Carbohydrate

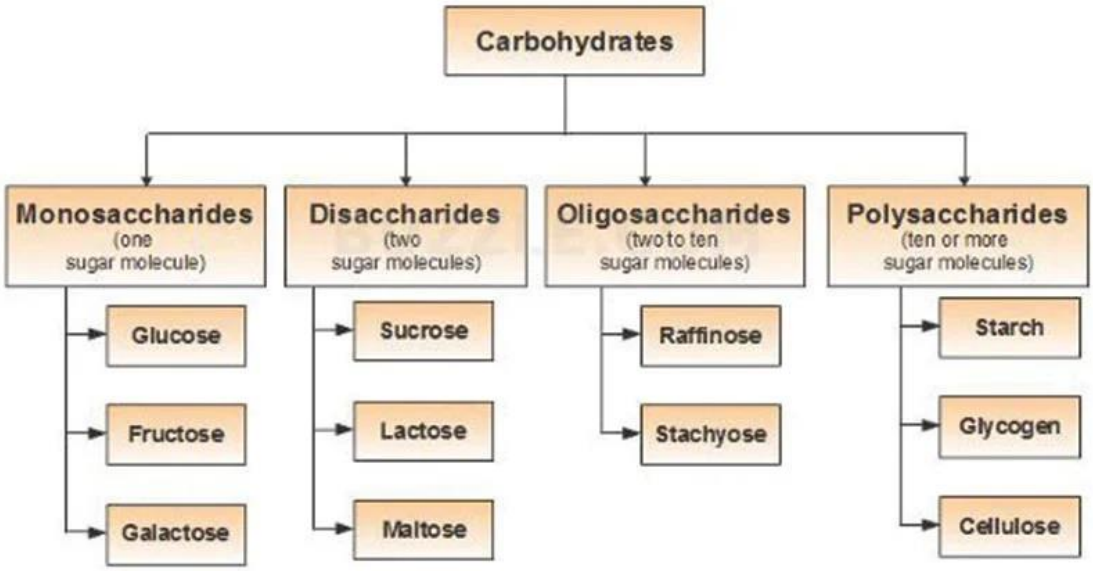
Introduction

Carbohydrates are organic compounds that contain large quantities of hydroxyl groups. It has the general formula ($C_nH_{2n}O_n$). The simplest carbohydrates also contain either an aldehyde moiety (these are termed **polyhydroxyaldehydes**) or a ketone moiety (**polyhydroxyketones**).

Importance of Carbohydrates:

1. Glucose is a major fuel of the tissues of mammals and can be stored as glycogen for energy.
2. Ribose and deoxyribose is a component of RNA and DNA, respectively.
3. Galactose is the main component of milk.
4. Many diseases associated with defects in CHO metabolism e.g. {diabetes mellitus (DM), glycogen storage diseases}.
5. Polysaccharides are structural elements in the cell wall of bacteria, plants, and exoskeleton of arthropods.
6. CHO play a key role in cell-cell recognition process.

Carbohydrate Classifications

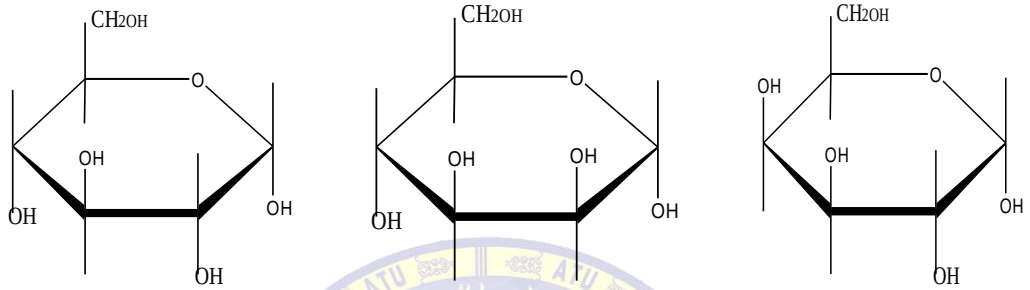


Carbohydrates can combine with lipid to form glycolipids or with protein to form glycoproteins.

I-Monosaccharides

The monosaccharides commonly found in humans are classified according to the number of carbons they contain in their backbone structures. The major monosaccharides contain four to six carbon atoms.

* The simplest carbohydrates encountered in the body are **glyceraldehyde** (aldotriose) and to the **dihydroxyacetone**(ketotriose).



α-D-Glucose **α-D-Mannose** **α-D-Galactose**

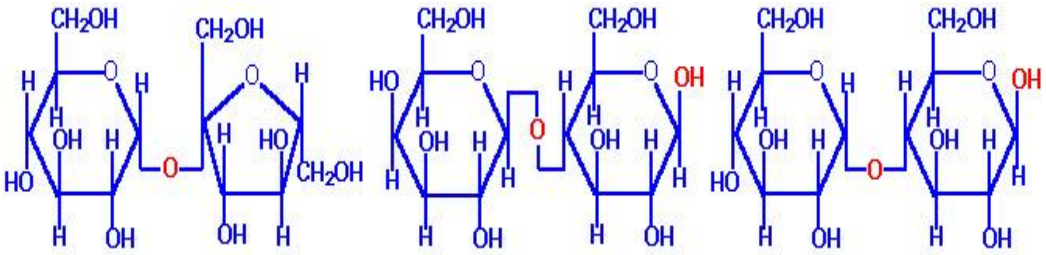
II-Disaccharides

Covalent bonds between the anomeric hydroxyl of a cyclic sugar and the hydroxyl of a second sugar (or another alcohol containing compound) are termed **glycosidic bonds**, and the resultant molecules are **glycosides**. The linkage of two monosaccharides to form disaccharides involves a glycosidic bond. Most important disaccharides are sucrose, lactose and maltose.

Sucrose: prevalent in sugar cane and sugar beets, is composed of glucose and fructose through an α (1,2) -glycosidic bond.

Lactose: is found exclusively in the milk of mammals and consists of galactose and glucose in a α -(1,4) glycosidic bond.

Maltose: the major degradation product of starch, is composed of 2 glucose monomers in an □-(1,4) glycosidic bond.



Sucrose **Lactose** **Maltose**

-Polysaccharides

Most of the carbohydrates found in nature occur in the form of high molecular weight polymers called **polysaccharides**.

The monomeric building blocks used to generate polysaccharides can be varied; in all cases, however, the predominant monosaccharide found in polysaccharides is D-glucose.

* **Homopolysaccharides:** polysaccharides are composed of a single monosaccharide building block, they are termed.

* **Heteropolysaccharides**: Polysaccharides composed of more than one type of monosaccharide are termed.

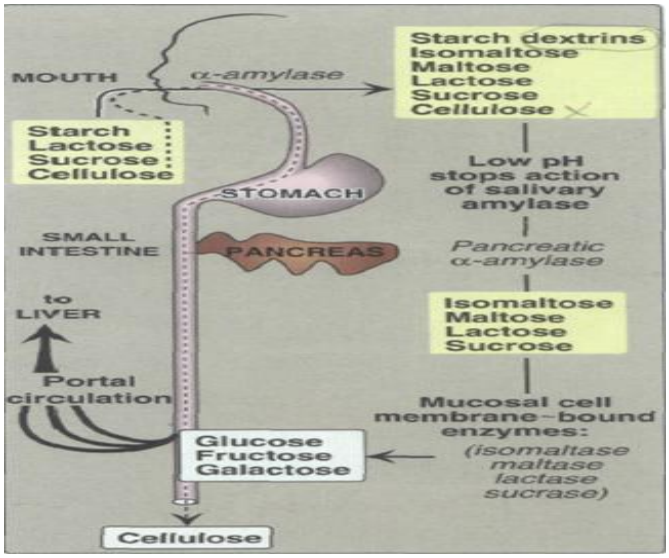
1. **Glycogen**: Glycogen is the major form of stored carbohydrate in animals. This crucial molecule is a homopolymer of glucose in α-(1,4) linkage; it is also highly branched, with □-(1,6) branch linkages occurring every 8-10 residues. Glycogen is a very compact structure that results from the coiling of the polymer chains. This compactness allows large amounts of carbon energy to be stored in a small volume, with little effect on cellular osmolarity.
2. **Starch**: Starch is the major form of stored carbohydrate in plant cells. Its structure is identical to glycogen, except for a much lower degree of branching (about every 20-30 residues). Unbranched starch (α-(1,4) linkage only) is called **amylase** (15-20%); branched starch (α-(1,4) and α-(1,6) linkages) is called **amylopectin**.
3. **Cellulose:** The main polysaccharide in plants. It is a homopolymer of glucose in β-glycosidic linkage**s.**

Definition of carbohydrate metabolism

The various biochemical processes responsible for the formation, breakdown and interconversion of carbohydrates in living organisms.

The most important carbohydrate is glucose, a simple sugar (monosaccharide) that is metabolized by nearly all known organisms. Glucose and other carbohydrates are part of a wide variety of metabolic pathways across species: plants synthesize carbohydrates from carbon dioxide and water by photosynthesis, storing the absorbed energy internally, often in the form of starch or lipids. Plant components are consumed by animals and fungi, and used as fuel for cellular respiration. Oxidation of one gram of carbohydrate yields approximately 4 kcal of energy, while the oxidation of one gram of lipids yields about 9 kcal. Energy obtained from metabolism (e.g., oxidation of glucose) is usually stored temporarily within cells in the form of ATP. Organisms capable of aerobic respiration metabolize glucose and oxygen to release energy with carbon dioxide and water as byproducts.

Carbohydrates can be chemically divided into two types: simple and complex. Simple carbohydrates consist of single or double sugar units (monosaccharides and disaccharides, respectively). Sucrose or table sugar (a disaccharide) is a common example of a simple carbohydrate. Complex carbohydrates contain three or more sugar units linked in a chain, with most containing hundreds to thousands of sugar units. They are digested by enzymes to release the simple sugars. Starch, for example, is a polymer of glucose units and is typically broken down to glucose.



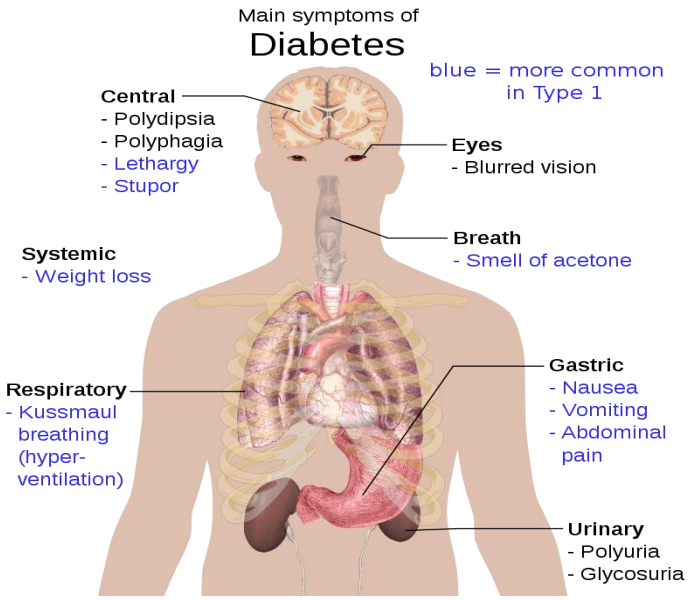
Diabetes mellitus

Diabetes mellitus refers to a group of diseases that affect how the body uses blood sugar (glucose). Glucose is an important source of energy for the cells that make up the muscles and tissues. It's also the brain's main source of fuel.

The main cause of diabetes varies by type. But no matter what type of diabetes you have, it can lead to excess sugar in the blood. Too much sugar in the blood can lead to serious health problems.

Chronic diabetes conditions include type 1 diabetes and type 2 diabetes. Potentially reversible diabetes conditions include **prediabetes and gestational diabetes. Prediabetes happens when blood sugar levels are higher than normal**. But the blood sugar levels aren't high enough to be called diabetes. And prediabetes can lead to diabetes unless steps are taken to prevent it. **Gestational diabetes** happens during pregnancy. But it may go away after the baby is born.

Type 2 diabetes is a condition that happens because of a problem in the way the body regulates and uses sugar as a fuel. Eventually, high blood sugar levels can lead to disorders of the circulatory, nervous and immune systems.



In type 2 diabetes, there are primarily two problems. The pancreas does not produce enough insulin — a hormone that regulates the movement of sugar into the cells. And cells respond poorly to insulin and take in less sugar.

Type 2 diabetes used to be known as adult-onset diabetes, but both type 1 and type 2 diabetes can begin during childhood and adulthood. Type 2 is more common in older adults. But the increase in the number of children with obesity has led to more cases of type 2 diabetes in younger people.There's no cure for type 2 diabetes. Losing weight, eating well and exercising can help manage the disease. If diet and exercise aren't enough to control blood sugar, diabetes medications or insulin therapy may be recommended.

Type 1 diabetes, once known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition. In this condition, **the pancreas makes little or no insulin**. **Insulin is a hormone the body uses to allow sugar (glucose) to enter cells to produce energy.**Different factors, such as genetics and some viruses, may cause type 1 diabetes. Although this type usually appears during childhood or adolescence, it can develop in adults.Even after a lot of research, type 1 diabetes has no cure. Treatment is directed toward managing the amount of sugar in the blood using insulin, diet and lifestyle to prevent complications.

Case	Fasting		Random	
	Mmol/l	**Mg/dl**	Mmol/l	**Mg/dl**
Normal	< 5.5	**< 99**	< 7.8	**< 140**
Prediabetes (IFG)	6-6.9	**108 -124**	< 7.8	< 140
DM	>7.0	**>126**	>11.1	**>199**
GDM	7.8	**140**	11.1	**200**

Glycosaminoglycans (GAGs)

Glycosaminoglycans, often called **GAGs**, are special carbohydrates found in the extracellular matrix. They are important for the strength, flexibility, and hydration of tissues. The structure of GAGs consists of:

- Long, unbranched polysaccharides.
- Made of repeating disaccharide units.
- Often contain sulfate groups, giving them a negative charge.
- Their negative charge attracts water, forming gels.

Types of GAGs

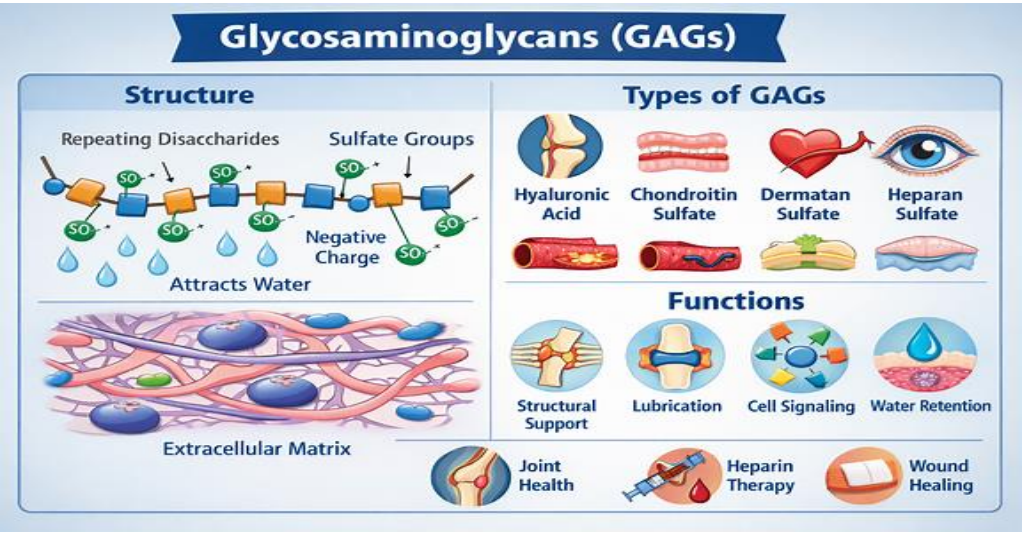
- **Hyaluronic acid** – found in skin and joints, helps with lubrication.
- **Chondroitin sulfate** – important in cartilage, provides resistance to compression.
- **Dermatan sulfate** – found in skin, blood vessels, and heart valves.
- **Heparan sulfate** – plays a role in cell signaling and blood clot regulation.
- **Keratan sulfate** – found in cornea, cartilage, and bone.

Functions

- Structural support: strength and elasticity of tissues.
- Lubrication: smooth movement of joints.
- Cell communication: regulation of growth and repair.
- Water retention: keeps tissues hydrated.

Clinical Importance

- Changes in GAGs are linked to diseases such as osteoarthritis.
- Some GAGs, like heparin, are used as medicines (blood thinners).
- Important in wound healing and tissue engineering.



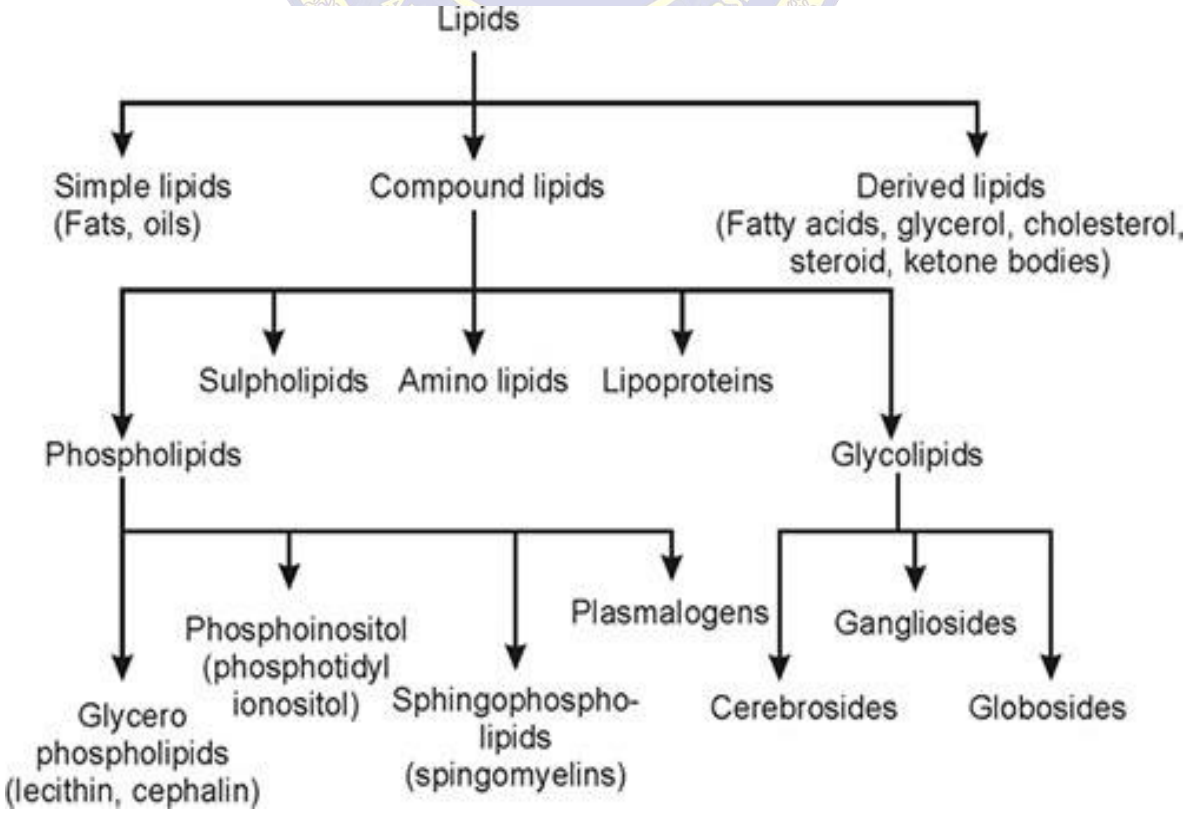
LIPIDS

Lipids: - are defined as compounds which are relatively insoluble in water, but freely soluble in non-polar organic solvent like benzene, chloroform, ether, hot alcohol, acetone, etc.

- **Functions of lipids:-**

1- Storage form of energy (triglycerides) firstly because of their high energy content. The calorific value is 9 Kcal\gm secondly they are storage in concentrated form in water in the tissues compared to carbohydrates which are highly hydrated and can not be stored in such concentrated form.
2- Structural components of biomembranes (phospholipids and cholesterol).
3- As protective coating on the surface of many organs such as kidney, against injury.
4- Facilitation the absorption of the fat soluble vitamins A, D, E and K.
5- Providing insulation against changes in external temperature (subcutaneous fat).
6- Metabolic regulators (steroid hormone and prostaglandins).
7- As transport forms of various metabolic fuel.
8- Acting as electric insulator in neurons.

- **Classification of lipids: -** we can classify lipid depending on the chemical nature.

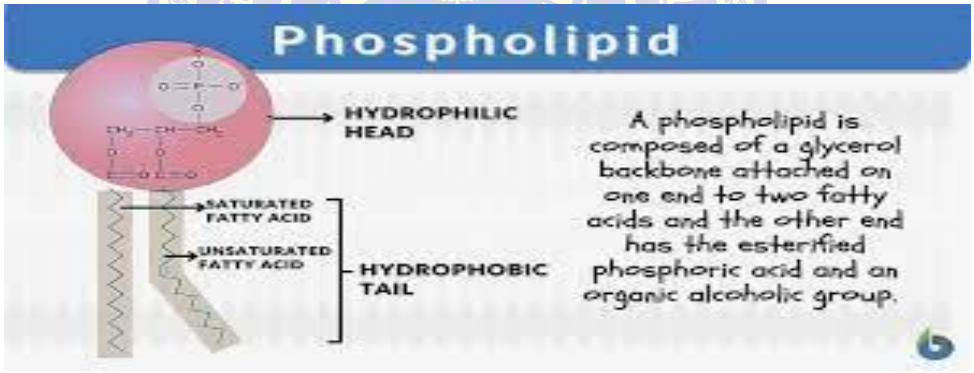


Fatty acids

- **Fatty acids:-** are the major unit of lipids. They are generally found in ester linkage in different glasses of lipids.
- Fatty acids are represented as general formula R─COOH.
- Numbers of carbon atoms of fatty acids are even or odd number, but odd number fatty acids are very rare.
- Fatty acids may be saturated or unsaturated.
- Fatty acids that have double bonds are said to be *unsaturated*. If they have more than one double bond but no more than four they are commonly referred to as polyunsaturated fatty acids.
- Fatty acids that contain only single bond are called *saturated fatty acids*. For example palmitic and stearic acids.

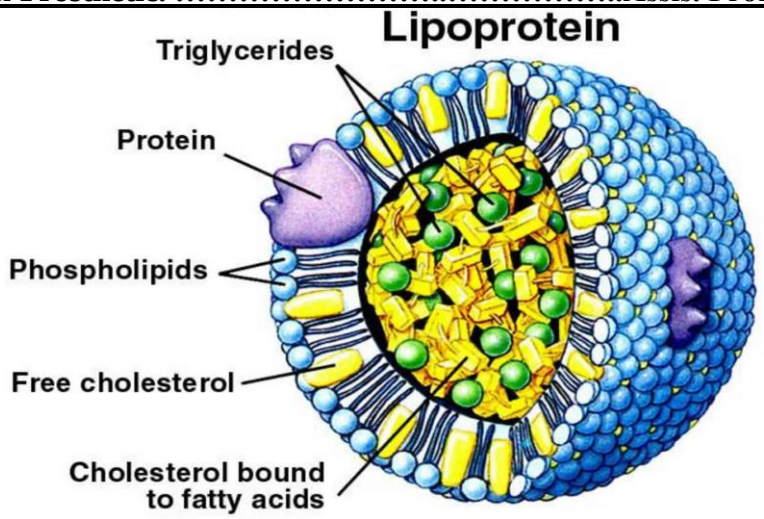
Compound lipids

1. **Phospholipids:-** They are also known as phosphatides. They are present in all plant and animal cells and are the primary components of cell membranes.



Phospholipids are unique and versatile molecules. They are of natural occurrence and the main components in cellular membranes. Arranged as a lipid bilayer, phospholipids play a significant role in the structure and functionality of biological membranes. They are amphiphilic and consist of a hydrophilic head group and a lipophilic/hydrophobic tail.

2. **Lipoproteins:** Cholesterol and triglycerides are insoluble in water and therefore these lipids must be transported in association with proteins. Lipoproteins are complex particles with a central core containing **cholesterol esters** and **triglycerides** surrounded by free **cholesterol**, **phospholipids**, and **apolipoproteins**, which facilitate lipoprotein formation and function.



There are four main types of Lipoproteins

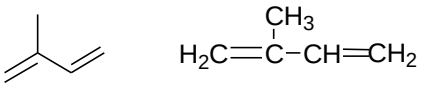
- **High-density lipoprotein (HDL)** is the "good cholesterol." It carries cholesterol back to your liver to be flushed out of your body. High levels of HDL reduce your risk of cardiovascular (heart) disease.
- **Low-density lipoprotein (LDL)** is the "bad cholesterol." It increases your risk of coronary artery disease, heart attacks and stroke. LDL carries **cholesterol** that accumulates as plaque inside blood vessels. Plaque buildup can make blood vessels too narrow for blood to flow freely. This condition is atherosclerosis.
- **Very low-density lipoproteins (VLDL)** are another type of "bad cholesterol." VLDLs carry triglycerides — and to a lesser degree, cholesterol — to your tissues.
- **Chylomicrons** are very large particles that also transport triglycerides.

3. Glycolipids:- this group of lipids do not contain phosphoric acid; instead they contain carbohydrates usually glucose or galactose bonded by glycosidic linkage.

Derived lipids

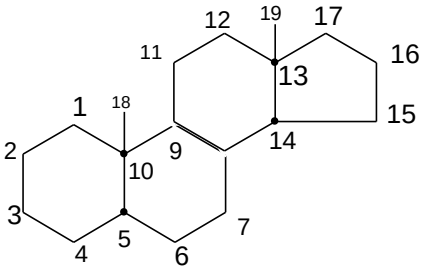
❖ **Derived lipids** are substances which are derived from other lipids by hydrolysis. These include fatty acids of various series, steroids, bile acids and substances associated with lipids in nature such as carotenes, vitamin A, D, E and K.

1. **Terpenes:-** they are linear or cyclic compounds formed by condensation of two or more isoprene units. Terpenoid compounds include, tocopherol (vitamin E), Coenzyme Q(ubiquinone), Vitamin K (a napthaquinon), carotenes, squalene etc.



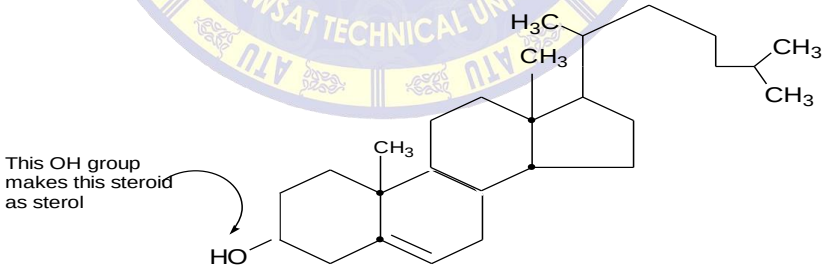
Isoprene unit

2. **Steroids:-** They are considered lipids because they are soluble only in organic solvents. They are derivatives of cyclopentano-perhydro-phenanthrene ring (consists of four fused rings). Steroids are steroidal alcohol.



Cyclopentano-perhydro-phenanathrene nucleus
(Steroid nucleus)

A. *Cholesterol* \ It is the most common steroid, found in large concentration in the brain and spinal cord. Cholesterol is found only in animal cells, although plants have their own steroids called "phytosterols". Cholesterol, as amphipathic lipid is an important component of membranes and starting material, or precursor, of all steroids of animal origin such as bile acids, vitamins, hormones and miscellaneous group of steroids. When present in excess it is deposited in the arteries as a component of a lipid- type **plaque** and in the gallbladder as **gallstone**. Some people suffer from hypercholesterolemia, an abnormally level of cholesterol in the body. Those people are more likely to develop cardiovascular disease, especially heart attacks. There are two sources of cholesterol. One is cholesterol from the diet. Secondly, it is synthesized from sugar in the body.



Cholesterol

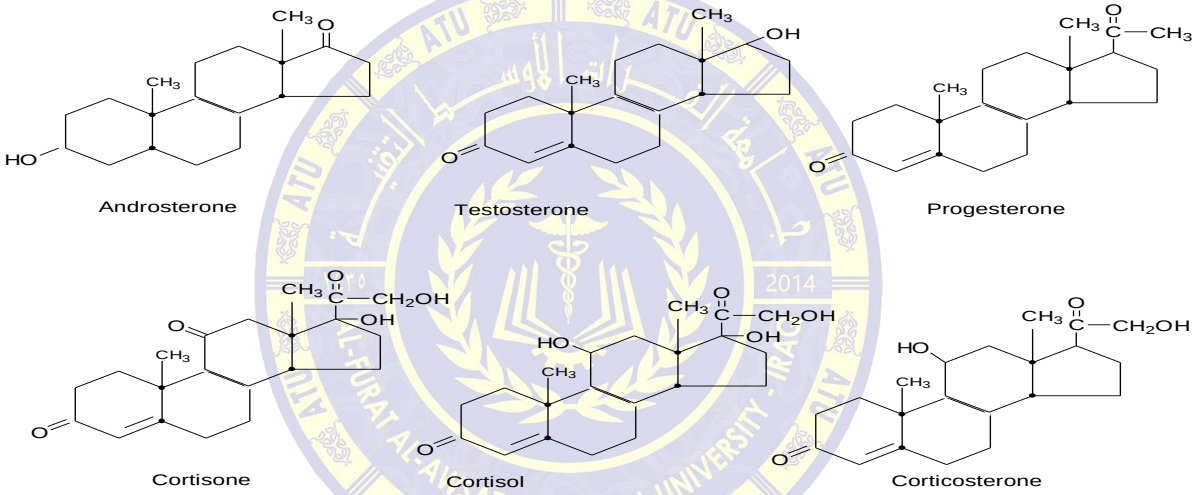
Function of Cholesterol

1. **Cell structure**: Cholesterol is a major component of **cell membranes**, giving them stability and flexibility.
2. **Hormone production**: It serves as a building block for **sex hormones** (estrogen, testosterone, progesterone) and **cortisol**.
3. **Vitamin D synthesis**: Cholesterol is converted into vitamin D when skin is exposed to sunlight.
4. **Bile formation**: The liver uses cholesterol to produce **bile acids**, which help digest and absorb dietary fats.

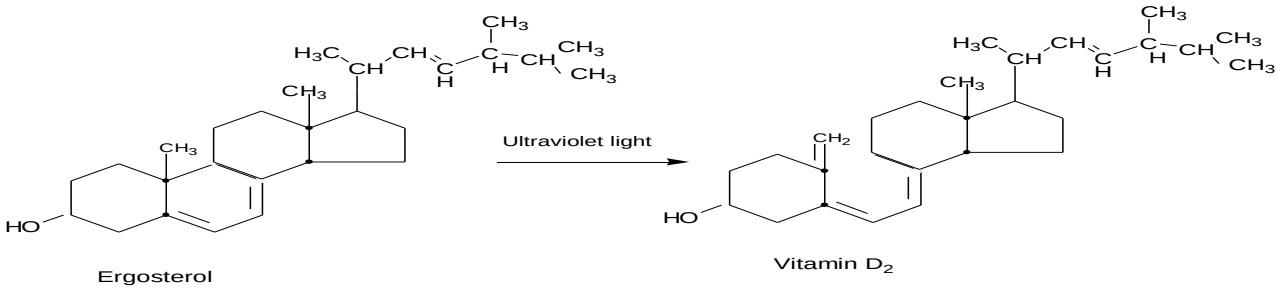
5. **Nerve function**: Cholesterol is part of the **myelin sheath**, which insulates nerve cells and supports signal transmission.

Bile acids are simply steroids with an acid group. Fat are insoluble in water. In order for fat to be transported through the walls of the intestine and absorbed, they must be emulsified. Bile acids secreted as components of bile serve this function. Although cholic acid is the most common bile acid, other bile acids contain amino acids.

B. *Steroid hormones*\ the most important group of steroids hormones are the sex hormones. Male sex hormones are called androgens; the two most important are *androsterone* and *testosterone.* Female hormones include *progesterone* and *estrogen*. Also (adrenal gland) cortisone, cortisol, aldosterone and corticosterone are steroids hormones.



C. *The fat soluble vitamins* \ The structure of many of the fat soluble vitamins does not include a sterol nucleus. For example, vitamin D_2, produced by the action of sunlight on the skin, has an open benzene ring in its structure and thus is not a true steroid. However, the compound ergosterol, which reacts with ultraviolet light to produce vitamin D_2, is a true steroid. Vitamin A, E and K are the other fat soluble vitamins.



Amino acids , Peptides & Proteins

Although more than 300 different amino acids have been described in nature, only 20 α-amino acids that are relevant to the make-up of mammalian proteins. Several other α-amino acids are found in the body free or in combined states (i.e. not associated with peptides or protein). These non-proteinamino acids perform specialized functions. E.g. γ-aminobutric acid (GABA): involved in neurotransmission of nerve pulses and β-alanine: part of structure of coenzyme A.

Several of the α-amino acids found in proteins also serve functions distinct from the formation of peptides and proteins: e.g. tyrosine in the formation of thyroid hormones and other example is glutamate acting as a neurotransmitter.

The amino acids in peptides and proteins (excluding proline) consist of a carboxylic acid (-COOH) and an amino ($-NH_2$) functional group attached to the same tetrahedral carbon atom. This carbon is theα-carbon. Distinct R-group, that distinguishes one amino acid from another, also are attached to the alpha-carbon (except in the case of glycine where the R-group is hydrogen).

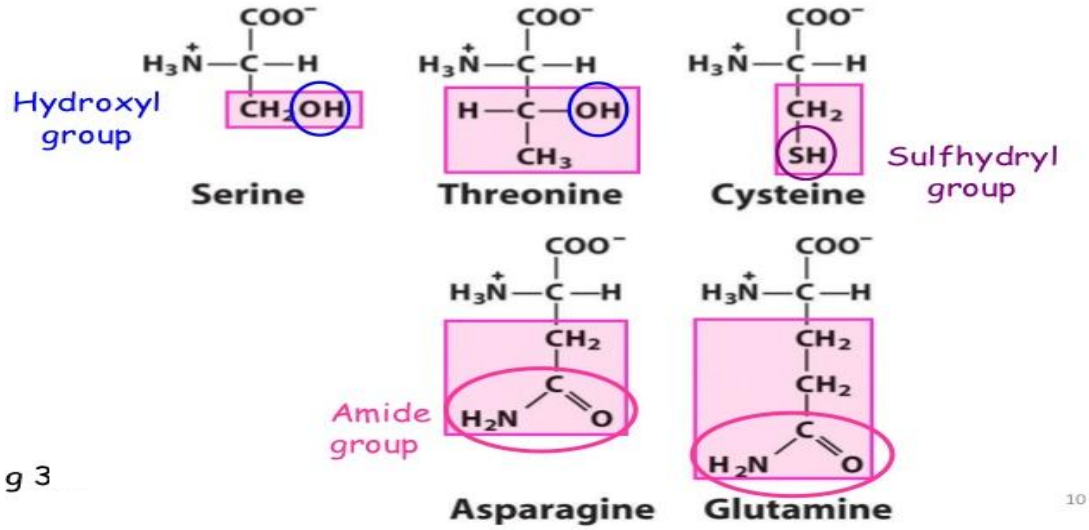
Amino acids are colorless, odorless, and melt with decomposition at temperature more than 200˚C. In aqueous solution amino acids exist predominantly in the form of zwitterion.

Classification of Amino Acid

Amino acids can be classified into four general groups based on the properties of the "R" group in each amino acid. Amino acids can be polar, non-polar, positively charge or negatively charged

1) **Polar Amino Acids**. Polar amino acids have "R" groups that are hydrophilic, meaning that they seek contact with aqueous solutions. The polarity of serine and threonins is contributed by their hydroxyl groups; that of cysteine by its sulphydryl (-SH); and that of asparagine and glutamine by their amide group.

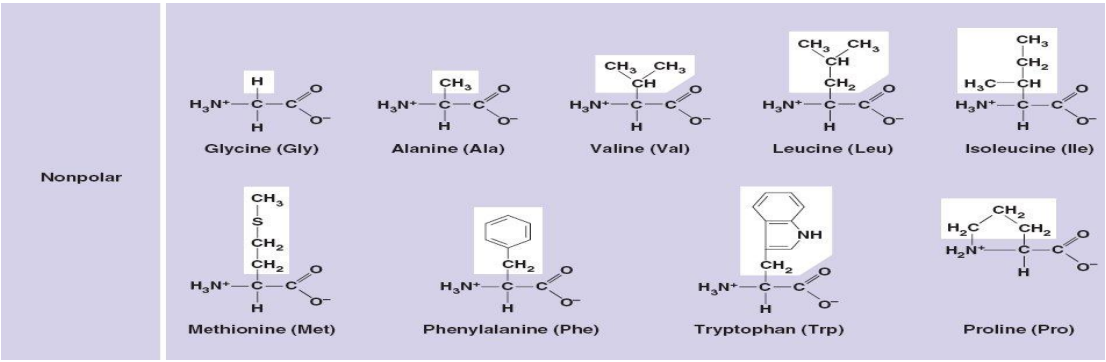
- **Ser:** Serine **Thr:** Threonine **Cys:** Cysteine **Asn:** Asparagine **Gln:** Glutamine



2) **Nonpolar amino acids** are the opposite (hydrophobic) in that they avoid contact with liquid. These interactions play a major role in protein folding and give proteins their 3-D structure. The nonpolar amino acids are hydrophobic.

Nonpolar Amino Acids

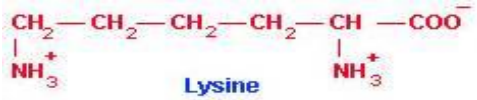
- **Ala:** Alanine **Gly:** Glycine **Ile:** Isoleucine **Leu:** Leucine
- **Met:** Methionine **Trp:** Tryptophan **Phe:** Phenylalanine **Pro:** Proline
- **Val**: Valine



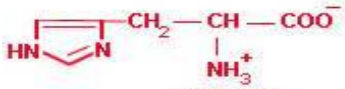
3) **Positively Charged (basic) Amino Acids**

The most hydrophilic R groups are those that are either positively ($-NH^{+}_{3}$) or negatively ($-COO^{-}$) charged. The amino acids in which the R groups have significant positive charges at pH7.0 are :

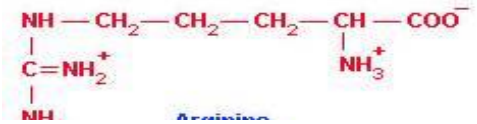
His: Histidine **Lys:** Lysine **Arg:** Arginine



Lysine



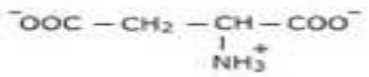
Histidine



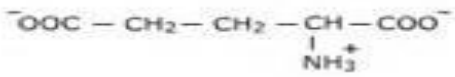
4) Negatively Charged (Acidic) Amino Acids

The two amino acids having R groups with a net negative charge at pH 7.0 are

- **Asp:** Aspartic acid **Glu:** Glutamic acid



Aspartic acid



Glutamic acid

Essential and non-essential amino acid

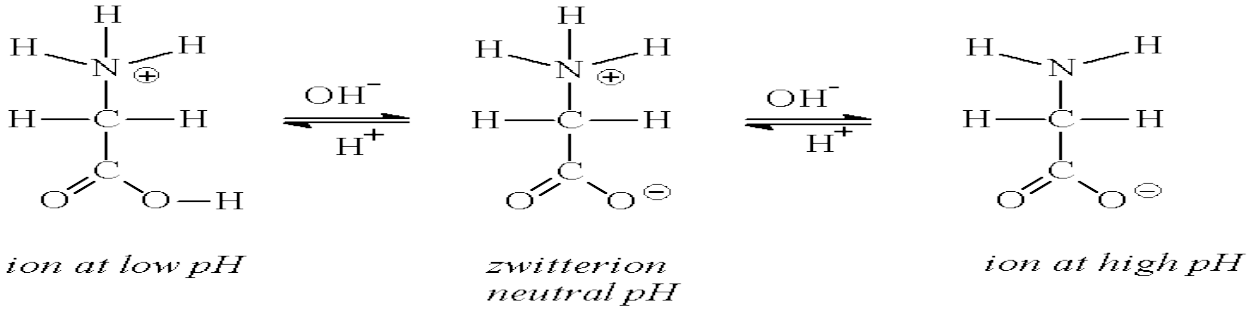
While amino acids are necessary for life, not all of them can be produced naturally in the body. Of the 20 amino acids, 10 can be produced naturally. These amino acids are alanine, proline, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, serine, glycine, and tyrosine. The amino acids that can not be produced naturally are called ***essential amino acids***. They are arginine (essential for children), histidine, threonine, isoleucine, methionine, leucine, lysine, phenylalanine, tryptophan, and valine. The essential amino acids must be acquired through diet. Unlike humans, plants are capable of synthesizing all 20 amino acids.

"**Non-essential" amino acids** are also required in order to make proteins. However, most organisms can synthesize these compounds, and therefore do not require dietary sources of these amino acids. In general, the synthesis pathways for the essential amino acids are complex, and involve a large number of reactions. Non-essential amino acids are at least as important as essential amino acids. In fact, they are so important

that animals have retained the enzyme pathways necessary to synthesize these compounds. "Nutritionally non-essential" is therefore a better term.

Acid-Base Properties of the Amino Acids

The α-COOH and α-NH_2 groups in amino acids are capable of ionizing (as are the acidic and basic R-groups of the amino acids). At physiological pH (around 7.4) the carboxyl group will be unprotonated and the amino group will be protonated. An amino acid with no ionizable R-group would be electrically neutral at this pH. This species is termed a **zwitterion**.



ion at low pH *zwitterion neutral pH* *ion at high pH*

Like typical organic acids, the acidic strength of the carboxyl, amino and ionizable R-groups in amino acids can be defined by the association constant, K_a or more commonly the negative logrithm of K_a, the pK_a. The **net charge** (the algebraic sum of all the charged groups present) of any amino acid, peptide or protein, will depend upon the pH of the surrounding aqueous environment. As the pH of a solution of an amino acid or protein changes so too does the net charge. This phenomenon can be observed during the titration of any amino acid or protein. When the net charge of an amino acid or protein is zero the pH will be equivalent to the **isoelectric point: pI**.

Functional Significance of Amino Acid R-Groups:

1. The imidazole ring of histidine is frequently found in the reactive center of enzymes.
2. The ability of histidines in hemoglobin to buffer the H^+ ions from carbonic acid ionization in red blood cells. It is this property of hemoglobin that allows it to exchange O_2 and CO_2 at the tissues or lungs, respectively.

3. The primary alcohol of serine and threonine as well as the thiol (-SH) of cysteine allow these amino acids to act as nucleophiles during enzymatic catalysis.
4. The thiol of cysteine is able to form a disulfide bond with other cysteines:

Cysteine-SH + HS-Cysteine <--------> Cysteine-S-S-Cysteine

(This simple disulfide is identified as cystine)

5. Disulfide bonding between cysteines in different polypeptide chains of oligomericproteins plays a crucial role in ordering the structure of complex proteins, e.g. the insulin receptor.

Q/ Why metal cations such as (Pb^{+2} and Hg^{+2}) are toxic to most living organisms?

A/ The metal cations can react with (-SH) group in cystine within peptides structure to produce insoluble mercaptides, or can react with a carboxyl group of other amino acids to form insoluble salts and cause the protein to precipitate out of solution

The Peptide Bond

The simplest peptide, a **dipeptide**, contains a single peptide bond formed by the condensation of the carboxyl group of one amino acid with the amino group of the second with the concomitant elimination of water. Peptide bond formation is a condensation reaction leading to the polymerization of amino acids into peptides and proteins. Peptides are small consisting of few amino acids.

Importance of Peptides:

1. A number of hormones (e.g. Insulin) and some neurotransmitters are peptides.
2. Several antibiotics (e.g. Gramacidin and Valinomycin) are peptides.
3. Some antitumor agents are peptides (e.g.Bleomycin).

- The presence of the carbonyl group in a peptide bond allows electron resonance stabilization to occur such that the peptide bond exhibits rigidity not unlike the typical -C=C- double bond. The peptide bond is, therefore, said to have **partial double-bond character**.
- Poly peptide chain is unbrabched chain, rigid, and planer.

- We started from amino group (N-terminal residue) from the left if we write the sequence of a.a. of P.P.Ch.

Naming peptides

With the exception of the C terminal amino acid, the names of all the other amino acids in a peptides end with *yl*. For example, a tripeptide consisting of alanine at the N terminal, glycine, and serine at the C terminal is named as one word; alanylglycylserine. For convenience, the order of amino acids in the peptide is often written as the sequence of three-letter abbreviations.

Proteins

Proteins are complex organic nitrogenous substances consist of C, N, O, H with high molecular weight and consist of α-amino acids(NH_2, COOH) joined by peptide linkages found in animal and plant tissues. Proteins make up to 12% of the protoplasm. They are not only responsible for comprising the structure of the cell but are concerned with every function of the cell such as respiration, catalysis of reactions by enzymes, transport of materials, regulation of metabolism and defence action.The sources of proteins are obtained from animal and plant. The animal sources of proteins include milk, egg, meat, fish, liver etc. plant sources of proteins are pulses, nuts and cereals.

2.1 Classification of proteins

Even though there is no universally accepted classification system, proteins may be classified on the basis of their composition, solubility, shape, biological function, and on their three dimensionl structure.

2.1.1**Composition:** In term of structure, proteins can also be classified as:

1- **Simple proteins**: Yield only amino acids when they are hydrolyzed such as Albumins(egg, serum) and Albuminoids (keratins, Collagen, Elastin).
2- **Conjugated proteins**:Yield amino acids and additional products when hydrolyzed. The main conjugated protein are:

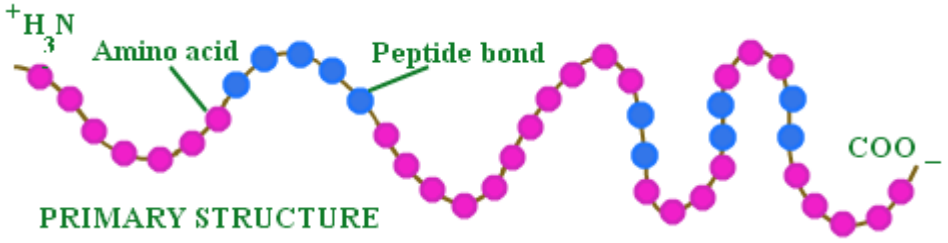
a) Nucleoprotein (a protein containing Nuclei acid)
b) Lipoprotein (a protein containing lipids)
c) Phosphoprotein (a protein containing phosphorous)
d) Metalloprotein (a Protein containing metal ion of Fe^{+2})
e) Glycoprotein (a protein containing carbohydrate)

2.1.2 **On their level of organization:**

Proteins can divided to four types according to their shapes: primary, secondary, tertiary, and quaternary.

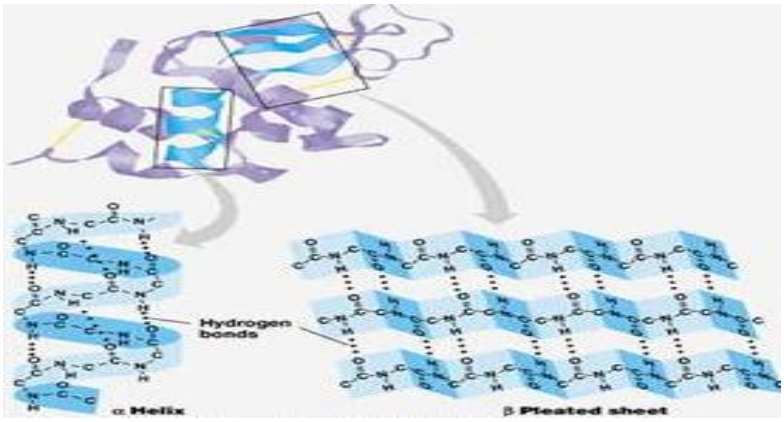
a) Primary structure of proteins

The primary structure is the linear sequence of amino acids. The primary structure of a protein is reported starting from the amino-terminal (N) end to the carboxyl-terminal (C) end.



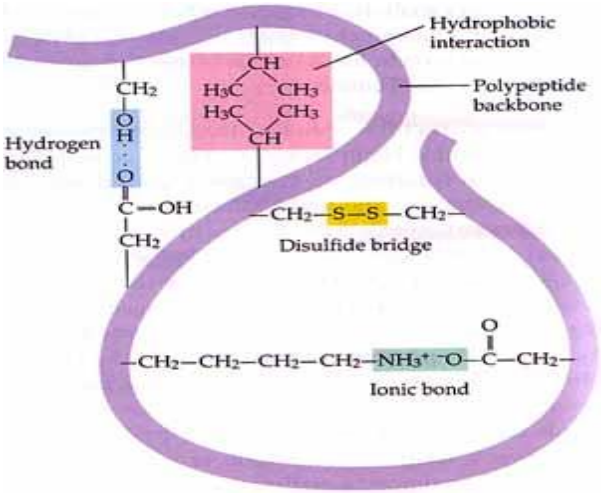
b) Secondary structure of proteins

Protein secondary structure is the three dimensional form of *local segments* of proteins. Secondary structure is formally defined by the pattern of hydrogen bonds between the amine hydrogen and carbonyl oxygen atoms in the peptide backbone. The two most common secondary structural elements are alpha helices and beta sheets.



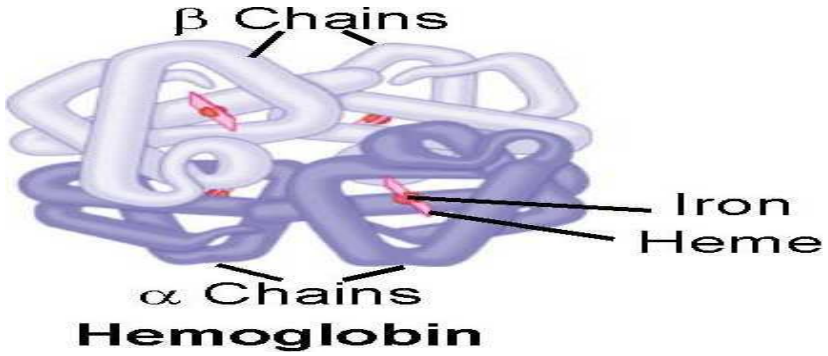
c) Tertiary structure proteins

The three dimensional, folded and biologically active conformation of a protein is referred to as tertiary structure. The structure reflects to overall shape of the molecule. The three-dimensional tertiary structure of a protein is stabilized by interactions between side. Chain functional group, covalent, disulfide bonds, hydrogen bonds, salt bridges, and hydrophobic interactions.



d) Quaternary structure of proteins

Many proteins are made up of multiple polypeptide chains, often referred to as *protein subunits*. These subunits may be the same (as in a homodimer) or different (as in a heterodimer). The ***quaternary structure*** refers to how these protein subunits interact with each other and arrange themselves to form a larger aggregate protein complex. The final shape of the protein complex is once again stabilized by various interactions, including hydrogen-bonding, disulfide-bridges and salt bridges.



2.1.3 Functions of proteins

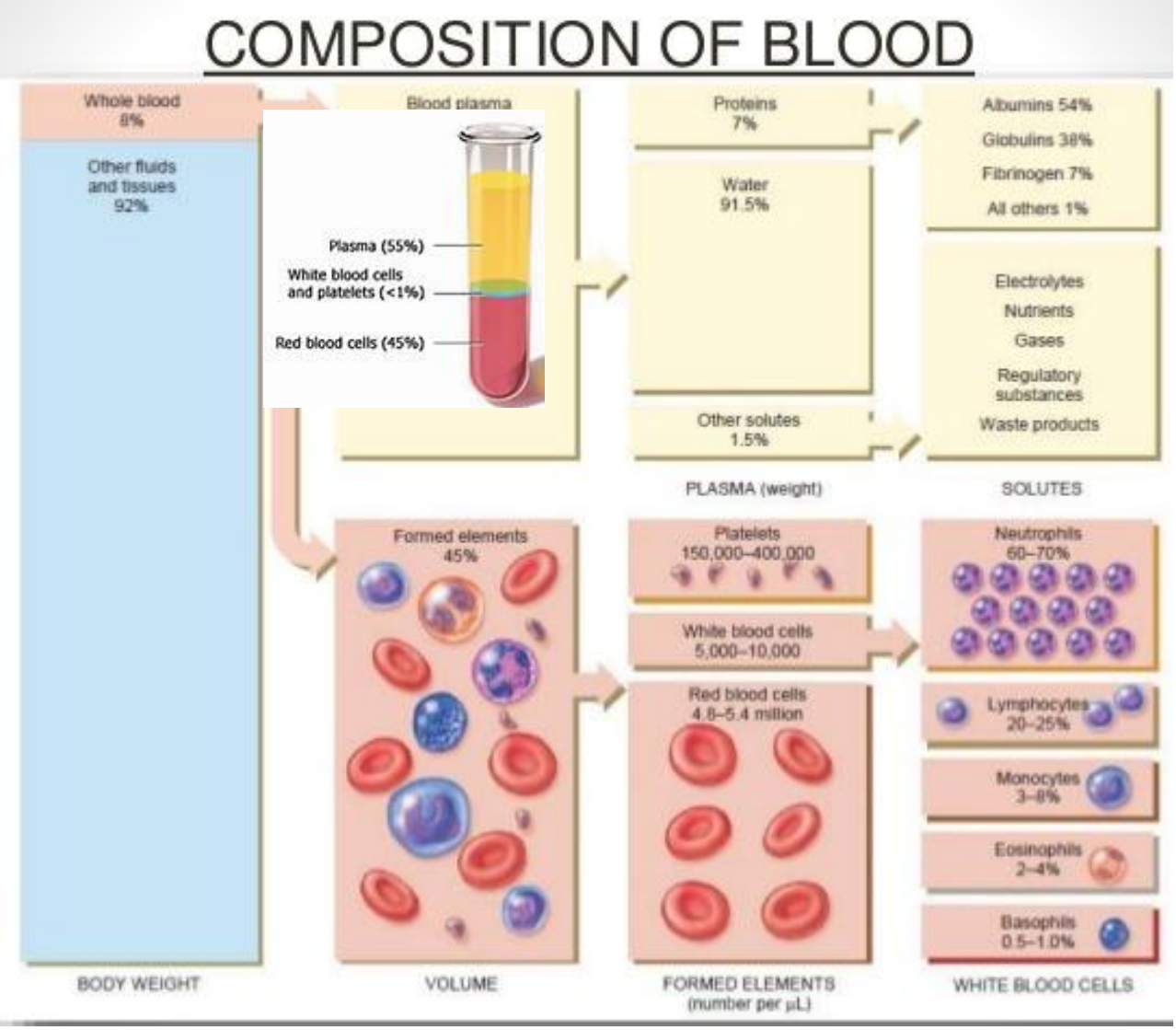
Class of Protein	Function in thebo	Examples
1) Structural	Provide structural	llagen is in tendons and cartilage components. Keratin isin hair, skin, wool, and nails
2) Contractile	Move muscles.	Myosin and Actin contract muscle fibers

3) Transport	Carry essential substances	Hemoglobin transports oxygen. throughot the body. Lipoproteins transport lipids
4) Storage	Store nutrien	Casein stores protein in milk. Ferritin stores iron in the spleen and liver.
5) Hormone	Regulate body metabolism	Insulin regulates blood glucose level. and nervous system. Growthhormone regulates body growth
6) Enzyme	Catalyze biochemic reactions in the cells.	Sucrasecatalyses the hydrolysis of sucrose.Trypsin catalyses the hydrolysis of proteins.
7) Protection	Recognize and destroy foreign substances	Immunoglobulins stimulate immune responses.

Serum Protein Components

Serum proteins (also blood or plasma proteins) are proteins present in blood that serve many different functions, including:

Transport of lipids, hormones, vitamins and minerals in the circulatory system.
The regulation of a cellular activity.
Functioning of the immune system.
Other blood proteins act as enzymes, complement components, protease inhibitors or kinin precursor.

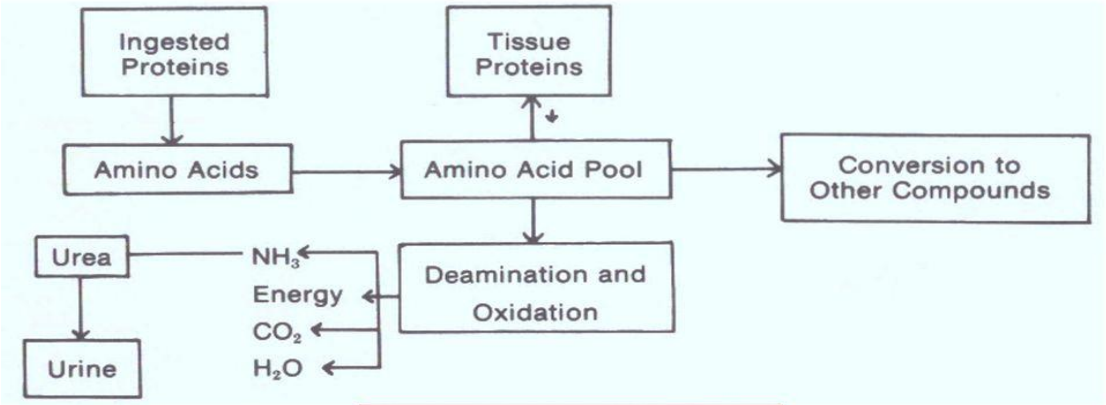


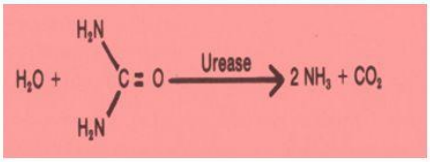
Although serum proteins have very high concentration, they exhibit an uneven distribution in terms of composition. That is, only about 22 proteins account for 99% of all the serum proteins. These include serum albumin, globulins and fibrinogen. The remainder 1% of blood proteins is composed of low abundance circulatory

proteins as well as proteins secreted by live, apoptotic and necrotic cells. Most of blood proteins are secreted by the liver and intestines except for the gamma globulins, synthesized by the immune system.

Overview of main serum protein components, function and relative abundance.

Fraction	Protein Type	Function	Abundance
Serum and Plasma	Albumin	• Prevention of blood vessel leakiness. • Blood carrier (transporter) of insoluble molecules. • Tissue growth and healing.	55%
Serum and Plasma	Alpha-1 globulin Fraction	• Contains high-density lipoprotein (HDL) known as "good" cholesterol.	38%
	Alpha-2 globulin Fraction	• Contains haptoglobin that binds hemoglobin and prevents loss of iron.	
	Beta globulin Fraction	• Carry substances, such as iron, through the bloodstream and help fight infection.	
	Gamma globulin Fraction	• Antibodies. Prevent and fight infection.	
Plasma	Fibrinogen	• Blood coagulation.	7%
Plasma	Clotting Factors	• Conversion of fibrinogen into fibrin	<1%
Serum and Plasma	Regulatory proteins	• Regulation of gene expression and other functions.	<1%





A) **Illustration of the fate of ingested protein**
B) **The reaction that occurs when urea is split by urease**

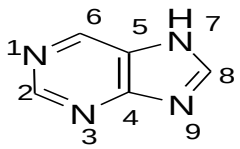
DNA and RNA

Ribonucleoside and deoxyribonucleoside phosphates (nucleotides) are essential for all cell. Without them, neither DNA nor RNA can be produced and, therefore, proteins cannot be synthesized or cells proliferate.

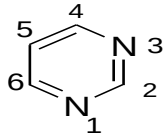
Nucleotides play an important role as "energy currency" in the cell. Finally, nucleotides are important regulatory compounds for many of the pathways of intermediary metabolism, inhibiting or activating key enzymes. The purine and pyrmidine bases found in nucleotides can be synthesized de novo, or can be obtained through salvage pathways that allow the reuse of the performed bases resulting from normal cell turnover or from the diet.

Nucleic acids are colorless, complex, amorphous compounds made up of 3 components:

a. Nitrogenous base, (a purine or a pyrimidine)
b. Pentose sugar, either ribose or deoxyribose
c. Phosphate group esterified to the sugar



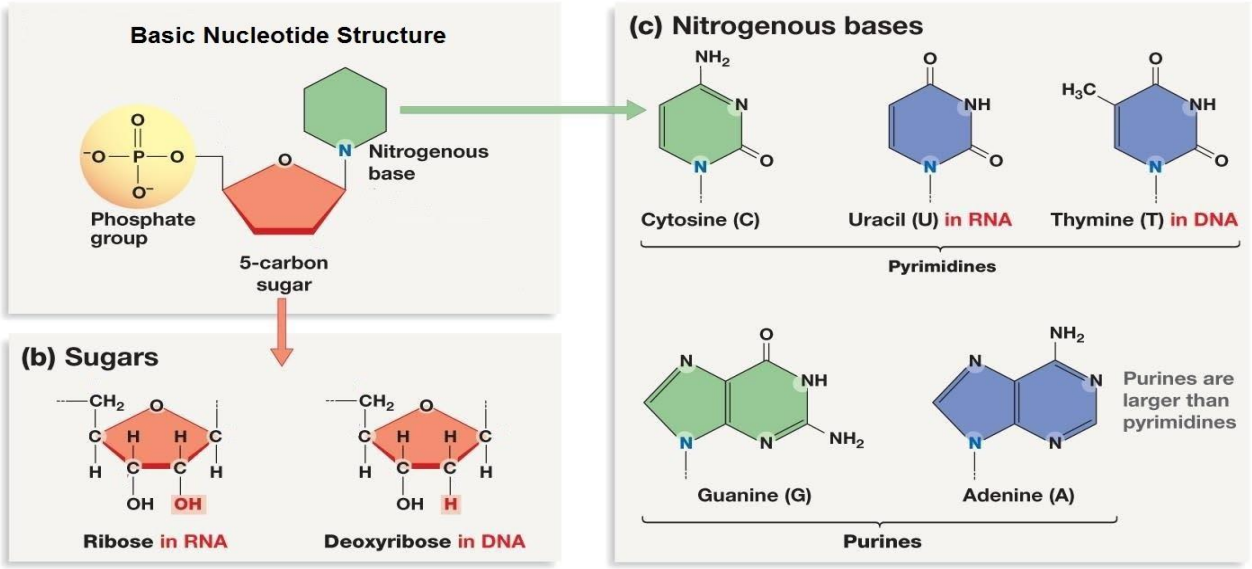
purine ring



pyrimidine ring

- Purine bases include: Guanine and Adenine
- Pyrimidine bases include : Cytosine, Uracil, and Thymine

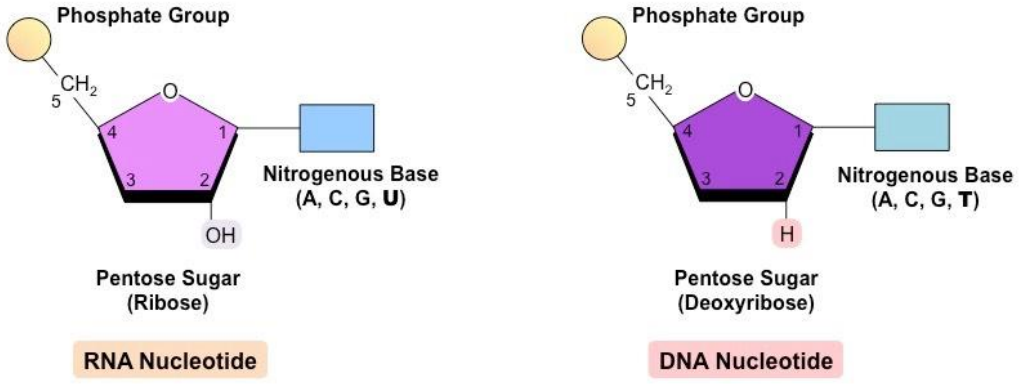
Both DNA and RNA contain the same purine bases: adenine (A) and guanine (G). Both DNA and RNA contain the pyrimidine cytosine (C), but they differ in their second pyrmidine base: DNA contais thymine (T), whereas RNA contains uracil (U). T and U differ by only one methyl group, which is present on T but absent in U (FIG below).



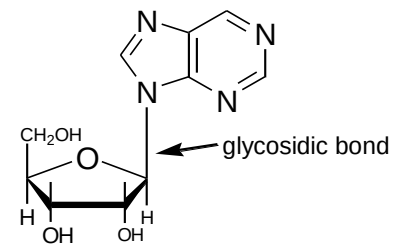
Nucliec acids

Two types of nucleic acids are present in all mammalian cells. They are DNA- deoxy ribonucleic acids and RNA- ribonucleic acid. DNA is present in the nucleus and mitochondria. RNA is present in the nucleus, ribosome and cytoplasm.

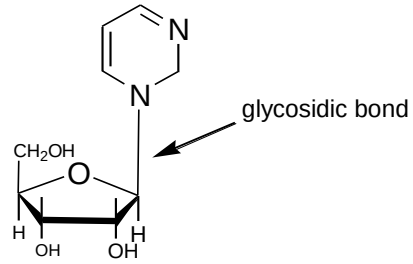
Nucleic acids are acidic substances containing nitrogenous bases, pentose sugar and phosphoric acid; both DNA and RNA are polynucleotides. They are polymers of mononucleotides. In nucleic acids, nucleotides are joined together by phosphodiester linkages.



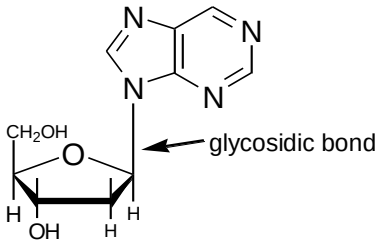
Nucleoside: When a base combines with a pentose sugar, by a beta-N-glycosidic bond, a nucleoside is formed. When the nucleoside is esterified to a phosphate group, it is called a nucleotide or nucleoside mono-phosphate.



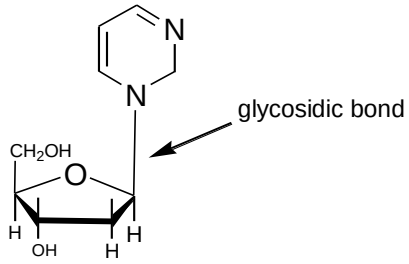
Purine nucleoside



Pyrimidine nucleoside



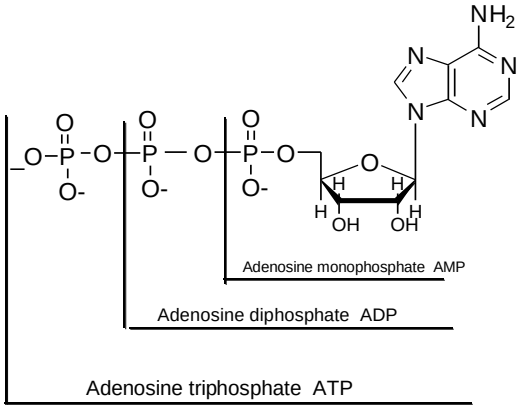
Purine deoxynucleoside



Pyrimidine deoxynucleoside

Adenine + Ribose sugar ⟶ Adenosine
Guanine + Ribose sugar ⟶ Guanosine
Uracil + Ribose sugar ⟶ Uridine
Cytosine + Ribose sugar ⟶ Cytidine
Adenine + Doxy ribose sugar ⟶ d-Adenosine
Guanine + Doxy ribose sugar ⟶ d-Guanosine
Cytosine + Doxy ribose sugar ⟶ d-Cytidine
Thymine + Doxy ribose sugar ⟶ d-Thymidine

❖ **Nucleoside triphosphate**:- corresponding nucleoside di- and tri- phosphates are formed by esterification of phosphate groups existing ones.



❖ **Replication of DNA:-** during cell division, each daughter cell gets an exact copy of the genetic information of the mother cell. This process of copying the DNA is known as DNA replication. In the daughter cell, one strand is derived from the mother cell; while

the other strand is newly synthesized. This is called semi-conservative type of DNA replication.

1. Each strand serves as a template or mould, over which a new complementary strand is synthesized.
2. The base pairing rule is always maintained. The new strand is joined to the old strand by hydrogen bonds between base pairs (A with T and G with C).
3. Polymerization of the new strand of DNA is taking place from 5' to 3' direction. This means that the template is read in the 3' to 5' direction.
4. Thus two double strands are produced. One double strand goes to one daughter nuclei, and the other to the second daughter nuclei. But each daughter cell gets only one strand of the parent DNA molecule. Old DNA strand is not degraded, but is conserved for the daughter cell, hence this is semi-conservative synthesis.
5. DNA polymerase synthesizes a new complementary strand of DNA, by incorporating dNMP sequentially in 5' to 3' direction, making use of single stranded DNA as template.

Functions of DNA

1- DNA is the genetic material of living organisms. It is the greatest super chip ever made by man.

2- DNA contain all the information required for the information of an individual organism.

3- The genetic information in DNA is converted to characterstic features of living organisms like color of the skin and eye, height, intelligence, ability to metabolize particular substance, ability to withstand stress, susceptibility to disease and ability to produce or synthesize certain substance.

4- DNA is the source of information for the synthesis of all cellular proteins. The segment of DNA that contain information for a protein is known as gene.

5- DNA is transmitted from parents to off springs and hence transmits genetic information from one generation to another.

6- The amount of DNA in any given species or cell is constant and is not affected by nutritional and metabolic states.

RNA structure

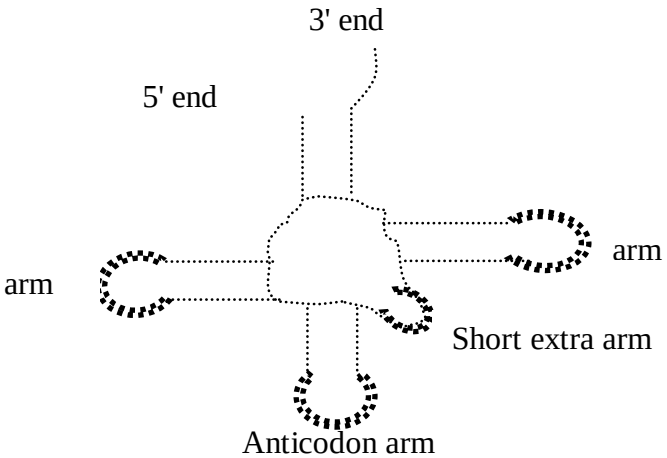
❖ RNA is polymer of nucleoside monophosphates. Cellular RNAs are of 5 types: (1)messengerRNA (mRNA) (2) heterogeneous nuclear or heteronuclear RNA (hnRNA) (3)transfer RNA (tRNA) (4) ribosomal RNA(rRNA) and (5)small nuclear RNA (snRNA).

❖ The differences between RNA and DNA are:-

RNA	DNA
1. Mainly seen in cytoplasm	Mostly inside nucleus
2. Usually 100-5000 bases	Million of base pairs
3. Generally single stranded	Double stranded
4. Sugar is ribose	Sugar is deoxyribose
5. Purines: adenine,guanine, pyrimidines: cytosine, uracil	Adenine, guanine,cytosine, thymine
6. Guanine content is not equal to cytosine and adinine is not equal to uracil.	Guanine is equal to cytosine and adenine is equal to thymine.
7. Easily destroyed by alkali	Alkali resistant

❖ **Messenger RNA (mRNA)**: it acts as a messenger of the information in the gene in DNA to the protein synthesizing machinery in cytoplasm. It carries the message to be translated to protein. The template strand of DNA is transcribed into a single stranded mRNA. Half-life of mammalian mRNA is a few hours; but some have only few minutes. In cytoplasm, mRNA is degraded by ribonucleases.

❖ **Transfer RNA (tRNA):-** they transfer amino acids from cytoplasm to the ribosomal protein synthesizing machinery; hence the names transfer RNA. The transfer RNAs show extensive internal base pairing and acquire clover leaf like structure.



The specificity of tRNA a residues in the anticodon site, which has base sequences complementary to that of mRNA codon. For example, if the mRNA has codon with the sequence UUU, the anticodon sequence of the tRNA will be AAA, by which it base pairs with mRNA codon. In this case, the UUU codon is translated as phenylalanine. Recognition of codon by the tRNA anticodon may be illustrated as follows:

Codon in mRNA 5' A-U-G 3'

Anticodon in tRNA 3' U-A-C 5'

- **Ribosomal RNA(rRNA):-** Ribosomes carry ribosomal RNA. Each ribosome contains a small number of specific RNAs. Ribosomes both aid in the interaction between tRNA and mRNA and also prove the enzyme, peptidyl transferase that catalyzes the peptide bond formation. The mammalian ribosome has a sedmination velocity of 80S unit. It has a larger 60S subunit and another smaller 40S subunit. They contain different rRNA and specific proteins.

Functions of RNA

- Facilitate the translation of DNA into proteins.
- Functions as an adapter molecule in protein synthesis.
- Serves as a messenger between the DNA and the ribosomes.
- They are the carrier of genetic information in all living cells.
- Promotes the ribosomes to choose the right amino acid which is required in building up of new proteins in the body.

Enzymes

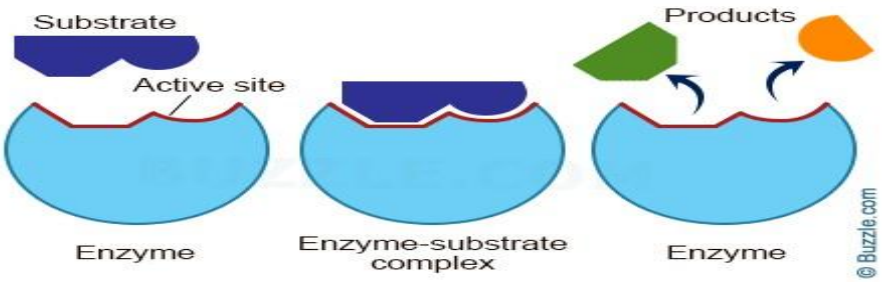
Enzymes are biocatalysts that increase the rate of biological reactions. They are produced by living organism and are usually present in only very small amounts in various cells.The entire enzyme are proteins where it catalyzed and enhance the rate of biochemical reactions occurring in various vital processes like breathing, digestion, pumping of heart, formation of body tissues, contractions of muscles, transport of ions across the plasma membranes etc. So without enzymes there is no life**.**

Enzymes are involved in **metabolic pathways &** many regulatorymechanisms that allow the metabolism to adapt to changing conditions.

- Enzyme molecules contain a special pocket or cleft called the active site. The active site contains amino acid side chains that create a three- dimensional surface complementary to substrate. The active site binds the substrate, forming an enzyme-substrate (ES) complex. ES is converted to enzyme-product complex (EP), which subsequently dissociated to enzyme and product.
- The substances on which the enzymes act are called as "***substrate***". Enzymes are highly specific in their action (i.e) an enzyme can act on a single or a small group of closely related substrate. During catalytic action, the enzymes do not undergo any permanent modification and regenerated at the end of the reaction. The general enzyme catalyzed reaction take place as per the equation.

E + S---------- ES

ES ---------- E + P



For example

α-amylase acts on starch and produce maltose units. In this reaction α-amylase is the enzyme, starch is the substrate and maltose is the product.

α –amylase (E) +starch(S)---------- α-amylase- (starch)(ES)

α -amylase-starch(ES)---------- α-amylase (E)+ maltose(P)

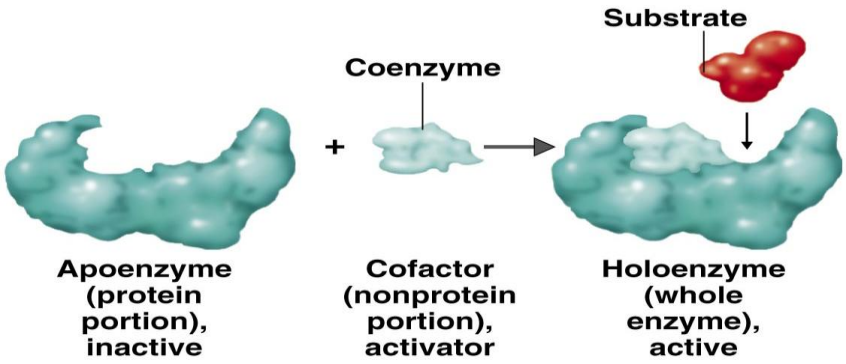
Chemical nature of enzyme

All the enzymes are proteins;some enzymes consist of only amino acids. Many enzymes are conjugated proteins and their molecules consist of **amino acids** and a **non-protein part** (not made up of amino acids). The non-protein part of the enzyme is known as **coenzyme** or prosthetic group without which the enzyme is inactive. The protein part of the enzyme (in conjugated type) is known as the **apoenzyme**. The coenzyme and the apoenzyme complex is called as **holoenzyme**.

Coenzyme + Apoenzyme---------- Holoenzyme

Holoenzyme = Apoenzyme + Cofactors

= protein part + nonprotein part



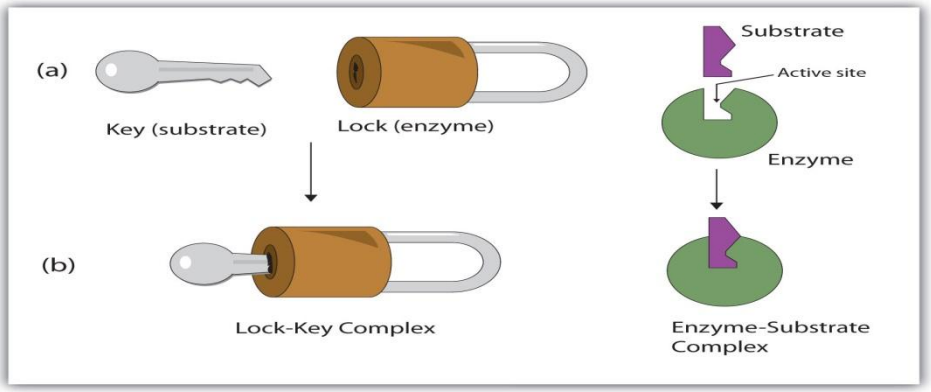
- **Enzyme activity**: is expressed as micromoles of substrate converted to product per minute under specified assay conditions. One standard unit (or International Unit) of enzyme activity (U) is that activity which catalyses transformation of 1 micromole of substrate per minute.
- **specific activity** : it is the number of enzyme units per mg of protein.

Q/ What are mechanisms for the binding between activesite of an enzyme & substrate?

There are two main theories for the explanation of binding between active site of an enzyme & substrate.

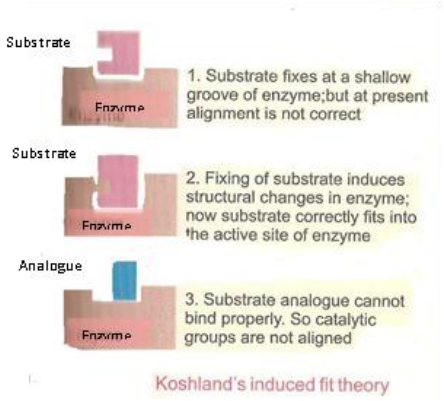
1- **Lock and key theory:**

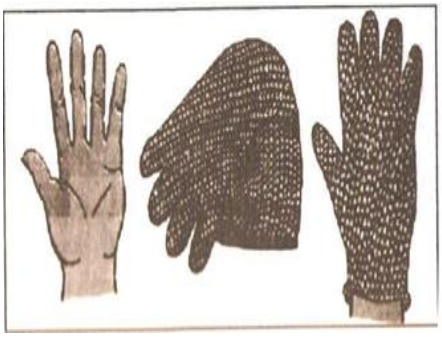
To fit the interaction between substrate & enzyme, the active sitesof enzymes are rigid in its shape where no change will happen before and after a chemicalreaction



2- **Koshland'sInduced fit theory (Hand and gloves)**

The active site of the enzyme changing to be complementary to that of the substrate only after the substrate is bound. The active site in this type will change the shape to allow enzyme and substrate to bind.



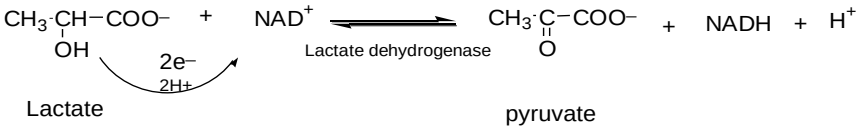


Classification of enzymes:-

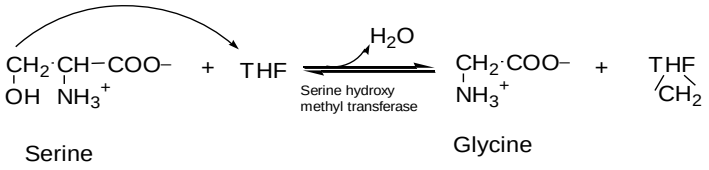
More than 2000 different enzymes are currently known.
Enzymes are classified according to the type of reaction they catalyze (six types). All enzymes have traditional names (e.g. trypsin, &chemotrypsin) or use the

Enzyme Catalogue with a four-digit Enzyme Commission number (**EC number**). The first digit indicates membership of one of the six **major classes**. The next two indicate subclasses and subsubclasses. The last digit indicates where the enzyme belongs in the subsubclass.

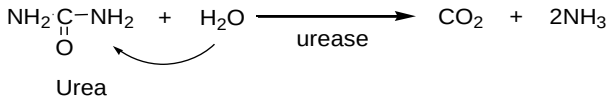
1.Oxidoreductase catalyze oxidation reduction reaction such as :



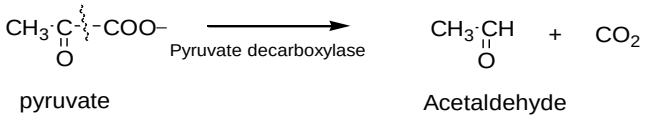
2.Transferase catalize transfer of C-, N-,or P- contaning groups, such as:



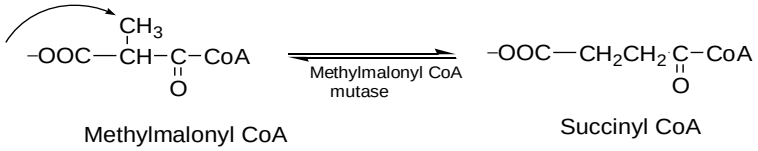
3.Hydrolase catalyze cleavage of bonds by addition of water, such as:



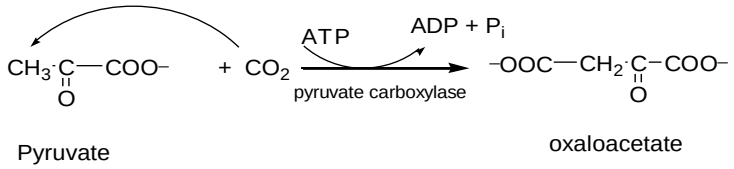
4.Lysase catalyze cleavage of C-C, C-S and certain C-N bonds, such as:



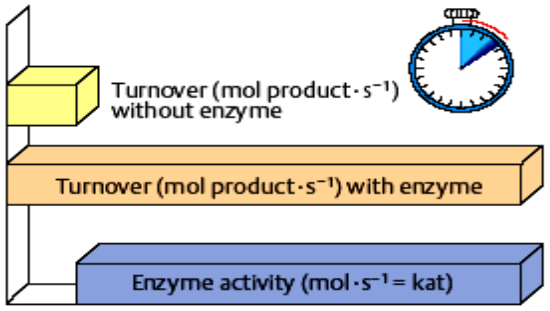
5.Isomerase catalyze racemization of optical or geometric isomers, such as:



6.Ligase catalyze formation of bonds between carbon and O,S,N coupled to hydrolysis of high energy phosphates, such as:

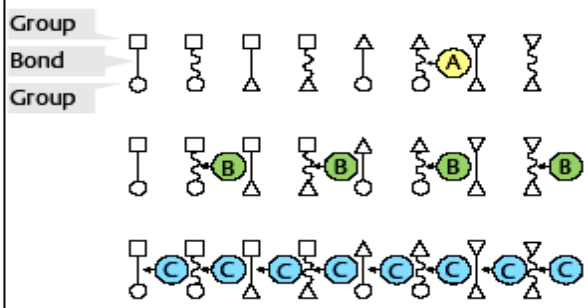


A. Enzymatic activity



1 Katal (kat): Amount of enzyme which increases turnover by 1 mol · s^{-1}

B. Reaction and substrate specificity



	Reaction specificity	Substrate specificity
A	High	High
B	High	Low
C	Low	Low

C. The enzyme classes

Class	Reaction type	Important subclasses
1 Oxidoreductases	○ = Reduction equivalent $A_{red} + B_{ox} \rightleftharpoons A_{ox} + B_{red}$	Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases	$A–B + C \rightleftharpoons A + B–C$	C_1-Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases	$A–B + H_2O \rightleftharpoons A–H + B–OH$	Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")	$A + B \rightleftharpoons A–B$	C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases	$A \rightleftharpoons$ Iso-A	Epimerases *cis trans* Isomerases Intramolecular transferases
6 Ligases ("synthetases")	$A + B + XTP \rightleftharpoons A–B + XDP + P$ X = A, G, U, C	C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

A system of *classification* has been developed that takes into account both their:1-*reaction specificity*and their 2-*substrate specificity*. Enzymes with similar reaction specificities are grouped into each of the six major classes.

Most commonly used enzyme names have the suffix "-ase" attached to the substrate of the reaction (for example, glucosidase, urease, sucrase) or a description of the action performed (for example, lactate dehydrogenase and adenylcyclase).[Note some enzymes retain their original trivial names, which give no hint of the associated enzymic reaction, for example, trypsin and pepsin.

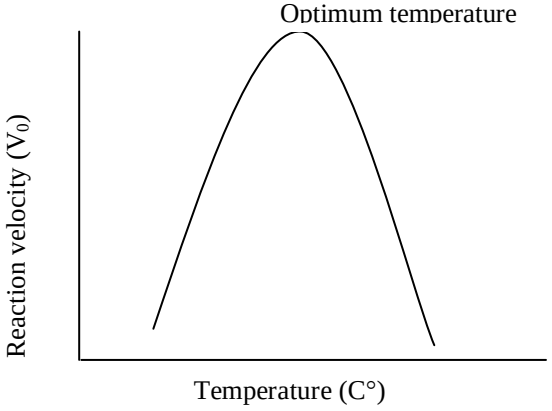
Factors influencing enzyme activity:-

Enzyme can be isolated from cells, and their properties in a test tube (that *invitro*). Different enzymes show different responses to changes in substrate concentration, temperature, and pH.Enzymic responses to these factors give us valuable clues as to how enzymes function in living cells.

1- Temperature

Rise in temperature cause increase in the rate of enzyme catalyzed reactions up to a certain temperature i.e about 45˚C. Above which the activity declines due to denaturation of enzymes (due to their protein nature). As the enzyme is denatured and inactivated, the reaction which it catalyzes slow down and ultimately stops. So the temperature at which the enzyme shows maximum activity is known as optimum temperature. The optimum temperature of most of the enzymes is found to be 37˚C.

Effect of temperature on an enzyme-catalyzed reaction

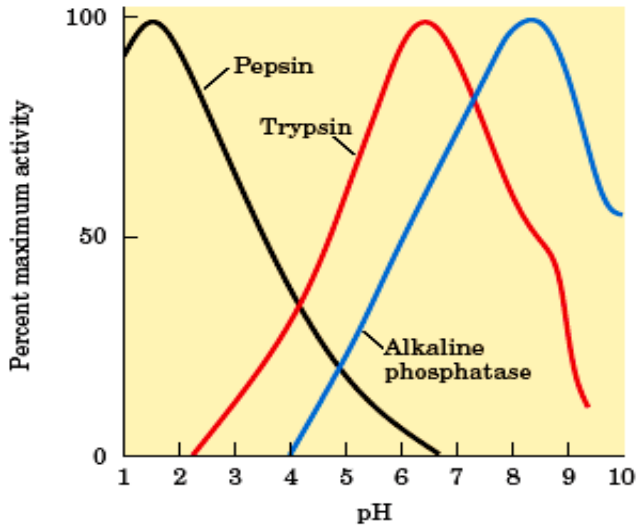


2 \ pH

PH: Every enzyme has an optimum pH (or pHrange) at which it has maximal activity.pH changes affects the ionic state of the enzymes & substrate. Optimal activity of enzymes is generally observed between pH values of (5-9). However, a few enzymes e.g. pepsin are active at pH values outside this range.The shape of pH activity shape (Figure) s determined by these factors

1/ Enzymes denaturation at high or low pH values.

2/ Effects on the charged state of the substrate or enzymes.

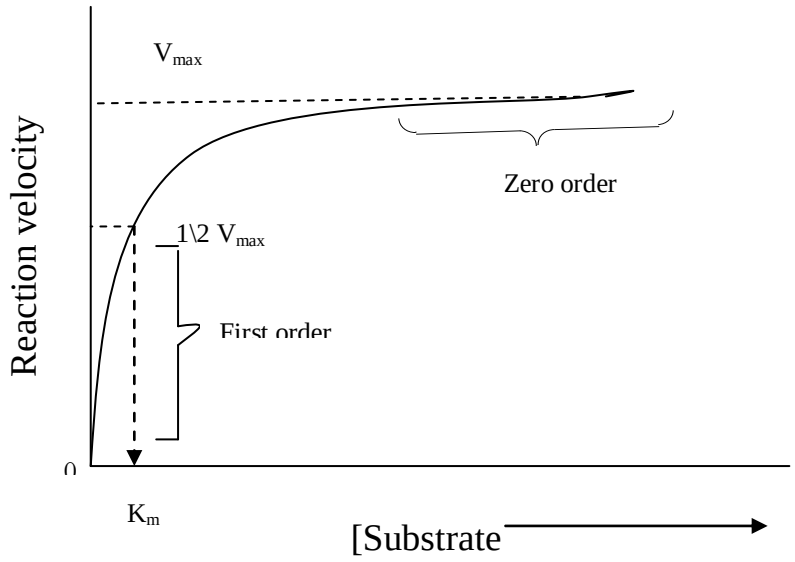


Pepsin=2.0
Trypsin=8.5
Alkaline phosphate=9.9

3 \ Substrate concentration

With s fixed amount of enzyme, the reaction rate is proportional to the concentration of substrate. But this is true up to a certain concentration after which the increase in concentration of substrate does not further increase in the velocity of the reaction.

Since the number of active sites on an enzyme molecule are limited, a stage will come when all of them have filled with the substrate molecules. This is known as saturation of enzyme. Now, since none of the active sites of the enzymes is free , further addition of the substrate molecule will not increase the product formation.



Effect of substrate concentration on reaction

4/ **Effect of activators**

Ions like Mg^{+2}, Cu^{+2}, Mn^{+2}, $Zn^{+2}$and monovalent ions such as Na^{+} and K^{+} are required for the activity of many enzymes. For example, amylase needs Cl^{-} ions, Zn^{+2} ions are required for carbonic anhydrase action, Fe^{+2} and Cu^{+2} ions are required for enzymes involved in redox reactions. Several peptidases are activated by Mn^{+2}, Zn^{+2} or Co^{+2}. Enzymes are requiring metal ions or enzymes which contain metal ions in their structure are called as **metalloenzyme**.

5/Enzyme concentration

The rate of an enzyme catalyzed reaction is directly proportional to the concentration of the enzyme. The greater the concentration of enzyme, the faster will be reaction taking place.

Michaelis-Menten Equation

It is an equation which describes how reaction velocity varies with substrate concentration.

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Where
V_o = initial reaction velocity
V_{max} = maximal velocity
K_m = Michaelisconstant = (k_{-1} + k_2)\ k_1
[S] = substrate concentration

- **K_m– the Michaelis constant** is characteristic of an enzyme and its particular substrate, and reflects the affinity of the enzyme for that substrate. Km is numerically equal to the substrate concentration at which the reaction velocity is equal to 1\2 V_{max}. K_m does not vary with the concentration of enzyme. Low K_m reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme- that is, reach a velocity that is 1\2 V_{max} . Large or high Km reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme.

- **Lineweaver-Burke plot :-** If 1\V_0 is plotted versus 1\[S], straight line is obtained. This plot, the Lineweaver-Burk plot can be used to calculate K_m and V_{max} as well as to determine the mechanism of action of enzyme inhibitor. The equation describing the Lineweaver-Burk plot is:-

$$\frac{1}{V_0} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$$

Oxidation: sulfhydryl (-SH) groups of many enzymes Are essential for enzymes. Activity. Oxidation of these (SH) groups forming disulfide linkages (S-S) leads to conformal changes.e.g: Dehydrogemases enzymes are active when (-SH) present in reduced form and inactive in (S-S) from coxidized form.

Radiation: Enzymes. Are highly sensitine to short waueleugth (high enemgy) such as (UV, X, β, or γ –rays). This due to oxidation of the enzymes.By peroxides formed by high energy radiation.

Type C: enzymes (with low reaction specificity *and* low substrate specificity, bottom) are very rare.

Importance of enzymes

1- Enzymes catalyze many biological reactions and enhance the rate of product formation in metabolic pathways.
2- Some enzymes in blood are used as diagnostic indicators of various diseases for example the level of transaminases are elevated in blood during jaundice.
3- Some enzymes are used for therapeutic purposes.
   a- Penicillinase- to treat patients allergic to penicillin.
   b- Asparaginase- to treat leukemia.
   c- Diastase- to treat indigestion.

Inhibition of enzyme activity

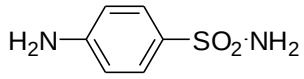
The rates of enzyme catalyzed reactions are decreased by specific inhibitors. Inhibitors are compounds that combine with enzymes and prevent enzyme and substrate from forming ES complex. The toxicity of many compounds such as hydrogen cyanide and hydrogen sulphide results from their action as enzyme inhibitors. Many drugs also act to inhibit specific enzyme. Thus, knowledge of enzyme inhibitors is vital to understand drug action and toxic agents.

Compounds which convert the enzymes into inactive substances and then adversely affect the rate of enzyme catalyzed reactions are called as enzyme inhibitors. Substances that inhibit enzymatic reactions are classified into three groups:-

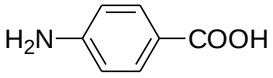
1. Competitive inhibition
2. Non-competitive inhibition
3. Uncompetetive inhibition

1. **Competitive inhibition:-**This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy and therefore, competes with the substrate for the site. In competitive inhibition, the inhibitor will be structural analogue of the substrate. The effect of a competitive inhibitor is reversed by increasing [s]. At a sufficiently high substrate concentration, the reaction velocity reaches the V_{max} observed in the absence of inhibitor. A competitive inhibitor increases the apparent K_m for given substrate. This means that, in the presence of a competitive inhibitor, more substrate is needed to achieve 1\2 V_{max} . Competitive inhibition shows a characteristic Lineweaver-Burk plot in which the plots of the inhibited and inhibited reactions intercept on the y axis at 1\V_{max} (V_{max}unchanged). The inhibited and inhibited reactions show different x axis intercepts, indicating that apparent K_m is increased in the presence of the competitive inhibitor.

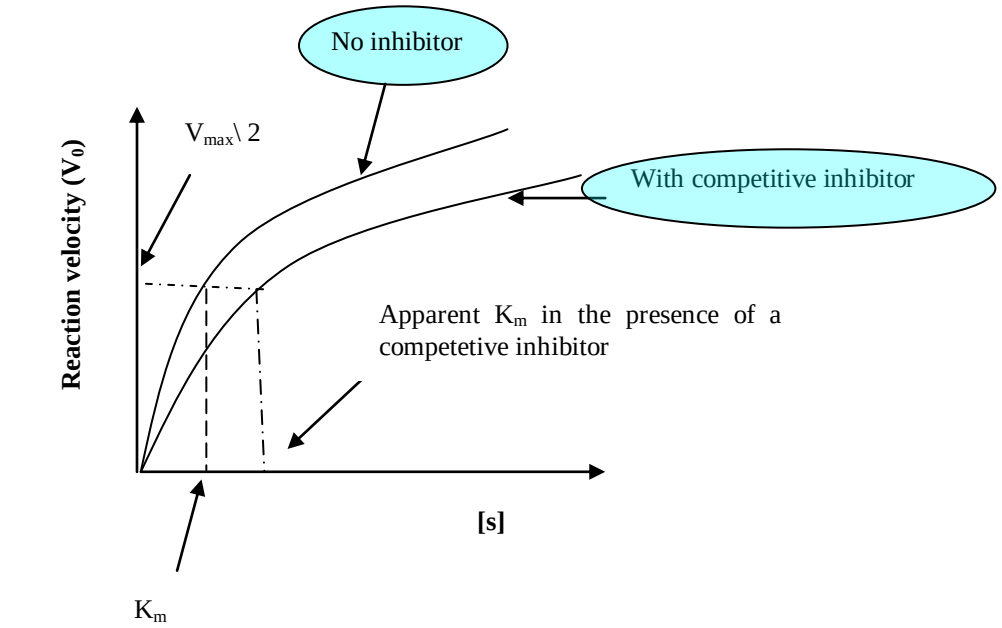
   Many drugs may be explained by the principle of competitive inhibition. For example sulfonamides are commonly employed antibacterial agents. Bacteria synthesize folic acid by combining PABA with pteroyl glutamic acid. Bacterial wall is impermeable to folic acid. Sulpha drug, being structural analogues of PABA, will inhibit the folic acid synthesis in bacteria, and they die. The drug is nontoxic to human cells, because human being cannot synthesis folic acid.

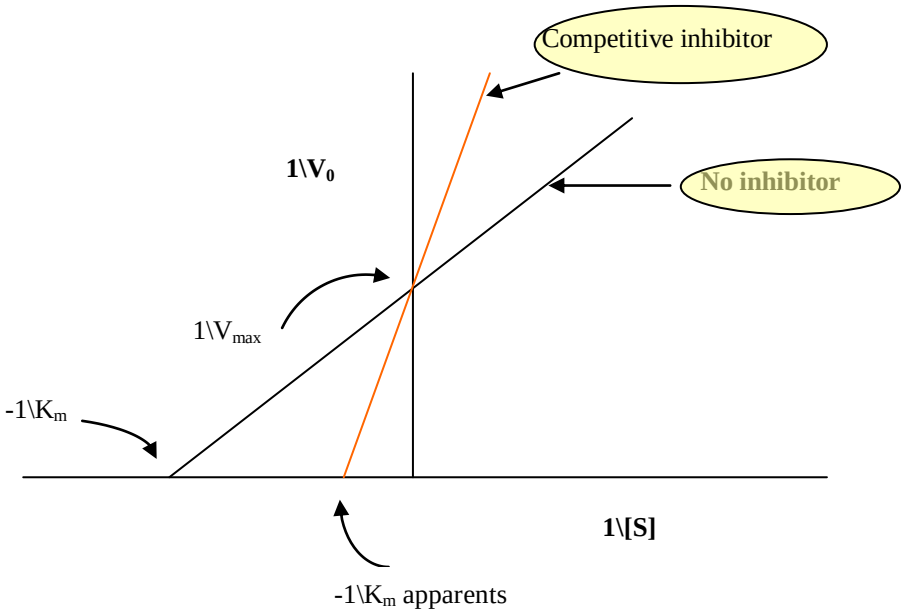


Sulphanilamide



PABA



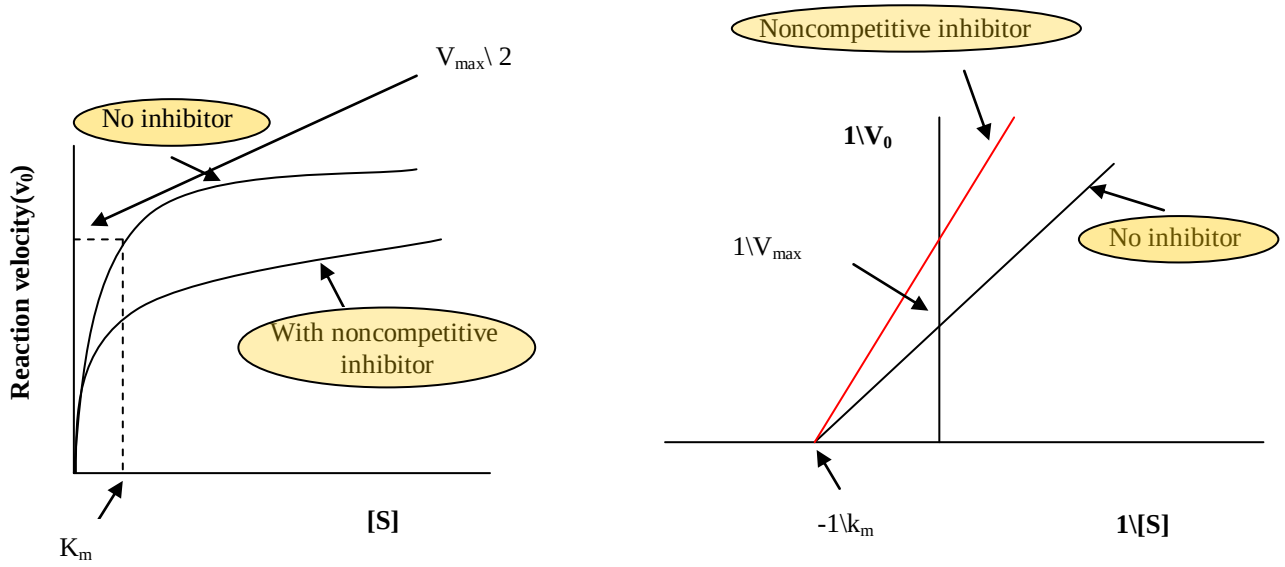


Lineweaver-Burk plot of competitive inhibition of an enzyme

2. Non- competitive inhibition:- As the name implies there is no competition between the substrate and the inhibitor molecules there is little or no structural resemblance between the substrate and the inhibitor molecules and hence they bind

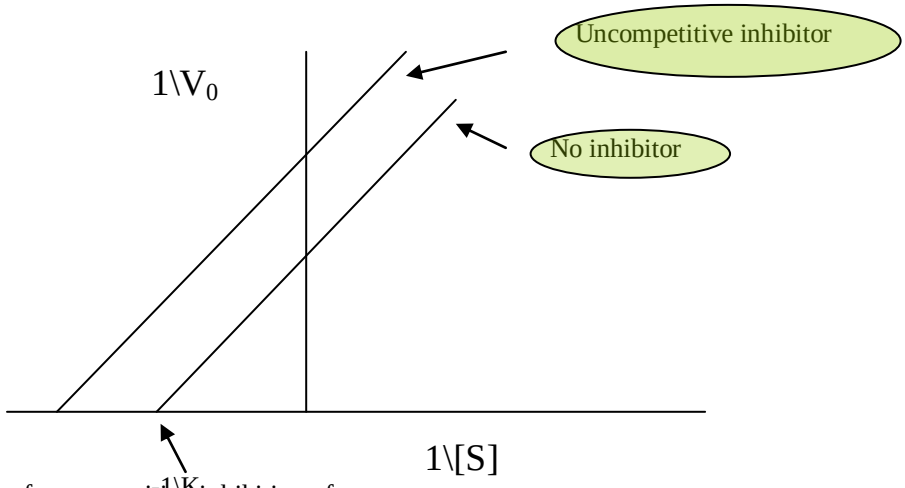
to the different sites of the enzyme. Inhibitors combine with the allosteric site of the enzyme, this combination results in the distortion of the active site. In non-competitive inhibition, the affinity of enzyme remains same but its efficiency decreases. This inhibition is also known as allosteric inhibition. Noncompetitive inhibition cannot be overcome by increasing the concentration of substrate. Thus, noncompetitive inhibitors decrease the $V_{max}$of the reaction. Noncompetitive inhibitors do not interfere with the binding of substrate to enzyme. Thus, the enzyme shows the same K_m in the presence or absence of the noncompetitive inhibitor. Noncompetitive inhibition is readily differentiated from competitive inhibition by plotting 1\v0 versus 1\[s] and noting that V_{max} decreases in the presence of a noncompetitive inhibitor, whereas K_m is unchanged.

A variety of poisons, such as iodoacetate, heavy metal ions (silver, mercury) and oxidizing agents acts as irreversible noncompetitive inhibitors. Cyanide inhibits cytochrome oxidase. Fluoride will remove magnesium and manganese ions and so will inhibit the enzyme, enolase and consequently the glycolysis.

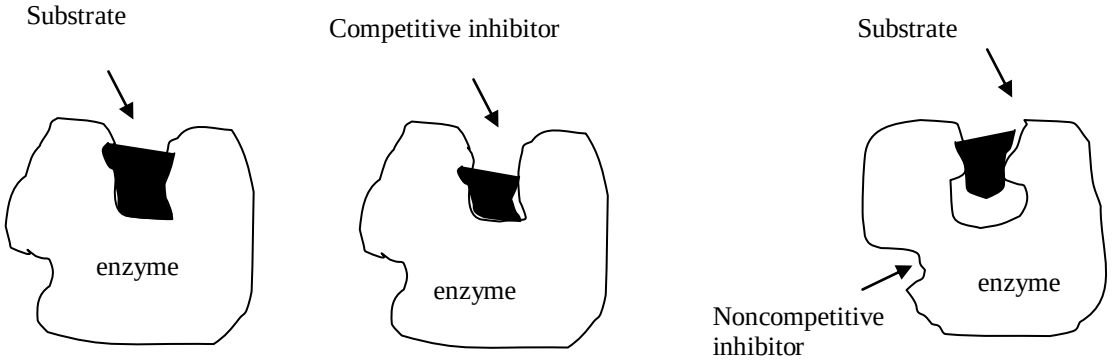


Lineweaver-Burk plot of noncompetitive inhibition of an enzyme

3. **Uncompetitive inhibition:-** this inhibitors combine only with the enzyme-substrate forming an irreversible complex. The inhibition is dependent only on the concentration of the inhibitor. In case of uncompetitive inhibition V_{max} is lower, slope is same, apparent $K_m<K_m$



Lineweaver-Burk plot of uncompetitive inhibition of an enzyme



Effect of Inhibitors

The catalytic enzymatic reaction may be inhibited by substances which prevent the formation of a normal enzyme-substrate complex. The level of inhibitionthen depends entirely upon the relative concentrations of the true substrate and the inhibitor. Such inhibition, which depends on competition with the substrate for the active sites of the enzyme, is termed competitive inhibition. In other cases, the inhibitor combines with the enzyme-substrate complex to give an inactive enzyme-

substrate-inhibitor complex, which cannot undergo further reaction to give the usual product. This is termed uncompetitive inhibition. Non competitive inhibition involves combination of the inhibitor with the enzyme or the enzyme substrate complex, to give inactive complexes. In this case, theinhibitor binds to sites, on the enzyme other than enzyme sites, resulting in deformation of the enzyme molecule so that the formation of the enzymesubstrate complex is slower than normal. Some enzymes undergo irreversible inactivation; reaction of the inhibitor with a functional group of the enzyme, resulting in a loss of its catalytic activity. Enzyme inhibitor plays a vital role in clinical utility and is listed below.

Sl.No	Enzymatic inhibitor/drug	Enzyme inhibited	Clinical use
1	Allopurinol	Xanthine oxidase	gout
2	Dicoumarol	Vitamin-K-epoxide-reductase	Anti-coagulant
3	Penicillin	Transpeptidase	Anti-bacterial
4	Sulphonamide	Pteroidsynthetase	Anti-bacterial
5	Pyrimethamine	FH2-reductase	Anti-malarial
6	5-fluorouracil	Thymidylatesynthetase	Anti-cancer

Isoenzymes:- also called isozymes. They are different images of the enzyme catalyze the same reaction. However, they do not necessarily have the same physical properties because of genetically determined differences in amino acid sequence. For this reason, isoenzymes may contain different numbers of charged amino acids and may, therefore, be separated from each other by electrophoresis. The patterns of isoenzymes are Lactate dehydrogenase (LDH) and creatine kinase (CK). LDH enzyme is a tetramer with 4 subunits. But the the subunit may be either H (heart) or M(muscle) polypeptide chains. These two are the products of 2 different genes. Although both of them have the same molecular weight (32 kD), there are minor amino acid variations. So 5 combination of H and M chains are possible; H_4, H_3M, H_2M_2, M_3H, M_4 varieties, forming 5 iso- enzymes. All these 5

form are seen in all persons. In myocardial infarction, total LDH activity is increased. CK is a dimmer; each subunit has a molecular weight of 40000. The units are called B for brain and M for muscle and three isozymes are seen in circulation. Also it is determined in the diagnosis of myocardial infarction.

❖ **Denaturation of proteins:-** heating, X-ray, ultraviolet rays, high pressure, vigorous shaking, pH change, organic solvents, and such other physiochemical agents produce non-specific alterations in secondary, tertiary and quaternary structures of protein molecules. This is called *denaturation.* Primary structure is not altered during the process of denaturation. It generally decreases the solubility, increases precipitability and often causes loss of biological activity.

Denatured proteins are sometimes re-natured when the physical agent is removed. Ribonuclease is a good example for such reversible denaturation. Protein kept in solution for long periods may denature, and lose their biological or enzymetic properties. Such loss of activity can be minimized when the solution is kept at low temperature.

Questions

Q1\ Define the following terms: (a) apoenzyme (b) holoenzyme (c) coenzyme

Q2\ Give three examples of metal ions that are activate specific apoenzymes.

Q3\ Name the six major classes of enzymes and give an example of each.

Q4\ Describe the effect of temperature on enzyme activity.

Q5\ Describe the lock- and-key model for enzyme activity. How does this model explain the following: (a) optical isomer specificity (b) substrate- complex theory?

Q6\ Distinguish between competitive and noncompetitive enzyme inhibition.

Q7\ Explain how enzymes regulate their own concentration.

Q9\ choose the one correct answer

1. A competitive inhibitor of enzyme

a. Increases K_m without affecting V_{max}.
b. Decreases K_m without affecting V_{max}.
c. Increases V_{max} without affecting K_m.
d. Decreases V_{max} without affecting K_m.
e. Decreases both V_{max} and K_m.