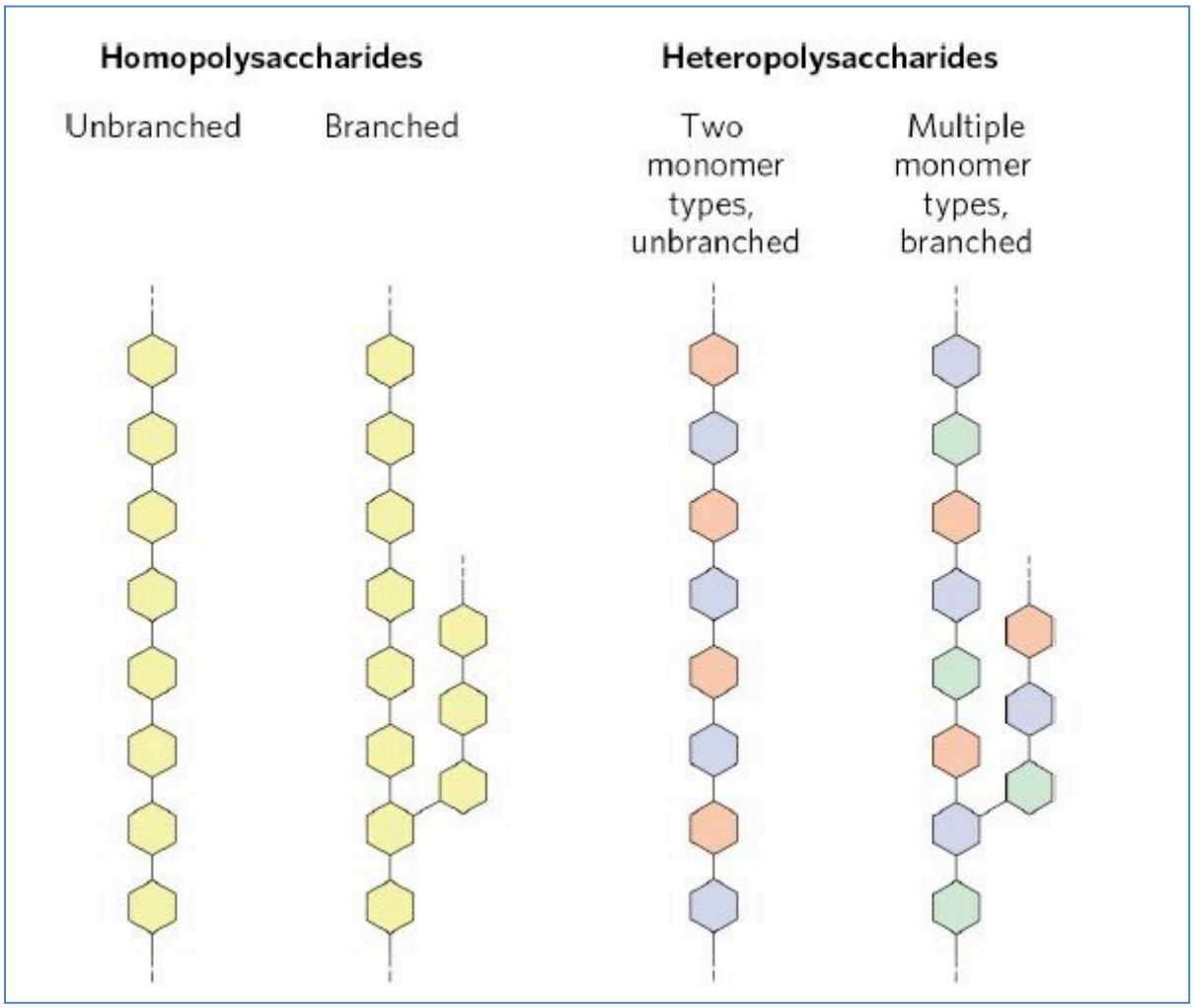


Figure 10: Homopolysaccharides and heteropolysaccharides.

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 intra cellularly 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 . 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.

A- Amylose : linear, a-l,4 glycosidic links. MW: 4,000-150,000. Deep blue col or with iodine due to coiled complex enclosing iodine-colour is lost with heating, returns when cooled. Amylopectin: branched every 30 or 50 units - linear a-l,4 chain of 30 gluresidues then a-l,6 branch point. Of course get branches on branches as well. MW: ->500,000. Broken down by - amylase (pancreas and salivary glands; random cleavage of a-l,4 links) to give glucose and maltose; or beta-amylase (plants; hydrolyses from reducing ends to give maltose). When either of these enzymes attack amylopectin, they are blocked when they reach or are near a branch, thus end up with a "limit dextrin."

Foods such as potatoes, rice, corn and wheat contain starch granules which are important energy sources for humans. The human digestive process breaks down the starches into glucose units with the aid of enzymes, and those glucose molecules can circulate in the blood stream as an energy source. An interesting example of this enzyme-catalyzed breakdown process is if you chew on a piece of bread for a while, it will begin to taste sweet because the enzymes in saliva are already beginning to break down the starch into glucose, a sugar.

B- Inulin

Inulin is a non-digestible fructosyl oligosaccharide found naturally in more than 36000 types of plants. It is a storage polysaccharide found in onion, garlic, chicory, artichoke, asparagus, banana, wheat and rye. It consists of mainly, if not exclusively, of β 2 1 fructosyl-fructose links. A starting glucose moiety can be present, but is not necessary.

Inulin is a soluble fibre that helps maintain normal bowel function, decreases constipation, lowers cholesrerol and triglycerides. It is used for fat replacement and fibre enrichment in processed foods.

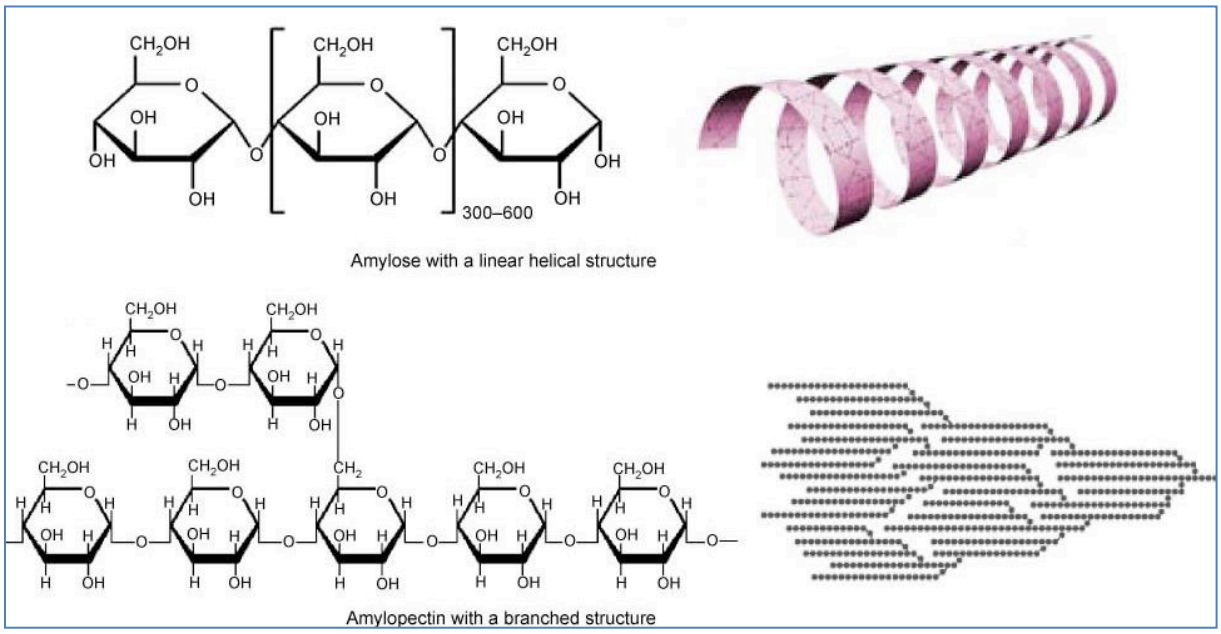


Figure 11: Amylose and amylopectin stuctures

C- Glycogen 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 12)**.

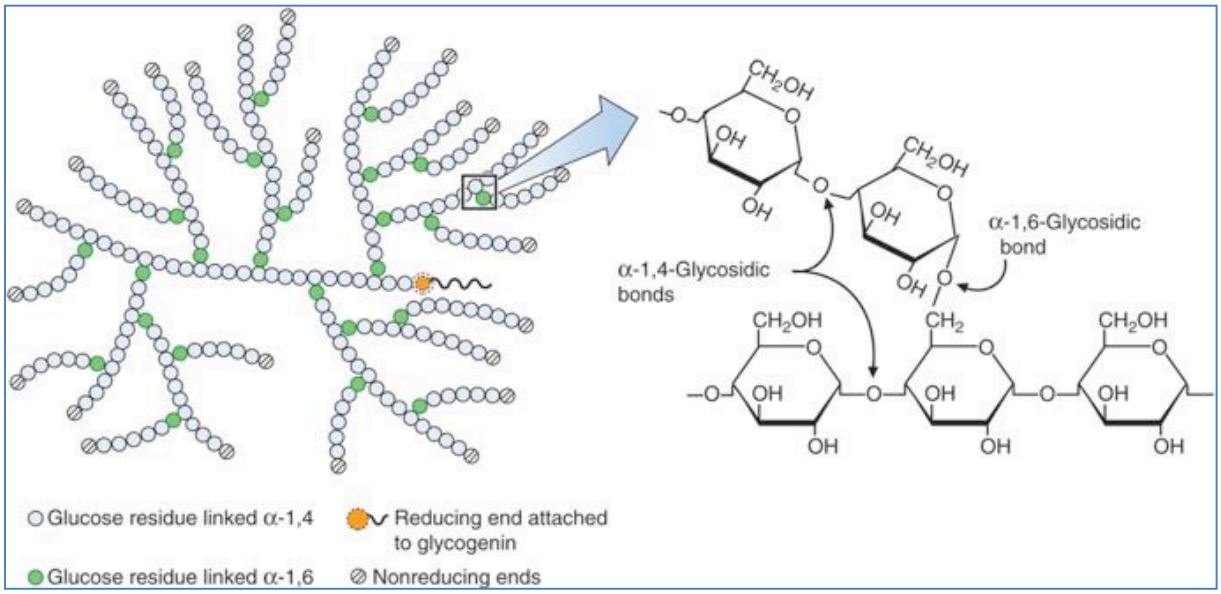


Figure 12: Glycogen is a polymer of (α1→4)-linked glucose subunits, with (α1→6)-linked branches

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.

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 readily digest cellulose (and therefore wood), but only because their intestinal tract harbors a symbiotic microorganism, Trichonympha that secretes cellulase, which hydrolyzes the (β1→4) linkages.

D-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.

E- Cellulose , 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 13)** .

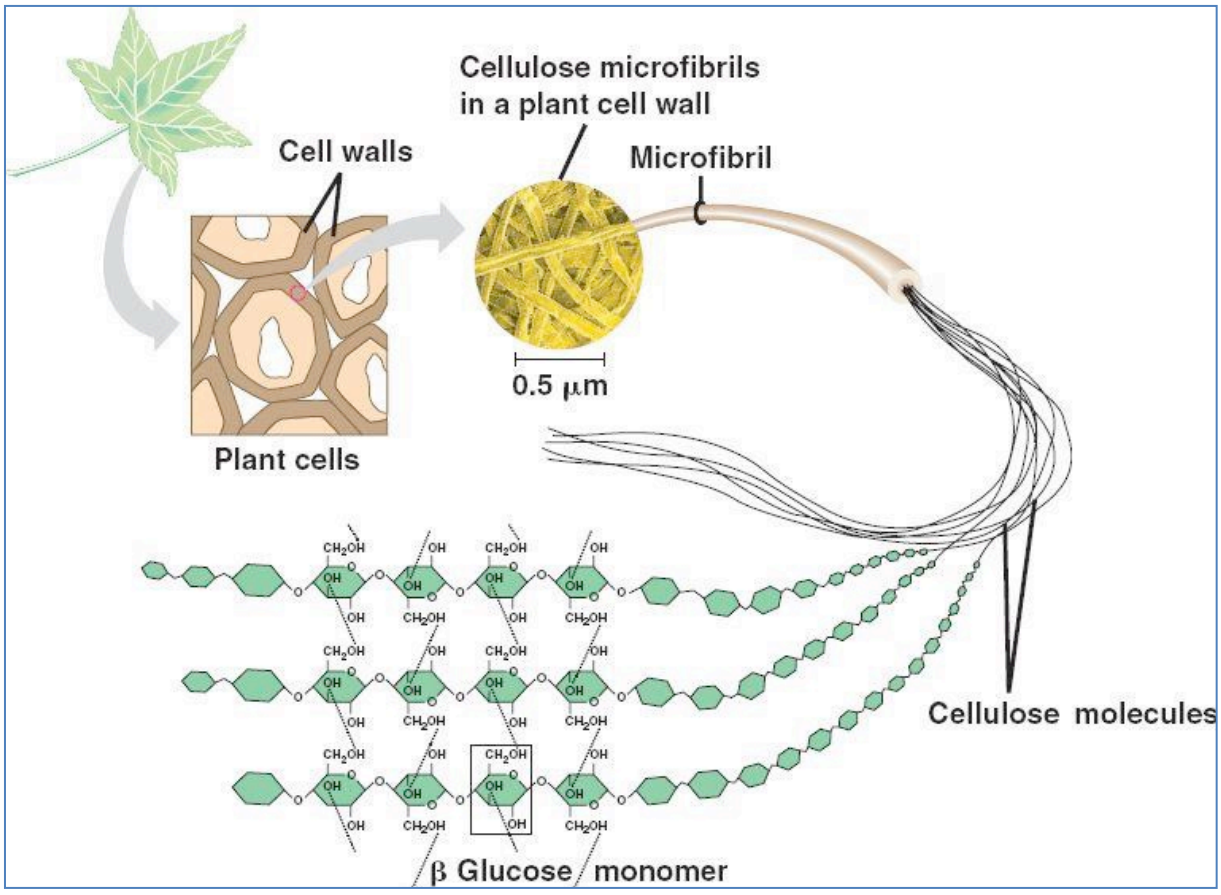


Figure 13: Cellulose structure

I-8 Functions of carbohydrates

1. Carbohydrates are the main sources of energy in the body. Brain cells and RBCs are almost wholly dependent on carbohydrates as the energy source. Energy production from carbohydrates will be 4 k calories/g (16 k Joules/g).

2. Storage form of energy (starch and glycogen).

3. Excess carbohydrate is converted to fat.

4. Glycoproteins and glycolipids are components of cell membranes and receptors.

5. Structural basis of many organisms:

Cellulose of plants; exoskeleton of insects, cell wall of microorganisms,mucopolysaccharides as ground substance in higher organisms.

6 . Energetic (major metabolic fuel);

7. Structural (glycosaminoglycans of connective tissues, glycolipids in membranes);

8. Metabolic (lipids and some aminoacids can synthesized from carbohydrates);

9. Protective (components of immunoglobulins);

10. Receptative (glycoproteins of membranes);

11. Antigenic (glycoproteins of erythrocytes define the group of blood);

12. Plastic (elements in the structure of DNA, RNA, FAD, NAD(P), etc);

13. Antitoxic.

1-9 Chemical Properties of Carbohydrates

Reactions of monosaccharides are due to the presence of hydroxyl (-OH) and the potentially free aldehyde (-CHO) or keto (>C=O) groups.

• Sugars in weak alkaline solutions undergo isomerization to form 1,2- enediol followed by the formation of a mixture of sugars.

• Under strong alkaline conditions sugar undergo caramelization reactions.

• Sugars are classified as either reducing or non-reducing depending upon the presence of potentially free aldehyde or keto groups. The reducing property is mainly due to the ability of these sugars to reduce metal ions such as copper or silver to form insoluble cuprous oxide, under alkaline condition. The aldehyde group of aldoses is oxidized to carboxylic acid. This reducing property is the basis for qualitative (Fehling's, Benedict's, Barfoed's and Nylander's tests) and quantitative reactions.

All monosaccharides are reducing. In the case of oligosaccharides, if the molecule possesses a free aldehyde or ketone group it belongs to reducing sugar (maltose and lactose). If the reducing groups are involved in the formation of glycosodic linkage, the sugar belongs to the non- reducing group (trehalose, sucrose, raffinose and stachyose).

• When reducing sugars are heated with phenylhydrazine at pH 4.7 a yellow precipitate is obtained. The precipitated compound is called as osazone. One molecule of reducing sugar reacts with three molecules of phenylhydrazine. D-mannose and D-fructose form same type of osazone as that of Dglucose since the configuration of C-3, C-4, C-5 and C-6 is same for all the three sugars.

The osazone of D-galactose is different. Different sugars form osazone at different rates. For example, D-fructose forms osazone more readily than D-glucose. The osazones are crystalline solids with characteristic shapes, decomposition points and specific optical rotations. The time of formation and crystalline shape of osazone is utilized for identification of sugars. If methyl phenylhydrazine is used instead of phenylhydrazine in the preparation of osazone, only ketoses react. This reaction serves to distinguish between aldose and ketose sugars.

• The hydroxyl group formed as a result of hemiacetal formation in monosaccharides react with methanol and HCl to form methyl a- and ~glycosides. The derivaties of each sugar are named according to the name of the sugar, that is, the derivaties of glucose as glucosides, of galactose as galactosides and of arabinose as arabinosides etc. Glycosides are acidlabile but are relatively stable at alkaline pH. Since the formation of glycosides convert the aldehydic group to- an acetal group, the glucosides are not a reducing sugars.

• **Glycosides** are also formed with a non-sugar component, the aglycone. The sugars which are connected to the non-sugar moiety, are pentoses, hexoses, branched sugars or deoxy or dideoxy sugars. The chain length varies from one to five monosaccharide sugar re si dues per glycosides.

Apart from O-glycosides, three other classes of glycosides are found in higher plants namely S-glycosides, N-glycosides and C-glycosides.

• **Monosaccharides** are generally stable to hot dilute mineral acids though ketoses are appreciably decomposed by prolonged action.

• Heating a solution of hexoses in a strong non-oxidising acidic conditions

hydroxy methyl furfural is formed. The hydroxymethyl furfural from hexose is usually oxidized further to other products When phenolic compounds such as resorcinol, a-naphthol or anthrone are added, mixture of coloured compounds are formed.

• The molisch test used for detecting carbohydrate in solution is based onn following principle. When conc. H2S04 is added slowly to a carbohydrate solution containing a-naphthol, a pink colour is produced at the juncture. The heat generated during the reaction hydrolyse and dehydrate it to produce furfural or hydroxymethyl furfural which then react with anaphthol to produce the pink colour.

• When sugars are treated with appropriate acid anhydride or acid chloride under proper conditions, the hydroxyl groups get esterified and form sugar esters.

I-10 1Digestion of carbohydrates

Dietary carbohydrates enter the gut as mono-, di-, and polysaccharides. Disaccharides and polysaccharides require hydrolytic cleavage prior to absorption. During the eating process and homogenization that occurs with mastication in the mouth and the action of gastric folds, dietary polysaccharides become hydrated.

Hydration of polysaccharides is essential for the appropriate action of amylase. This enzyme is specific for internal α1→4-glycosidic linkages. The cleaved units thus formed are the trisaccharide maltotriose, the disaccharide maltose, and an oligosaccharide with one or more α1→6 branches, and containing on average eight glycosyl units termed the "α-limit dextrin". These compounds are then further cleaved to glucose units by oligosaccharidase and α-glucosidase, the latter removing single glucose residues from α1→4-linked oligosaccharides (including maltose) from the nonreducing end of the oligomer. A sucrase-isomaltase complex activated to two separate active polypeptide enzymes, one of which (isomaltase) is responsible for the hydrolytic cleavage of α1→6 glycosidic linkages. Disaccharides are acted upon by membrane-bound disaccharidases on the intestinal mucosal surface.

Dietary disaccharides such as lactose, sucrose, and trehalose are hydrolyzed to their constituent monomeric sugars by a series of specific disaccharidases, which are attached to the small intestinal brush-border membrane.

2-1 -Glucose Metabolism

2-1-1 Absorption of monosaccharides in the small intestine

There are active and passive transport systems which transport carbohydrates across the brush-border membrane. Glucose, fructose, and galactose are the primary monosaccharides produced by the digestion of dietary carbohydrates. The absorption of these sugars occurs via

specific carrier-mediated mechanisms. In addition, all monosaccharides can cross the brush-border membrane by a simple diffusion process, although it is extremely slow.

At least two carrier-mediated transport systems for monosaccharides exist – an Na+-dependent co-transporter and an Na+-independent transporter. At the brush-border membrane both **glucose** and **galactose** are transported by the **Na+-dependent glucose transporter**. This membrane-linked protein binds with glucose (galactose) and Na+ at separate sites and transports both into cytosol. The Na+ is thus transported down its concentration gradient, carrying glucose against its concentration gradient. This transport mechanism is linked to Na+-dependent ATPase, which then removes Na+ from the cell in exchange for K+, with the concomitant hydrolysis of ATP. The transport of glucose (galactose) is thus an **indirect active process**.

Fructose is transported across the brush-border membrane by an Na+-independent facilitated diffusion process involving a specific membrane-associated protein, possibly glucose transporter (GLUT 5), which is present on the luminal side of the enterocyte, and GLUT 2 present on the anti luminal side.

2-1-2 Transport of glucose from the bloodstream to the tissues

Glucose transporters are essential for facilitated diffusion of glucose into cells. According to the latest data, the glucose transporter family comprises 15 major species, named GLUT. Cells in insulin-sensitive tissue such as muscle and adipose have GLUT 4. Insulin stimulates translocation of GLUT 4 from intracellular vesicles to the plasma membrane, facilitating glucose uptake during meals **(Figure 14)**.

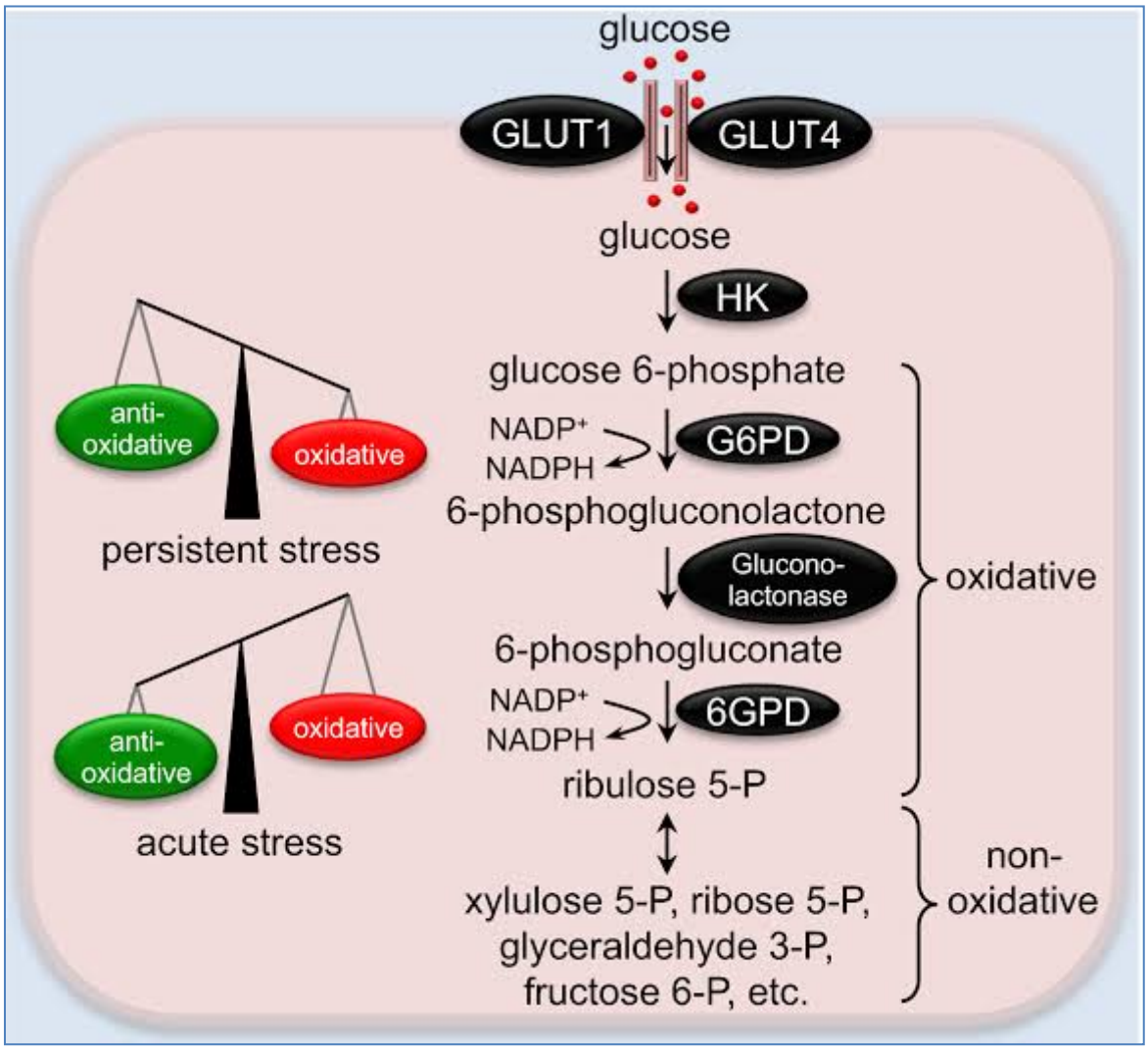


Figure 14: Glucose transport

2-1-3 Pathways of glucose metabolism

Glucose is the major carbohydrate on Earth, the backbone and monomer unit of cellulose and starch. Glucose is the most important carbohydrate. Most dietary carbohydrates are absorbed into the bloodstream as glucose, and other sugars are converted to glucose in the liver. Glucose is the major metabolic fuel of mammals. It is the precursor for synthesis of all other carbohydrates in the body, including glycogen for storage; ribose and deoxyribose for nucleotides; galactose in lactose of milk, in glycolipids, glycoproteins and proteoglicans. Glucose enters metabolism by phosphorylation to glucose 6-phosphate, catalyzed by **hexokinase**. Hexokinase has a high affinity (low Km) for glucose, and is saturated under all normal conditions and so acts at a constant rate to provide glucose 6-phosphate to meet the cell's need. Hexokinase is inhibited allosterically by its product, glucose 6-phosphate.

Liver and pancreatic β islet cells also contain an isoenzyme of hexokinase, **glucokinase**. The glucokinase differs from hexokinase:

- The glucose concentration at which glucokinase is half-saturated (about 10 mM) is higher than the usual concentration of glucose in the blood. When blood glucose is high, as it is after a meal rich in carbohydrates, excess glucose is transported into hepatocytes, where glucokinase converts it to glucose 6-phosphate, which will be used for glycogen synthesis;
- glucokinase is not inhibited by glucose 6-phosphate, and it can therefore continue to operate when the accumulation of glucose 6-phosphate completely inhibits hexokinase;
- glucokinase is subject to inhibition by the reversible binding of a regulatory protein specific to liver. Glucokinase is also regulated at the level of protein synthesis. Circumstances that call for greater energy production or a high-carbohydrate diet cause increased transcription of the glucokinase gene **(Figure 15)**.

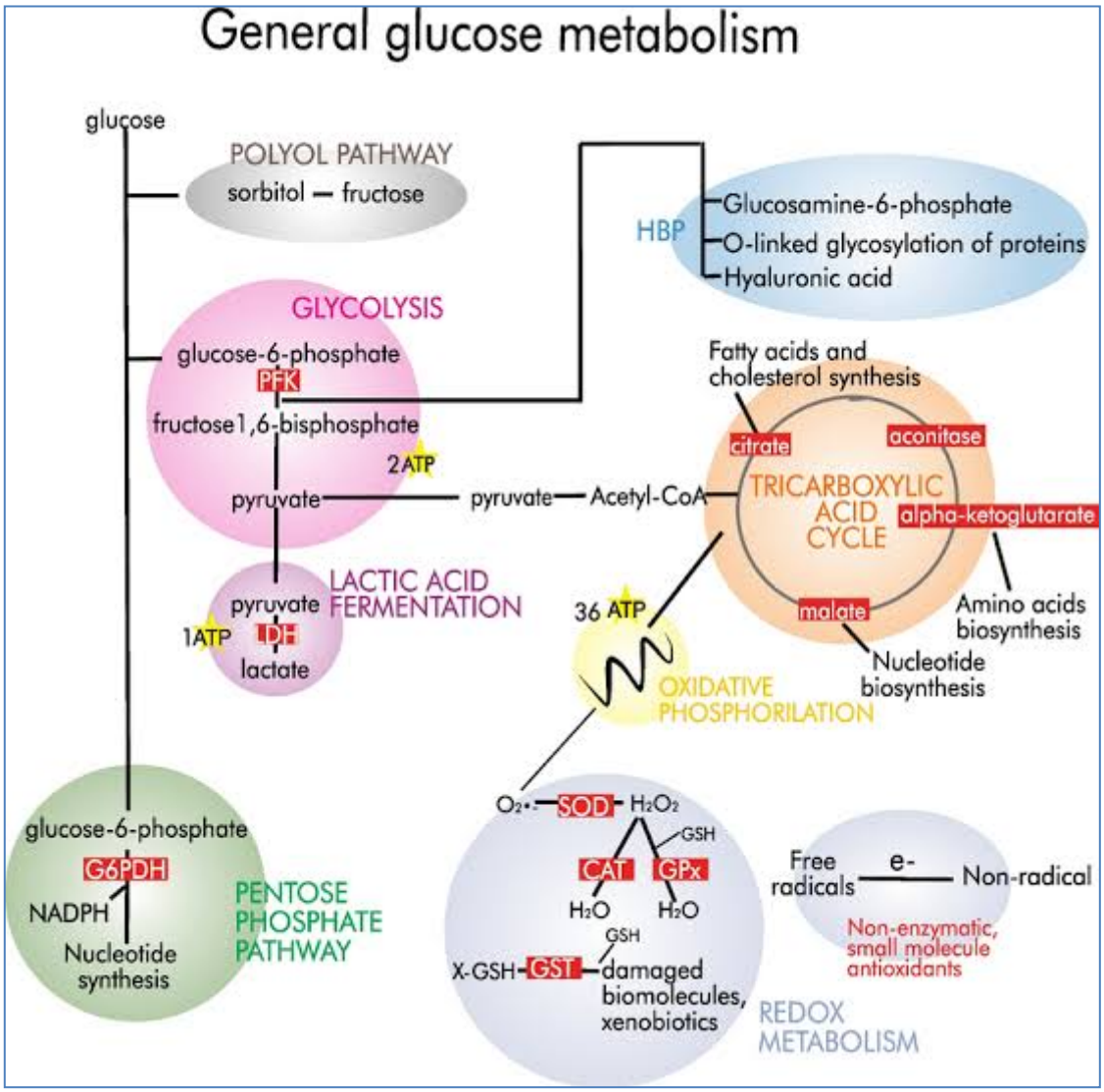


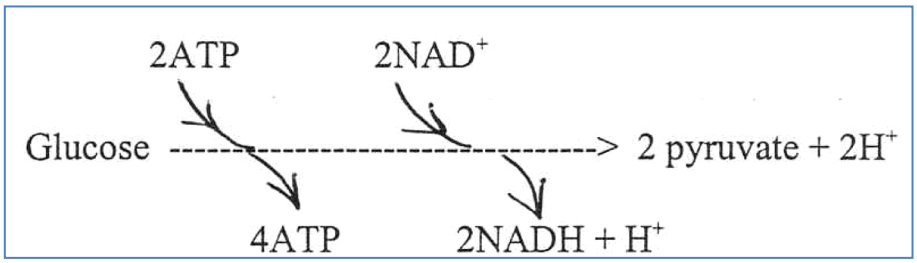
Figure 15: General glucose metabolism

2-2 Glycolysis

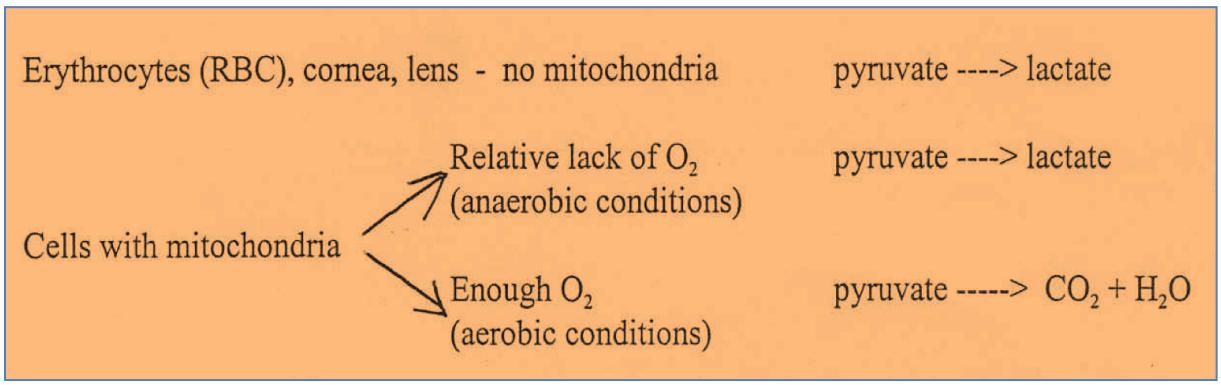
Glycolysis is a set of reactions that converts glucose to pyruvate or lactate. This is the first metabolic pathway to be elucidated and hence is considered as a paradigm of metabolic pathways. Glycolysis is also called Embden-Meyerhoff pathway. The complete set of reactions occurs in the cytoplasm of virtually every animal cell. The entire process occurs without molecular oxygen. .

2-2-1 The roles of glycolysis:

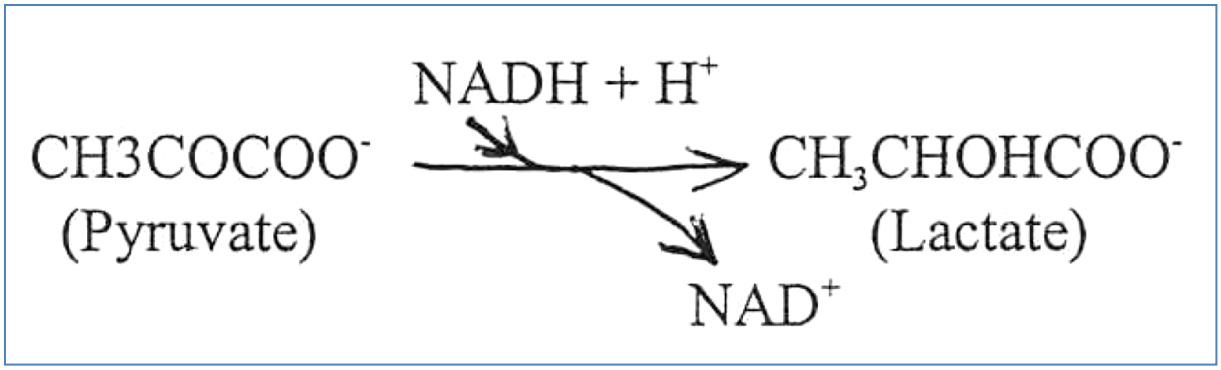
• to produce energy;
• to produce intermediates for metabolic pathways.



Glycolysis consumes 2 ATP and generates 4ATP. Thus, the process results in the generation of 2 net ATP. The process also generates 2 NADH. What happens to pyruvate depends upon the presence or absence of mitochondria in the cell or upon the availability of oxygen in mitochondria – containing cells.



For the glycolytic pathway to continue, NAD^+ has to be regenerated. In erythrocytes (no mitochondria) and in mitochondria – possessing cells under anaerobic conditions, NAD^+ is regenerated from NADH during the conversion of pyruvate to lactate **(Figure 16) (Figure 17)** .

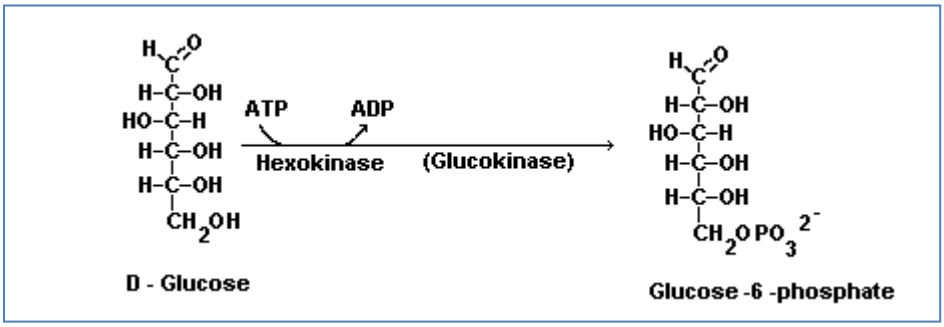


In mitochondria – possessing cells under aerobic conditions, NAD^+ is regenerated by either malate – aspartate shuttle or α-glycerophosphate shuttle, which transfer the reducing

equivalents from NADH into mitochondria for electron transport chain, thus regenerating NAD^+ in the cytoplasm.

2-2-2 Reactions

Glycolysis consists of two phases. In the first phase, glucose is broken down to two molecules of glyceraldehyde-3-phosphate in a series of five reactions. In the second phase, another series of five reactions convert these two molecules of glyceraldehyde-3-phosphate into two molecules of pyruvate. Phase I consumes 2 ATP and Phase II generates 4 ATP. The net ATP production in the entire process is 2.



This is the first intracellular reaction of glycolysis (remember all reactions are in the cytoplasm).

b. Requires an ATP (Mg). This is one of the investment reactions.

c. The phosphorylation of glucose traps the glucose inside the cell.

d. The reaction is considered irreversible.

e. Hexokinase has a K_m for glucose of less than 0.1 mM (high affinity). It is also inhibited by the product glucose-6-phosphate

f. Liver hepatocytes and pancreatic β cells contain another enzyme Glucokinase.

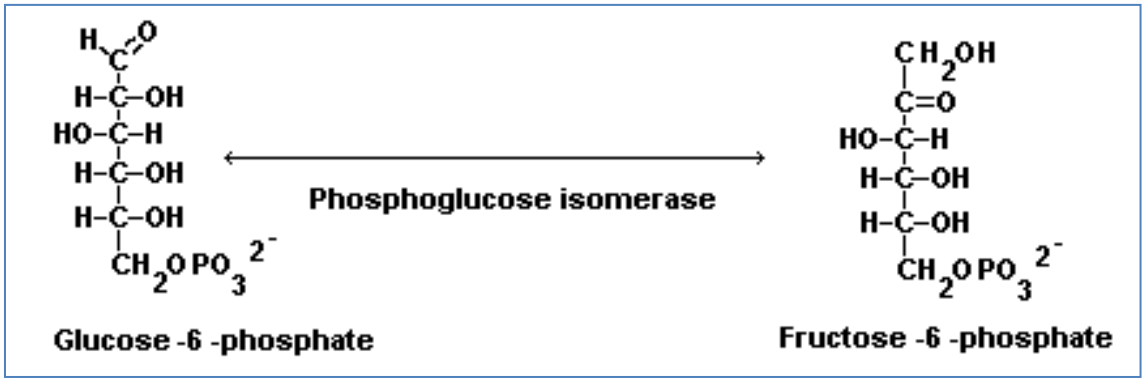
Glucokinase has a K_m for glucose of about 10 mM (low affinity).

Glucokinase is not inhibited by its product glucose-6-phosphate.

Glucokinase is induced by insulin. The levels of glucokinase in the liver of untreated Type 1 diabetics are lower than normal.

Hexokinase and glucokinase are isoenzymes. Therefore, irrespective of the isoenzyme catalyzing the reaction, the Keq, ΔG, and ΔG^o for the reaction remain the same.

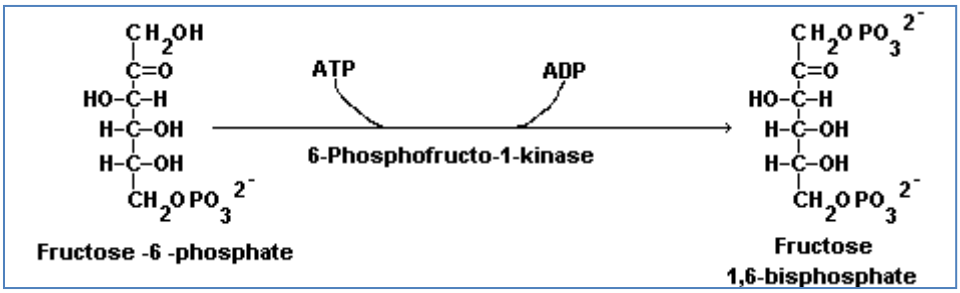
Reaction 2: Phosphoglucose isomerase.



a. This reaction is readily reversible (not a controlling step) and functions in both glycolysis and gluconeogenesis.

b. Conversion of an aldose to a ketose.

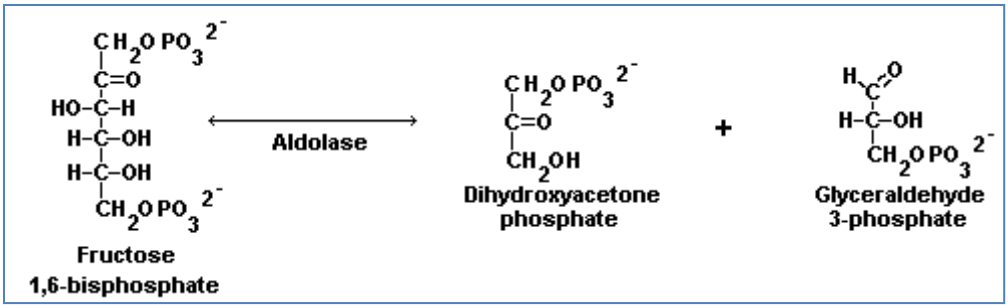
Reaction 3: 6-Phosphofructo-1-kinase (PFK-1) or **Phosphofructokinase-1**



a. Reaction is the rate-limiting step of glycolysis.

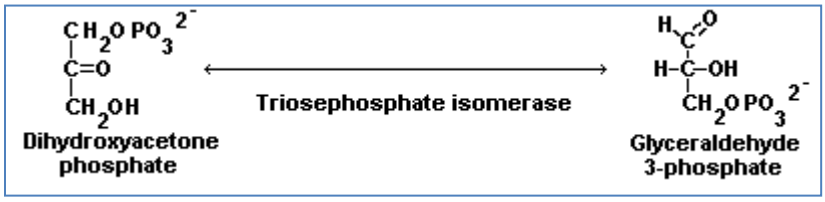
b. It is irreversible, and the committed step. It is an allosteric enzyme and also a major regulatory enzyme.

c. We have invested our second ATP molecule.



a. We now have two phosphorylated trioses.

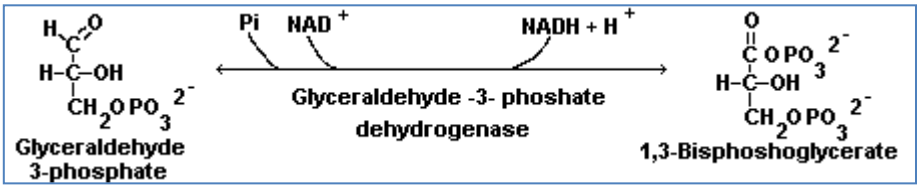
b. Only glyceraldehyde-3-phosphate is used in glycolysis. Therefore, dihydroxyacetone phosphate has to be converted into glyceraldehyde-3-phosphate. This occurs in the next step.



Reaction 5: Triose phosphate isomerase.

a. Catalyzes the interconversion of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate.
b. Because of the interconversion, one glucose molecule can be converted to two glyceraldehydes 3-phosphate molecules.

Reaction 6: **Glyceraldehyde-3-phosphate dehydrogenase**



a. The enzyme oxidizes the number one carbon aldehyde and then adds a phosphate group. We have an acid anhydride in the product 1,3-bisphophoglycerate. Remember from bioenergetics that acid anhydrides are high-energy bonds.

b. The phosphate on the number 3 carbon is not an high-energy bond

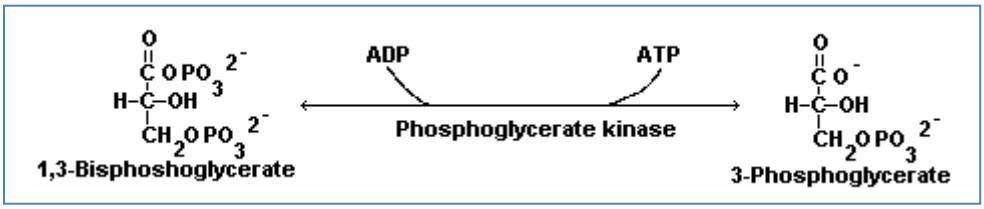
c. We have used an NAD^+ for the oxidation reaction. The cell has limited amounts of NAD^+, so somewhere along the line we have to regenerate it or glycolysis will stop.

d. This reaction is a target for Arsenate (AsO_4^{3-}). The arsenate resembles inorganic phosphate (Pi). In the presence of arsenate, the product of the reaction is 1-arseno-3-phosphoglycerate. This product is unstable and decomposes into arsenate and 3-phosphoglycerate with no ATP formation. After this step, glycolysis continues.

e. The enzyme contains an essential thiol (cysteine-SH) group at the active site. Iodoacetic acid (ICH_2COOH) is also an inhibitor of this reaction. It reacts with the active site SH group and inhibits the enzyme.

Reaction 7:

Phosphoglycerate kinase

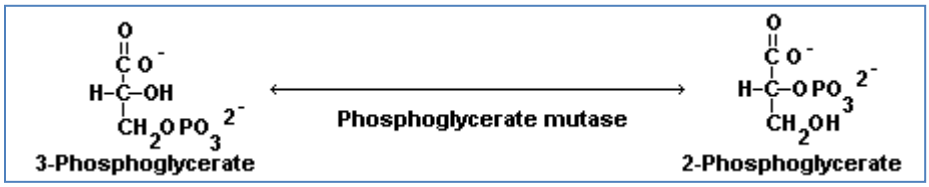


a. This is the first step of energy production.

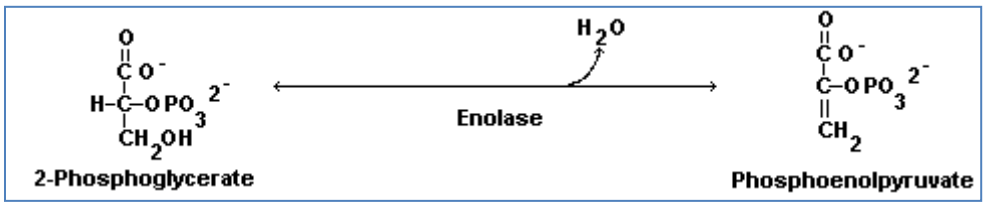
b. This is referred to as substrate-level phosphorylation as opposed to oxidative phosphorylation that occurs in mitochondrial ATP production.

d. We have recovered both ATP that were invested. Remember that each glucose gives 2 phosphoglycerate molecules.

Reaction 8: Phosphoglycerate mutase.



Reaction 9: Enolase

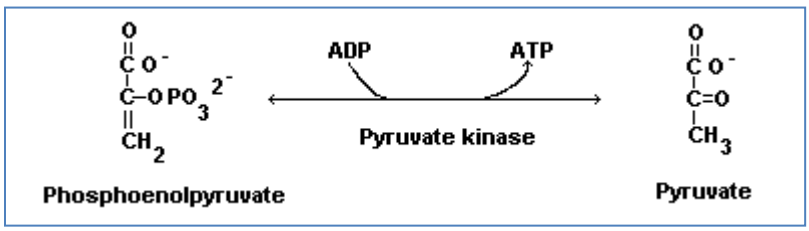


a. Catalyzes the dehydration of 2-phosphoglycerate to form phosphoenolpyruvate (PEP).

c. Recall from the bioenergetics lecture that PEP contains an high-energy bond.

d. This reaction is inhibited by Fluoride.

Reaction 10: Pyruvate Kinase



a. Catalyzes the transfer of the phosphate from PEP to ADP to generate ATP and 9

pyruvate.

b. Again, this is substrate-level phosphorylation.

c. This reaction completes that part of glycolysis that is common to both anaerobic and aerobic metabolism.

d. Under aerobic conditions and the presence of mitochondria, pyruvate can enter the citric acid cycle. NAD^+ is regenerated by malate/aspartate shuttle or by α-glycerophosphate shuttle

e. Under anaerobic conditions or in the absence of mitochondria, pyruvate is reduced to lactate via the following reaction and regenerate NAD^+.

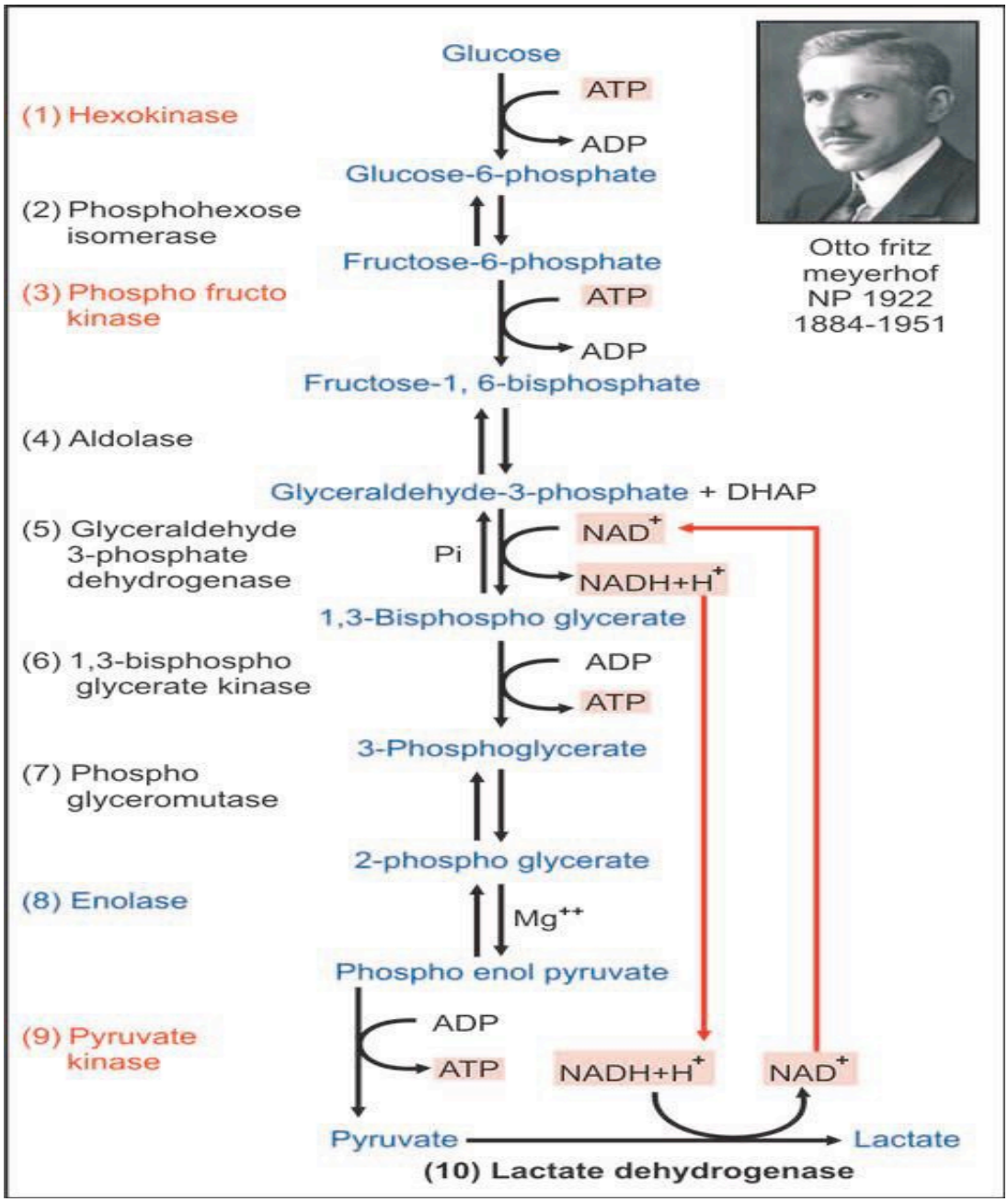
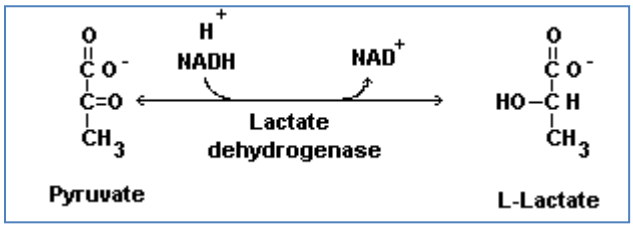


Figure 16: Summary of glycolysis (Embden-Meyerhof pathway).

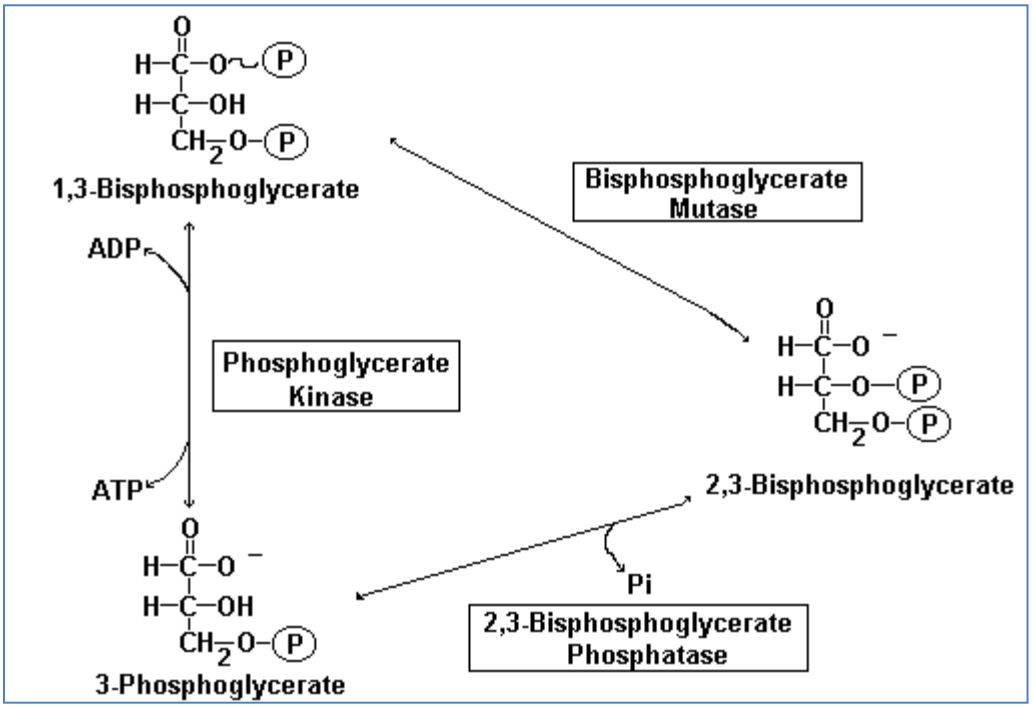
Steps 1, 3 and 9 are key enzyems; thesereactions are irreversible. Steps 5, 6 and 9 produce energy. Steps 5 and 10 are coupled for regeneration of NAD+

❖ **Anerobic Glycolysis**

When pyruvate cannot be oxidized within mitochondria for some reason (e.g., hypoxia, genetic defects in pyruvate dehydrogenase or citric acid cycle enzymes, genetic defects in electron transport chain), pyruvate is reduced to lactate by lactate dehydrogenase.



Synthesis of 2, 3-bisphosphoglycerate



a. All cells contain a catalytic amount of 2,3-BPG.

b. Red blood cells contain a high concentration of 2,3-BPG (~ 4mM). It facilitates the release of oxygen from hemoglobin**.**

c. ~20% of glucose goes through this shunt in RBC.

d. No net ATP is generated if 1,3-BPG is converted to 3-PG via 2,3-BPG. If the entire glycolytic pathway in RBC occurs via the formation of 2,3-BPG, glycolysis will yield no net ATP because

the 2 ATP produced by pyruvate kinase will be equal to 2 ATP consumed in Phase I of glycolysis.

e. 2, 3-BPG levels in erythrocytes increase in high altitude where partial pressure of oxygen is low.

f. Loss-of-function mutations in pyruvate kinase increase the levels of 2,3-BPG in RBC, thus shifting the sigmoidal curve of oxygen binding to hemoglobin to the right. If loss-of-function mutations occurs in any of the enzymes upstream of the step involved in the formation of 1,3-BPG, the levels of 2,3-BPG in RBC will be lower than normal, thus shifting the curve to the left.

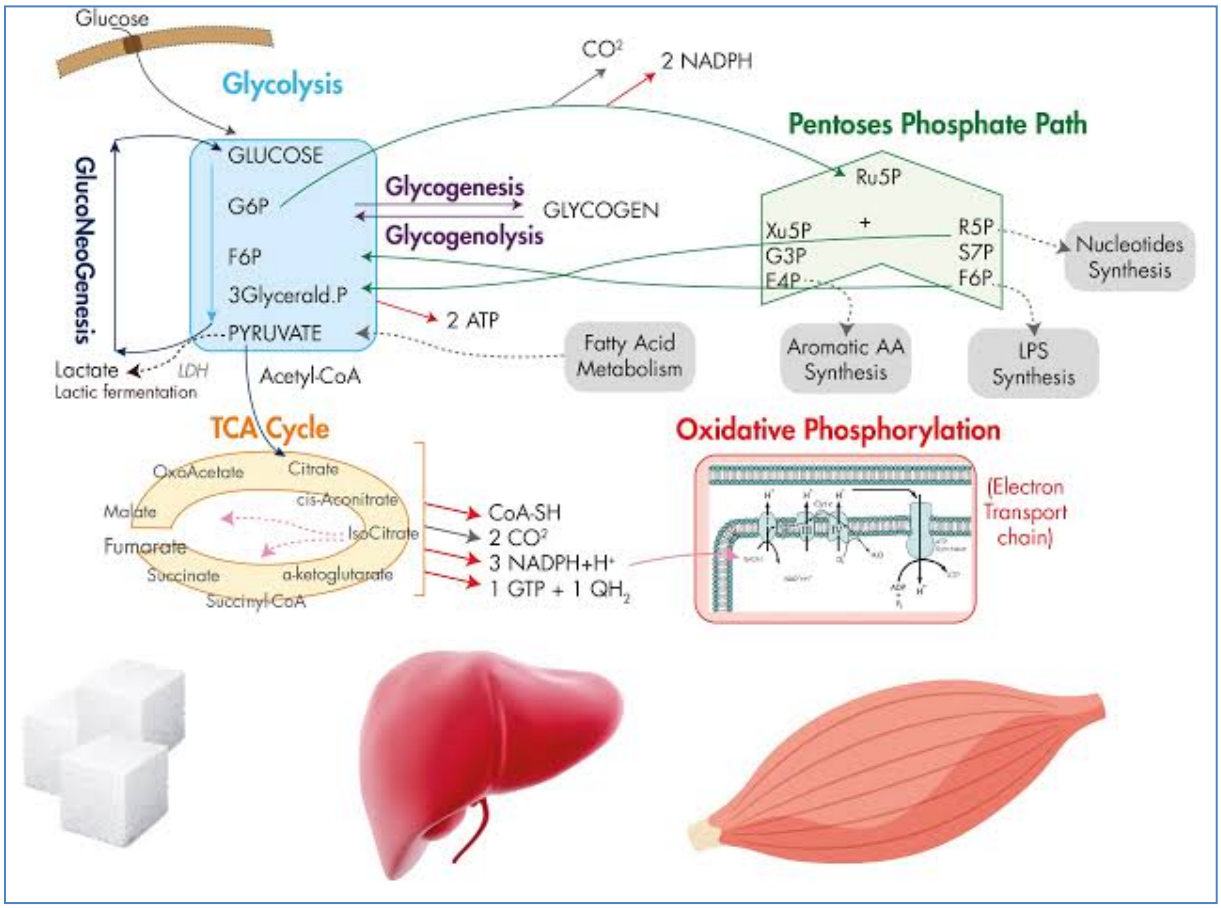


Figure 17: Glucolys stapes

CHAPTER II AMINO ACIDS, PEPTIDES, AND PROTEINS.

II -1 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.

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 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 **(Figure 18)**.

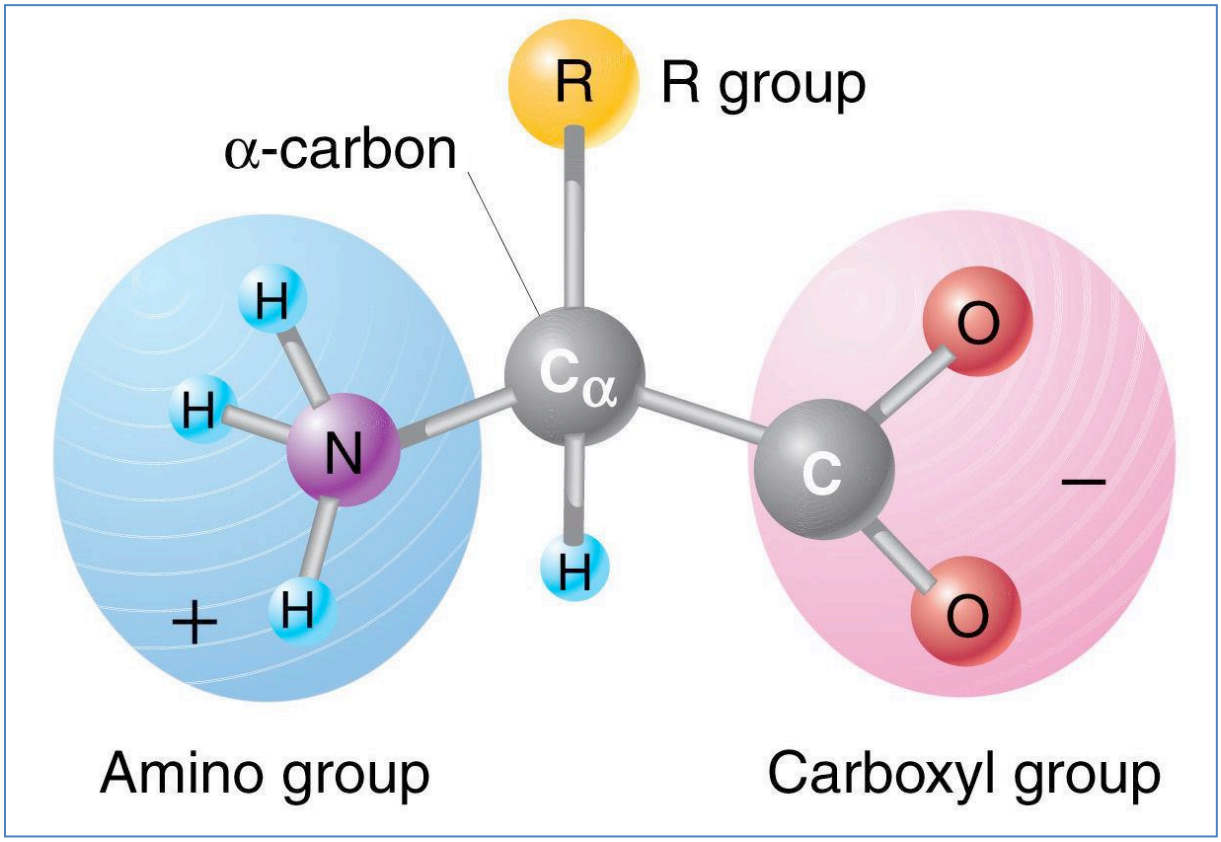


Figure 18:Amino acid structure

Because of the tetrahedral arrangement of the bonding orbitals around the carbon atom, the four different groups can occupy two unique spatial arrangements, and thus amino acids have two possible stereoisomers. Since they are non superimposable mirror images of each

other ,the two forms represent a class of stereoisomers called **enantiomers** . All molecules with a chiral center are also **optically active** that is, they rotate plane-polarized light .

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 , 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 non polar 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.

II -1-1 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 **(Figure 19)**.

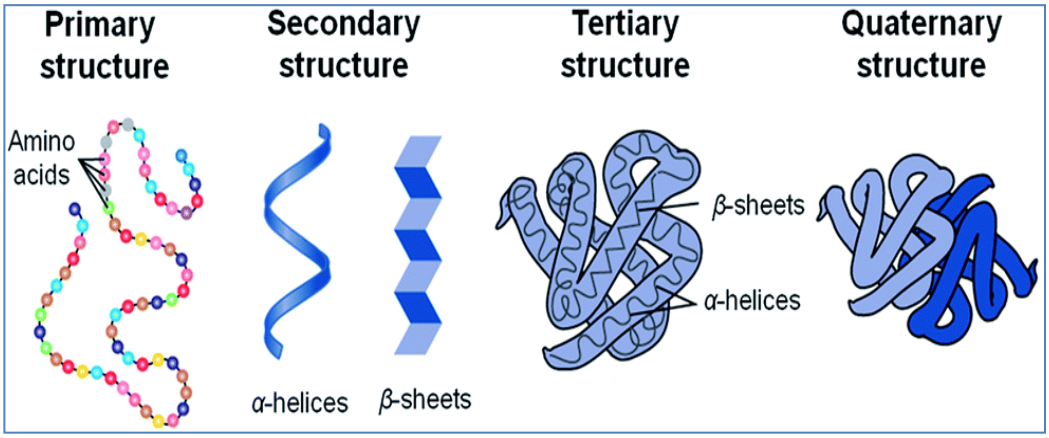


Figure 19: Summary of the Four Levels of Protein Structure

II -1-1 -1 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 **(Figure 20).** . 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.

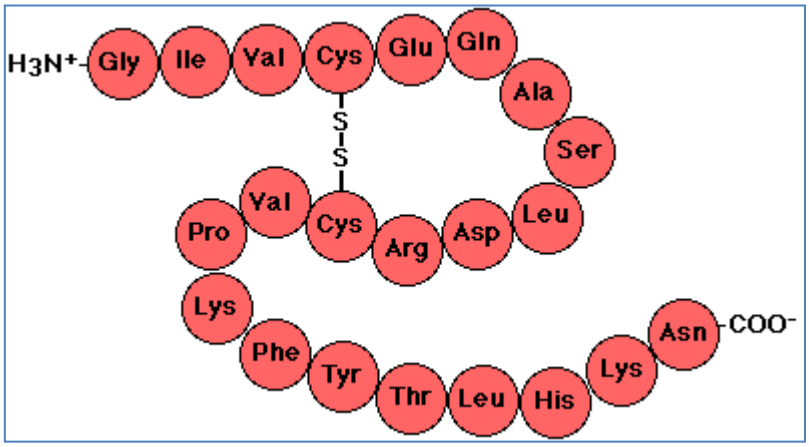


Figure 20: Amino acid sequence with disulfide bonds

II -1-1 -2 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. 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 21).**

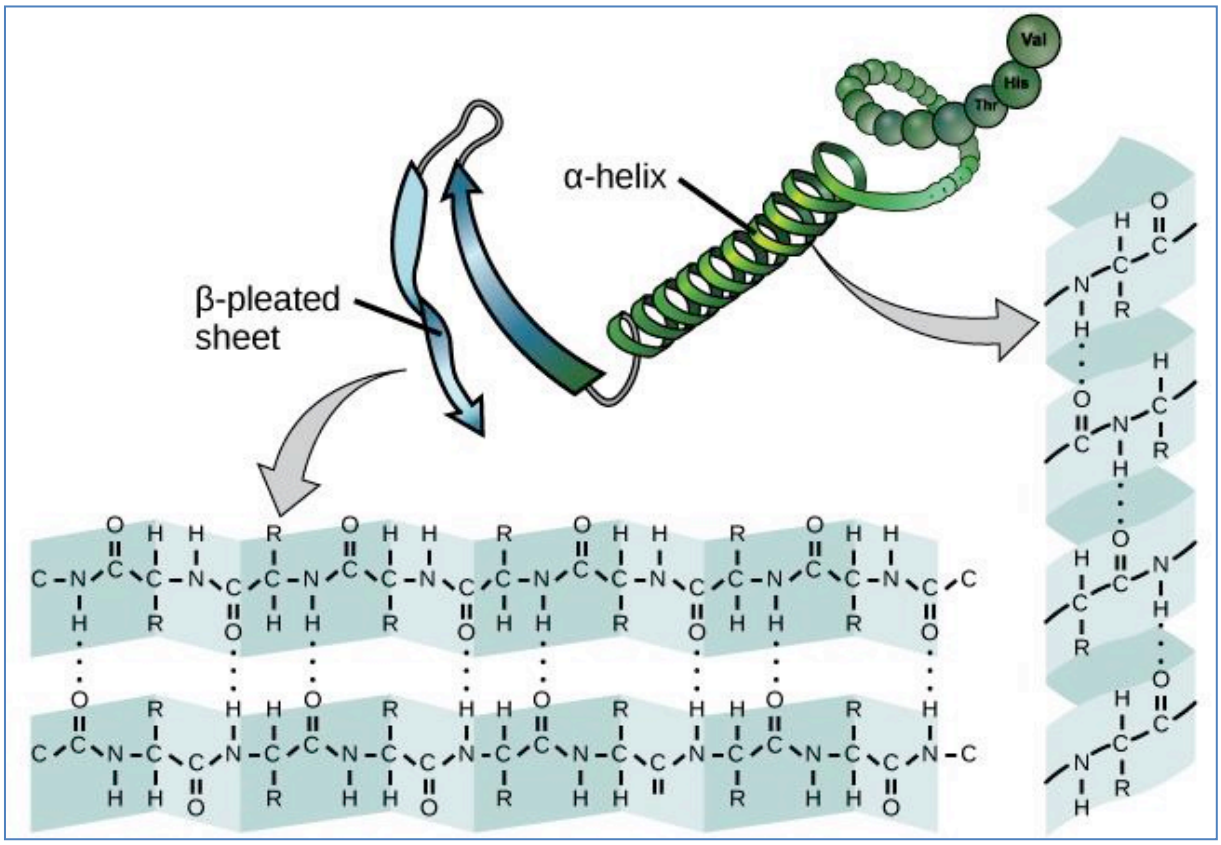
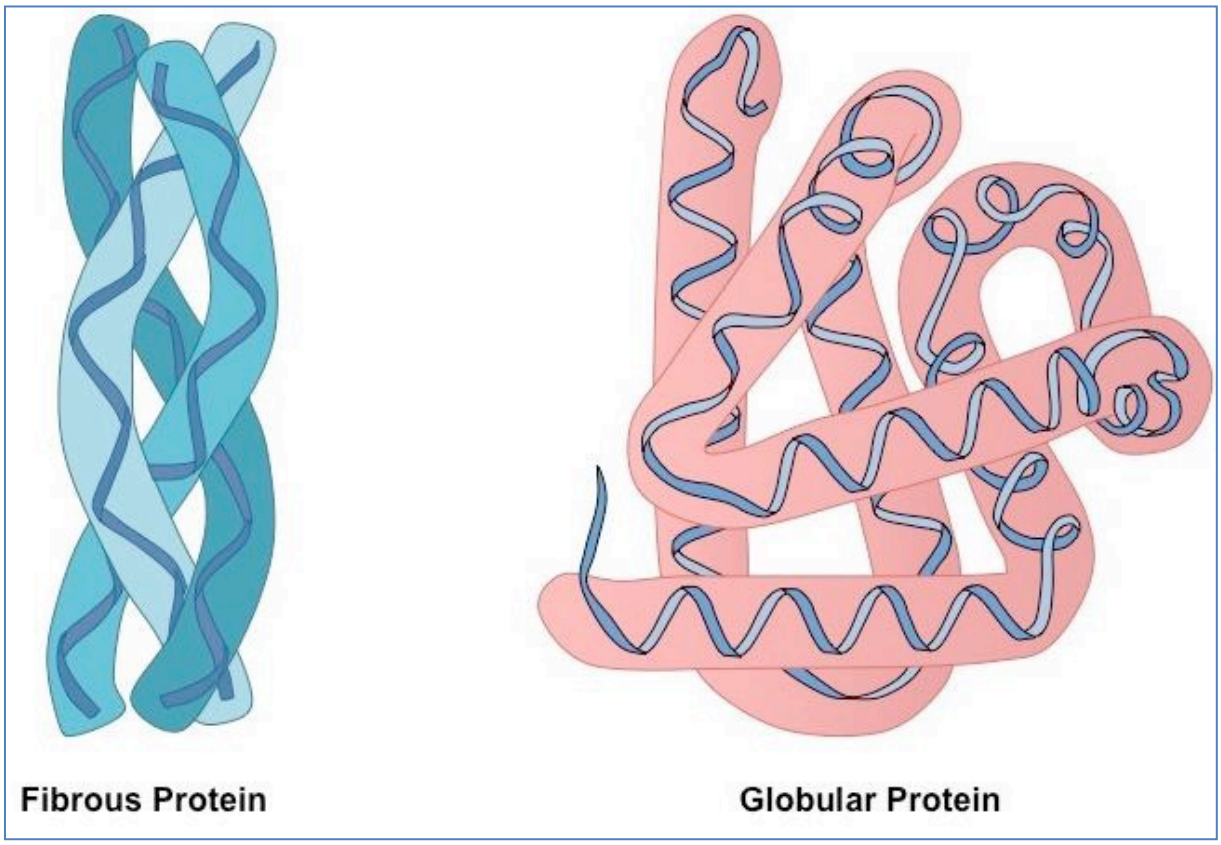


Figure 21: The two main types of secondary structure - the α-helix and the ß-sheet

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.

II -1-1 -3 Tertiary structure

It 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.



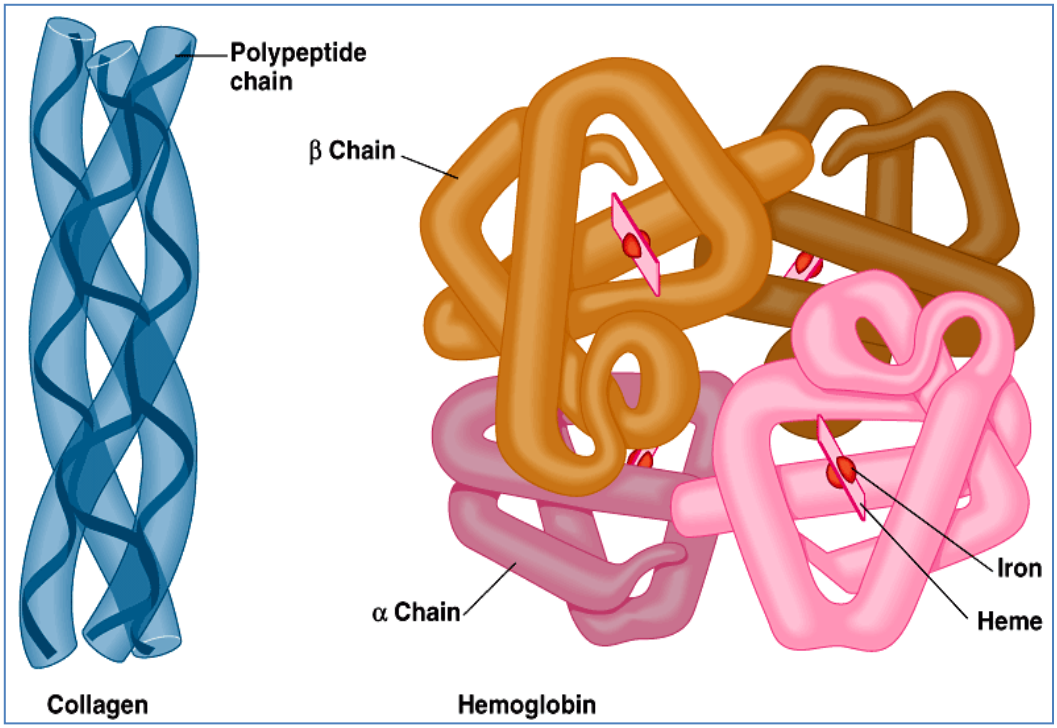


Figure 22: Two major groups fibrous and globular proteins: Collagen is fibrous and hemoglobin is globular protein.

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 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 **(Figure 23)**.

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. 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.

Collagen is founding 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.

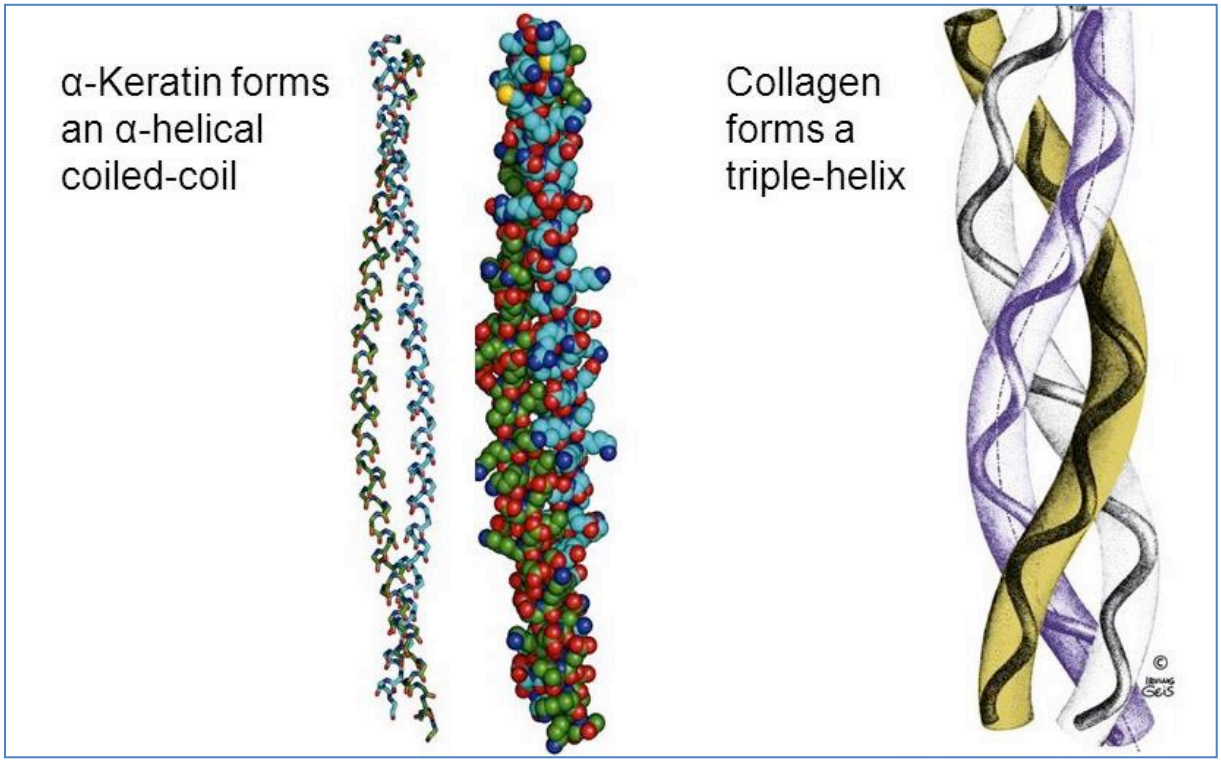


Figure 23: The secondary structure α-Keratin and collagen

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

II -1-1 -4 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 **(Figure 24)**.

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. In the whole human body the proteins account for 45% of dry mass.

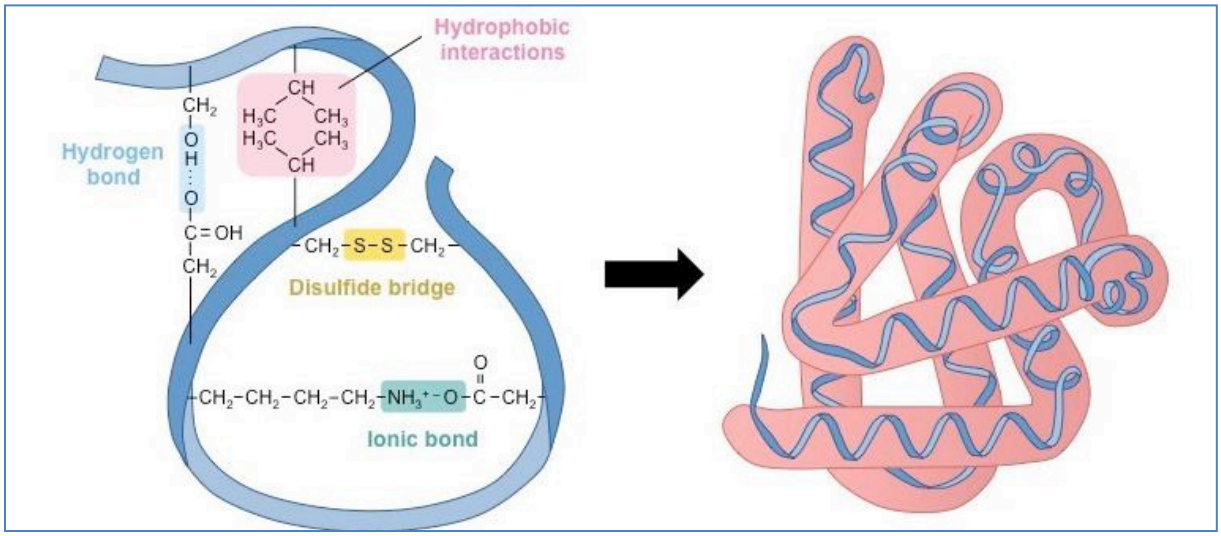


Figure 24: Tertiary structure: types of interaction

II -1-1 –5 Quaternary structure

Quaternary structure refers to a complex or an assembly of two or more separate peptide chains that are held together by non- covalent or, in some case, covalent interactions. If the subunits are identical, it is a homogeneous quaternary structure; but if there are dissimilarities, it is heterogeneous.

For instance insulin consists of A and B chain which are different. Hemoglobin has 4 chains, two of them are α and two are β. these, the polymers may be dimers, trimers, tetramers and so on.

i. Certain polypeptides will aggregate to form one functional protein. This is referred to as the quaternary structure.

ii. The protein will lose its function when the subunits are dissociated.

iii. The forces that keep the quaternary structure are hydrogen bonds, electrostatic bonds, hydrophobic bonds and van der Waals forces.

iv. Depending on the number of polypeptide chain, the protein may be termed as monomer (1 chain), dimer (2 chains), tetramer (4 chains) and so on. Each polypeptide chain is termed as subunit or monomer. Homodimer contains two copies of the same polypeptide chain. Heterodimer contains two different types of polypeptides as a functional unit.

v. For example, 2 alpha-chains and 2 beta-chains form the Hemoglobin molecule. Similarly, 2 heavy chains and 2 light chains form one molecule of immunoglobulin G. Creatine kinase (CK) is a dimer. Lactate dehydrogenase (LDH) is a tetramerf **(figure below)**

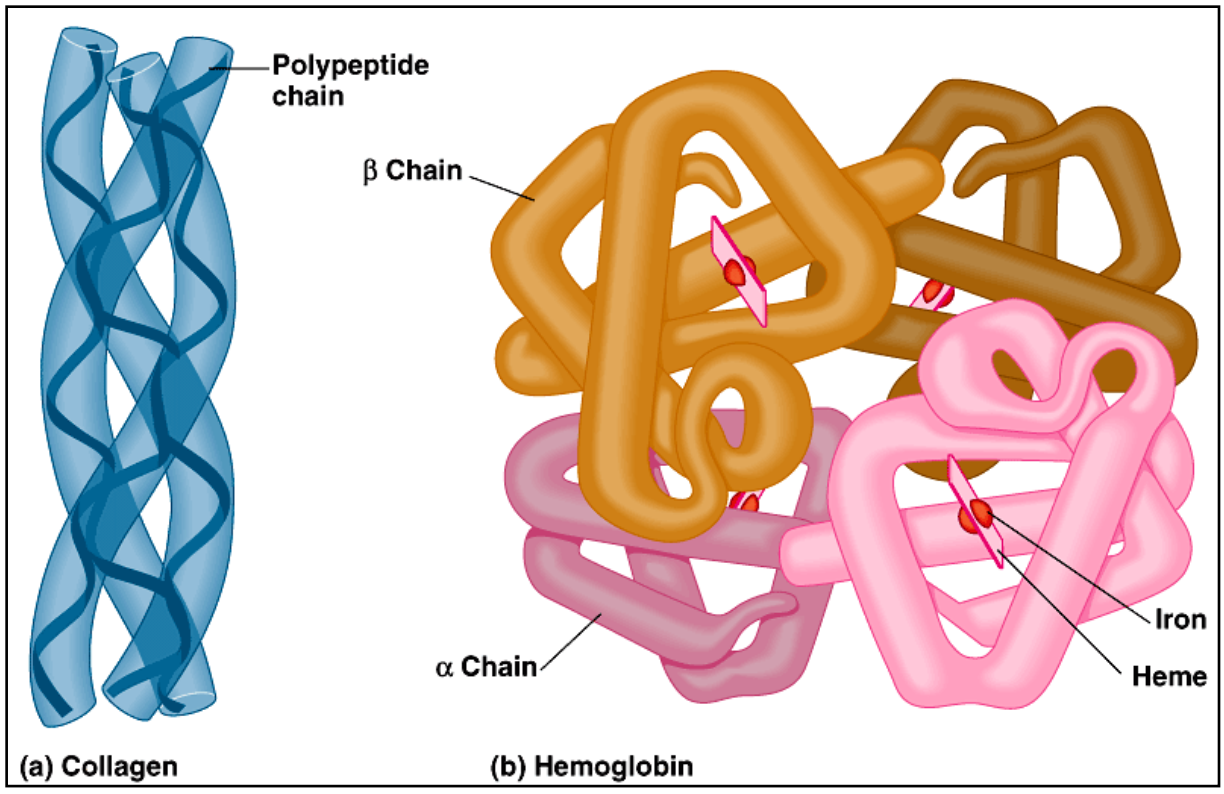


Figure 25 : Quaternary structure

II -2- 1 Physico-chemical properties of proteins

- high molecular weight;
- shape and size of molecules;
- high viscosity in solution;

- inability to penetrate semipermeable membranes
- pronounced swelling ability;
- optical activity;
- carge;
- mobility in electric field;
- low osmotic and high oncotic pressures;
- solubility;
- ability to absorhb UV light at 280 nm;
- amphoteric;
- denaturation.

II – 2 – 2 Properties of proteins in solutions

The **solubility** of proteins is highly variable. Some are completely insoluble (such as keratin, elastin) or show negligible solubility (e.g. collagen). Others dissolve very well (e.g. haemoglobin, albumin). The following are solvents for proteins: water or aqueous solutions of salt, acids and alkalis, urea or detergents. Solubility depends on the presence of polar amino acids in the protein molecule. Proteins with a high content of polar amino acid dissolve in a water environment. Polar groups of amino acid side chains produce hydrogen bonds with water molecules. The protein molecule is surrounded by a water jacket. Proteins, which are dominated by non-polar amino acids, have a limited ability to bind water and are therefore insoluble.

Protein solutions are generally **real solutions** with a monomolecular degree of dispersion. Sometimes, however, protein molecules associate to form aggregates composed of two or several particles. The protein solution then takes on the characteristics of the **colloidal solution**. preventing acidification, and protons separated during the dissociation of the **COOH** groups neutralize the **OH-** ions preventing alkalization.

This characteristic of amino acids, peptides and proteins has been important in maintaining the **acid-alkaline balance** of tissues and body fluids. The acidic characteristics are given to protein primarily by the **β**carboxyl groups of aspartic acid residues and the **γ**-carboxyl groups of the glutamic acid residues, which dissociate releasing (H+) protons and create the negatively charged -COO- group. An alkaline environment is conducive to the dissociation of the carboxyl groups and the transformation of proteins into the anionic form. The ε-amino group of lysine residues, the guanidine groups of the arginine residues and the imidazole rings of histidine residues give protein.

II -2-3 Proteins exhibit amphoteric properties.

In the solution (depending on pH), they act like acids or bases. This characteristic is mainly conditioned by the presence of polar groups with the electric charge in the side chains of certain amino acids. The **NH2** groups bind the **H+** ions present in the solutionalkaline characteristics. They can bind protons (H+), giving the protein molecule a positive charge. An acidic environment is conducive to the binding of protons by the before mentioned groups and the transformation of protein into cationic form.

Single α-amino and α-carboxyl groups found in N-terminal and C-terminal amino acids have little influence on the net electric charge of the protein molecules.

Each protein has its own characteristic pH value, where the number of positive and negative charges on the surface of a particle offset each other. This pH value is called the **isoelectric point - pI.** In the isoelectric point, the net electric charge of a protein molecule equals zero. In these conditions, protein does not move in the electric field, and its solubility is the lowest.

In an environment with a pH above the isoelectric point, proteins show a **negative** net charge and can form compounds with cations of heavy metals. The acidic action impedes the course of these reactions, because the dissociation of the carboxyl groups reverses. Protein loses anionic properties and does not react with cations.

II -2-4 Denaturation of proteins.

Destruction of the three-dimensional structure of a protein molecule with loss of its specific properties (solubility, electrophoretic mobility, biological activity and other) is called denaturation.

Most proteins can be denatured:

- by heat, which affects the weak interactions in a protein;
- by extremes of pH;
- by certain miscible organic solvents such as alcohol or acetone;
- by certain solutes such as urea and guanidine hydrochloride;
- by detergents;
- by heavy metal salts.

Molecular weight of proteins varies from 6,000 Da to over 1,000,000 Da. For determination of molecular weight are used:

- sedimentation analysis;
- gel chromatography;

- gel electrophoresis.

II -2 -5 Protein functioning

Biologically active proteins are polymers consisting of amino acids linked by covalent peptide bonds. Many different conformations (three-dimensional structures) are possible for a molecule as large as a protein. Of these many structures, one or (at most) a few have biological activity; these are called the native conformations**.** Many proteins have no obvious regular repeating structure. As a consequence, these proteins are frequently described as having large segments of "random structure" (also referred to as *random coil*). The term *random* is really a misnomer, because the same non repeating structure is found in the native conformation of all molecules of a given protein, and this conformation is needed for its proper function. Because proteins are complex, they are defined in terms of four levels of structure.

Protein function often entails interactions with other molecules. A molecule bound by a protein is called a ligand. The site to which it binds is called the binding site. Binding site is complementary to the ligand in size, shape, charge, and hydrophobic or hydrophilic character. A ligand may be any kind of molecule, including another protein. Interaction protein – ligand is specific: the protein can discriminate among the thousands of different molecules in its environment and selectively bind only one or a few. The given protein may have separate binding sites for several different ligands.

Proteins may undergo conformational changes when a ligand binds, a process called induced fit. In a multi-subunit protein, the binding of a ligand to one subunit may affect ligand binding to other subunits. Interactions between ligands and proteins may be regulated, usually through specific interactions with one or more additional ligands. These other ligands may cause conformational changes in the protein that affect the binding of the first ligand.
Protein-ligand interactions can be described quantitatively.

A quantitative description of this interaction is therefore a central part of many biochemical investigations. In general, the reversible binding of a protein (P) to a ligand (L) can be described by a simple equilibrium expression:
P + L = PL The reaction is characterized by an equilibrium constant, Ka (an association constant).

Proteins are sometimes described as the "workhorses" of the cell because they do
So many things Like:
Enzymes: kinases, transaminases etc.

Storage proteins: myoglobin, ferretin

Regulatory proteins : peptide hormones, DNA binding proteins

Structural protein : collagen, proteoglycan

Protective proteins: blood clotting factors, Immunoglobins,

Transport protein : Hemoglobin, plasma lipoproteins

Contractile or motile : Proteins Actin, tubulin

II -2 -6 Biologically active peptides

Naturally occurring peptides vary in length from two to many thousands of amino acids. These peptides functions as hormones, neurotransmitter, opiate, antibiotics etc. Here we describe the structure and function of some of the biologically active peptides. Most of the chemical reactions of the cell are carried out by proteins.

Proteins also are the major structural components of most cells and tissues. Proteins are often called polypeptides in reference to the fact that they are composed of amino acids held together by peptide bonds. Peptide bonds actually are amide bonds which are formed by the condensation of the carboxyl groups and amino groups of consecutive amino acids in the polymer chain. The socalled peptide backbone of a protein is a monotonous, regularly repeating structure. Projecting out from the backbone are the R-groups which are the side-chains of the amino acids.

The enzymes comprise one subclass of proteins. These proteins carry out chemical reactions with extraordinary specificity and speed (up to 1017-fold enhancement in reaction rate). Specificity is achieved because the binding site for reactants--the active site--is highly complementary in shape to the reactants and products.

A stereoview of the active site of lysozyme **.** This enzyme binds to and cleaves the polysaccharide portion of the bacterial cell wall. Cleavage leads to osmotic lysis of the affected bacterium. Lysozyme is present in tears and egg whites where it helps protect against unwanted bacterial growth and infection. We will discuss the structure and function of many medically and otherwise relevant proteins and enzymes such as myoglobin, hemoglobin, collagen, trypsin, insulin receptor, glycogen phosphorylase, plasma lipoproteins, and DNA polymerase in this course.

II -3 Classification of protein

II -3-1 Simple and conjugated proteins

Depending on their composition proteins can be classified as simple and conjugated. Simple proteins are composed of amino acid residues only. Conjugated proteins are composed of a simple protein and a non-protein moiety.

II-3-2 Simple proteins

II-3- 2-1 Protamines – proteins of a small molecular size, display pronounced basic properties due to the occurrence of large amounts of arginine, readily soluble in water.

II-3-2-2 Histones – proteins of a small molecular size, with basic properties (major constituents – arginine and lysine), play an important role in the metabolic control of genome activity.

II-3- 2-3 Prolamines and Glutelins – proteins of vegetal origin, soluble in 60-80% ethanol solution.

II-3-2 -4 Albumins and Globulins – widely occur in animal organs and tissues.

II -3 -4Conjugated proteins

The classification of conjugated proteins is based on the chemical nature of their non-protein components.

II -3 -5 Chromoproteins.

These are proteins in combination with a proshetic group that is a pigment. The examples are the respiratory pigments hemoglobin and hemocyanin, visual purple or rhodopsin found in the rods of the eye, flavoproteins and cytochromes. In this group are present:

- hemoproteins; (containing heme as prostetic group); includes hemoglobin, myoglobin;

- flavoproteins; (FMN, FAD as prostetic group);

constituents of oxidoreductases.

II -3-6-Lipoproteins.

These are proteins conjugated with lipids. There are five types of lipoproteins:

-High density lipoproteins (HDL);

-Intermediate density lipoproteins (IDL);

-Low density lipoproteins (LDL);

-Very low density lipoproteins (VLDL);

-Chylomicrons.

II -3 -7 Glycoproteins.

These are proteins conjugated with carbohydrates. Glycoproteins are of two main categories, intracellular and secretory. Intracellular glycoproteins are present in cell membranes and have an important role in membrane interaction and recognition. Secretory glycoproteins are: plasma glycoproteins, secreted by the liver, thyroglobulin, secreted by the thyroid gland, immunoglobins, secreted by plasma cells, ovoalbumins, secreted by the oviduct in the hen, ribonuclease, theenzyme which breaks down RNA, and deoxyribonuclease, which breaks down DNA.

II -3 -8 Phosphoproteins

Phosphoproteins are proteins in combination with a phosphate-containing radical other than a nucleic acid or a phospholipid. The examples of phosphoproteins are casein of milk and ovovitelline in eggs.

II -3 -9 Nucleoproteins

They are proteins in combination with nucleic acids. There are two types of nucleoproteins:

- deoxyribonucleoproteins (chromatin);
- ribonucleoproteins (ribosomes).

II -3 -10 Metalloproteins

They are proteins conjugated to metal ion(s) which are not part of the prosthetic group. They include ceruloplasmin, an enzyme with oxidase activity that may transport copper in plasma, siderophilin, ferritin, hemosiderin. Functions of metalloproteins: transport, depot, structural and enzymatic functions.

II -4 Protein Metabolism

The digestion of proteins begins in the stomach. When protein-rich foods enter the stomach, they are greeted by a mixture of the enzyme pepsin and hydrochloric acid (HCl; 0.5 percent). The latter produces an environmental pH of 1.5–3.5 that denatures proteins within food. Pepsin cuts proteins into smaller polypeptides and their constituent amino acids. When the food-gastric juice mixture (chyme) enters the small intestine, the pancreas releases sodium bicarbonate to neutralize the HCl. This helps to protect the lining of the intestine. The small intestine also releases digestive hormones, including secretin and CCK, which stimulate digestive processes to break down the proteins further. Secretin also stimulates the pancreas to release sodium bicarbonate.

The pancreas releases most of the digestive enzymes, including the proteases trypsin, chymotrypsin, and elastase, which aid protein digestion. Together, all of these enzymes break

complex proteins into smaller individual amino acids ,which are then transported across the intestinal mucosa to be used to create new proteins, or to be converted into fats or acetyl CoA and used in the Krebs cycle.

II -4 -1 Protein degradation:

•Degradation of proteins proceeds at different rates which vary , depending on the physiological needs and roles of proteins.

•Protein degradation will result in amino acids

•The first step in catabolism of all amino acids involves the removal of amino group.

The first step in the catabolism of all amino acids involves the removal of the amino group through:

A.Transamination

B. Deamination

C. Transdeamination

D. Transamination:

It is the transfer of the amino group from an **α-amino acid** to an **α-keto acid** with the production of a **new amino** acid and **new keto acid**.

-Transamination reactions are carried out by **Transaminases (Aminotransferases)**

Transaminases (aminotransferases) are present in cytoplasm and mitochondria of all tissues especially liver.

•Vitamin B6 (PLp) (pyridoxal phosphate) is essential for transamination reaction.

•All amino acid can do transamination except :

–Proline

–Hydroxyproline

–Threonine

–Lysine

The commonest α-keto acid used is α-ketoglutaric acid which can accept amino group from all amino acids converting them to **Glutamic acid.**

•This is important because **Glutamic acid** is the only amino acid that undergoes oxidative deamination at an appreciable amounts in liver by (L-glutamate dehydrogenase).

A. Amino acid pool

Free amino acids are present throughout the body, for example, in cells, blood, and the extracellular fluids. For the purpose of this discussion, envision all these amino acids as if they belonged to a single entity, called the amino acid pool.

This pool is supplied by three sources:

1) amino acids provided by the degradation of body proteins,

2) amino acids derived from dietary protein, and

3) synthesis of nonessential amino acids from simple intermediates of metabolism.

Conversely, the amino pool is depleted by three routes:

1) synthesis of body protein, 2) amino acids consumed as precursors of essential nitrogen-containing small molecules, and 3) conversion of amino acids to glucose, glycogen, fatty acids, ketone bodies, or $CO_2 + H_2O$.

Although the amino acid pool is small (comprised of about 90–100 g of amino acids) in comparison with the amount of protein in the body (about 12 kg in a 70-kg man), it is conceptually at the center of whole-body nitrogen metabolism.

B. Protein turnover

Most proteins in the body are constantly being synthesized and then degraded, permitting the removal of abnormal or unneeded proteins. For many proteins, regulation of synthesis determines the concentration of protein in the cell, with protein degradation assuming a minor role. For other proteins, the rate of synthesis is constitutive, that is, relatively constant, and cellular levels of the protein are controlled by selective degradation.

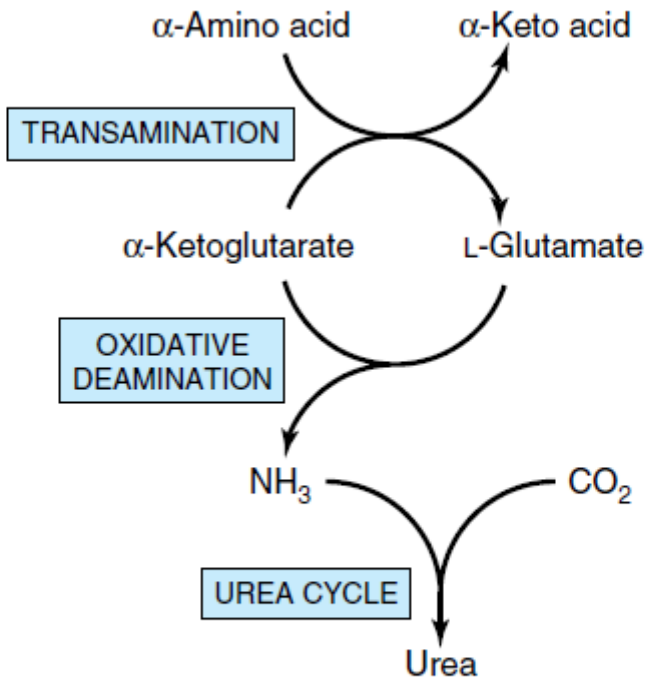


Figure 29–2. Overall flow of nitrogen in amino acid catabolism.

Figure 26: Overall flow of nitrogen in amino acid

C -Nutritional function of protein

Proteins play a major role in ensuring your health well being. There are innumerable functions of proteins in the body.

-Building and repairing of body tissues.

Protein makes up nearly 17 percent of the total body weight. For example: muscle contains about 1/3 protein, bone about 1/5 part and skin consists of 1/10 portion. The rest part of proteins is in the other body tissues and fluids.

-Take part in some kinds of important physiological activities

Oxidation and supply energy regulation of body processes and formation of enzymes and hormones, antibody.

D- Nutrition value of proteins

Essential amino acids :

some Amino acids that cannot be synthesized by the body and must be obtained from the diet.

- **Nutrition value**

A protein's nutritional value is judged by how many of the essential amino acids it provides and in what quantity. Different foods contain different numbers and amounts of the essential amino acids

- **Complementary effect of dietary proteins**

Two or more plant proteins are consumed together which complement each other in essential amino acid content.

CHAPTER III LIPIDS

III – I Definition

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. 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, lightabsorbing pigments, hydrophobic anchors for proteins, "chaperones" to help membrane proteins fold, emulsifying agents in the digestive tract, hormones, and intracellular messengers.

III – 2 Proprieties

A lipids are non-polar organic compounds that is insoluble in water and soluble in alcohol, ether, and chloroform.

•Lipids are an important constituent of the structure of living cells. Together with carbohydrates and proteins, lipids are the main constituents of plant and animal cells.

•Lipids are easily stored in the body and serve as a source of Energy/Fuel.

•Lipids includes fatty acids, neutral fats, waxes and steroids (like cortisone) etc. Almost all fat in your diet comes in the form of triglycerides

•Fatty acidsrarely occur as free molecules in nature. it is found in complex molecules such as fats(energy-storage compounds) and phospholipids(the primary lipid components of cellular membranes).

III -3 Lipids Classification

III -3-1 Simple lipids

Esters of fatty acids with various alcohols. They are Saturated and Unsaturated(Ex. Fats, oil and Waxes).

III -3-2 Complex Lipids

Esters of fatty acids with various alcohols + Other Groups like phosphoric acids, sugar residue etc.

A. Phospholipids (glycerophospholipids and sphingophospholipids)

B. Glycolipids (glycosphingolipids): Carbohydrates containing lipids.

C. Other complex lipids: Sulfolipids, aminolipids, and Lipoproteins.

III -3-3 Precursor and derived Lipids:

Steroids, other alcohols, fatty aldehydes, and ketonebodies, lipid-soluble vitamins, and hormones.

III -4 Structure of lipids

Fatty acids are composed of ahydrocarbon chain with one terminal carboxyl group (COOH).At physiological condition hydrogen ion (H+) lost to form a negatively charged carboxylategroup (COO−).

-Most biological fatty acids contain an even number of carbonatoms, because the biosynthetic pathway is common to all organisms.

-Fatty acids are amphipathicin nature.

-They are found in two form saturated (only single bond), Ex. myristicacid (C14), palmiticacid (C16), Stearicacid (C18),etc.and Unsaturated (double bond), Ex. palmitoleicacid (C16), Oleic acids (C18) etc.

III -4-1 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 . A few contain three-carbon rings, hydroxyl groups, or methyl-group branches.

A simplified nomenclature for un branched 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 , 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).

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

III-4 -2 Saturated fatty acids

Swedish scientist Scheele isolated glycerol in 1779. Chevreul ME isolated oleic, stearic, butyric and caproic acids in 1823. In 1898, Edmed FG identified the structure of oleic acid. Linoleic acid was prepared from linseed oil by Sacc F in 1844. Lauric acid (12:0) was discovered in *Lauraceae* seeds by Marsson T in 1849. Franz Soxhlet, a German chemist,

invented the Soxhlet apparatus in 1879. This apparatus was used first to separate fats from food.

i. They have the general formula CH3-(CH2)n- COOH. For example,

Acetic acid CH3—COOH

Butyric acid CH3(CH2)2—COOH

Palmitic acid CH3—(CH2)14—COOH

Stearic acid CH3—(CH2)16—COOH

ii. They are named by adding the suffix ‘anoic' after the hydrocarbon.

iii. The two carbon acetic acid and 4 carbon butyric acid are important metabolic intermediates.

iv. The C16 (palmitic acid) and C18 (stearic acid) are most abundant in body fat.

v. Each animal species will have characteristic pattern of fatty acid composition. Thus human body fat contains 50% oleic acid, 25% palmitic acid 10% linoleic and 5% stearic acid.

III-4-3 Unsaturated fatty acids

They are named by adding the suffix ‘enoic' after the systematic name. They are similar to saturated fatty acids in the reaction of the carboxylic group but also show properties due to presence of the double bond. Unsaturated fatty acids exhibit geometrical isomerism at the double bonds (Fig.7.1). All the naturally occurring fatty acids have the cisconfiguration. However, in the body during metabolism trans fatty acids are formed .

-The polyunsaturated fatty acids (PUFA) exist in cis-configuration in naturally occurring lipids.

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.

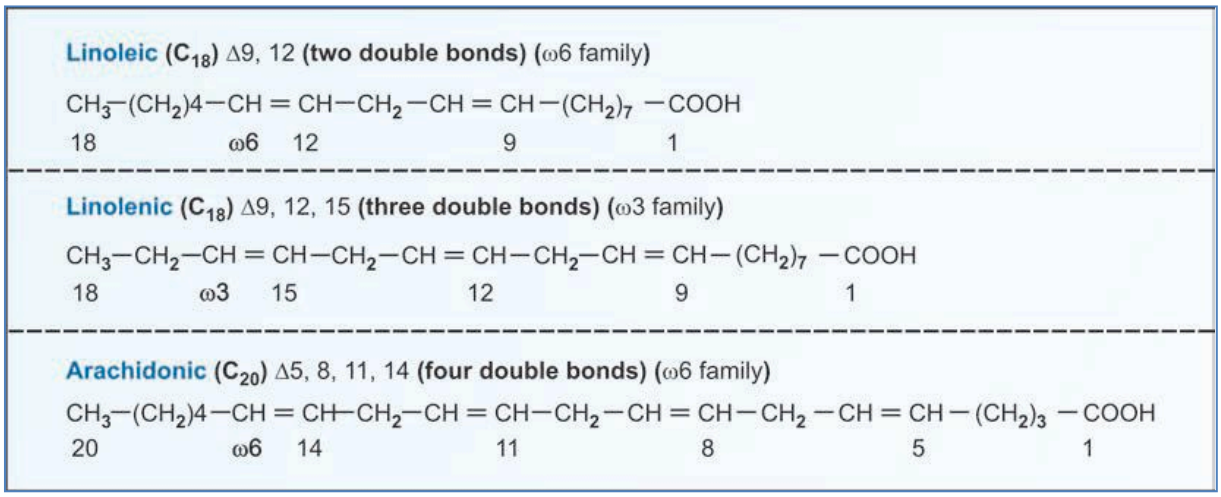


Figure 27: Polyunsaturated fatty acids (PUFA)

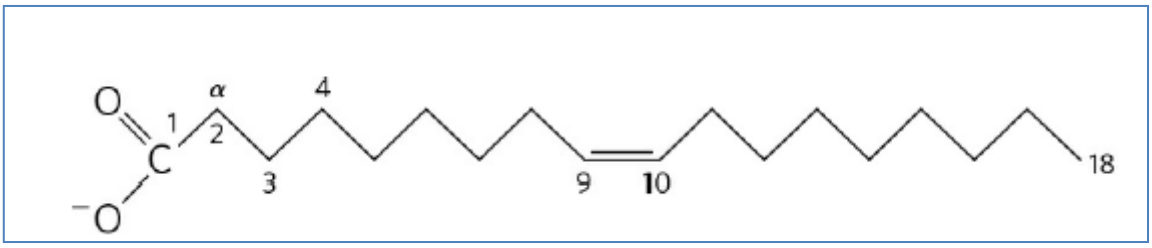


Figure 28: The 18-carbon oleic (octadecenoic) acid

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 , 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.

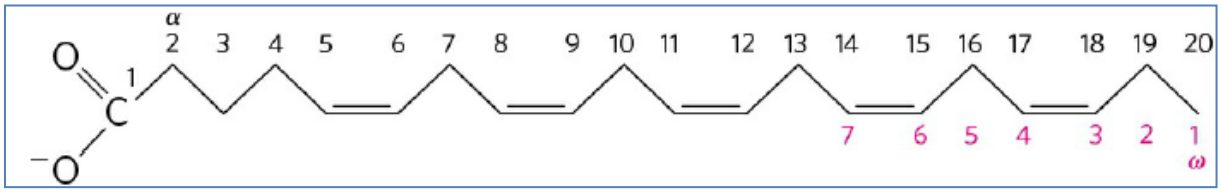


Figure 29: Eicosapentaenoic acid

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. 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 (**Figure 29)**.

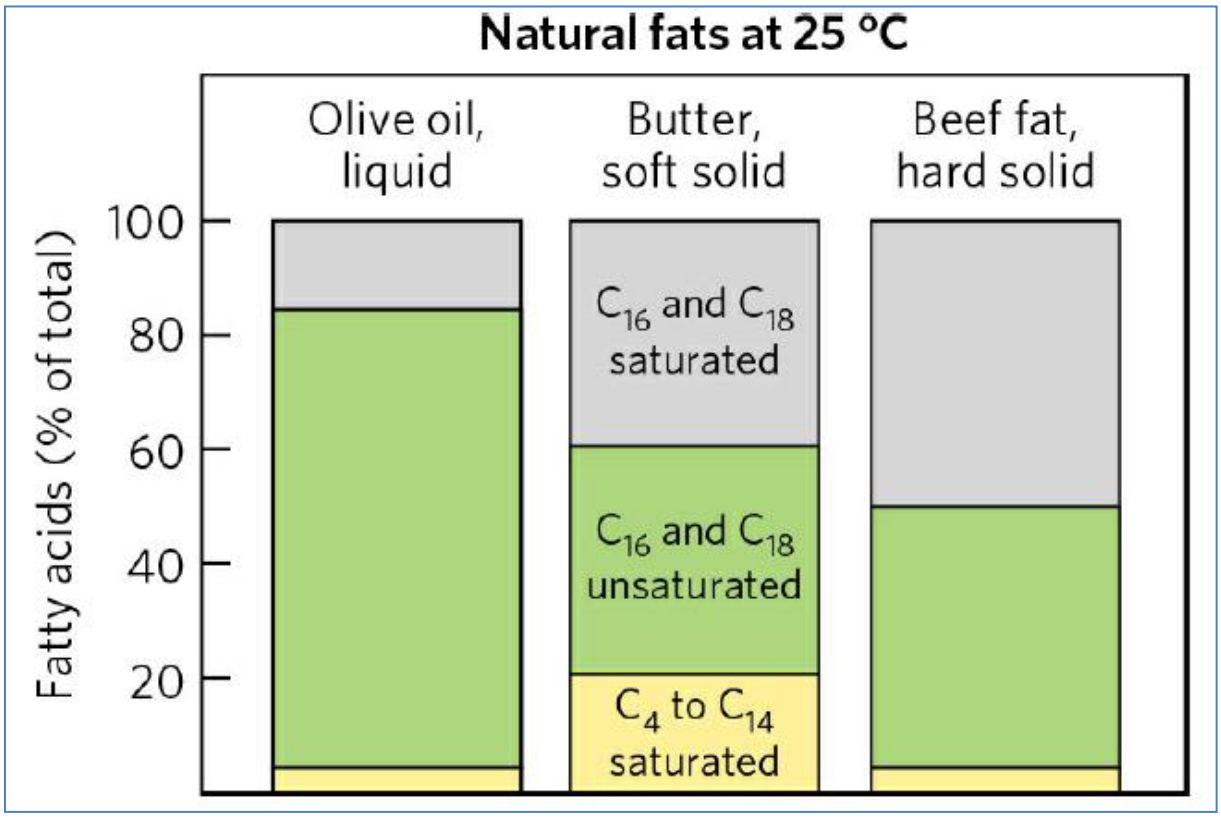


Figure 30: Fatty acid composition of three food fats.

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 **(Figure 30)** .

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 . 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.

$$H_2C{=}CH_2 + H_2 \rightarrow CH_3CH_3$$

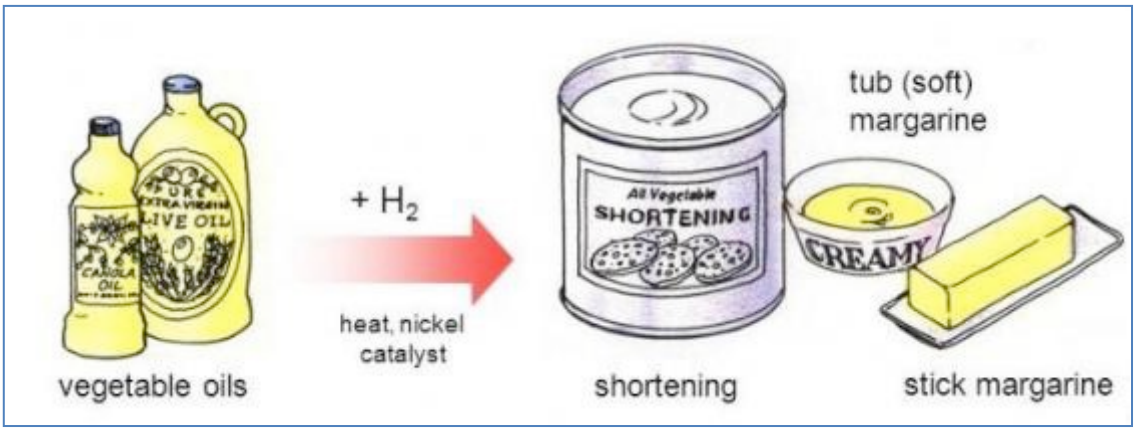


Figure 31: Hydrogenation reaction.Hydrogenation of a double bond.

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 31)**.

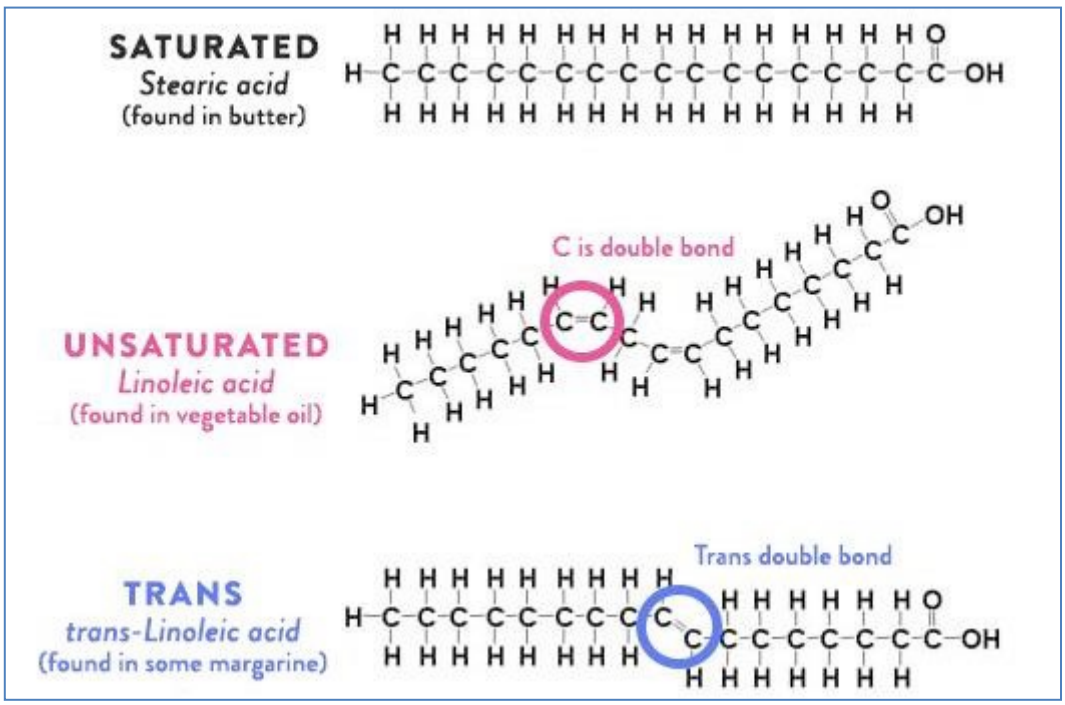


Figure 32: Converting cis double bonds to trans double bonds

III-5 Trans Fatty Acids (TFA)

They are present in dairy products and in hydrogenated edible oils. They are generally considered to be injurious to health. However, they are used in food industry as they increase the shelf life of the fried food. Oils containing PUFA also have high content of TFA. Fast food preparations have a high TFA content. Trans fatty acids adversely affect multiple risk factors, including plasma lipids and lipoproteins, systemic inflammation, endothelial dysfunction, insulin resistance, diabetes and adiposity. It is high in processed foods and bakery products, where partially hydrogenated vegetable oils are used for cooking.

III- 5-1 Clinical Significance of PUFA

1. Linoleic and Linolenic acids are polyunsaturated fatty acids.

2. They are called essential fatty acids, because they cannot be synthesized by the body and have to be supplied in the diet.

3. Unsaturated fatty acids are also designated ω3 (omega 3) family—Linolenic acids ω6 family—Linoleic and Arachidonic acids .ω9 family—Oleic acid

4. Arachidonic acid is the precursor of prostaglandins. Arachidonic acid can be synthesized in the body, if the essential fatty acids are supplied in the diet.

5. The pentaenoic acid present in fish oils is of great nutritional importance (ω3 unsaturated fatty acid).

6. Eicosanoids (eicosa = twenty) are derived from 20 C arachidonic acid. They are polyenoic fatty acids. They are prostanoids (prostaglandins, prostacyclins, thromboxanes) and leukotrienes.

III- 6 Essential Fatty Acids

Human body is unable to synthesise all fatty acids found in the body. Those fatty acids that are not synthesized in the body but required for normal body growth and maintenance are called as essential fatty acids. These fatty acids are to be supplied through diet. Linoleic and linolenic acids are essential fatty acids. The longer chain fatty acids can be synthesized by the body from dietary linoleic and ex-linolenic acids. Arachidonic acid is essential but it can be synthesised by the body from linolenic acid. It is also present in the meat. Linoleic acid is grouped under n-6 family because the 6th carbon from methyl end possesses the double bond.

III -7 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 **(Figure 32)**.

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) and wax extracted from the seeds of the jojoba bush are widely used in the manufacture of lotions, ointments, and polishes.

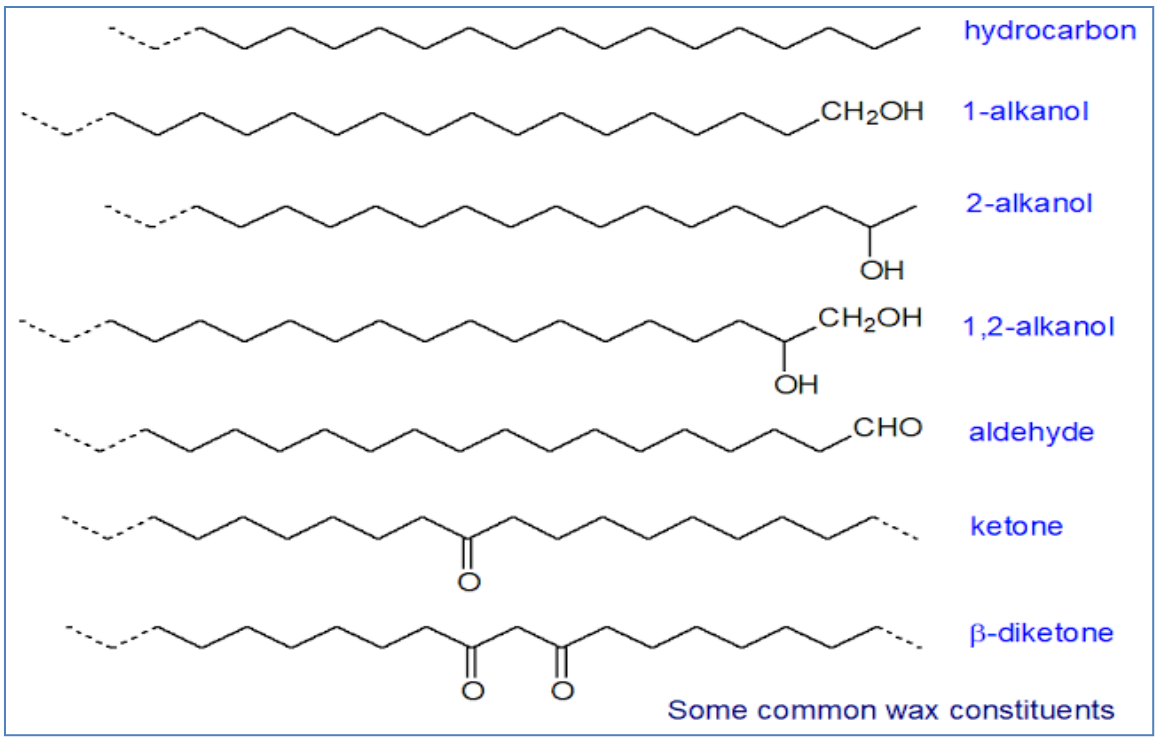


Figure 33: Some common wax constituents

III-7-1 Properties of Fat Physical

• They are insoluble in water and soluble in organic solvents.

• Pure triacylglycerols are tasteless, odourless, colourless and neutral in reaction.

• They have lesser specific gravity (density) than water and therefore float in water.

• Though fats are insolu1;>le in water, they can be broken down into minute droplets and dispersed in water. This is called emulsification **(Figure 33)** .

• They contain hydrophilic colloidal particles such as proteins, carbohydrates and phospholipids which act as stabilizing agents.

• Emulsification greatly increases the surface area of the fat and this is an essential requisite for digestion of fat in the intestine.

III-7-2 Chemical

• Fat is hydrolysed to yield three molecules of fatty acid and one molecule of glycerol. The hydrolysis of fat is effected by alkali and enzyme.

• The process of alkali hydrolysis is called 'saponification'.

• The alkali salt of fatty acid resulting from saponification is soap

• Hydrolysis of triacylglycerol may be accomplished enzymatically through the action of lipases.

• Development of disagreeable odour and taste in fat or oil upon storage is called rancidity. Rancidity reactions may be due to hydrolysis of ester
bonds (hydrolytic rancidity) or due to oxidation of unsaturated fatty
acids (oxidative rancidity).

• The partial hydrolysis of the triacylglycerol to mono and diacylglycerol is called Hydrolytic rancidity. The hydrolysis is hastened by the presence of moisture, warmth and lipases present in fats or air. In fats like butter which contains a high percentage of volatile fatty acids, hydrolytic rancidity produces disagreeable odour and taste due to the liberation of the volatile butyric acid. Butter becomes rancid more easily in summer.

• The unsaturated fatty acids are oxidised at the double bonds to form peroxides, which then decompose to form aldehydes and acids of objectionable odour and taste (Oxidative rancidity).

III- 8 Functions of Lipids

1. Storage form of energy (triglycerides)

2. Structural components of biomembranes (phospholipids and cholesterol)

3. Metabolic regulators (steroid hormones and prostaglandins)

4. Act as surfactants, detergents and emulsifying agents (amphipathic lipids)

5. Act as electric insulators in neurons

6. Provide insulation against changes in external temperature (subcutaneous fat)

7. Give shape and contour to the body

8. Protect internal organs by providing a cushioning effect (pads of fat)

9. Help in absorption of fat soluble vitamins (A, D, E and K)

10. Improve taste and palatability of food.

III – 9 Properties of Fatty Acids

1. Hydrogenation

Unsaturated fatty acids may be converted to the corresponding saturated fatty acids by hydrogenation of the double bond. (+)2H (+)2H (+)2H

Linolenic —→ Linoleic —→ Oleic —→ Stearic

Hydrogenation of oils can lead to solidification and saturation, e.g. Vanaspathi.

2. Halogenation

When treated with halogens under mild conditions, the unsaturated fatty acids can take up two halogenatoms, at each double bond to form the halogenated derivative of the fatty acid. For example, Oleic acid + I2 → Di-iodo oleic acid The number of halogen atoms taken up will depend on the number of double bonds and is an index of the degree of unsaturation. (See iodine number, under triglycerides).

3. Melting Point

The short and medium chain fatty acids are liquids, whereas long chain fatty acids are solids at 25oC. The solubility in water decreases, while melting and boiling points increase, with increase in chain length. The unsaturated fatty acids have lower melting point compared to saturated fatty acids with the same chain length. For example, stearic acid (C18 fatty acid, no double bond) has the melting point 69oC, oleic acid (C18, 1 double bond) has 13oC; linoleic acid (C18, 2 double bonds) has –5oC and linolenic (C18, 3 double bonds) has –10oC.

4. Salt Formation

Saturated and unsaturated fatty acids form salts with alkali.

CH3—COOH + NaOH → CH3—COONa + H2O

Sodium and potassium salts of long chain fatty acids are called soaps. Calcium and magnesium soaps are insoluble. Calcium soaps are used in grease.

Alkyl sulfate (R—CH2—O—SO2—ONa) and alkyl sulfonate (R—CH2—SO2—O—Na) are not precipitated by hard water and are used as detergents**.**

5. Ester Formation

Both saturated and unsaturated fatty acids form esters with alcohols, especially with glycerol. Fatty acids can form mono-, di- or tri- esters with alcohol groups of glycerol. Triglycerides or triacyl glycerols are also known as **neutral fat** .

Glycerol + fatty acid → Mono acyl glycerol Monoglyceride + fatty acid → Di acyl glycerol Diglyceride + fatty acid → Triglyceride or tri acyl glycerol

6. Oxidation of Fatty Acids

All fatty acids undergo oxidation in the body to give energy. Beta-oxidation is the major process by which acids are oxidized (Chapter 11). However, the unsaturated fatty acids can undergo auto oxidation, due to the presence of the highly reactive double bonds and a variety of products.

- **Neutral fats**

Neutral fats are also called as triacylglycerols (TAG) or triglycerides (TG). These are esters of the trihydric alcohol, glycerol with fatty acids .

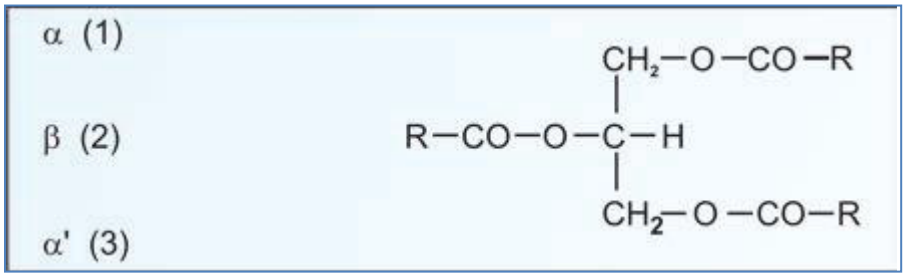


Figure 34:Triacylglycerol (TAG) (triglyceride)

III -9 -1 Clinical Applications

1. Excessive fat deposits cause obesity. Truncal obesity is a risk factor for heart attack.

2. Abnormality in cholesterol and lipoprotein metabolism leads to atherosclerosis and cardiovascular diseases.

3. In diabetes mellitus, the metabolisms of fatty acids and lipoproteins are deranged, leading to ketosis.

III - 10 Biological membrane.

III – 10-1 The Composition and Architecture of Membranes

One approach to understanding membrane function is to study membrane composition—to determine, for example, which components are common to all membranes and which are unique to membranes with specific functions. So before describing membrane structure and function we consider the molecular components of membranes: proteins and polar lipids, which account for almost all the mass of biological membranes, and carbohydrates, present as part of glycoproteins and glycolipids **(Figure 34)**.

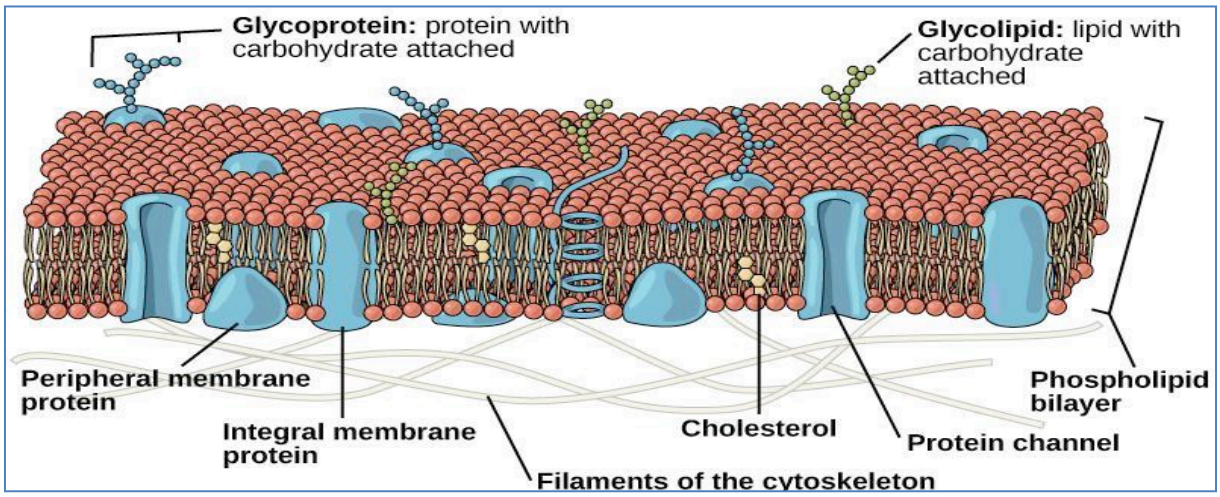


Figure 35:Proteins and lipids form complexes with carbohydrates

III – 10-2 A Lipid Bilayer Is the Basic Structural Element of Membranes

Glycerophospholipids, sphingolipids, and sterols are virtually insoluble in water. When mixed with water, they spontaneously form microscopic lipid aggregates in a phase separate from their aqueous surroundings, clustering together, with their hydrophobic moieties in contact with each other and their hydrophilic groups interacting with the surrounding water. Recall that lipid clustering reduces the amount of hydrophobic surface exposed to water and thus minimizes the number of molecules in the shell of ordered water at the lipid-water interface , resulting in an increase in entropy. Hydrophobic interactions among lipid molecules provide the thermodynamic driving force for the formation and maintenance of these clusters **(Figure 35)** .

III – 10-3 Structural Lipids in Membranes

The polar lipids, with polar heads and nonpolar tails, are major components of membranes. The most abundant are the glycerophospholipids, which contain fatty acids esterified to two of the hydroxyl groups of glycerol, and a second alcohol, the head group, esterified to the third hydroxyl of glycerol via a phosphodiester bond. Other polar lipids are the sterols.

- Glycerophospholipids differ in the structure of their head group; common glycerophospholipids are phosphatidylethanolamine and phosphatidylcholine. The polar heads of the glycerophospholipids carry electric charges at pH near 7.

-Chloroplast membranes are remarkably rich in galactolipids.

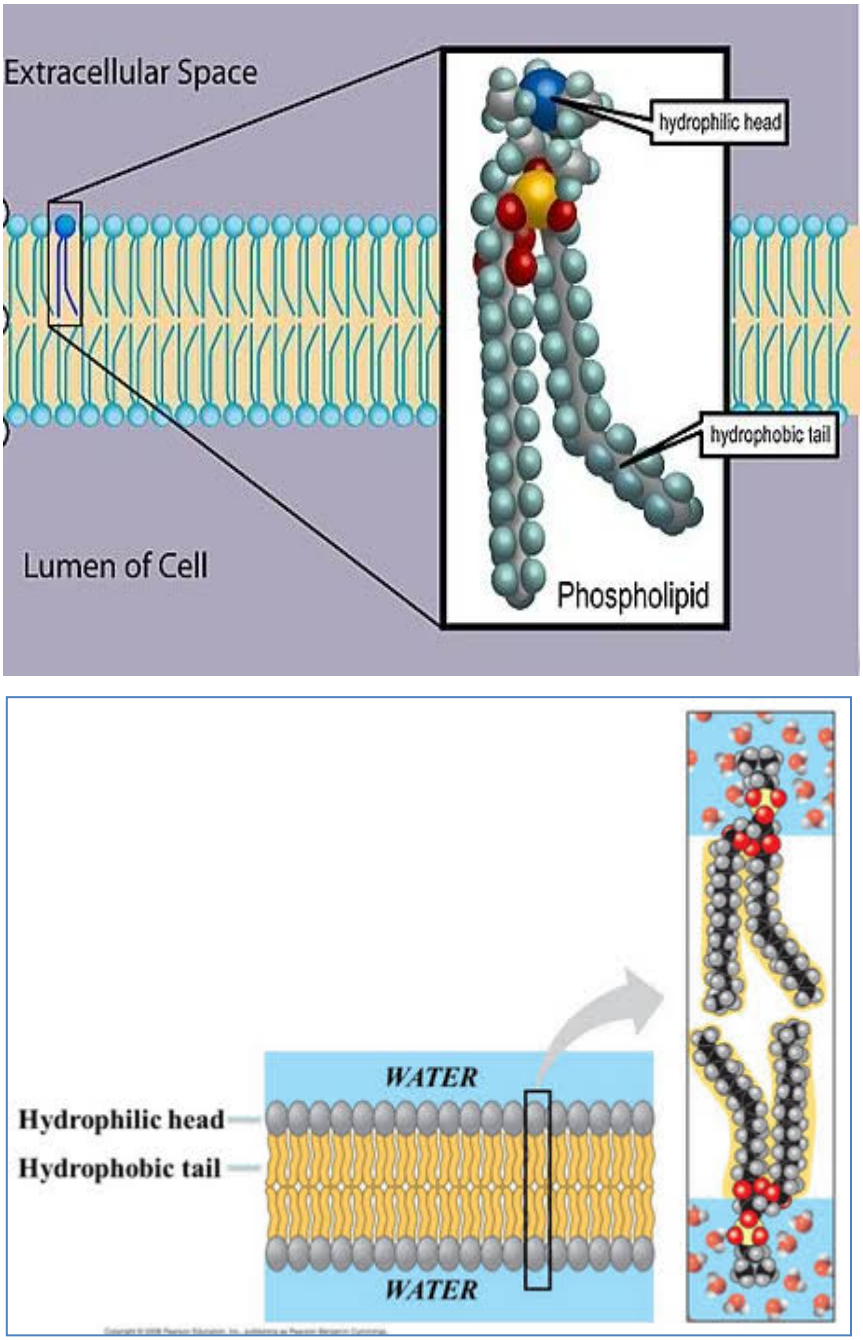


Figure 36: Phospholipids structure

III-10-4 Lipid Metabolism

Lipid metabolism is referred to the synthesis and degradation of lipids within the cells, either break down or storage of fats for energy.

•These fats are obtained from consuming food and absorbing themor they are synthesized by an animal's liver.

•Minimal amount of fat is essential in our food :

-For essential fatty acids synthesis .

-Help Fat-soluble vitamins absorption.

-As a Source of energy: 1 gm supplies 9.1 calories

Just like glucose Metabolism, the end-products of fatty acid metabolism are carbon dioxide, water and ATP. However, complete combustion of fatty acids requires glucose to convert it in to carbon dioxide, water and ATP, otherwise ketones are produced.

III – 10-5 Digestion and Absorption of the Lipids

There are following steps that start from mouth:

Table 1. Steps of lipid digestion and absorption

Step	Location	Enzymes
1. Minor digestion (TAGs → DAGs + FFA)	mouth and stomach	lingual/ gastric lipase
2. Major digestion (all) TAG → MAG + 2FFA (PL) CE → chol. + ester (CE) PL → FA + lysoPL (PLA)	lumen of the small intestines	pancreatic lipase (+colipase) cholesterol esterase phospholipase A_2
3. Formation of mixed micelles (uses bile salts as biological detergent)	lumen of the small intestines	N/A
4. Passive absorption of lipolytic products	into intestinal epithelial cell	N/A
5. Assembly and export of chylomicrons	from intestinal cells to the lymphatics	N/A

Absorption of Lipids

The end products of lipid digestion are: monoglycerides, FA, glycerol, cholesterol & lysophospholipids.

1-Glycerol and short chain FA :

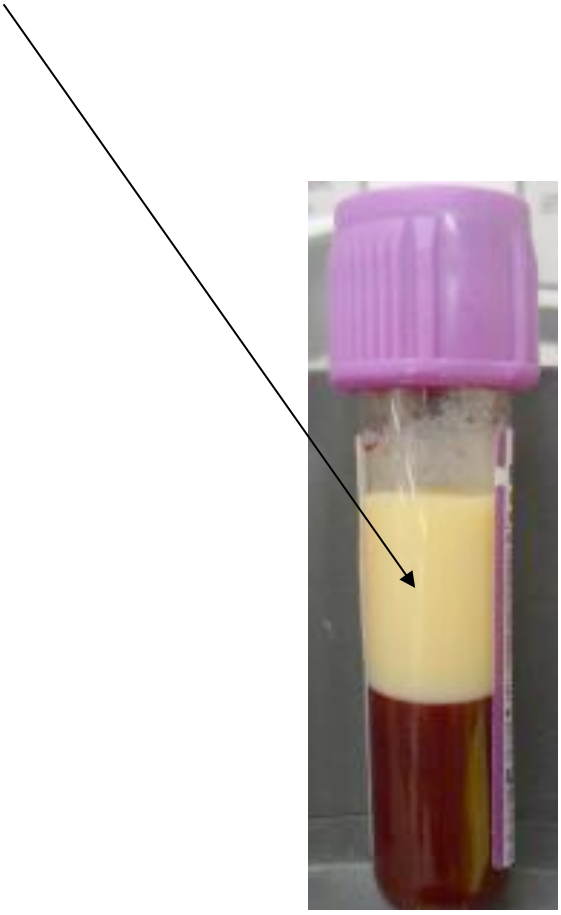
Water soluble carried through portal circulation

2-Long chain FA, monoglycerides, cholesterol et lysophospholipids: Need bile salts to be absorbed **(Figure 36)** .

Note : Immediately after absorption of lipids there is turbidity in plasma observed due to circulating chylomicrons(appear in plasma up to 2 hours after meals).

•Chylomironsare ultra low-density lipoproteins (ULDL), that consist of triglycerides (85–92%), phospholipids (6–12%), cholesterol (1–3%), and proteins (1–2%).

• This turbidity is soon cleared by lipoprotein lipase enzyme(clearing factor). Lipoprotein lipase breaks Chilomicrons into Glycerol and FA.



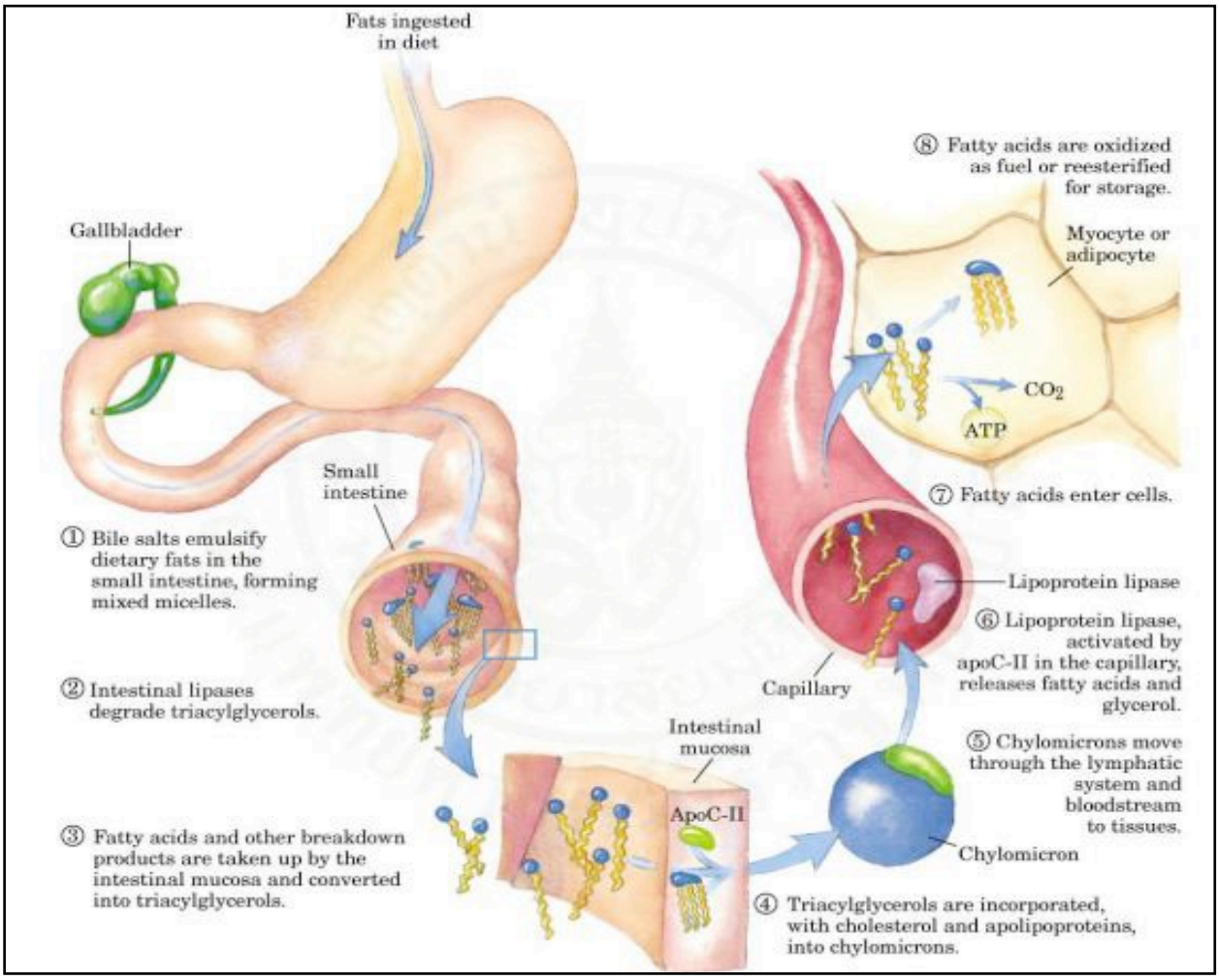


Figure 37: Absorption of Lipids

III – 10-6 Lipoproteins

Lipids are insoluble in water and therefore cholesterol and triglycerides need to be transported in association with proteins (ie, lipoproteins) in the bloodstream. Lipoproteins play a crucial role in the transport of dietary lipids from the small intestine to the liver, muscle, and adipose tissue, in the transport of hepatic lipids to peripheral tissues, and the transport of cholesterol from peripheral tissues to the liver and intestine (ie, reverse cholesterol transport). Lipoproteins may have additional functions and studies have suggested that they may play a role in protection from disease.1 For example, lipoproteins bind endotoxin (LPS) from gram-negative bacteria and lipoteichoic acid from gram-positive bacteria thereby reducing their toxic effects.

In addition, apolipoprotein L1, associated with HDL particles, has lytic activity against the parasite Trypanosoma brucei and lipoproteins can neutralize viruses. Thus, while this article

will focus on the transport properties of lipoproteins the reader should recognize that lipoproteins may have other important functions.

III – 10-6 -1 Lipoprotein structure

The surface of lipoproteins is a hydrophilic membrane consisting of phospholipids, free cholesterol, and apolipoproteins which surrounds a central hydrophobic core of nonpolar lipids, primarily cholesteryl esters and triglycerides. Lipoprotein particles are divided into 7 classes based on size, apolipoprotein composition, and lipid composition.

High-density lipoprotein particles are enriched in cholesterol and phospholipids and apolipoproteins A-I, A-II, A-IV, C-I, C-II, C-III, and E are associated with HDL particles.5,6 The core structural protein is Apo A-I and each HDL particle may contain multiple Apo A-I proteins. In addition, using mass spectrometry proteins involved in proteinase inhibition, complement activation, and the acute-phase response have been found associated with HDL particles.

HDL particles may be classified based on density, size, charge, and apolipoprotein composition and are very heterogeneous . HDL particles mediate the transport of cholesterol and other compounds from peripheral tissues to the liver (ie, reverse cholesterol transport), which is one of the several potential mechanisms by which HDL may be anti-atherogenic. In addition, HDL particles have anti-oxidant, anti-inflammatory, anti-thrombotic, and anti-apoptotic properties, which may also contribute to their ability to prevent atherosclerosis.

A / Chylomicrons

Chylomicrons are large triglyceride-rich particles secreted by the small intestine, which play a key role in the transport of dietary triglycerides and cholesterol to peripheral tissues and liver.8 Each chylomicron particle contains one Apo B-48 molecule, which is the core structural protein. In addition, chylomicron particles also contain A-I, A-II, A-IV, A-V, B-48, C-II, C-III, and E. Chylomicrons vary in size depending on the amount of fat ingested with a high-fat meal leading to the formation of large chylomicron particles with an increased quantity of triglyceride, whereas in the fasting state or with the consumption of a low-fat meal the chylomicron particles are small carrying decreased quantities of triglyceride. Similarly, the amount of cholesterol transported in chylomicrons can also vary.

B/ Chylomicron Remnant Particles

Chylomicron remnants are formed by the removal of triglyceride from chylomicrons by muscle and adipose tissue lipoprotein lipase (LPL) resulting in smaller particles. These particles are enriched in cholesterol and are pro-atherogenic.

III – 10-6 -2 Very Low-Density Lipoprotein Particles

Very low-density lipoprotein particles are produced by the liver and contain apolipoprotein B-100, C-I, C-II, C-III, and E. The core structural protein is Apo B-100 and each VLDL particle contains one Apo B-100 protein. The size of the VLDL particles varies and when triglyceride production in the liver is increased the secreted VLDL particles are large.

III – 10-6-3 Intermediate-Density Lipoprotein Particles; Very Low-Density Lipoprotein Remnant Particles

Intermediate-density lipoprotein particles are formed by the removal of triglycerides from VLDL by muscle and adipose tissue LPL resulting in the formation of IDL particles which are enriched in cholesterol and are pro-atherogenic. These IDL particles contain apolipoprotein B-100 and E **(Figure 37)** .

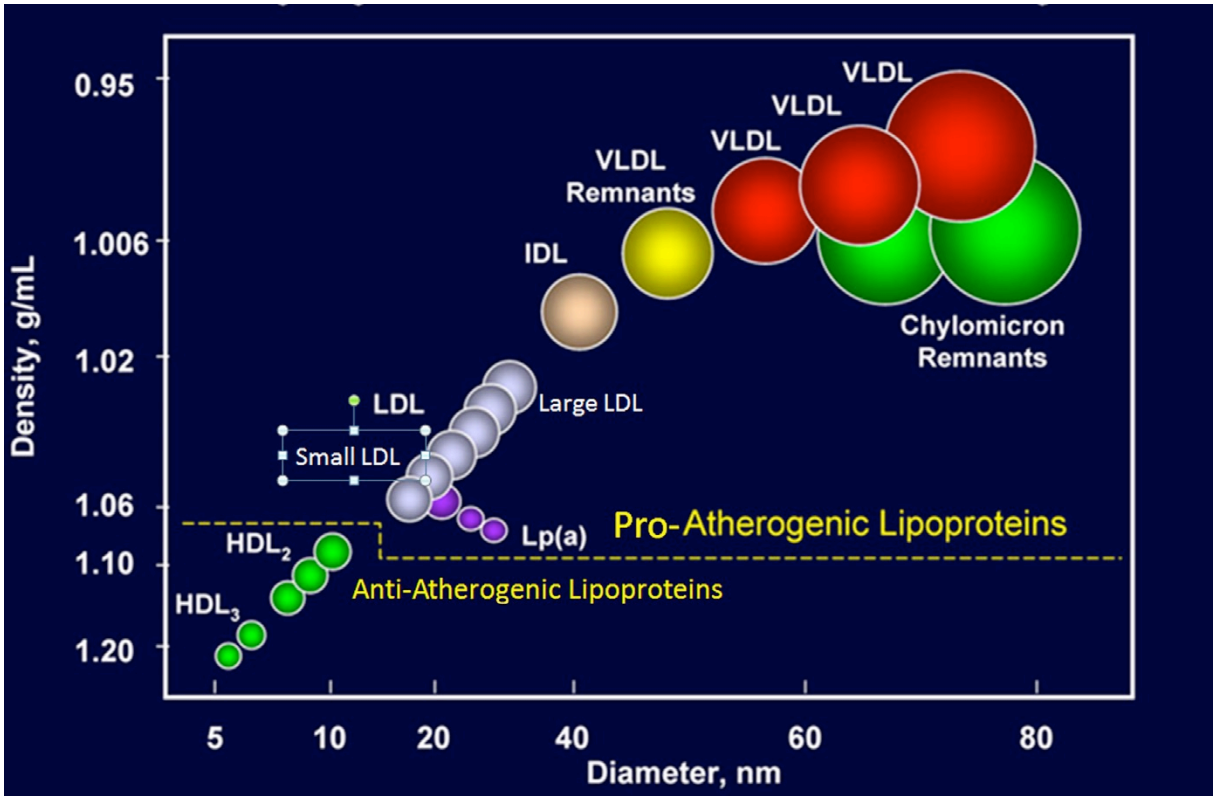


Figure 38: Intermediate-Density Lipoprotein Particles; Very Low-Density Lipoprotein

•Lipoprotein classes

•chylomicrons: take lipids from small intestine through lymph cells

•very low density lipoproteins (VLDL)

•intermediate density lipoproteins (IDL)

•low density lipoproteins (LDL)

•high density lipoproteins (HDL)

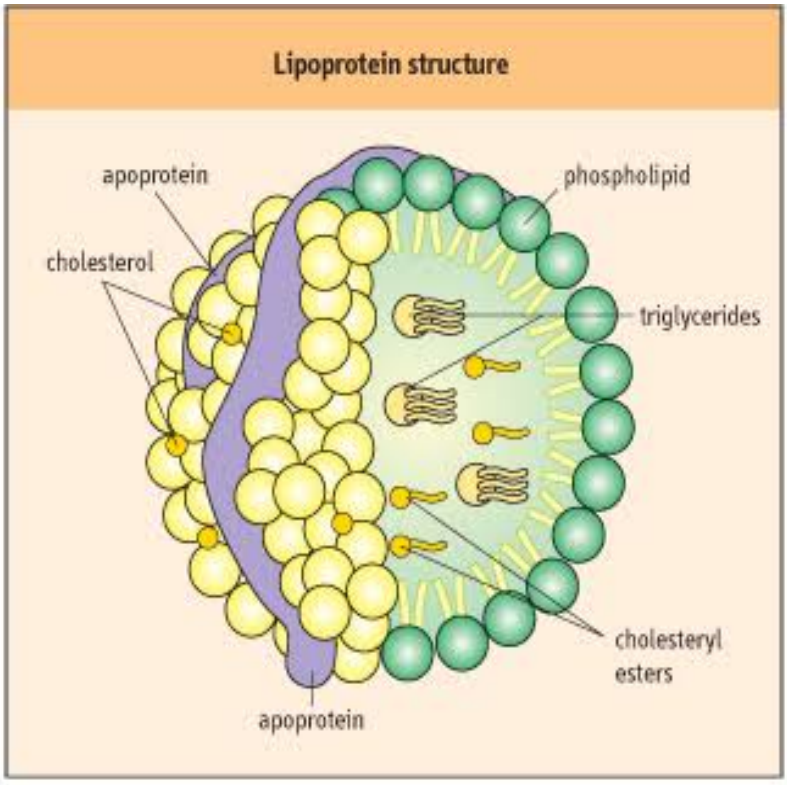
Spherical particles with a hydrophobic core (TG and esterified cholesterol)

•Apolipoproteins on the surface

•large: apoB (b-48 and B-100) atherogenic

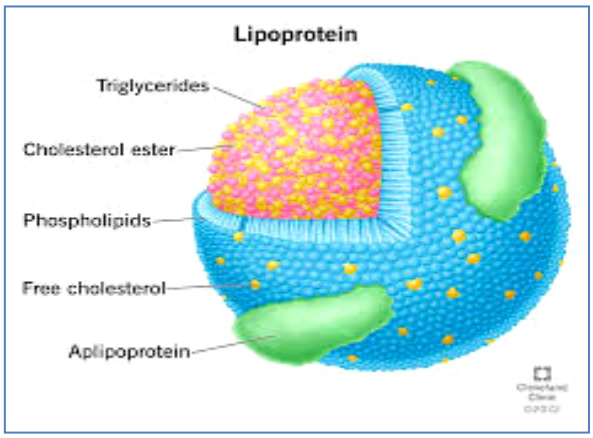
•smaller: apoA-I, apoC-II, apoE

Classified on the basis of density and electrophoretic mobility (VLDL; LDL; IDL;HDL; Lp(a)



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Figure 39: Lipopotein structure



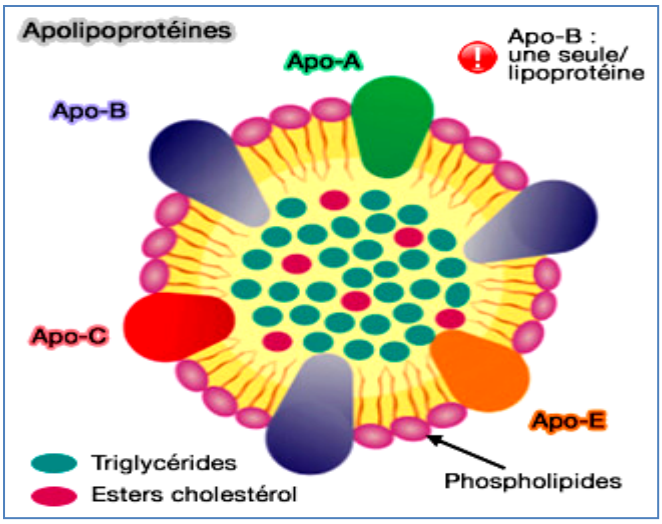


Figure 40: Apolipoproteins structure

- **Apolipoproteins**

Apolipoproteins are lipid-binding proteins in the blood, responsible for the transport of triacylglycerols, phospholipids, cholesterol, and cholesteryl esters between organs. Apolipoproteins ("apo" means "detached" or "separate," designating the protein in its lipid-free form) combine with lipids to form several classes of lipoprotein particles, spherical aggregates with hydrophobic lipids at the core and hydrophilic protein side chains and lipid head groups at the surface. Various combinations of lipid and protein produce particles of different densities, ranging from chylomicrons and verylow- density lipoproteins (VLDL) to very-high-density lipoproteins (VHDL), which can be separated by ultracentrifugation **(Figure 39)** .

Apolipoproteins role

Apolipoproteins play an essential role in lipoprotein metabolism . They have 4 key functions :

1. Guiding the synthesis of lipoproteins

2. Serving in a structural role

3. Serving as ligands for lipoprotein receptors

4. Activating or inhibiting enzymes involved in the metabolism of lipoproteins

•Major components of lipoproteins

•Often referred to as apoproteins

•Classified by alphabetical designation (A thru E)

A, B, C, D, E are major classes

•Subclasses: apo A-1, apo C-II

•N.B. function of all apolipoproteins are not yet known

•The use of roman numeral suffix describes the order in which the apolipoprotein emerge from a chromatographic column.

•Responsible for recognition of particle by receptors

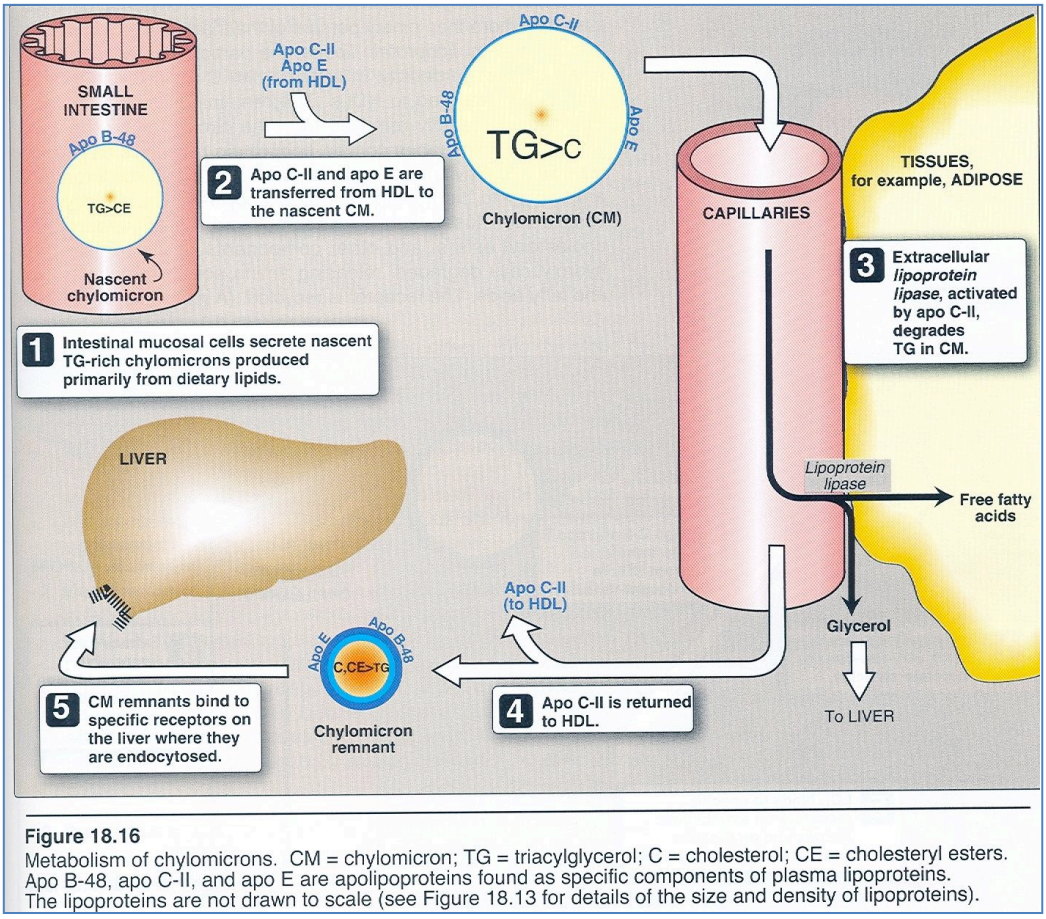


Figure 18.16
Metabolism of chylomicrons. CM = chylomicron; TG = triacylglycerol; C = cholesterol; CE = cholesteryl esters. Apo B-48, apo C-II, and apo E are apolipoproteins found as specific components of plasma lipoproteins. The lipoproteins are not drawn to scale (see Figure 18.13 for details of the size and density of lipoproteins).

Figure 41:Metabolism of chylimicron

CHAPTER IV: 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. 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.

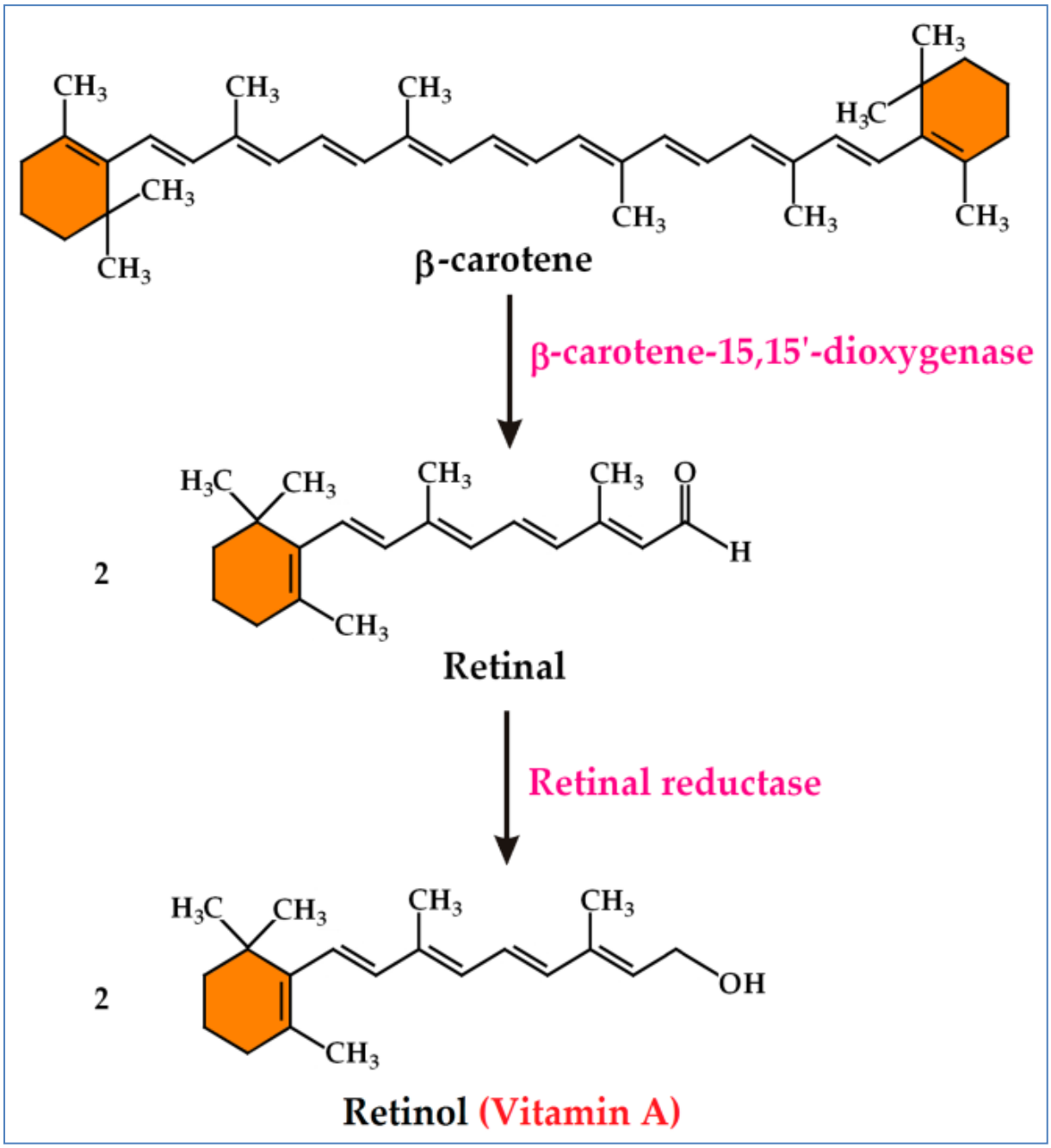
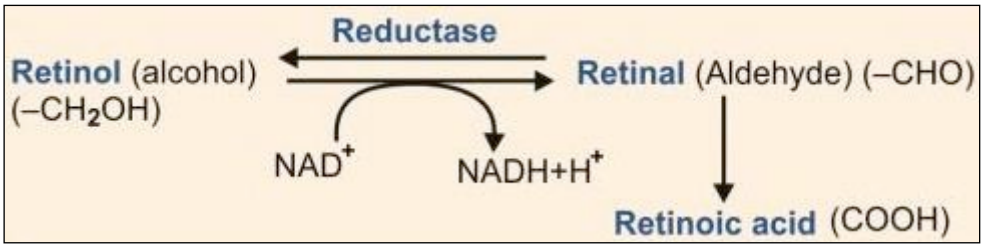


Figure 42: Vitamin A structure

Beta-carotene has two beta ionone rings. 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 . 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.



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 100

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 .

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).

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.

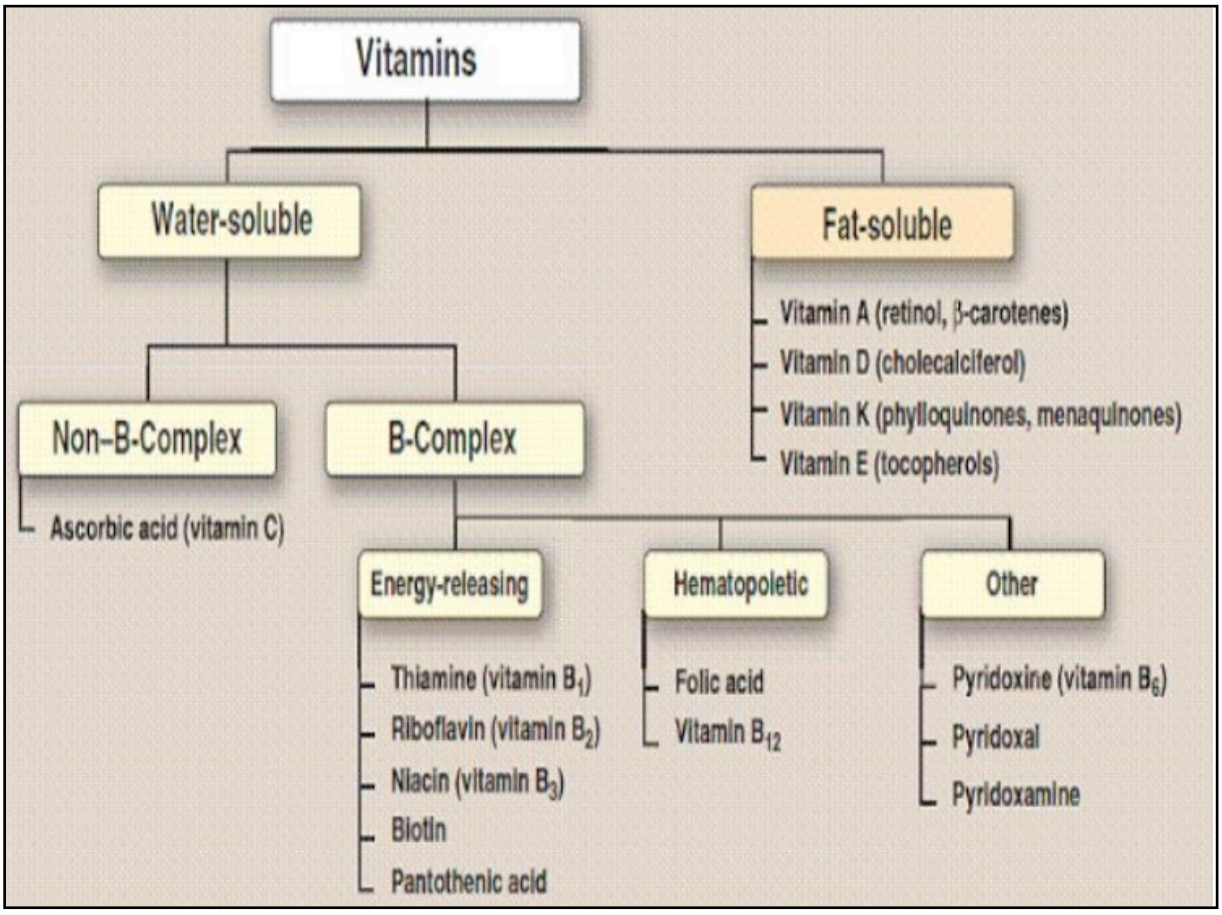


Figure 43: Classification of vitamins

CHAPTER V: 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 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.

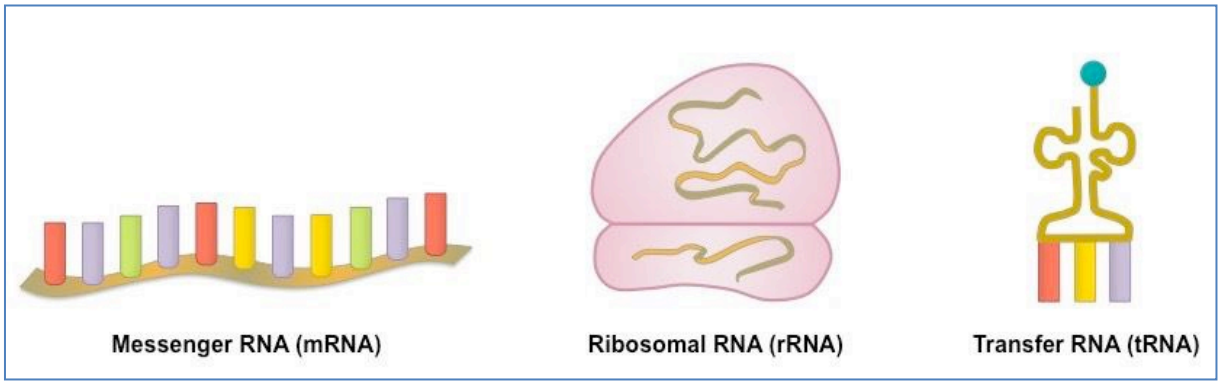
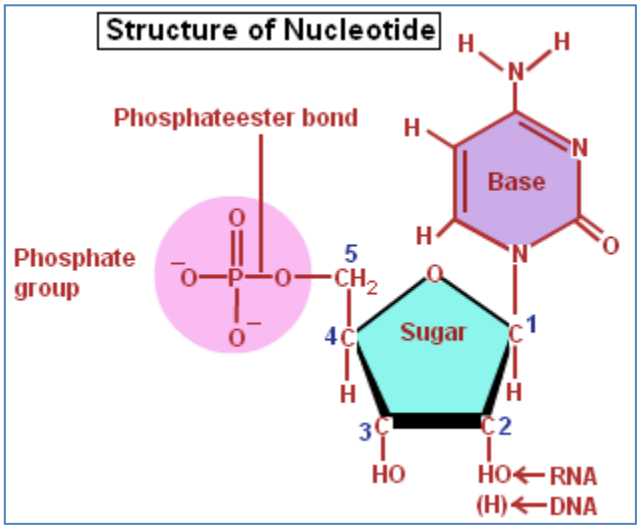


Figure 44: Types of RNA: mRNA, rRNA and tRNA Nucleic acids

V – 1 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 . The nitrogenous bases are derivatives of two parent compounds, pyrimidine and purine.



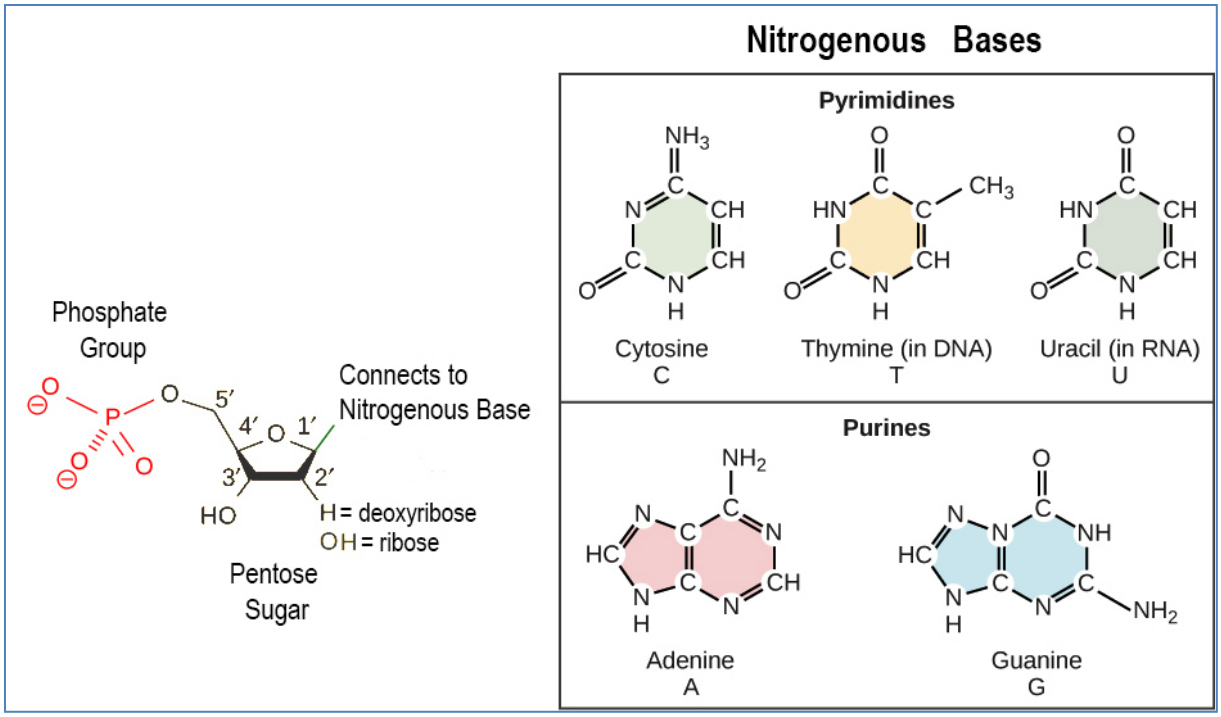


Figure 45: Structure of nucleotide and nitrogenous bases

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 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.

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.

V-2 Structure and function of DNA

DNA contains and stores the genetic information.

• It is the source of information for the synthesis of all proteins of the cell and organism.

• Provides the information inherited by daughter cell.

DNA has primary, secondary, and tertiary structures. The primary structure of DNA is a linear sequence of mononucleotides in a polynucleotide chain. Erwin Chargaff and his colleagues found that in all cellular DNAs, regardless of the species, the number of adenosine residues is equal to the number of thymidine residues (A = T), and the number of guanosine residues is equal to the number of cytidine residues (G = C). The sum of the purine residues equals the sum of the pyrimidine residues: A + G = T + C. These quantitative relationships, sometimes called “Chargaff’s rules”, were a key to establishing the three-dimensional structure of DNA.

The secondary structure of DNA is presented by two strands, wound around each other in a right-handed, helical structure with the base pairs in the middle and the deoxyribosylphosphate chains on the outside. The orientation of the DNA strands is antiparallel (i.e. the strands run in opposite directions). The nucleotide bases on each strand interact with the nucleotide bases on the other strand to form base pairs (adenine with thymine, guanine with cytosine).

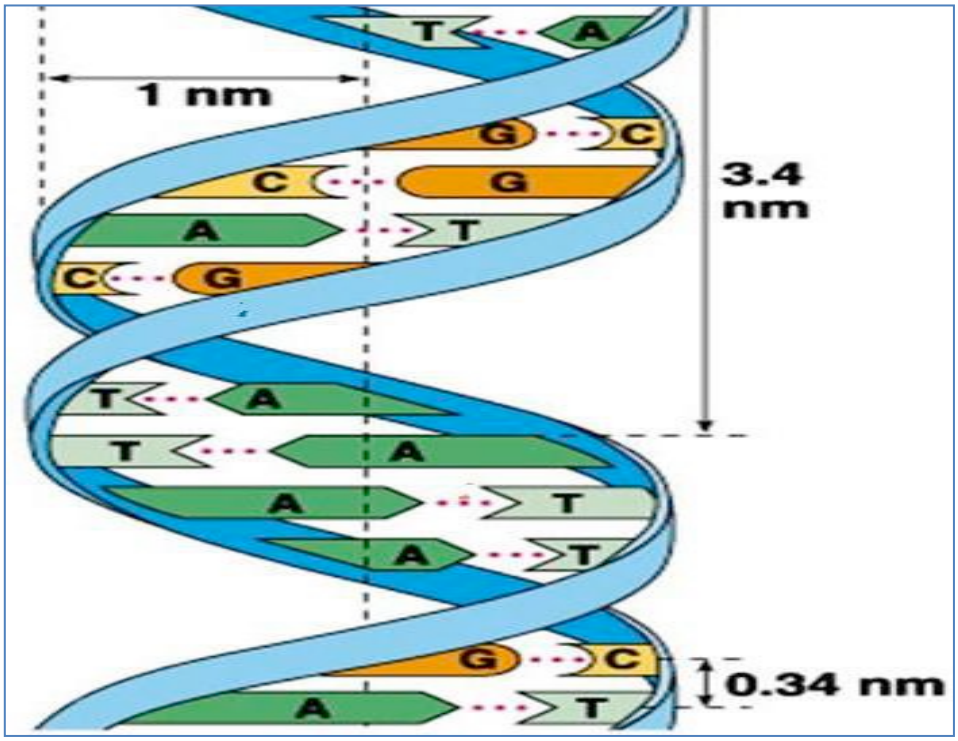


Figure 46: Secondary structure of DNA

Tertiary structure of DNA is different in prokaryotic and eukaryotic cells. In prokaryotes, there are different types of supercoiling. In eukaryotes, DNA is complexed with RNA and an approximately equal mass of protein. These DNA-RNA-protein complexes are termed chromatin.

Functions of chromatin:

- to package DNA into a smaller volume to fit in the cell,
- to strengthen the DNA to allow mitosis,
- to prevent DNA damage,
- control of gene expression and DNA replication.

There are four levels of chromatin organization:

The histone proteins associate into a complex termed a **nucleosome (1)**. Each of these complexes contains two molecules each of H2A, H2B, H3, and H4, and one molecule of H1. The nucleosome protein complex is encircled with about 200 base pairs of DNA that form two coils around the nucleosome core. The H1 protein associates with the outside of the nucleosome core to stabilize the complex. The nucleosome particles themselves are also organized into other, more tightly packed structures, termed 30-nm **chromatin filaments (fibers)**. These filaments are constructed by winding the nucleosome particles into a spring-shaped **solenoid**

(2) structure with about six nucleosomes per turn. The solenoid is stabilized by head-to-tail associations of the H1 histones. Nucleosomes can be organized into 30 nm fibers *in vitro*, but this structure has not been found in cells. Higher-order folding of chromosomes involves attachment to a chromosomal scaffold, notably topoisomerase II and SMC proteins. In interphase chromosomes chromatin fibers are organized into **loops (3),** anchored in a scaffold. The DNA in a loop may contain a set of related genes. The final level of chromatin organization **is metaphase chromosome (4)**.

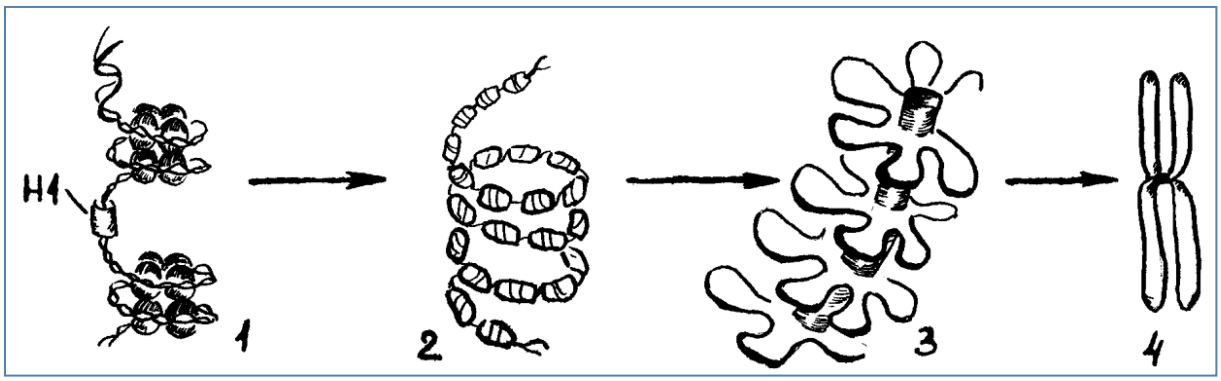


Figure 47: Levels of chromatin organization

Individual chromosomes are constrained within nuclear subdomains called territories. Histone H1,topoisomerase II, and SMC proteins (structural maintenance of chromosomes) play organizational roles in chromosomes. The SMC proteins, principally cohesins and condensins, have important roles in keeping the chromosomes organized during each stage of the cell cycle.

RNAs

The RNAs produced by prokaryotic and eukaryotic cells are single-stranded molecules. Even though most RNAs are single stranded, they exhibit extensive secondary structures. Single RNA strands can fold into hairpins, double-stranded regions, or complex loops that are important to RNAs function. These secondary structures are the product of intramolecular base pairing that occurs between complementary nucleotides within a single RNA molecule. All eukaryotic cells have two major classes of RNA, protein coding RNAs (messenger RNAs) and non-protein coding RNAs (ribosomal, long noncoding, transfer, small nuclear, micro and silencing RNAs). Each class has a distinctive size and function. The messenger, transfer, and ribosomal RNAs are involved in protein synthesis. The other RNAs are participate in either RNA processing or modulation of gene expression.

Messenger RNAs (mRNAs) represent the most heterogeneous class. They are:
- messenger conveying the information from the gene to the protein synthesizing machinery;
- template for protein synthesis.

All eukaryotic mRNAs contain a methylated guanine nucleotide “cap” at their 5' end. The function of this structure is two-fold; it is essential for ribosomal binding and it protects the mRNA from attack by 5' exonucleases. The 3' end is modified by the addition of a number of adenine residues - known as a polyA tail. The number of adenine residues added to a particular transcript can vary from as few as 30 to more than 300 residues. This structure protects the mRNA from attack by 3' exonucleases, increasing its half-life.

Transfer RNAs (tRNAs)

Transfer RNAs (tRNAs) transfer a specific amino acid to a growing polypeptide chain at the ribosomal site of protein synthesis during translation. They function as adapter molecules that translate the information stored in the mRNA nucleotide sequence to the amino acid sequence of proteins. Length of molecules is from 65 to 110 nucleotides.

They exhibit extensive secondary structure and contain several ribonucleotides that differ from the usual four by a variety of modifications. All tRNAs have a similar folded structure, with four distinctive loops (arms), that have been described as clover leafs (Fig.5.3). The D loop contains several modified bases, including methylated cytosine and dihydrouridine, for which the loop is named. The anticodon loop is the structure responsible for recognition of the complementary codon of an mRNA molecule. A variable loop exists in most tRNAs, but its function is unknown. Finally, there is a TψC loop, which is named for the presence in this loop of the modified base, pseudouridine.

Another prominent structure found in all tRNA molecules is the acceptor stem.

This structure is formed by base pairing between the nucleotides found at the 5' and 3' ends of the tRNA. The last three bases found at the extreme 3' end remain unpaired, and always have the same sequence: 5'-CCA-3'. The 3' end of the acceptor stem is the point at which an amino acid is attached via an ester bond between the 3'-hydroxyl group of the adenosine and the carboxyl group of an amino acid.

Ribosomal RNAs (rRNAs)

Ribosomal RNAs (rRNAs) are the structural and catalytic component of the ribosomes. rRNA from eukaryotes consists of four different sizes of RNA (5S, 5.8S, 18S, 28S). These RNAs interact with each other, and with proteins, to form a ribosome that provides the basic machinery on which protein synthesis takes place.

CHAPTER VI: Enzymes.

VI-1 Definition

Enzymes are protein catalysts for chemical reaction in biological systems. They increase the rate of chemical reactions taking place within living cells with out changing them selves. The breakthrough in understanding globular protein structure came from x-ray diffraction studies of the protein myoglobin carried out by John Kendrew and his colleagues in the 1950. 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.

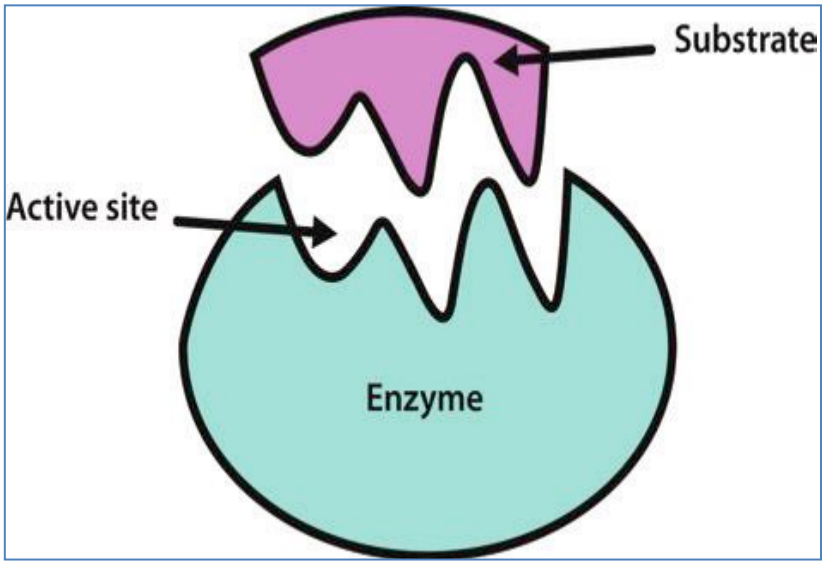


Figure 48: Enzyme and the active site

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.)

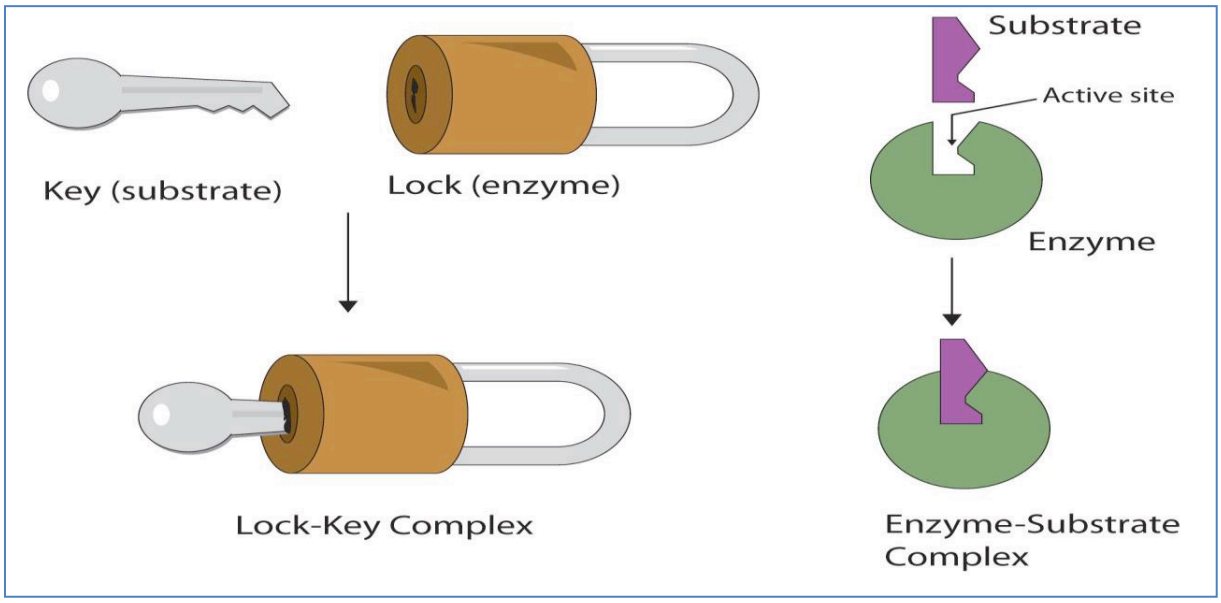


Figure 49: The lock and key model of enzyme activity

VI-2 Characteristics of Enzymes

i. Almost all enzymes are proteins. Enzymes follow the physical and chemical reactions of proteins.

ii. They are heat labile.

iii. They are water-soluble.

iv. They can be precipitated by protein precipitating reagents (ammonium sulfate or trichloroacetic acid).

v. They contain 16% weight as nitrogen.

VI-3 Active site

Enzyme molecules contain a special pocket or cleft called the active site. The active site contains amino acid chains that create a three-dimensional surface complementary to the substrate. The active site binds the substrate, forming an enzyme-substrate (ES) complex. ES is converted to enzyme-product (EP); which subsequently dissociates to enzyme and product.
For the combination with substrate, each enzyme is said to possess one or more active sites where the substrate can be taken up.

The active site of the enzyme may contain free hydroxyl group of serine, phenolic (hydroxyl) group of tyrosine, SH-thiol (Sulfhydryl) group of cysteine or imindazolle group of histidine to interact with there is substrates. It is also possible that the active site (Catalytic site) is different from the binding site in which case they are situated closely together in the enzyme molecule.

B. Catalytic efficiency/ Enzyme turnover number

Most enzyme- catalyzed reactions are highly efficient proceeding from 103 to 108 times faster than uncatalyzed reactions. Typically each enzyme molecule is capable of transforming 100 to 1000 substrate molecule in to product each second. Enzyme turn over number refers to the amount of substrate converted per unit time (carbonic anhydrase is the fastest enzyme).

C. Specificity

Enzymes are specific for their substrate. Specificity of enzymes are divided into:

a. Absolute specificity: this means one enzyme catalyzes or acts on only one substrate. For example: Urease catalyzes hydrolysis of urea but not thiourea.

b. Stereo specificity- some enzymes are specific to only one isomer even if the compound is one type of molecule:

For example: glucose oxidase catalyzes the oxidation of β-D-glucose but not α-Dglucose, and arginase catalyzes the hydrolysis of L-arginine but not D-arginine.

*Maltase catalyzes the hydrolysis of α- but not β –glycosides.

Bond Specificity

* Enzymes that are specific for a bond or linkage such as ester, peptide or glycosidic belong to this group

Examples:

1. Esterases- acts on ester bonds
2. Peptidases-acts on peptide bonds

3. Glycosidases- acts on glycosidic bonds.

D. Regulation

Enzyme activity can be regulated- that is, enzyme can be, activated or inhibited so that the rate of product formation responds to the needs of the cell.

E. **Zymogens** (- inactive form of enzyme)

Some enzymes are produced in nature in an inactive form which can be activated when they are required. Such type of enzymes are called Zymogens (Proenzymes).

Many of the digestive enzymes and enzymes concerned with blood coagulation are in this group Examples: Pepsinogen - This zymogen is from gastric juice.When required Pepsinogen converts to Pepsin

Trypsinogen - This zymogen is found in the pancreatic juice, and when it is required gets converted to trypsin.

* The activation is brought about by specific ions or by other enzymes that are proteolytic.

Pepsinogen + H+ Pepsin

Trypsinogen Enteropeptidase Trypsin

Zymogen forms of enzymes a protective mechanism to prevent auto digestion of tissue producing the digestive enzymes and to prevent intravascular coagulation of blood.

F. Isoenzymes (Isozymes)

These are enzymes having similar catalytic activity, act on the same substrate and produces the same product but originated at different site and exhibiting different physical and chemical characteristics such as electrophoretic mobilities, amino acid composition and immunological behavior. Example: LDH (Lactate dehydrogenase) exists in five different forms each having four polypeptide chains. H= Heart and M=Muscle.

VI-4 Classification of enzymes

Enzymes are classified on the basis of the reactions they catalyze- Each enzyme is assigned a four-digit classification number and a systematic name, which identifies the reaction catalyzed.

The international union of Biochemistry and Molecular Biology developed a system of nomenclature on which enzymes are divided in to six major classes, each with numerous sub groups. Enzymes are classified based on the reactions they catalyze. Each enzyme is characterized by a code number comprising four digits separated by points. The four digits characterize class, sub-class, sub-sub-class, and serial number of a particular enzyme.

When early workers isolated certain enzymes, whimsical names were given. Some of these, such as Pepsin, Trypsin, Chymotrypsin, etc. are still used. Later, it was agreed to call the enzymes by adding the suffix **"-ase"** to the substrate. Thus, enzyme **Lactase** acts on the substrate lactose, and the products glucose and galactose are formed. Enzymes that hydrolyse starch (amylose) are termed as amylases; those that dehydrogenate the substrates are called dehydrogenases. These are known as the **trivial names** of the enzymes.

1 - Class I: Oxidoreducatases-

Enzymes catalyzing oxidation reduction reactions.

Example: Lactate-dehydrogenase

1- Lactic acid + NAD+ Pyruvic acid + NADH+H+

2- Class II.: Transferases:

Enzymes catalyzing a transfer of a group other than hydrogen (methyl, acyl, amino or phosphate groups)

Example: Enzymes catalyzing transfer of phosphorus containing groups.

ATP: D-hexose-6 phosphotransferase (Hexokinase)

ATP+D-Hexose ADP+D-hexose-6-phosphate

Example: Acetyl-CoA: Choline D-acyltransferase (Choline Acyltransferase).

3 Class III : Hydrolases:

Enzymes catalyzing hydrolysis of ester, ether, peptido, glycosyl, acid-anhydride, C-C, C-halide, or P-N-bonds by utilizing water.

Example: Enzymes action on glycosyl compounds β-D- Galactoside galactohydrolase (β -Galactosidase) α β -D- Galactoside+H2O alcohol +D-Galactose

4 Class IV: Lyases:

Enzymes that catalyze removal of groups from substances by mechanisms other than hydrolysis, leaving double bonds.

5 Class V: Isomerases:

Includes all enzymes catalyzing interconversion of optical, geometric, or positional isomers.

Example: Enzymes catalyzing interconversion of aldose and ketoses

D - Glyceraldehyde-3- phosphate ketoisomerase (triosephosphate isomerase)

D - Glyceraldehyde-3phosphate Dihydroxyacetone phosphate.

6 Class VI: Ligases or synthetases.

Enzymes catalyzing the linking together of 2 compounds coupled to the breaking of a pyrophosphate bond in ATP or similar trinucleotides: GTP, UTP etc. included are enzymes catalyzing reactions forming C-O, C-S, C-N, and C-C bonds,

Example: Enzymes catalyzing formation of C-N-bonds.

L- Glutamine: ammonia ligase (ADP) [Glutamine Synthetase]

ATP + L-Glutamate + NH4 = ADP + orthophosphate + L-Glutamine

Example: Enzymes catalyzing formation of C-C bonds

Acetyl-CoA: CO2 ligase (ADP) [acetyl-CoA carboxylase] ATP+ Acetyl-COA-CO2

⟶ Malonyl-CoA+ADP+pi.

VI -5 Tow types of enzymes

1. Simple enzyme: It is made up of only protein molecules not bound to any nonproteins**.**

Example: Pancreatic Ribonuclease.

2. Holo enzyme is made up o protein groups and non-protein component.

- The protein component of this holo enzymes is called apoenzyme
- The non-protein component of the holo enzyme is called a cofactor. I

If this cofactor is an organic compound it is called a coenzyme and if it is an inorganic groups it is called activator. (Fe 2+, Mn 2+, or Zn 2+ ions). If the cofactor is bound so tightly to the apoenzyme and is difficult to remove without damaging the enzyme it is sometimes called a prosthetic group.

VI -6 Coenzymes

Coenzymes are derivatives of vitamins without which the enzyme cannot exhibit any reaction. One molecule of coenzyme is able to convert a large number of substrate molecules with the help of enzyme.

- Coenzyme accepts a particular group removed from the substrate or donates a particular group to the substrate
- Coenzymes are called co substrate because the changes that take place in substrates are complimentary to the changes in coenzymes.

The coenzyme may participate in forming an intermediate enzyme-substrate complex

Example: NAD, FAD, Coenzyme A

VI -7 Metal ions in enzymes

Many enzymes require metal ions like **ca2+, K+, Mg2+, Fe2+, Cu2+, Zn2+, Mn2+** and **Co2+**for their activity.

Metal-activated enzymes-form only loose and easily dissociable complexes with the metal and can easily release the metal without denaturation. Metalloenzymes hold the metal tightly on the molecule and do not release it even during extensive purification.

Metal ions promote enzyme action by:

a. Maintaining or producing the active structural conformation of the enzyme (e.g. glutamine synthase)

b. Promoting the formation of the enzyme-substrate complex (Example: Enolase and carboxypeptidase A.)

c. Acting as electron donors or acceptors (Example: Fe-S proteins and cytochromes)

d. Causing distortions in the substrate or the enzyme Example: phosphotransferases).

VI -8 Mechanism of action of enzymes

Enzymes are called specific proteins that are part of all cells and tissues of living organisms which serve as biological catalysts.

Common properties of enzymes and inorganic catalysts:

1. Not consumed in the reaction.
2. Exert their effects at low concentrations.
3. Do not affect the value of the equilibrium constant of the reaction.
4. Their action is subject to the law of mass action.

VI -9 Differences between enzymes and inorganic catalysts:

1. Thermolability of enzymes.

2. The dependence of enzyme activity on pH.

3. Specificity of action of enzymes.

4. Rate of enzymatic reactions is subject to certain kinetic regularities.

5. Enzyme activity depends on the action of regulators - activators and inhibitors.

6. Several enzymes in the formation of tertiary and quaternary structures are subjected to postsynthetic modification.

7. Size of enzyme molecules is usually much larger than their substrates.

VI -10 The structure of the enzyme molecules

By the structure all enzymes can be simple or complex proteins. The enzyme is a complex protein called **holoenzyme**. The protein part of the enzyme is called **apoenzyme**, non-protein part is called **cofactor**. There are two types of cofactors:

1. Prosthetic group – strongly associated with the apoenzyme, often by covalent bonds.

2. Coenzyme – non-protein part is easily separated from the apoenzyme. Coenzymes are often vitamin derivatives.

Coenzymes include the following compounds:

- Vitamin derivatives;
- Hemes that are parts of cytochromes, catalases, peroxidases, guanylatecyclase, NO-synthase and the enzyme prosthetic group are nucleotides;
- Donors and acceptors phosphoric acid residue;
- Ubiquinone or coenzyme Q, involved in the transfer of electrons and protons in the chain of tissue respiration;
- Phoshoadenosylphosphosulfat (PAPS) involved in the transport of sulfate;
- Glutathione involved in redox reactions.

VI -11 Metal ions as cofactors

More than 25% of all enzymes exhibit full catalytic activity of metal ions needs. Consider their role in enzymatic catalysis.

VI -11 -1 Role in joining metal substrate in the active site of the enzyme.

The metal ions act as stabilizers of the substrate molecule, the active center of the enzyme and protein conformation of the enzyme molecule, namely the tertiary and quaternary structures.

VI -11 -2 Metal ions are stabilizers of substrate molecule.

For some enzymes, a substrate is the complex attached to the metal ion. For example, in most kinases ATP should combine with Mg2+ and form Mg2+-ATP complex. In this case, the Mg2+ ions do not interact directly with the enzyme but are involved in the stabilization of ATP molecules and neutralizing the negative charge of the substrate, which facilitates its connection to the active center of the enzyme. Schematically, cofactor role in the interaction of enzyme and substrate may be present as a complex ES-Me, where E - the enzyme, S - substrate Me - metal ion.

VI -11 -3 Metal ions are the active center of the enzyme stabilizers.

In some cases, metal ions serve as a "bridge" between the enzyme and the substrate. They act as stabilizers of the active center, facilitating accession to the substrate and chemical reaction. In some instances, the metal ion may facilitate adherence of the coenzyme. The above functions are performed by metals such as Mg2+, Mn2+, Zn2+, Co2+, Mo2+. In the absence of metal, these enzymes have no activity. Such enzymes are called "metalloenzymes". Metalloenzymes include, for example, the enzyme pyruvate kinase.

VI -11 -4 The role of metals in the stabilization of the enzyme structure.

Metal ions ensure the preservation of the secondary, tertiary, quaternary structure of the enzyme molecule. Such enzymes in the absence of metal ions are capable of chemical catalysis, however, they are unstable. Their activity is reduced or even disappears completely for small changes in pH, temperature, and other minor changes in the external environment. Thus, metal ions function as stabilizers of the protein molecule. Sometimes alkaline earth metal ions are involved in stabilizing the secondary and tertiary structures. Thus, to maintain the tertiary structures pyruvate kinase requires ions K+.

To stabilize the quaternary structure of the alcohol dehydrogenase, which catalyzes the reaction of oxidation of ethanol, Zn2+ ions are required.

VI -11 -5 The role of metals in enzyme catalysis

Not less important role in the implementation of the metal ions plays enzymatic catalysis.

VI -11 -6 Participation of metal in electrophonic catalysis.

Most often this function is performed by metal ions with variable valence, having a free d-orbital and act as electrophiles. This is, primarily, metals such as Zn2+, Fe2+, Mn2+, Cu2+. Alkali metal ions such as Na+ and K+, do not possess this property. During catalysis electrophilic metal ions are often involved in the stabilization of intermediates.

VI -11 -7 Participation of metals in redox reactions.

Metal ions with variable valency may also participate in electron transfer. For example, in cytochromes (heme-containing proteins), the iron ion can bind and release one electron. Due to this property cytochromes are involved in redox reactions.

The enzyme active site Part of the enzyme molecule which specifically interacts with the substrate is called an active center. Active site is a unique combination of amino acid residues in the enzyme molecule, providing direct interaction with its substrate molecule and participating directly in the act of catalysis. In complex enzymes cofactor is a part of the active center**.**

VI -12 Properties of the active sites of enzymes:

1. On the active site accounts for a relatively small portion of the total enzyme.

2. Active center is in the form of a narrow recess or gap in the globule enzyme.

3. Active site – a three-dimensional formation, which is involved in the formation of functional groups – linearly spaced apart amino acids.

4. Substrates are relatively weakly bound to the active site.

5. The specificity of binding of the substrate depends on a well-defined arrangement of atoms and functional groups in the active center.

In some regulatory enzymes there is another center called allosteric (regulatory) center. It is spatially separated from the active center.

Allosteric center is the portion of the enzyme molecule, which normally binds certain small molecules (allosteric regulators), molecules which are not similar in structure to the substrate.

Accession of allosteric regulator to the center results in a change of the tertiary and quaternary structure of the enzyme molecule and accordingly, the conformation of the active site, resulting in reduced or increased enzymatic activity.

VI -12 -1 The mechanism of enzymes action

Any enzymatic reaction includes the following stages :

$$E + S \quad [ES] \quad E + P$$

Where:

E - enzyme, S - substrate, [ES] - enzyme - substrate complex, P - product.

The mechanism of enzymes action can be considered from two perspectives: in terms of changing the energy of chemical reactions as well as in terms of events in the active site.

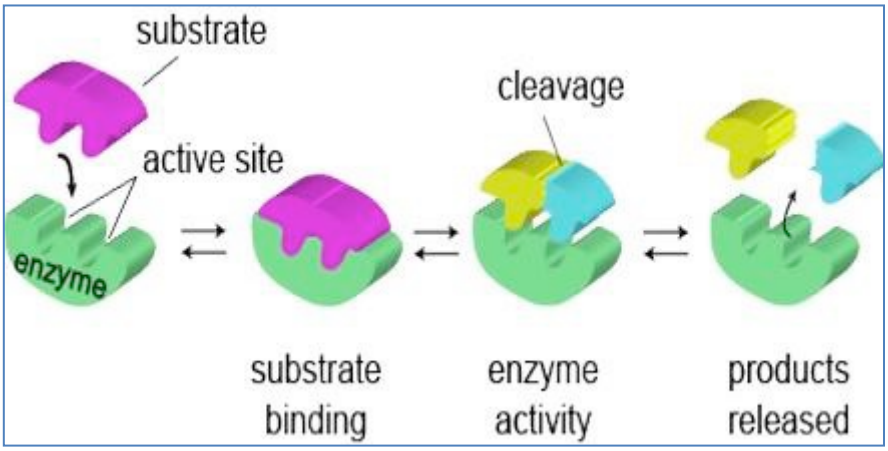


Figure 49: Mechanism of catalysis

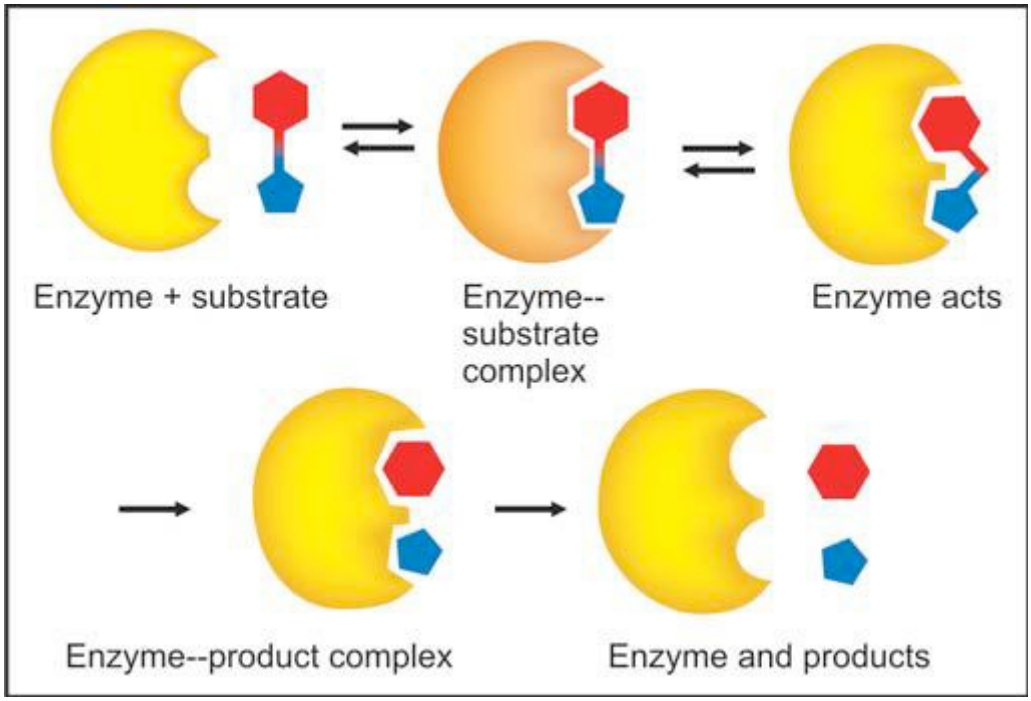


Figure 50: Enzyme substrate complex

VI -12 -2 Energy changes in chemical reactions

Any chemical reactions taking place are subject to two basic laws of thermodynamics: energy conservation law and the law of entropy. Under these laws, the total energy of a chemical system and its environment remains constant, while the chemical system tends to reduce order (increasing entropy). To understand the chemical reaction energy is not enough to know the energy balance of incoming and outgoing of the reactants. It is necessary to take into account changes in the energy of the chemical reaction process and the role of this enzyme in the process dynamics.

The larger molecules have an energy level higher than Ea (activation energy), the higher the rate of a chemical reaction. Heat can increase the rate of a chemical reaction. It increases the energy of the reacting molecules. However, living organisms detrimental high temperatures, so the cell to accelerate chemical reactions using enzymes. Enzymes provide high-speed reactions under optimal conditions in the cell, by lowering Ea. Thus, enzymes reduce the energy barrier height, thereby increasing the amount of reactive molecules and, consequently, increases the reaction rate.

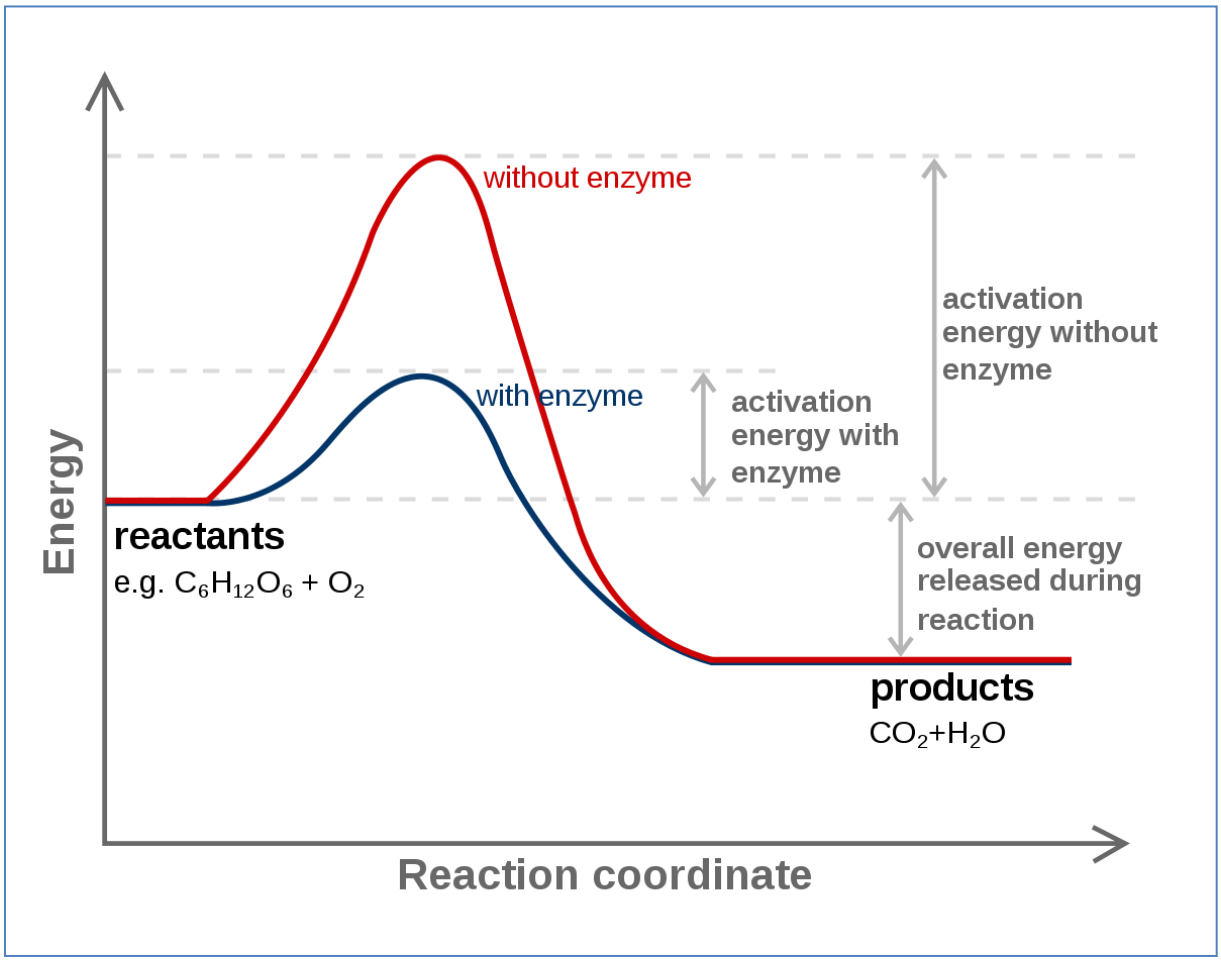


Figure 51: The mechanism of enzymes action

VI -12-3 The role of the active center in enzymatic catalysis

Investigations have shown that the molecule is an enzyme, usually much larger than the substrate molecule, undergoes chemical conversion by this enzyme. In contact with the substrate it enters only a small portion of the enzyme molecule, typically from 5 to 10 amino acid residues forming the active site of the enzyme. The role of other residues is to provide proper conformation of the enzyme molecule for optimum chemical reaction.

The active site at all stages of enzymatic catalysis can't be regarded as a passive site for substrate binding. This is a complex molecular "machine" using a variety of chemical mechanisms that facilitate the transformation of substrate to product.

In the active site of the enzyme substrates are arranged in such a way, allowing to participate in the reaction; functional groups of the substrates were in close proximity to each other. This property is called the effect of the active center of convergence and orientation of the reagents. Such an orderly arrangement of substrates causes a decrease in entropy and, consequently,

lower activation energy (Ea), which determines the catalytic efficiency of enzymes. The active site of the enzyme promotes destabilization of the interatomic bonds in the molecule of the substrate, which facilitates the chemical reaction and product formation. This property is called the active site of the effect of deformation of the substrate.

VI -12 -4 Molecular mechanisms of enzymatic catalysis

Mechanisms of enzymatic catalysis are determined by the role of the functional groups in the active center of the enzyme reaction, a chemical conversion of the substrate into the product. There are two main mechanisms of enzymatic catalysis: acid-base catalysis and covalent catalysis.

VI -12 -4 -1 Acid-base catalysis

The concept of acid-base catalysis explains enzymatic activity involving chemical reaction of acid groups (proton donors) and / or basic groups (proton acceptors). Acid-base catalysis is a frequent phenomenon. Amino acid residues belonging to the active center have functional groups that exhibit properties of both acids and bases.

Such amino acids as Cys, Tyr, Ser, Lys, Glu Asp, and His participate in the acid-base catalysis most often. Radicals of these amino acids in the protonated form are acids (proton donor), while in the deprotonated form are bases (proton acceptor). Due to this property of the functional groups enzyme active center becomes unique biological catalysts, in contrast to the non-biological catalysts, capable of exhibiting either acidic or basic properties.

VI -12 -4-2 Covalent catalysis

In covalent catalysis based on nucleophilic or electrophilic attack groups of the active center of the enzyme molecules form a covalent bond between the substrate and cofactor. The action of serine proteases such as trypsin, chymotrypsin, and thrombin - example of covalent catalysis mechanism, when covalent bond is formed between the substrate and the amino acid residue serine active site of the enzyme. The term "serine protease" is associated with the fact that the amino acid residue serine is included in the active center of these enzymes directly involved in catalysis.

Consider the mechanism of covalent catalysis by chymotrypsin example, performing the hydrolysis of peptide bonds in the digestion of proteins in the duodenum. Substrates of chymotrypsin are peptides containing amino acids with aromatic and cyclic hydrophobic radicals (Phe, Tyr, Trp), indicating that the hydrophobic forces are involved in the formation of enzyme-substrate complex.

VI -12 -4-3 Specificity of enzyme action

Enzymes have a higher specificity of action than inorganic catalysts. There exists specificity towards the type of chemical reaction catalyzed by the enzyme and specificity relative to the substrate. There are two kinds of specificity for each characteristic of the enzyme.

Specificity with respect to the substrate is enzyme's substrate preference for a particular structure in comparison with other substrates. There are 4 types of substrate specificity of enzymes:

1. Absolute specificity is the ability of the enzyme to catalyze the conversion of only one substrate. For example, glucokinase phosphorylates only glucose, arginase cleaves only the arginine, urease – urea.

2. Relative specificity is the ability of the enzyme to catalyze the conversion of several substrates having the same type of bond. For example, lipase cleaves the ester bond in any triacylglycerols.

3. Relative group specificity. The enzyme catalyzes the conversion of several substrates having one type of bond, but requires the presence of certain functional groups. For example, all proteolytic enzymes cleave the peptide bond, but pepsin breaks the bond only between aromatic amino acids. Chymotrypsin cleaves the bonds between amino acids with carboxyl groups.

4. **Stereochemical specificity** (stereospecificity) is the property of the enzyme to convert only one stereoisomer. For example, bacterial aspartate decarboxylase catalyzes the decarboxylation of L-spartate only and doesn't act on the D-aspartic acid.

VI -12 -4-4 Specificity with respect to the reaction

Each enzyme catalyzes the reaction of one or a group of the same type of reactions. Often, the same chemical compound acts as a substrate for different enzymes, each of which is specific to the catalytic reaction leading to the formation of different products. Specificity for the type of reaction is the basis of a uniform classification of enzymes.

- **Group Specificity**

Enzymes acting on a group of substances that possess a particular type of linkage common to that group of substances are said to exhibit group specificity. Amylase hydrolyses the group of substances like starch, dextrin and glycogen, which have the same type of glycosidic linkages (α. 1,4).

- **Optical Specificity**

Almost all enzymes show a high degree of optical specificity. Thus, there are certain enzymes which catalyse the hydrolysis of same group of substances possessing same optical activity i.e., D-amino acid oxidase acts on D-amino acid and L-amino acid oxidase acts on L-amino acid. Maltase catalyses the hydrolysis of a.-but not 13- glycosides.

VI -13 Mechanism of Enzyme Action

A chemical reaction such as A → P takes place because a certain fraction of the substrate possesses enough energy to attain an activated condition called the transition state. This transition state is at the top of the energy barrier separating the reactants and products. The rate of a given chemical reaction is proportional to the concentration of this transition state species. The energy of activation is the amount of energy required to bring all the molecules in 1 mole of a substance at a given temperature to the transition state. Enzymes combine transiently with the substrate to produce a transition state intermediate having a lower energy of activation than the un catalysed reaction. Thus, they accelerate chemical reactions by lowering the energy of activation. It is generally believed that the catalytic reactions occur in at least two steps:

Step 1: A molecule of enzyme (E) and a molecule of substrate(S) collide and react to form an intermediate called the enzyme-substrate complex (E5).

Step 2: The decomposition of E5 complex to give product(s) and the active enzyme

[S] + [E] ⟶ [ES] ⟶ P + [E]

The formation of an ES complex affords a lower activation energy.

Factors influencing enzyme activity

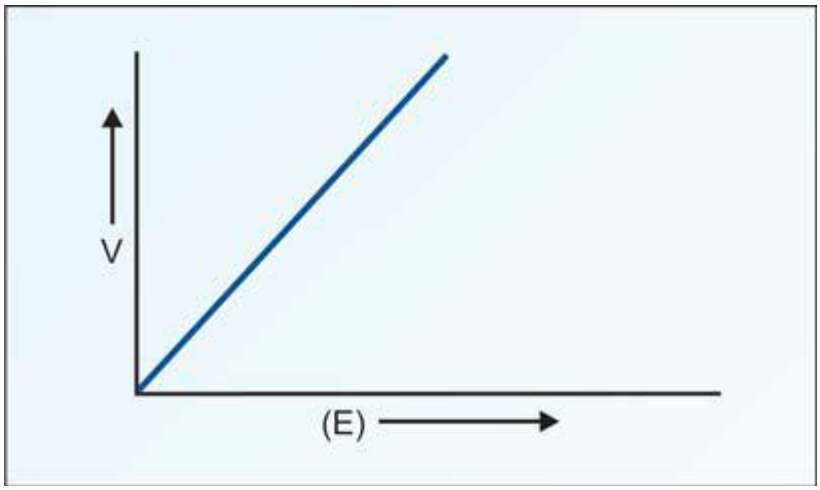
The various factors which affect enzyme activity are enumerated in Box 5.7. These are explained below.

1. Enzyme Concentration

i. Rate of a reaction or velocity (V) is directly proportional to the enzyme concentration, when sufficient substrate is present. Velocity of reaction is increased proportionately with the concentration of enzyme, provided substrate concentration is unlimited

ii. Hence, this property is made use of in determining the level of particular enzyme in plasma, serum or tissues.

iii. Known volume of serum is incubated with substrate for a fixed time, then reaction is stopped and product is quantitated (**end point method***)*. Since the product formed will be proportional to the enzyme concentration, the latter could be assayed.



2. Effect of Substrate Concentration

In a series of test tubes, equal volume of enzyme solution is taken, but increasing quantity of substrate is added and the rate of reaction is assayed in each tube. The velocity (v) is expressed in micromoles of substrate converted per minute. If the velocity is plotted against the substrate concentration, a typical curve will be obtained. As substrate concentration is increased, the velocity is also correspondingly increased in the initial phases; but the curve flattens afterwards. At lower concentrations of substrate (point A in the curve), some enzyme molecules are remaining idle. As substrate is increased, more and more enzyme molecules are working. At half-maximal velocity, 50% enzymes are attached with substrate (point B in the curve). As more substrate is added, all enzyme molecules are saturated (point C).

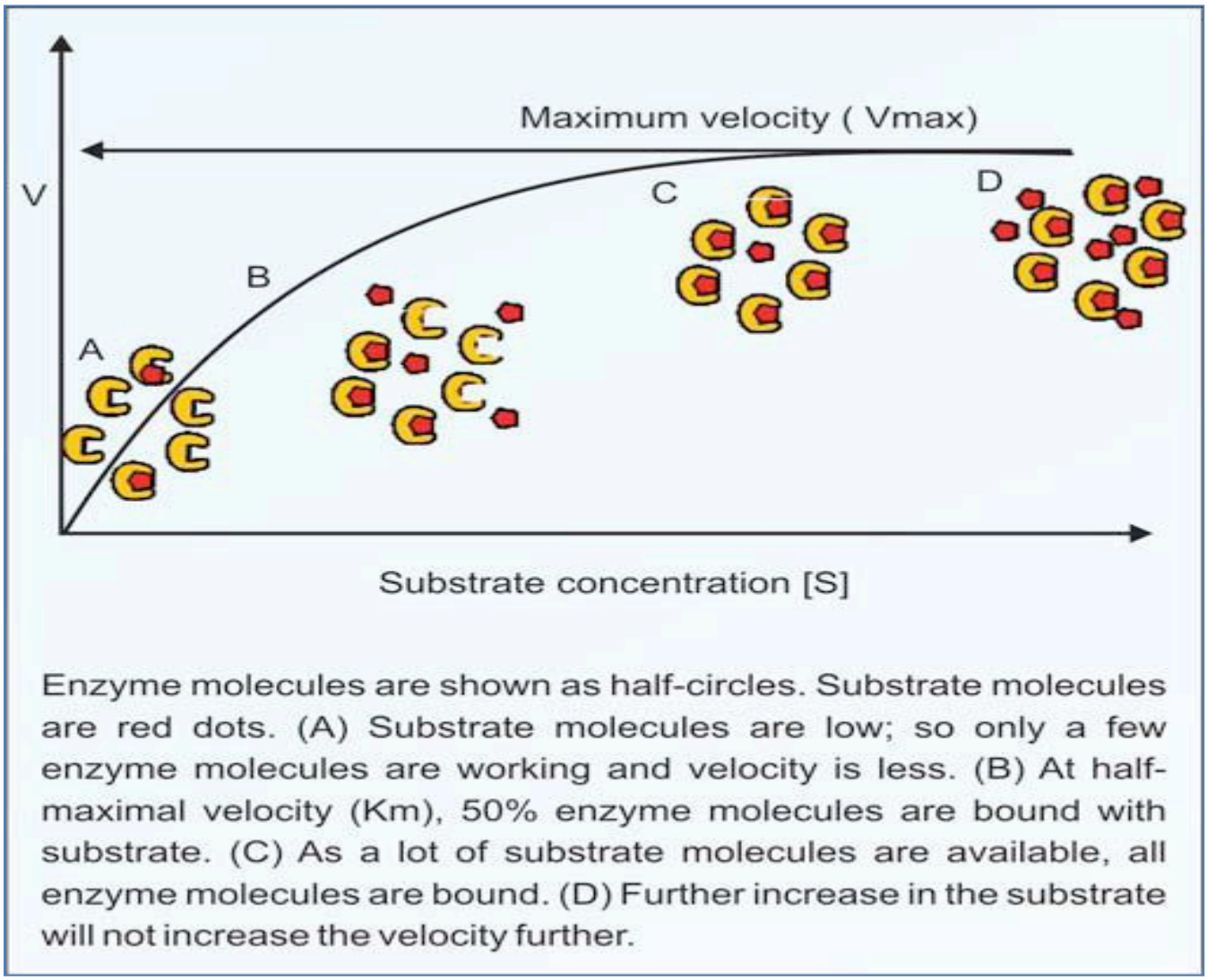


Figure 52: Effect of substrate concentration on enzyme activity

CONCLUSION

Biochemistry is the science concerned with studying the various molecules that occur in living cells and organisms and with their chemical reactions. Because life depends on biochemical reactions, biochemistry has become the basic language of all biologic sciences.

Biochemistry is concerned with the entire spectrum of life forms, from relatively simple viruses and bacteria to complex human beings. Biochemistry and medicine are intimately related. Health depends on a harmonious balance of biochemical reactions occurring in the body, and disease reflects abnormalities in biomolecules, biochemical reactions, or biochemical processes.

Biochemistry asks how the remarkable properties of living organisms arise from the thousands of different lifeless biomolecules. When these molecules are isolated and examined individually, they conform to all the physical and chemical laws that describe the behavior of inanimate matter as do all the processes occurring in living organisms. The study of biochemistry shows how the collections of inanimate molecules that constitute living organisms interact to maintain and perpetuate life animated solely by the physical and chemical laws that govern the nonliving universe. 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, principles we refer to collectively as *the molecular logic of life.* Although biochemistry provides important insights and practical applications in medicine, agriculture, nutrition, and industry, its ultimate concern is with the wonder of life itself.

Advances in biochemical knowledge have illuminated many areas of medicine. Conversely, the study of diseases has often revealed previously unsuspected aspects of biochemistry. The determination of the sequence of the human genome, nearly complete, will have a great impact on all areas of biology, including biochemistry, bioinformatics, and biotechnology. Biochemical approaches are often fundamental in illuminating the causes of diseases and in designing appropriate therapies.

In this document we have presented to our students the essential chapters of the programmed of biochemistry, hob it well be interesting to help them to understanding biochemistry very assay.

References

Ahern, K., Rajagopal, I. & Tan, T. Biochemistry: free for all. United States: Oregon StateUniversity, 2018

Berg JM, Tymoczko JL and Stryer L 2002. Biochemistry, 5th edition, W H

CBS Publishers & Distributors, Delhi.

College Publishing, New York.

D M Vasudevan; S Sreekumari; Kannan Vaidyanathan. Textbook of biochemistry for medicalstudents: New Delhi ; Philadelphia, 2016.

David W Ball, John W Hill, Rhonda J Scott. The Basics of General, Organic, and BiologicalChemistry: Saylor Academy, 2012.

dedicated to analyses of the human genome.)

Devlin TM 2001. Textbook of Biochemistry with Clinical Correlations Fifth

edition, John Wiley & Sons, Inc. Publication, New York.

Emily Irwin, "The Spermaceti Candle and the American Whaling Industry," Historia 21, 2012

Freeman & Company, England.

Fruton JS: *Proteins, Enzymes, Genes: The Interplay of Chemistry and Biology.* Yale Univ Press, 1999. (Provides the historical background for much of today's biochemical research.)

Garrett RH and Grisham CM 1999. Biochemistry. Second edition, Saunders

Garrod AE: Inborn errors of metabolism. (Croonian Lectures.) Lancet 1908;2:1, 73, 142, 214.

Gerald F. Combs. The Vitamins. Fundamental aspects in nutrition and health 3 edition: ElsevierInc. New York, 2008

Goodwin TW and Mercer El 1983. Introduction to Plant Biochemistry,

Gurr, M.I., Harwood, J.L., & Frayn; K.N. (2002) *Lipid Biochemistry: An Introduction,* 5th edn, Blackwell Science, Oxford, UK.

Hames BD and Hooper NM 2003. Instant Notes Biochemistry, Third edition,

Harborne JB and Dey PM 1997. Plant Biochemistry, First edition, Academic

Inc., USA.

International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. Nature 2001:409;860. (The issue [15 February] consists of articles

Karp G 1999. Cell and Molecular Biology, 2nd Edition, John Wiley & Sons,

Kornberg A: Basic research: The lifeline of medicine. FASEB J 1992;6:3143.

Kornberg A: Centenary of the birth of modern biochemistry. FASEB J 1997;11:1209.

L.L. Dilworth, D.K. Stennett. Plant Constituents: Pharmacognosy, Elsevier Inc. Academic Press.Jamaica, 2017.

Langin, D., Holm, C., & Lafontan, M**.** (1996) Adipocyte hormone-sensitive lipase: a major regulator of lipid metabolism. *Proc. Nutr. Soc.* **55,** 93–109.

Lehninger AL, Nelson DL and Cox MM 1993. Principles of Biochemistry,

Lehninger principles of biochemistry (4th ed.): Nelson, D., and Cox, M, W.H. Freeman and Company, New York, 2005 .

Manuel Megías Pacheco, M. Diego, P. M. García. Atlas of plant and animal histology:University of Vigo. Spain, 2017.

Muhammad Akram, Naveed Munir, Muhammad Daniyal, Chukwuebuka Egbuna, Mihnea-Alexandru Găman, Peculiar Feenna Onyekere, and Ahmed Olatunde. Vitamins and minerals:types, sources and their functions: Springer, 2020

Murray, Robert K.; Victor W Rodwell; David Bender; Kathleen M Botham; P Anthony Weil.Harper's Illustrated Biochemistry, 28th Edition, 2009

Nelson, D.L. and Cox, M.M. (2017) Lehninger Principles of Biochemistry. 7th Edition, W.H.Freeman, New York, 1328.

Press, San Diego.

Reginald H. Garrett and Charles M. Grisham. Biochemistry. 4th edition. University of Virginia.:Boston, MA, 2010

Sagar Aryal. Enzymes- Properties, Classification and Significance. Online Microbiology andBiology Study Notes, 2018. 7.https://kids.britannica.com/students/article/cell/273572

Scriver CR et al (editors): *The Metabolic and Molecular Bases of Inherited Disease,* 8th ed. McGraw-Hill, 2001.

Second edition, Pergamon Press, New York.

Venter JC et al: The Sequence of the Human Genome. Science 2001;291:1304. (The issue [16 February] contains the Celera draft version and other articles dedicated to analyses of the human genome.) Williams DL, Marks V: *Scientific Foundations of Biochemistry in Clinical Practice,* 2nd ed. Butterworth-Heinemann, 1994.

Viva Books Private Limites, New Delhi.

Wang, C.S., Hartsuck, J., & McConathy, W.J. (1992) Structure and functional properties of lipoprotein lipase. *Biochim. Biophys. Acta* 1123**,** 1–17.

1.https://lubrizolcdmo.com/technical-briefs/protein-structure/

2.https://www.khanacademy.org/science/biology/macromolecules/proteins-and-amino-acids/a/orders-of-protein-structure

3.https://www.liposomes.ca/publications/190%20Fenske%20et%20al%201995.pdf

4.https://www.nchmd.org/education/mayo-health-library/details/CON-20257401

5.https://www.scientificamerican.com/article/how-do-arctic-animals-stay-warm/

6 https://biochimia.usmf.md/sites/default/files/inline-files/Hydrosoluble-vitamines_0.pdf

APPENDIX

Table 3.4
Classes of proteins based on their biological functions

Protein	Biological Function
	Enzymes
Phosphofructokinase	An enzyme in carbohydrate metabolism that catalyzes phosphate group transfer from ATP to fructose-6-phosphate
Trypsin	A digestive enzyme in vertebrates that catalyzes protein hydrolysis
Adenylate cyclase	An enzyme that catalyzes the formation of the second messenger cyclic AMP
RNA polymerase	An enzyme present in all organisms that catalyzes DNA-directed RNA synthesis
Reverse transcriptase	An enzyme found in HIV (the virus that causes AIDS) that catalyzes RNA-directed DNA synthesis
	Structural Proteins
Collagens	Fibrous proteins found in all animals; form cable networks that serve as scaffolding for support of tissues and organs
Elastins	Fibrous proteins found in connective tissue of the lungs and in large blood vessels such as the aorta, which have rubberlike properties that allow them to stretch to several times their normal length
Keratins	Mechanically durable fibrous proteins present in vertebrates as major components of the outer epidermal layer and its appendages such as hair, nails, and feathers
	Defense (Immune) Proteins
Antibodies	Globular proteins produced by the immune system of higher animals that participate in the destruction of biological invaders
Interferons	Proteins, produced by higher animals, that interfere with viral replication
	Transport and Storage Proteins
Hemoglobin	Globular heme-containing protein that carries oxygen from the lungs to other tissues of vertebrates
Apolipoproteins	Components of lipoproteins such as low density lipoprotein (LDL) that participate in triacylglycerol and cholesterol transport
Casein	Protein found in milk that stores amino acids
Ferritin	Widely distributed protein that stores iron
Myoglobin	A heme-containing protein found in vertebrates that binds oxygen
Glucose permease	Carries glucose into erythrocytes
	Regulatory and Receptor Proteins
Lac repressor	Genetic switch that turns off bacterial genes involved in lactose catabolism
Insulin	A protein synthesized in the pancreas that acts as a signal for the fed state in higher animals
Insulin receptor	A membrane protein that binds insulin and sends a message inside the cell to regulate carbohydrate metabolism
	Muscle Contraction and Mobility Proteins
Actin	Component of skeletal muscle
Myosin	Component of skeletal muscle
Dynein	Protein that causes movement of sperm and protozoa by their flagella and cilia