

<b>COURSE CODE:</b>	<i>VBB 201</i>
<b>COURSE TITLE:</b>	<i>Biochemistry of Enzymes, Carbohydrates &amp; lipids</i>
<b>NUMBER OF UNITS:</b>	<i>3 Units</i>
<b>COURSE DURATION:</b>	<i>Three hours per week</i>

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### COURSE DETAILS:

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<b>Other Lecturers:</b>	<b>Dr Babatunde Samuel Okediran</b> <i>B.Sc., DVM., M.Sc</i>

### COURSE CONTENT:

Relevance of biochemistry to veterinary medicine. Biochemistry of the living cell. PH and buffers. Enzymes: nature, properties and functions; enzyme kinetics; Allosteric effects; Co-enzymes; structure and role in cellular metabolism. Enzyme assay in clinical veterinary medicine. Chemistry and biochemistry of carbohydrates of importance in vet. medicine; Metabolism of carbohydrates: Glycolysis [Embden-Meyerhof pathway]. Metabolism of fructose and galactose. Krebs's(citric acid) cycle, Glyoxylate cycle. Alternate pathways of carbohydrate oxidation. The Uronic acid pathway. Metabolism of glycogen. Disorders of carbohydrate metabolism. The electron transport chain and oxidative phosphorylation. Lipids; classification, chemistry and functions; digestion, absorption and transportation. Lipoproteins etc. Biosynthesis of fatty acids; the triacylglycerols. Degradation of triacylglycerols, phospholipids and sphingolipids.  $\beta$ -oxidation of fatty acids. Ketone bodies and ketosis. Metabolism of cholesterol. Primary disorders of plasma lipoproteins (dyslipoproteinemias). Biochemistry of prostaglandins.

## COURSE REQUIREMENTS:

This is a compulsory course for all students of Veterinary Medicine. In view of this, Veterinary students are expected to participate in all the course activities and have a minimum of 75% attendance to be able to write the final examination.

## READING LIST:

1. V.K Malhotra. Biochemistry for students tenth edition Jaypee brothers medical publishers (p) ltd.
2. J.Jerry Kaneko., John W. Harvey., Michael L. Bruss. Clinical Biochemistry of Domestic Animals Sixth edition. Academic Press.
3. Harvey Lodish., David Baltimore., Arnold Berk., S. Lawrence Zipursky., Paul Matsudaira and James Darnell. Molecular Cell Biology. Third Edition. By Scientific American Books, Inc.
4. David A. Bender., Kathleen M. Botham., Daryl K. Granner., Frederick W. Keeley., Peter A. Mayers., Robert K. Murray., Victor W. Rodwell., P. Anthony Weil.(2006). Harpers Illustrated Biochemistry. 27<sup>th</sup> Edition. Published By Mc Graw Hill

## LECTURE NOTES

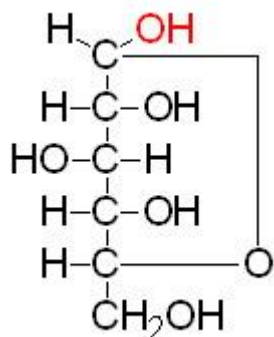
### **CHEMISTRY AND BIOCHEMISTRY OF CARBOHYDRATES OF IMPORTANCE IN VETERINARY MEDICINE E.G OLIGOSACCHARIDES, ANTIBIOTICS, HEPARIN, AGAR, CHONDROITIN, DEXTRANS ETC**

#### **CHEMISTRY AND BIOCHEMISTRY OF CARBOHYDRATES**

Carbohydrates are carbon compounds that have aldehyde (C-H=O) or ketone (C=O) moiety and comprises polyhydroxyl alcohol (polyhydroxyaldehyde or polyhydroxyketone); their polymers ,

which also contain an hemiacetal glycosidic linkage. Carbohydrates have the general molecular formula  $CH_2O$ .

For example:



A sugar is a Carbohydrate which is sweet to taste ,soluble in water an chars on heating.

### Classification of carbohydrates

3 major classes of CHO

- Monosaccharides-a single unit of polyhydroxylketone or aldehyde which cannot be broken down to simpler substances on acid hydrolysis.
- Oligosaccharides- two to ten monosaccharide units, linked by glycosidic bonds
- Polysaccharides- contain hundreds of monosaccharide units

### Monosaccharides

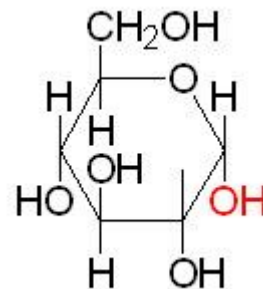
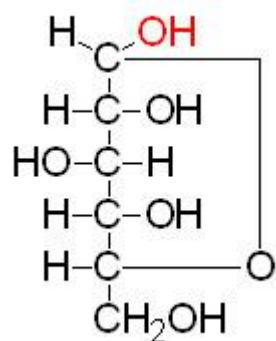
The monosaccharides commonly found in humans are classified according to the number of carbons they contain in their backbone structures. The major monosaccharides contain four to six carbon atoms.

## Carbohydrate Classifications

No of Carbon-atoms	Category Name	Relevant examples
3	Triose	Glyceraldehyde, Dihydroxyacetone
4	Tetrose	Erythrose
5	Pentose	Ribose, Ribulose, Xylulose
6	Hexose	Glucose, Galactose, Mannose, Fructose
7	Heptose	Sedoheptulose
9	Nanose	Neuraminic acid, also called sialic acid

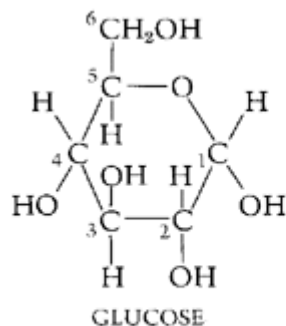
The aldehyde and ketone moieties of the carbohydrates with five and six carbons will spontaneously react with alcohol groups present in neighboring carbons to produce intramolecular hemiacetals or hemiketals, respectively. This results in the formation of five- or six-membered rings. The five-membered ring structures are termed **furanoses**. Those with six-membered rings are termed **pyranoses**.

Such structures can be depicted by either **Fischer (linear)** or **Haworth (cyclic)** style diagrams. The numbering of the carbons in carbohydrates proceeds from the carbonyl carbon, for aldoses, or the carbon nearest the carbonyl, for ketoses.

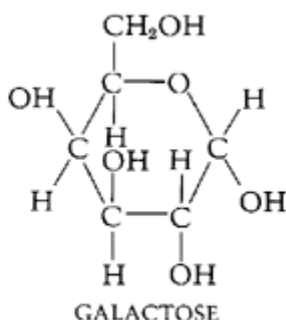


**Cyclic Fischer Projection of  $\alpha$ -D-Glucose**

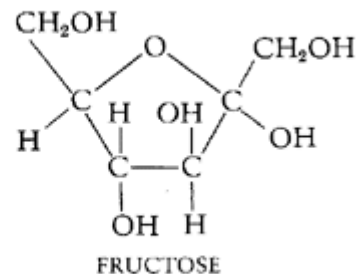
**Haworth Projection of  $\alpha$ -D-Glucose**



GLUCOSE



GALACTOSE



FRUCTOSE

The rings can open and re-close, allowing rotation to occur about the carbon bearing the reactive carbonyl yielding two distinct configurations ( $\alpha$  and  $\beta$ ) of the hemiacetals and hemiketals. The carbon about which this rotation occurs is the **anomeric carbon (that is carbonyl C-atom)** and the two forms are termed anomers. Carbohydrates can change spontaneously between the  $\alpha$  and  $\beta$  configurations: a process known as **mutarotation**. When drawn in the Fischer projection, the  $\alpha$  configuration places the hydroxyl attached to the anomeric carbon to the right, towards the ring. When drawn in the Haworth projection, the  $\alpha$ -configuration places the hydroxyl downward.

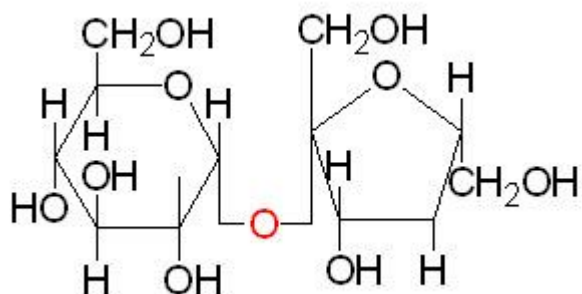
## Properties

- CHOs possess asymmetric carbon-atom that is C-atom that has 4 different atoms or functional groups attached to it.
- Isomers exist due to the presence of asymmetric C-atom, and number of isomers a compound has depends on the number of asymmetric C-atoms present and is given by  $2^n$  where  $n$  indicates number of asymmetric C-atom in that compound.
- CHOs that differ in their configuration about a specific C-atom other than the carbonyl C-atom are called epimers. Example; glucose and galactose.
- If the hydroxyl group on the highest **asymmetric C-atom** or on the penultimate C-atom is on the right side then the compound will belong to the D-series while on the left side it's L-series.
- CHOs that have amino group within their structure are called amino sugars common ones are glucosamine and galactosamine and they occur as N-acetyl compounds. These compounds are important components of the body; glucosamine is present in chitin shells of insects and mammalian polysaccharides whereas galactosamine is present in polysaccharides of cartilage and chondroitin.
- Monosaccharides are able to:- undergo dehydration on treatment with strong acids to give furfural derivatives; mutarotation; reduce heavy metals metallic cations like  $\text{Cu}^{++}$  in alkaline solution and high temperature (hence termed reducing sugars); form osazones with phenylhydrazines; react with dilute alkali to have reducing action; aldoses can be oxidized to yield aldonic acids (first C-atom oxidized to carboxyl group), uronic acids (terminal C-atom oxidized to carboxyl group), and aldaric or saccharic acids (first and terminal C-atom are oxidized to carboxyl groups); undergoes reduction, glucose to sorbitol and fructose to sorbitol and mannitol; form glycoside by the replacement of the H-atom of the hemiacetal/ketal group with an organic moiety with the loss of a molecule of water. When the organic moiety is not a carbohydrate it's called **Aglycone**. Examples are the cardiac glycosides digoxin, indicant (CHO + indoxyl), amygdalin (CHO+benzaldehyde) etc

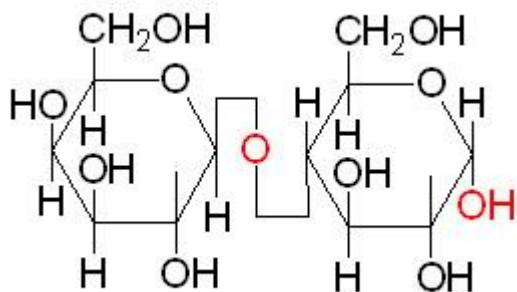
## Disaccharides

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. Several physiologically important disaccharides are sucrose, lactose and maltose.

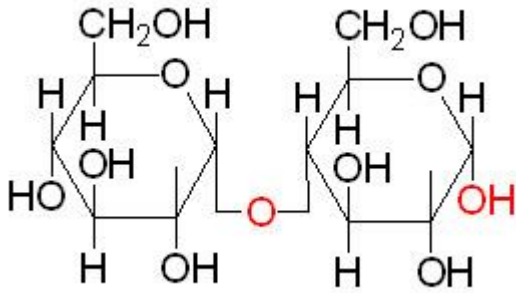
- **Sucrose:** prevalent in sugar cane and sugar beets, is composed of glucose and fructose through an  $\alpha$ -(1,2)- $\beta$ -glycosidic bond. On partial hydrolysis, sucrose, yields levorotatory (sucrose is dextrorotatory) equimolar amounts of glucose and fructose called invert sugar.



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



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



## Maltose

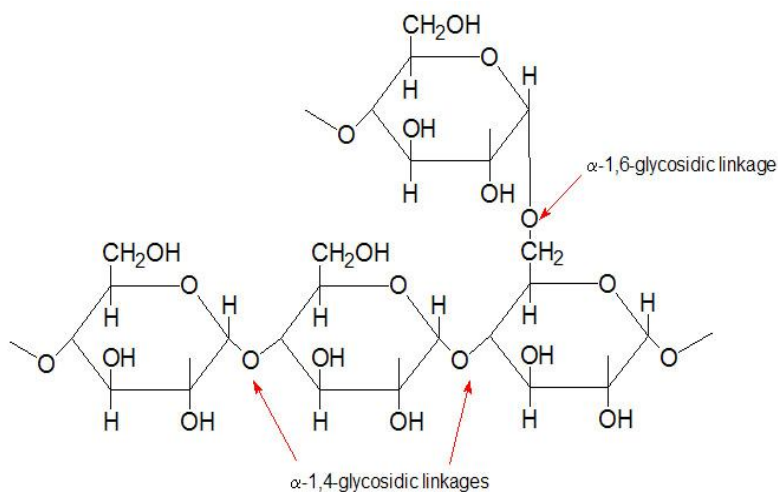
## Polysaccharides

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. When polysaccharides are composed of a single monosaccharide building block, they are termed **homopolysaccharides**. Polysaccharides composed of more than one type of monosaccharide are termed **heteropolysaccharides**.

## Glycogen

Glycogen is the major form of stored carbohydrate in animals. This crucial molecule is a homopolymer of glucose in  $\alpha$ -(1,4) linkage; it is also highly branched, with  $\alpha$ -(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. The structure of glycogen is similar to that of amylopectin, although the branches in glycogen tend to be shorter and more frequent. The liver and skeletal muscles are major depots of glycogen. Glycogen is broken back down into glucose when energy is needed (a process called glycogenolysis).





### Section of Glycogen Showing $\alpha$ -1,4- and $\alpha$ -1,6-Glycosidic Linkages

#### 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).

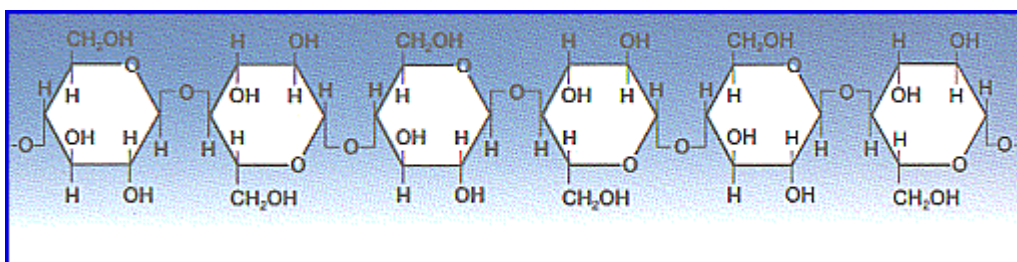
Unbranched starch is called **amylose**; branched starch is called **amylopectin**. Starches are insoluble in water and thus can serve as storage depots of glucose. Plants convert excess glucose into starch for storage. Before starches can enter (or leave) cells, they must be digested. The hydrolysis of starch is done by amylases. With the aid of an **amylase** (such as pancreatic amylase), water molecules enter at the 1  $\rightarrow$  4 linkages, breaking the chain and eventually producing a mixture of **glucose** and **maltose**. A different amylase is needed to break the 1  $\rightarrow$  6 bonds of amylopectin.

**Amylose** consists of linear, unbranched chains of several hundred glucose residues (units). The glucose residues are linked by a glycosidic bond between their number 1 and 4 carbon atoms.

**Amylopectin** differs from amylose in being highly branched. At approximately every thirtieth residue along the chain, a short side chain is attached by a glycosidic bond to the #6 carbon atom (the carbon above the ring). The total number of glucose residues in a molecule of amylopectin is several thousands.

## Cellulose

Cellulose is probably the single most abundant organic molecule in the



biosphere. It is the major structural material of which plants are made. Wood is largely cellulose while cotton and paper are almost pure cellulose. Like starch, cellulose is a polysaccharide with glucose as its monomer. However, cellulose differs profoundly from starch in its properties.

- Because of the orientation of the glycosidic bonds linking the glucose residues, the rings of glucose are arranged in a flip-flop manner. This produces a long, straight, rigid molecule.
- There are no side chains in cellulose as there are in starch. The absence of side chains allows these linear molecules to lie close together.
- Because of the many -OH groups, as well as the oxygen atom in the ring, there are many opportunities for hydrogen bonds to form between adjacent chains.

It is the main constituent of supporting tissues of plants and therefore forms a considerable part of animal vegetable diet. Herbivores with the help of bacteria can utilize a considerable proportion of cellulose. It adds bulk to the intestinal contents (roughage) thereby stimulating peristalsis and elimination of indigestible food residues.

## Dextrins

Partial hydrolytic products of starch by  $\alpha$ -amylase,  $\beta$ -amylase or acids give what is called dextrins that have lower molecular weight but resemble starch in being precipitable by alcohol and have a free sugar group and can thus reduce alkaline copper sulphate solution.

Dextrin solutions are often used as mucilages (sticky gums) and are widely used in infant feeding. Limit dextrans are well defined dextrans formed on total hydrolysis of starch by  $\beta$ -amylase.

### **Inulin**

It is a polymer of D-fructose with low molecular weight of about 5000. It occurs in tubers of *Dehlia*, bulbs of onions and garlic etc. It can be hydrolysed by acid and inulinase to D-fructose. It is used in physiological investigation for determination of glomerular filtration rate and in estimation of total body water.

### **Dextrans**

Synthesized by the action of *leuconostoc mesenteroides* (a bacterium) in sucrose medium, it is a polymer of D-glucose. They are different from dextrans in structure but have D-glucose units joined by  $\alpha$ 1-4,  $\alpha$  1-6,  $\alpha$ ; 1-3 glycosidic linkages joined together to form a network. Dextran solutions have a mol. wt of about 75000 and are used as plasma expanders because they have the ability to increase blood volume because high viscosity, low osmotic pressure, slow disintegration and utilization and slow elimination from the body they remain in the blood for many hours to exert its effect.

### **Agar**

It is a homopolysaccharide made up of repeating units of galactose which is sulphated. It is present in sea weed. It is used as a laxative in constipation because like cellulose it is not digested hence adds bulk to feces and helps propulsion. In microbiology it is purified and dissolved in hot water and on cooling sets like gel and used in culture of bacteria.

### **Heteropolysaccharides/mucopolysaccharides**

These are made up of mixed mixed disaccharide repeating units and on hydrolysis gives a mixture of more than one products of monosaccharides and their derivatives of amino sugars and uronic acids. The hexamine present is usually acetylated and these compounds are essentially components of tissues where they are generally present either in free form or in combination with proteins.

There are 2 major classes of MPS (mucopolysaccharides)

- Neutral MPS e.g. blood group substances
- Acidic MPS

Acid MPS are present in connective tissue and have hexamine as the repeating disaccharide units with alternating 1,4 and 1,3 linkages.

- Hyaluronic acid; it is sulphate free and found in vitreous humour, synovial fluid, skin, umbilicalcord and rheumatic nodule. it occurs as both free and in salt like combination with proteins hence called the ground substance of mesenchyme and intergral part of the gel-like ground substance of C.T and other tissues. Composed of repeating units of N-acetylglucosamine and D-glucuronic acid.
- Heparin

It is an anticoagulant present in liver and is produced mainly by mast cells of liver. It can also be found in lungs, thymus, spleen, walls of large vessels, skin etc. both the hydroxyl and the amino groups are combined with sulphuric acid. Repeating disaccharide units are D-glucosamine and either D-glucuronic acid or L-iduronic acid. There is a  $\alpha$ ;1-4 linkage from the anomeric C-atom of the glucuronic acid to the C4 hydroxyl group of the glucosamine.

- Chondroitin sulphates

They are present in connective tissue and serve as structural material such as cartilage, tendons and bones. It differs from hyaluronic acid in that it has N-acetyl galactosamine instead of N-acetyl glucosamine. there are 4 different types of chondroitin SO<sub>4</sub>s named as Chondroitin

Sulphate-A,B,C and D. these differ at points of C-atom linkages, positions and /or number of sulphate groups etc

- Heparin sulphate

It has been isolated from amyloid liver (a diseased state) and certain normal tissue such as human and cattle aorta. It has negligible anticoagulant activity and though structurally similar to heparin has a lower mol. wt. unlike heparin its predominant uronic acid is D-glucuronic acid

- Sialic acids

These are N-acetyl derivatives of neuraminic acid and are widely distributed in tissues and are present in blood group substances. Neuraminic acid is a condensation product of pyruvic acid and mannosamine.

### **Proteoglycans**

These are conjugated proteins. Core proteins are covalently linked to glycosaminoglycans (GAGS). The amount of carbohydrates in proteoglycans is up to 95%, higher than that in glycoproteins. These compounds are constituents of extracellular matrix or ground substance; they interact with collagen and elastin. They also act as polyanions, barriers in tissues, lubricants, in release of hormones etc

### **METABOLISM OF CARBOHYDRATES**

- This begins in the mouth where on contact with salivary amylase and maltase starch and glycogen is split into constituent monosaccharides.
- In the stomach acid hydrolysis may occur sparingly whilst the action of saliva enzymes is arrested by the acid pH.
- Carbohydrate splitting enzymes contained in pancreatic juice is released into small intestine where they rapidly break polysaccharides to Monosaccharides.

- Monosaccharides (predominantly glucose, fructose and galactose) are absorbed into the mucosa cells of the intestine through  $\text{Na}^+$  dependent-active-transport mechanism using a glucose co-transporter; leave the cells into the portal circulation via facilitated diffusion in the presence of glucose-transporters.
- In the liver two major mechanisms operate, glucose(carbohydrate is either withdrawn from blood into the cells, to further undergo breakdown for ultimate release of energy to power cellular processes or it is converted to storage forms to be drawn upon when the body later needs energy (including conversion to fatty acids and storage as triglycerides).
- For energy production, glucose is oxidized in a series of steps and pathways that leads to its complete catabolism to yield ATPs, carbon dioxide and water. The initial pathway involved in this energy production step is the pathway known as GLYCOLYSIS or the EMBDEN-MEYERHOF pathway.
- Glucose also provide the carbon skeleton backbone for synthesis of non-essential amino acids and as well other biologically useful carbohydrates such as mucopolysaccharides, glycoproteins etc.
- Fructose and galactose are absorbed into the liver where they are intergrated into the metabolic pathway of glucose (converted to glucose) through a number of steps starting with phosphorylation. They may also be used for specific functions in some special cells e.g fructose is required for metabolism of spermatozoa in the seminiferous tubules and fructose can also be formed from glucose through the Sorbitol (polyol) pathway in the seminiferous tubular epithelia cells. Galactose is used in the synthesis of lactose in the mammary gland for milk production.
- Uronic acid pathway is another alternative pathway of glucose metabolism that utilizes glucose for the production of glucuronic acids which can be further used for synthesis of mucopolysaccharides
- Through the hexose monophosphate shunt/pentose phosphate pathway,another alternative pathway of glucose metabolism, glucose is used in the formation of 5-carbon atom monosaccharides e.g. ribose, ribulose, deoxyribose which is required in the synthesis of nucleic acids.

### **Glycolysis/Embden –Meyerhof Pathway.**

This pathway cleaves the six carbon glucose molecule ( $C_6H_{12}O_6$ ) into two molecules of the three carbon compound pyruvate ( $C_3H_3O_3^-$ ). This oxidation is coupled to the net production of two molecules of ATP/glucose. The pathway was described by Embden, Meyerhof and Parnas.

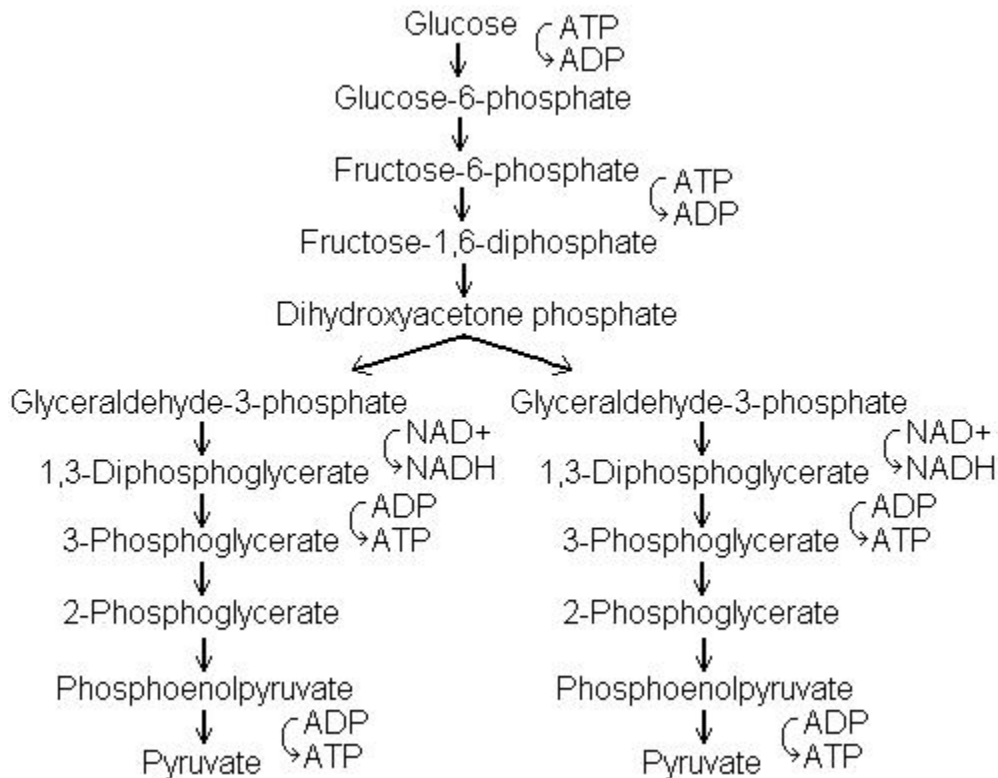
Glycolysis can be broken down into 2 steps Glycolysis I which is endothermic activation that uses ATP) and the second is glycolysis II which is an Exothermic reaction that creates ATP and pyruvate. Glycolysis does not need oxygen for any of its chemical reactions and takes place in the cytoplasm of cells. Glycolysis is the one metabolic pathway found in all living organisms, and in virtually all cells of the body. Particularly in the erythrocytes and nervous tissue, energy is derived mainly from glycolysis. This pathway may utilize oxygen or occur in the absence of oxygen.

- In the presence of oxygen, oxidation is carried out with the production of reducing equivalents NADH (nicotinamide dinucleotide hydrogen) from  $NAD^+$  which is further reoxidized in the electron transport chain (ETC) to yield ATP (adenosine triphosphate)
- In the anaerobic phase the NADH cannot be oxidized in the (ETC) but is oxidized by the conversion of pyruvate to lactate without the production of ATP.
- The above phase is important in skeletal muscle for the provision of energy even in the absence of oxygen, with the accumulation of lactate which can then be transported to the liver for reconversion to glucose in a series of steps known as the CORI cycle.

## GLYCOLYSIS

- The first reaction of Glycolysis; During this reaction an enzyme transfers a  $P_i$  from one substrate to another (An ATP molecule is used to add a phosphate to glucose to form glucose 6 phosphate). The negative charge of the phosphate group does not permit the glucose to flow out of the cell. Catalysed by enzyme Glucokinase in the liver or Hexokinase in other cells with  $Mg^{2+}$  as a coenzyme. The G-6-P can undergo one of at least five different reactions depending on particular cellular needs at the time, which are:
  - I. reconverted back to free glucose that can enter systemic circulation,
  - II. glycogenesis( synthesis of glycogen)
  - III. glycolysis
  - IV. hexomonophosphate shunt
  - V. glucuronate pathway
- G-6-P is then converted to Rfructose -6-p under the influence of the enzyme phosphohexose isomerase as indicated from the enzyme name it is an isomerisation reaction.
- With the addition of Another phosphate group from ATP, by the enzyme phosphofructokinase and coenzyme  $Mg^{2+}$ , F-6-P converted to F-1,6- bisphosphate. This is the rate limiting step of glycolysis and is stimulated by F-6-P, AMP (adenosine monophosphate) and ADP( adenosine diphosphate) and inhibited by ATP i.e AMP, ADP, AND F-6-P are activators of the enzyme Phosphofructokinase while ATP is and inhibitor.this step is irreversible just as hexokinase.

- Aldolase catalyses the splitting of F-1,6-bisphosphate to two 3-carbon atom compounds namely glyceraldehyde -3-phosphate (GA-3-P) and dihydroxyacetone phosphate (DHAP). Dihydroxyacetone phosphate is then converted back to glyceraldehydes -3-phosphate by triose isomerase.



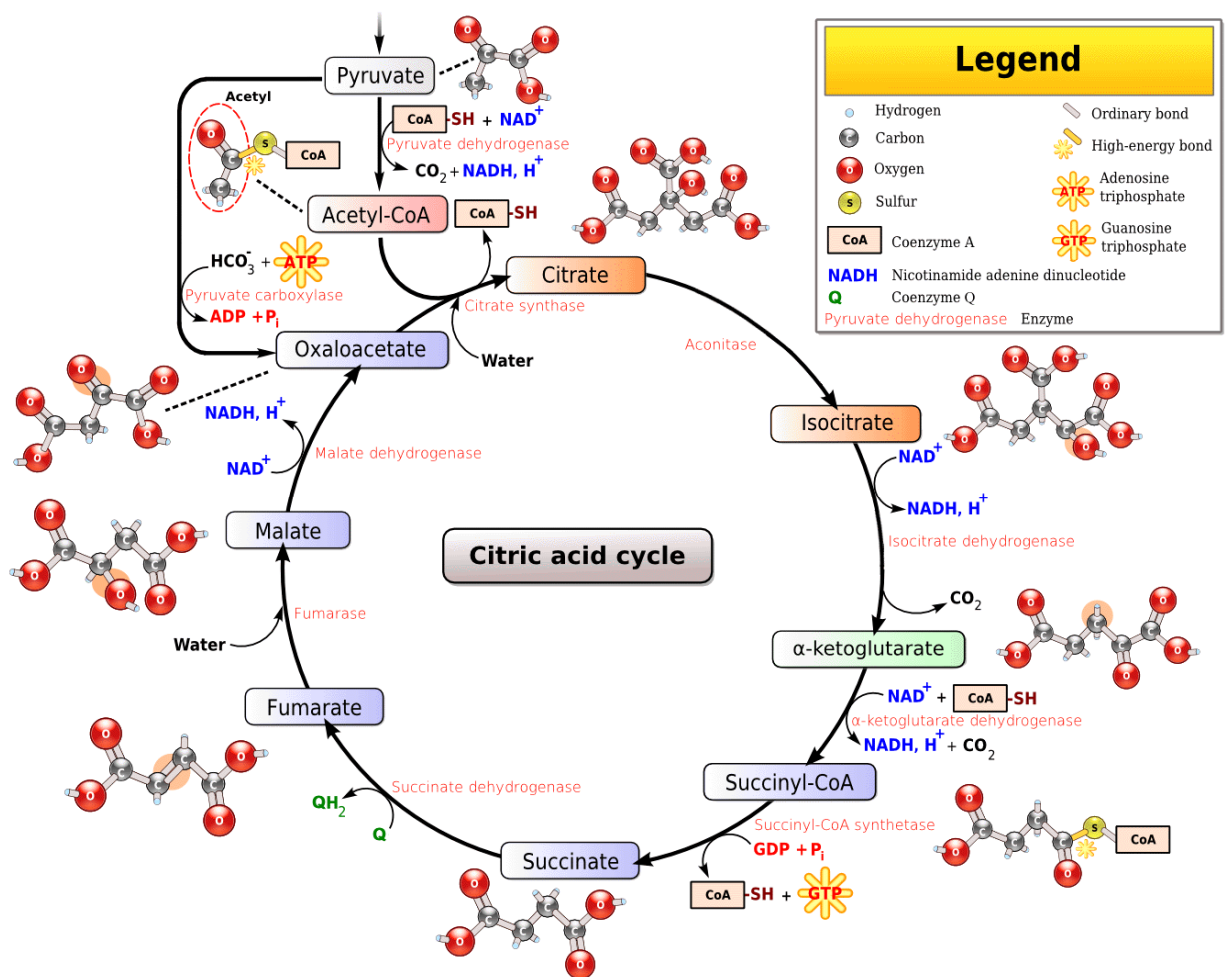
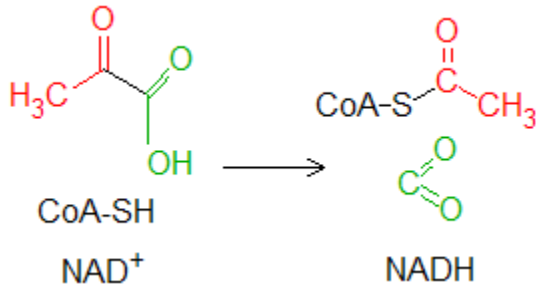
A total of 4 ATPs are produced directly however during oxidation of GA-3-P two NADHs are produced ( from both sides) and on oxidation in the ETC 6 molecules of ATP results, hence a total of 10 ATP results from aerobic oxidation, while a net of 8 ATPs remains. In anaerobic condition lactate is formed, oxidizing the NADH back to  $\text{NAD}^+$  by the enzyme lactate dehydrogenase.

### **TRICARBOXYLIC ACID CYCLE, CITRIC ACID CYCLE OR KREBS CYCLE**

Following the production of pyruvate from glycolysis in the cytosol of cell, a next cycle of events necessary for further energy production must take place within the mitochondrial correctly delineated powerhouse of the cell. Hence there is transport of pyruvate from the cytosol into the matrix of the mitochondria via special carriers, where it is then oxidatively decarboxylated in a series of steps to yield acetyl CoA, reaction catalysed by the multi enzyme complex called pyruvate dehydrogenase which is made up of pyruvate dehydrogenase( E1), dihyrolipoate transactylase (E2) and dihyrolipoate



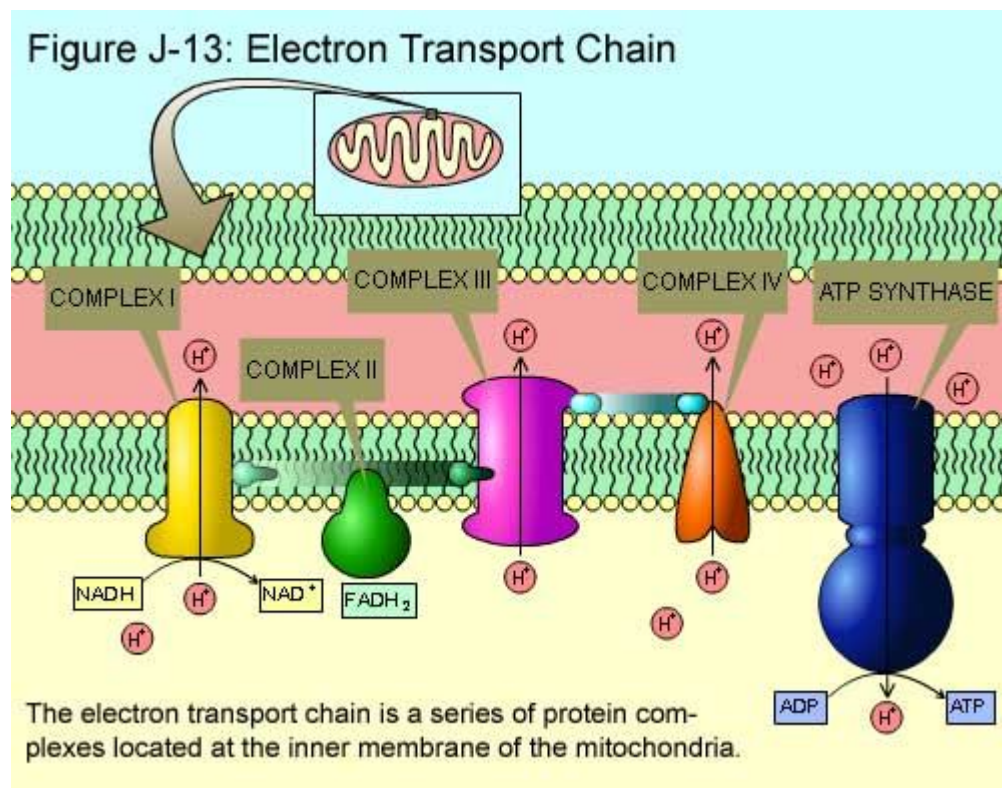
dehydrogenase (E3). Co factors involved in this reaction are  $Mg^{2+}$ , thiamine pyrophosphate. Lipoic acid, coenzyme A (CoA-SH).



The citric acid cycle.

The CAC has dual roles catabolic and anabolic.

The catabolic role involves oxidation of acetyl coA produced from carbohydrate, lipid and protein metabolism to produce ATP. While for the synthetic role involves the production of intermediates for synthesis of various compounds for example synthesis of non-essential amino acids from transamination of alanine, aspartate and glutamate to produce pyruvic acid, oxaloacetate and ketoglutarate respectively.; formation of glucose in the gluconeogenesis pathway via pyruvate., fatty acid, cholesterol and steroid synthesis through acetyl coA, heme synthesis form succinyl coA ,



The electron transport chain is a series of electron transfer from NADH and FADH<sub>2</sub> to oxygen occurring in the inner mitochondrial membrane and coupled to the generation of proton motive force., the energy stored in the electrochemical proton gradient is then used for ATP synthesis by the F<sub>0</sub>F<sub>1</sub> ATPase complex or ATP synthetase. The reactions in the glycolytic pathway and citric acid cycle result in the conversion of one glucose molecule to 6 CO<sub>2</sub> molecules and the concomitant reduction of 10 NAD<sup>+</sup> to 10 NADH molecules and 2 FAD to FADH<sub>2</sub> molecules.

These reduced co-enzymes are reoxidized in the ETC. components of the ETC are located on the inner mitochondrial membrane and are 4 distinct multiprotein complexes; complex I (NADH-Co Q reductase or NADH dehydrogenase), complex II (succinate-Q-reductase), complex III (cytochrome reductase, cytochrome b-c-1 complex) and complex IV (cytochrome oxidase) as well as two mobile elements coenzyme Q and cytochrome C. terminally there is the ATP synthase complex sometimes called complex V.

Complex I; it contains about 25 different proteins and contains a flavoprotein consisting of FMN and an iron sulphur protein (Fe-S). NADH donates 2 electrons to the FMN moiety which gets reduced to FMNH<sub>2</sub>. The 2 electrons are then transferred from the FMNH<sub>2</sub> to Fe-S from where the e<sup>-</sup>s are transferred to coenzyme Q (ubiquinone), in this process protons are driven out of the mitochondria matrix into the intermembrane space.

Complex II; here electrons from FADH<sub>2</sub> enter the ETC (FADH<sub>2</sub> bypasses the complex I, hence less ATP is generated from this, 2 instead of 3). It also receives the 2 electrons carried by coenzyme Q,

Coenzyme Q is reduced to semiquinone (QH) and then to quinol (QH<sub>2</sub>), these coenzymes can diffuse through the lipid layer, accept electrons from complex I and complex II and transfer them to complex III

Complex III; it is a multiprotein complex having a Cyt b and Cyt c 1 component, electrons are transferred from the quinol compound to the Cyt b and Cyt C1 respectively with shuttle of the iron in heme between Fe<sup>2+</sup> and Fe<sup>3+</sup> forms and the electrons are finally transferred to cytochrome C (the second mobile constituent). Protons are pumped out of the matrix too.

Cytochrome C is a single electron carrier and collects electrons from complex III and delivers it to complex IV. It has only one heme prosthetic group.

Complex IV it contains cytochrome a and cytochrome a<sub>3</sub>. It accepts 4 electrons from Cyt c and passes it to molecular oxygen to yield 2 molecules of water. It contains 2 heme groups and 2 copper ions. Protons are pumped out of matrix.



ATP synthase complex/F<sub>0</sub>F<sub>1</sub> ATPase; it is sometimes referred to as the fifth complex, it has two units F<sub>0</sub> and F<sub>1</sub>. The F<sub>0</sub> unit serves as a proton channel that channels the H<sup>+</sup> back into the matrix of the mitochondria. The F<sub>1</sub> unit catalyzes the synthesis of ATP from ADP and Pi. It has several chains that have binding sites for ATP, ADP and Pi and also catalytic sites.

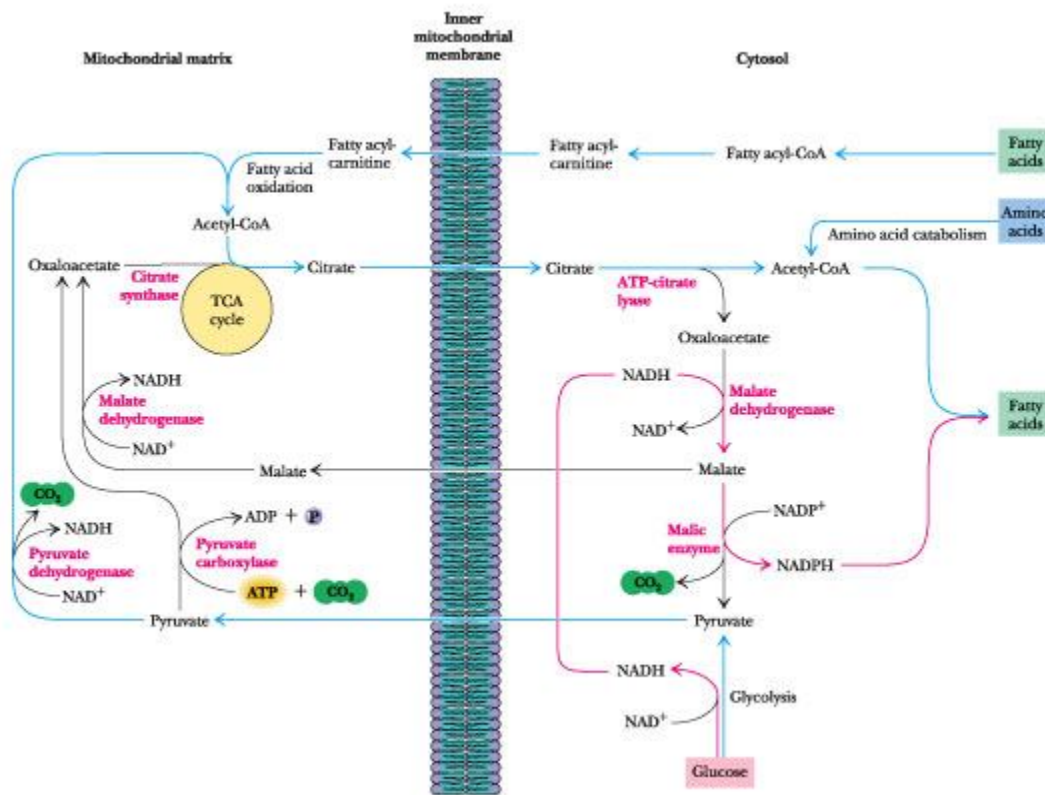
The accumulated protons in the inter-membrane space produce a gradient and an electrochemical potential difference, this proton motive force drives the synthesis of ATP when the protons are channeled back into the matrix through the ATP synthase.

Hence the oxidation of coenzymes or electrons in the chains is coupled to phosphorylation of ADP to yield ATP hence the term oxidative phosphorylation.

Defects in coupling of oxidation /phosphorylation may occur and this uncoupling causes a yield of heat.

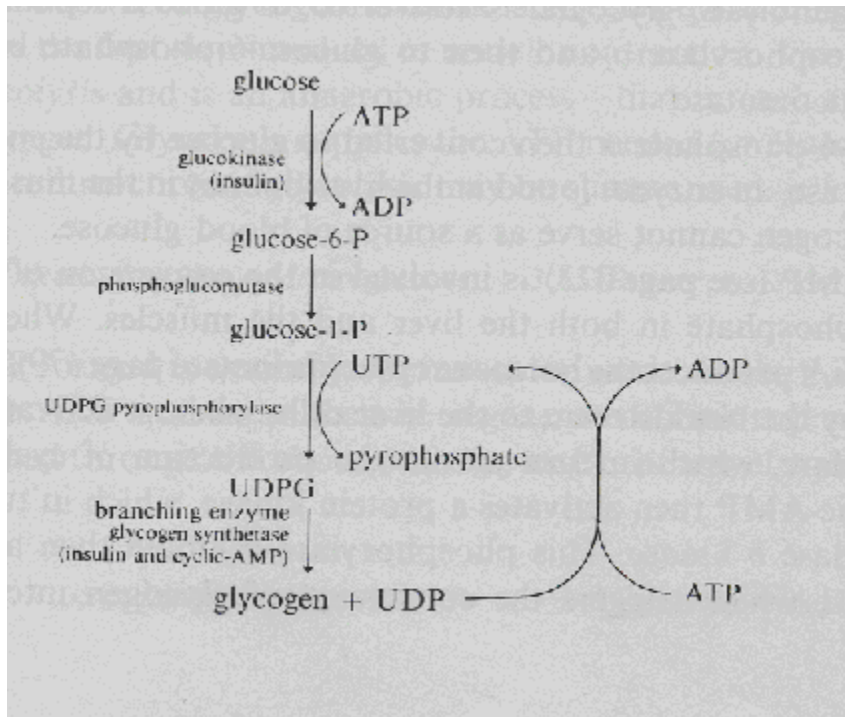
A total of 38 ATPs are produced from the complete oxidation of one molecule of glucose as well as 2 molecules of water and 6 CO<sub>2</sub>. Generation of ATP along the glycolytic pathway is called substrate level phosphorylation. While the same, from the ETChain (electron transport chain) is oxidative phosphorylation.

The malate-aspartate shuttle.



Malate-aspartate shuttle.

## GLYCOGENESIS



Glycogenesis: this is the formation of glycogen from glucose. It occurs majorly in liver and skeletal muscles but can also occur in some tissues to some extent (see pathway above)

- Glycogen is the storage form of carbohydrate in the animal body especially in the liver and muscles from where it is mobilized as glucose whenever tissue requires.
- Glycogen is insoluble and exerts no osmotic pressure and does not diffuse from its storage sites. It is also readily broken down under the influence of hormones and enzymes to either glucose (in the liver) and lower intermediates in skeletal muscles and other tissues for energy.
- Liver glycogen is the only immediately available source of glucose to the blood, glycogen is broken down to glucose -1-phosphate and then glucose for maintenance of blood glucose levels, and also serves as protection of liver from toxic substances in high concentration.
- Certain detoxification reactions e.g conjugation of compounds with glucuronic acids are influenced by liver glycogen levels.

## GLYCOGENOLYSIS

This is the breakdown of glycogen to glucose. This process is initiated by the phosphorylase enzyme which brings about phosphorylytic cleavage of the  $\alpha$ 1-4 linkage to yield G-1-P. The enzyme can exist as active or inactive forms (phosphorylated or dephosphorylated).

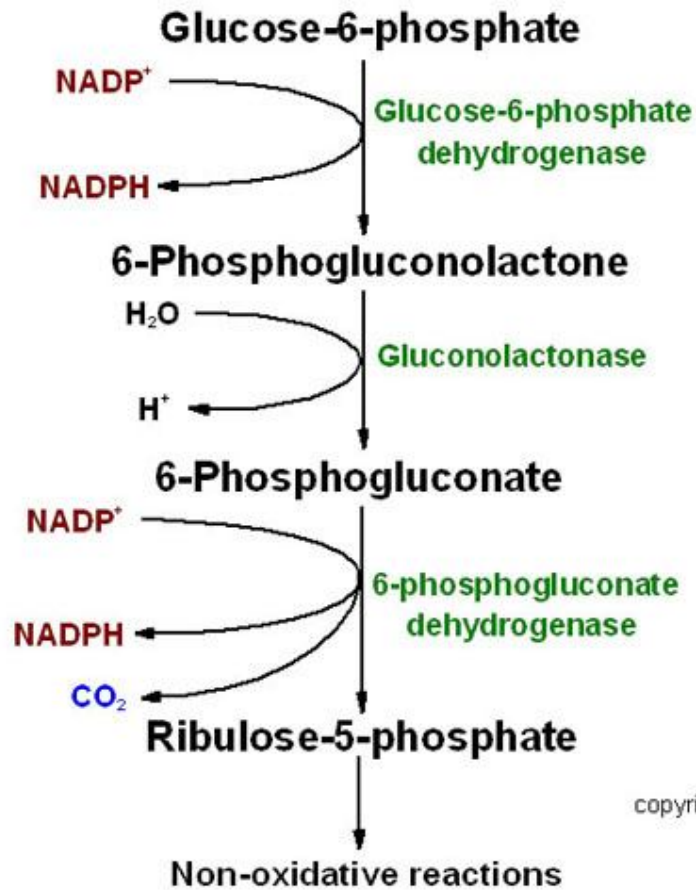
- The phosphorylase step is the first step and rate limiting and key enzyme in glycogenolysis with proper activation and in the presence of inorganic phosphate (Pi) the enzyme breaks down the glucosyl  $\alpha$ 1-4 linkages and removes by phosphorylytic cleavage the 1-4 glucosyl residues from outermost chains of the glycogen molecule until approximately 4 glucose residues remain on either side of a  $\alpha$ 1-6 branch (limit dextran).
- The phosphorylase liberates glucose as G-1-P.
- When 4 glucose residues are left from the branch point the another enzyme  $\alpha$ 1,4-1,6 glucan transferase transfers a trisaccharide unit from one side to the other thus exposing  $\alpha$ 1-6 branch point.
- The hydrolytic splitting of the  $\alpha$ 1-6 glucosidic linkage requires the action of a specific debranching enzyme (amylase 1-6 glucosidase).
- As the  $\alpha$ 1-6 linkage is hydrolytically split one molecule of free glucose is produced rather than one molecule of G-1-P as the case of phosphorylase cleavage.

## PENTOSE PHOSPHATE PATHWAY/HEXOSE MONOPHOSPHATE SHUNT/PENTOSE CYCLE/PHOSPHOGLUCONATE PATHWAY.

This is an alternate pathway of glucose metabolism. Energy is however not produced from this pathway but rather NADPH which is required for various reductive syntheses in the body; pentoses (5-carbon sugars) required for nucleic acid synthesis.

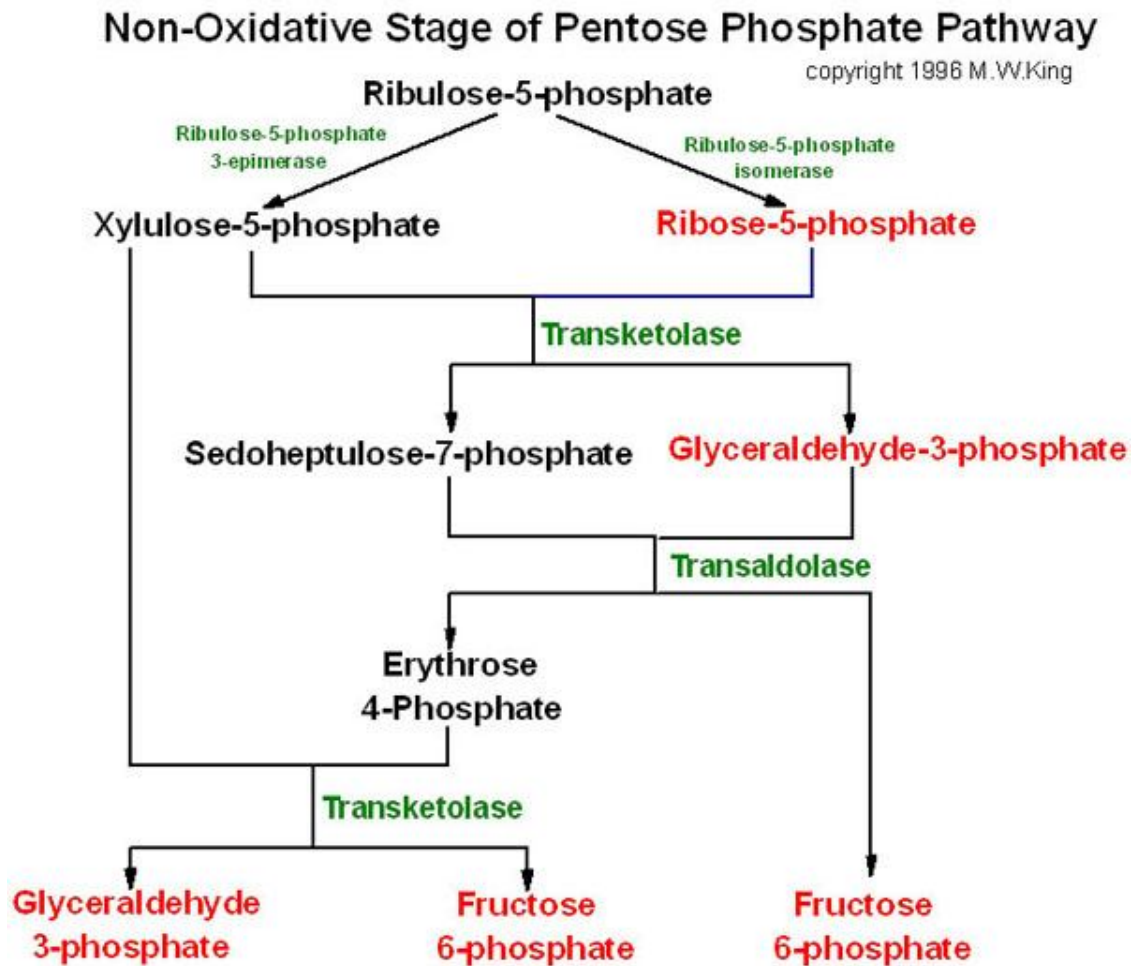
NADPH is used as an electron donor in such pathways as extra-mitochondrial de novo fatty acid synthesis, cholesterol and steroid synthesis, in conversion of oxidized glutathione G-S-S-G to reduced glutathione GSH ( which serves to remove  $H_2O_2$  from RBCs, maintenance of lens proteins), in synthesis of sphingolipids, microsomal denaturation of fatty acids, cytoplasmic synthesis of L-glutamate by the L-glutamate dehydrogenase, conversion of phenylalanine to tyrosine, as coenzyme for methemoglobin reductase for its conversion to Hemoglobin(Hb), in the uronic acid pathway, for production of superoxide ion by NADPH reductase useful for leucocytes phagocytosis etc

## Oxidative Stage of Pentose Phosphate Pathway



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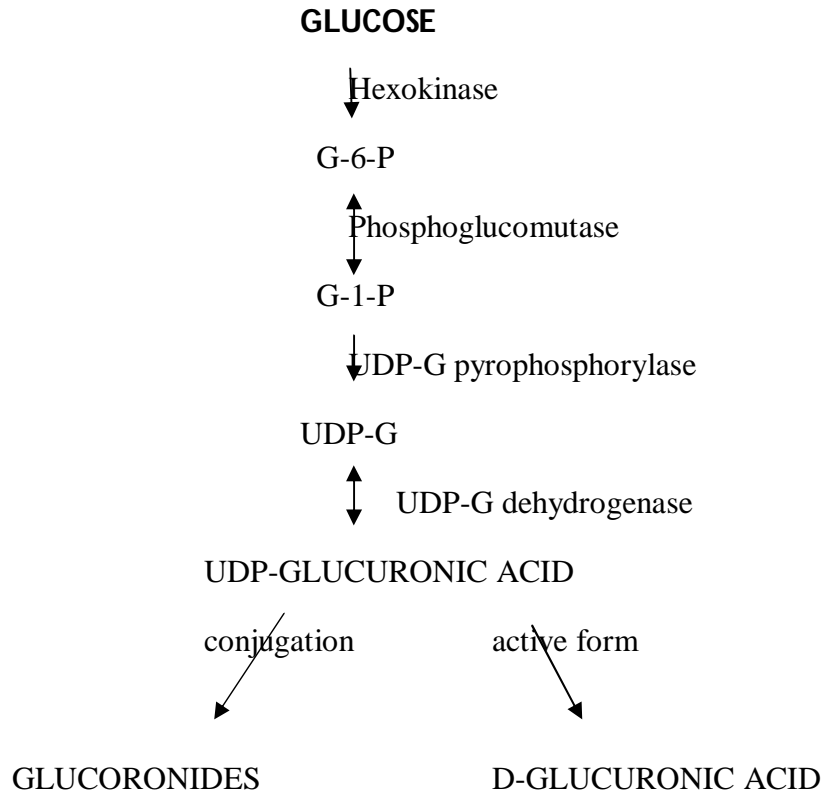


#### URONIC ACID PATHWAY.

Another alternative pathway of glucose oxidation also devoid of energy production but useful for the production of glucuronic acid which is mainly utilized for detoxification of foreign chemicals (xenobiotics). It is also important in synthesis of mucopolysaccharides.

Inherited deficiency of an enzyme in this pathway leads to a condition called inherited pentosuria.



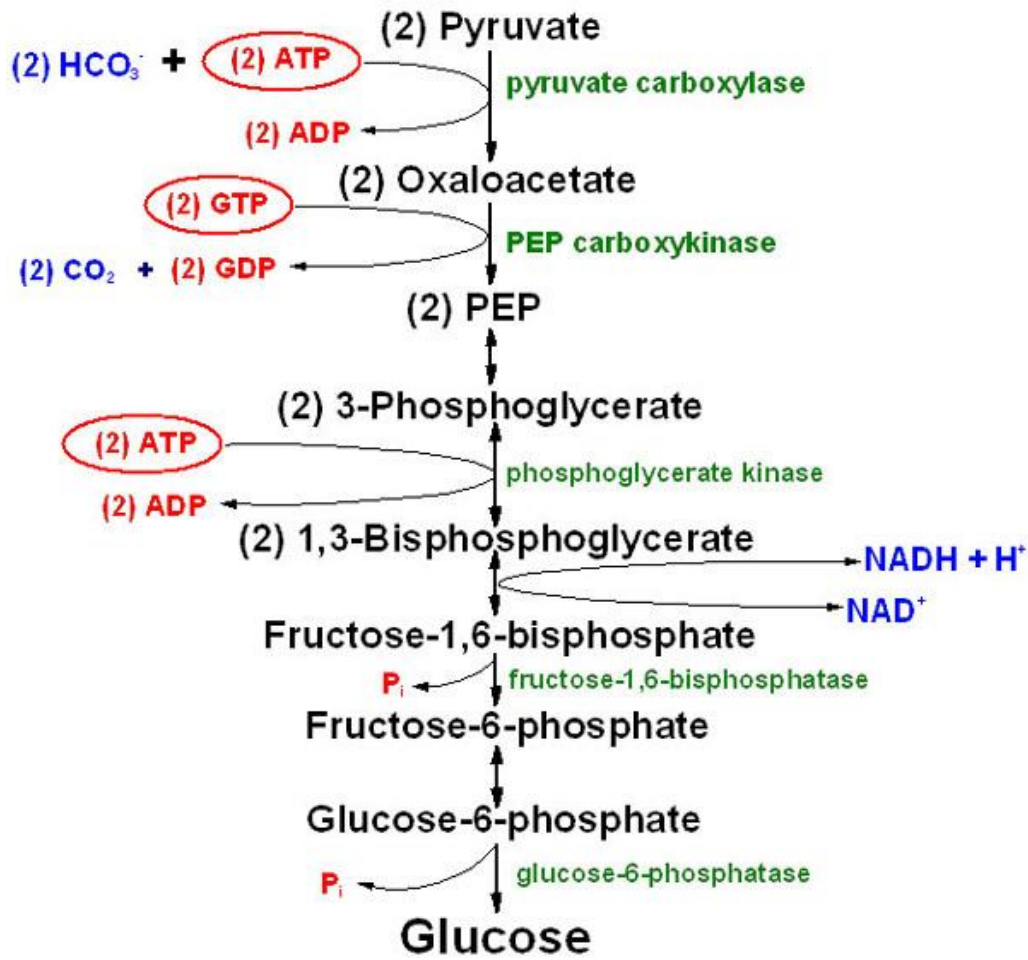


## GLUCONEOGENESIS

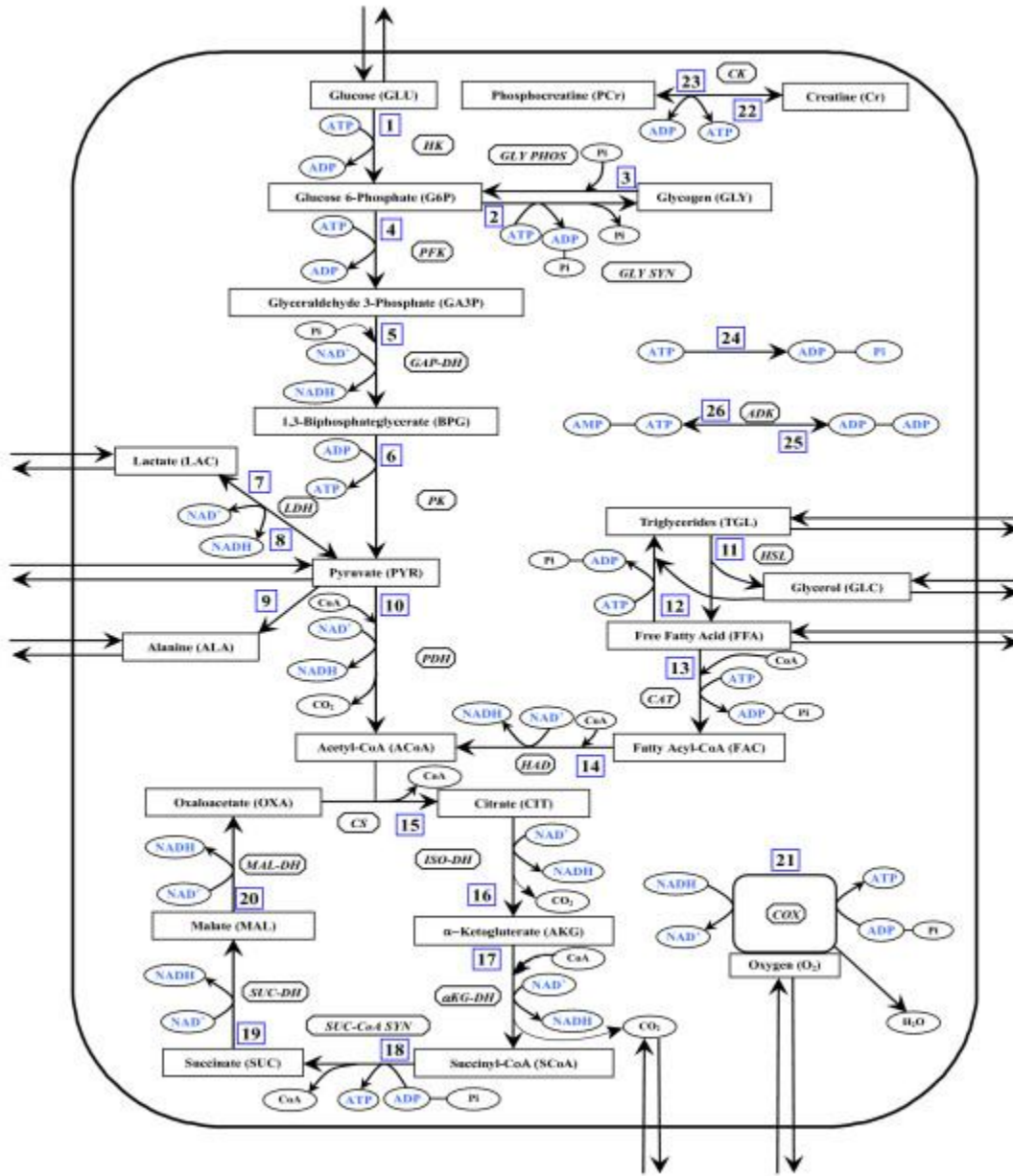
This is the formation of glucose from non-carbohydrate sources. It is required to meet the body's need for glucose when carbohydrates are not available or insufficient. And is also required for the maintaining levels of intermediates of the CAC and to clear products of metabolism of other tissues from blood e.g. lactic acid produced by muscles, glycerol by adipose tissue etc.

The principal site of gluconeogenesis is liver, kidney as they possess the full complement of enzymes necessary for the pathway.

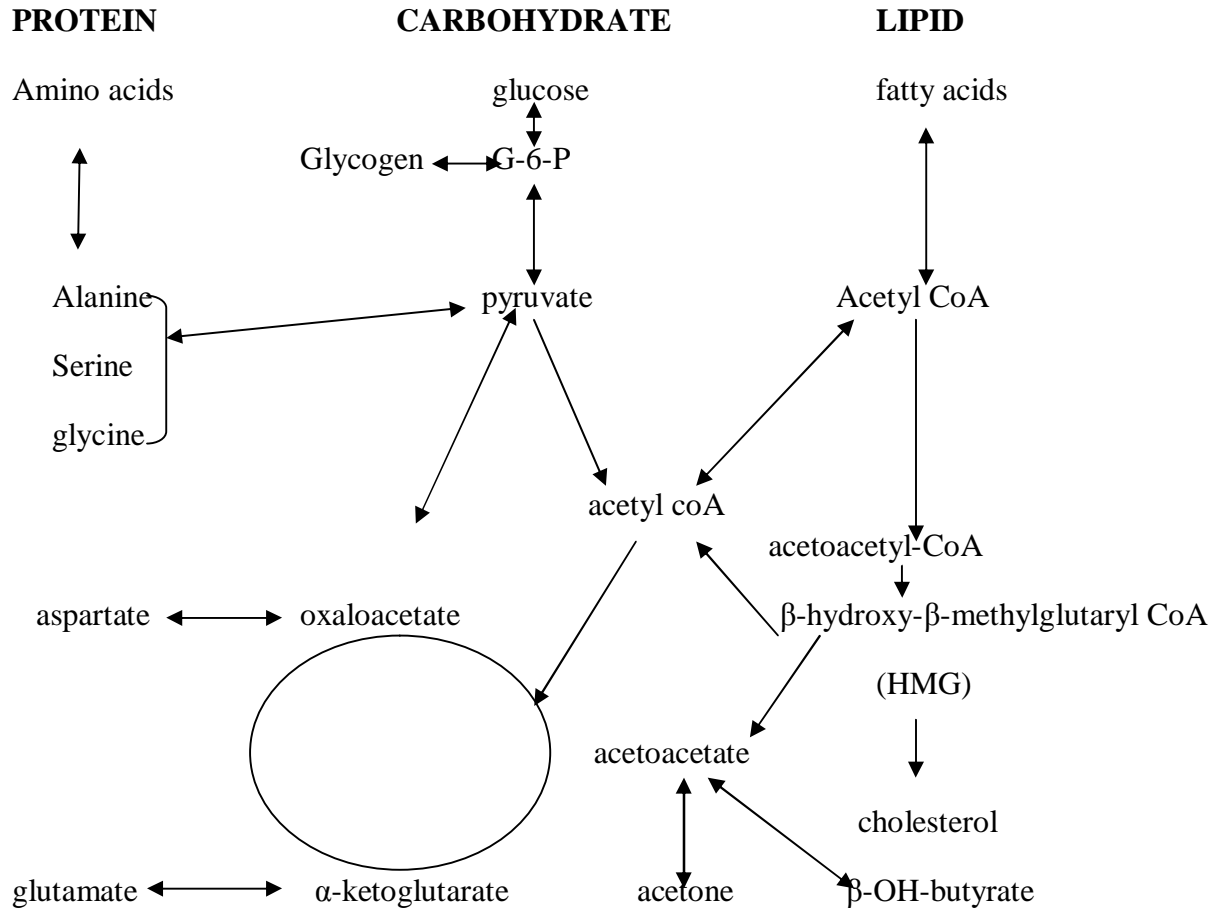
Gluconeogenesis substrates gluconeogenic amino acids forming pyruvate, oxaloacetate and  $\alpha$ -ketoglutarate (aspartate, glycine, alanine, serine, cysteine, threonine, glutamate, glutamine, proline, arginine, histidine, lysine); lactates and pyruvate, glycerol and propionic acid.



# INTERRELATIONSHIP BETWEEN CARBOHYDRATE, LIPID AND PROTEIN METABOLISM



Intergration of carbohydrate, lipid and protein metabolism.



## **DISEASES/DISORDERS OF CARBOHYDRATE METABOLISM**

- Diabetes mellitus;
- Hyperinsulinism
- Hypoglycemia of pigs (undeveloped gluconeogenic mechanisms)
- Glycogen storage diseases (GSD).

## **ENZYMES**

### **OCCURRENCE**

Enzymes are produced by all living organisms including humans and present only in small amounts.

### **MEDICAL AND BIOLOGICAL IMPORTANCE**

1. Enzymes are the chemical work horses of the body. Enzymes are biological catalysts that speed up the pace of chemical reactions.
2. A chemical reaction without an enzyme is like a drive over a mountain. The enzyme bores a tunnel through it so that passage is far quicker and takes much less energy.
3. Enzymes make life on earth possible, all biology from conception to the dissolution that follows death depends on enzymes.
4. Enzymes regulates rate of physiological process. So, defects in enzyme function cause diseases.
5. When cells are injured enzymes leak into plasma. Measurement of activity of such enzymes in plasma is an integral part of modern day medical diagnosis.
6. Enzymes are used as drugs.
7. Immobilized enzymes, which are enzymes attached to solid supports are used in clinical chemistry laboratories and in industry. For example glucose in blood or urine is detected by using immobilized glucose oxidase. In pharmaceutical industry, glucose isomerase is used to produce fructose from glucose.
8. Enzymes are used as biosensors.
9. AIDS detection involves use of enzyme dependent ELISA technique.
10. Enzymes are used as cleansing agents in detergent industry.

### **CHEMICAL NATURE OF ENZYMES (PROPERTIES)**

1. All the enzymes are proteins except ribozymes and a number of enzymes are obtained in the crystalline form.
2. In 1878, Kuhne, introduced term 'Enzyme' to indicate biological catalyst.
3. Enzymes cut big molecules apart and join small molecules to form big molecules.
4. Most of the chemical reactions in the body are enzyme catalysed.

5. The substance upon which an enzyme acts is called as substrate. By the action of enzyme it is converted to product. An enzyme-catalysed reaction consist of substrate, enzyme and product as shown below.

Substrate → Product

6. The enzymes are big particles. Their molecular (size) weight ranges from few thousands to millions.

7. Enzymes have enormous power of catalysis. They increase rate of reaction to  $10^5$  to  $10^{10}$  folds. For example, carbonic anhydrase can hydrate to  $10^6$  molecules of  $\text{CO}_2$  per second. In the absence of enzyme hydration of  $\text{CO}_2$  is 10–1 per second.

8. Enzymes are far more efficient compared to non-enzyme (man made) catalysts.

9. Enzymes are not consumed in the overall reaction.

10. Enzymes accelerate the rate of reaction but does not alter the equilibrium constant ( $K_{eq}$ ).

To know how enzymes work, physical chemistry of catalysts must be explored because enzymes are catalysts.

### **Catalysts**

A catalyst does not change the chemical reaction but it accelerates the reaction. They are not consumed in overall reaction. But they undergo chemical or physical change during reaction and returns to original state at the end of reaction. Transition state theory was proposed to explain action of catalyst.

For a chemical reaction  $A \rightarrow B$  to occur, energy is required. When enough energy is supplied. A undergoes to transition state which is an unstable state. So, it gets converted to product B which is more stable. The amount of energy needed to convert a substance from ground state to transition state is called *activation energy*. In presence of catalyst, A undergoes to transition state very fast and requires less energy . Hence, a catalyst accelerate the rate of reaction by decreasing the energy of activation. Likewise enzymes also speed up reaction by lowering energy of activation. Further, the activation energy is very much less for a reaction in presence of enzyme than non-enzyme catalyst . Therefore enzymes are more efficient than non-enzyme catalyst.

### **ENZYME SPECIFICITY**

Enzymes are highly specific compared to other catalyst. An enzyme catalyzes only specific reaction. Some general types of enzyme specificity are:

#### **1. Substrate Specificity**

Enzymes are specific towards their substrates. For example, glucokinase catalyzes the transfer of phosphate from ATP to glucose. Galactokinase catalyzes transfer of phosphate from ATP to galactose. Though both enzymes catalyzes transfer of phosphate from ATP they act only on specific substrate. Similarly, transaminase which catalyze transfer of amino group are specific to substrate. Aspartate transaminase catalyzes the transfer of amino group from

aspartate and alanine transaminase catalyzes transfer of amino group from alanine only. So, they are specific towards substrate.

## **2. Reaction Specificity**

A given enzyme catalyze only one specific reaction. For example, lipases only hydrolyze lipids, urease hydrolyzes urea. They do not catalyze any other type of reaction. Likewise amino acid oxidase catalyze oxidation of amino acid and decarboxylase catalyze only decarboxylation of amino acids.

## **3. Group Specificity**

Some lytic (hydrolases) enzymes act on specific groups. Proteases are specific for peptide groups, glycosidases are specific to glycosidic bonds.

Proteins Amino acids

## **4. Absolute Group Specificity**

Certain lytic enzymes exhibit high order group specificity. For example, chymotrypsin is protein splitting enzyme *i.e.*, it hydrolyzes peptide bonds. But it preferentially hydrolyzes peptide bonds in which carboxyl group is contributed by aromatic amino acids phenylalanine, tyrosine and tryptophan. Likewise, trypsin another peptide bond hydrolyzing enzyme preferentially hydrolyzes peptide bonds in which carboxyl group is contributed by basic amino acids.

Amino peptidase Chymotrypsin Trypsin Carboxypeptidase

H N Ala 2 Gly Val Tyr Glu Ala Ile Arg Ala Gln Asp COOH

Similarly, carboxy peptidase removes one amino acid each time from carboxy terminus and amino peptidase removes one amino acid each time from N-terminus. Thrombin of blood clotting process is highly specific for Arg-Gly-bonds.

## **5. Optical Specificity**

Several enzymes exhibit optical specificity of substrate on which they act. It means enzymes are able to recognise optical isomers of the substrate. For example, enzymes of amino acid metabolism act only on L-isomers (L-amino acid) but not D-isomers (D-amino acids). Likewise enzymes of carbohydrate metabolism act only on D-sugars but not on L-sugars.

## **Enzyme Classification and Nomenclature**

International Union of Biochemistry classified all enzymes into six major classes based on the type of reaction they catalyze and reaction mechanism.

### **Nomenclature**

The name of an enzyme has two parts. The first part indicates name of its substrate and second part ending in 'ase' indicates the type of reaction it catalyzes. Further, each enzyme has code (EC) number. It is a four-digit number. The first digit indicates major class, second digit indicates sub class, third digit denotes sub sub class and final digit indicates specific enzyme.

The six major classes of enzymes with some example are:

### **1. Oxidoreductases**

They catalyze oxidation and reduction reactions.

## **2. Transferases**

They catalyze transfer of groups

## **3. Hydrolases**

They catalyze hydrolysis of peptide, ester, glycosyl etc. bonds.

## **4. Lyases**

They catalyze removal of groups from substrates by mechanisms other than hydrolysis forming double bonds.

## **5. Isomerases**

They catalyze interconversion of optical, functional and geometrical isomers.

## **6. Ligases**

They catalyze linking together of two compounds. The linking is coupled to the breaking of phosphate from ATP.

## **MECHANISM OF ENZYME ACTION**

The mechanism of enzyme action deals with molecular events associated with conversion of a substrate to product in an enzymatic reaction.

### **Medical Importance**

1. Some drugs are designed based on mechanism of enzyme action. For example, X-ray crystallographic studies on mechanism of carboxy-peptidase action lead to design of specific inhibitor to angiotensin converting enzyme like captopril which is used in treatment of hypertension.
2. Enzymes with specific properties can be designed based on mechanistic studies. They may be introduced into humans to correct specific abnormalities associated with disorders.

The larger size of an enzyme molecule relative to smaller size of its substrate always puzzled biochemists. Ultimately it led to the concept that small portion of enzyme is required for enzyme action. This part of the enzyme is known as active site.

## **CHARACTERISTICS OF AN ENZYME ACTIVE SITE**

1. It consists of two parts.
  - (a) **Catalytic site.** It is the portion (part) of the enzyme that is responsible for catalysis. It determines reaction specificity. Occasionally, catalytic site and active site are used synonymously.
  - (b) **Binding site.** It is the part of the enzyme that binds with substrate. It determines substrate specificity.
2. The active sites of enzyme are clefts within the enzyme molecule. For example, the active site of ribonuclease lies within cleft .
3. Active site consists of few amino acid residues only.
4. Active site is three dimensional.
5. The active site is contributed by amino acid residues that are far apart in the enzyme



molecule. During catalysis, they are brought together.

6. The amino acids at the active site are arranged in a very precise manner so that only specific substrate can bind at the active site.

7. Usually serine, histidine, cysteine, aspartate or glutamate residues make up active site.

Enzymes are named according to the active site amino acid. For example, trypsin is a serine protease and papain is cysteine protease.

## **MODELS OF ACTIVE SITE**

Some active site models are proposed to explain enzyme specificity.

### **A. Lock and Key Model**

1. According to this model the active site is a rigid portion of the enzyme molecule and its shape is complementary to the substrate like lock and key.

2. The complimentary shape of substrate and active site favours tightly bound enzyme. Substrate complex formation followed by catalysis.

3. This model was unable to explain the possibility of rigid active site combining with the product to form substrate in reversible reaction.

### **B. Induced Fit Model**

1. According to this model, the active site is flexible unlike rigid type of the lock and key model.

2. In the enzyme molecule the amino acid residues that make up active site are not oriented properly in the absence of substrate.

3. When substrate combines with enzyme, it induces conformational change in the enzyme molecule in such way that amino acids that make active site are shifted into correct orientation to favour tightly bound enzyme-substrate complex formation followed by catalysis.

4. The enzyme molecule is unstable in the induced conformation and returns to its native conformation in the absence of substrate.

## **FACTORS AFFECTING ENZYME ACTION**

Rates of enzyme catalyzed reactions are affected by:

1. Enzyme concentration
2. Temperature
3. Hydrogen ion concentration or pH
4. Substrate concentration
5. Inhibitors and cofactors

## **MEDICAL AND BIOLOGICAL IMPORTANCE**

1. For normal health, all enzymatic reactions must occur in the body and they must proceed at appropriate rates. Alterations in the rates of enzymatic reactions may disturb tissue homeostasis.
2. Any alteration in intracellular pH disturbs rates of enzyme reactions.
3. Organs for transplantation, blood and serum are preserved at low temperature as soon as they are removed from body because enzymatic reactions proceed at much lower rate at low temperature. Under such conditions, O<sub>2</sub> demand of cells decreases, so cells of the organs or fluids survive with available O<sub>2</sub> for some time.
4. Rates of enzymatic reactions are altered in fever and hypothermia because temperature influences rate of enzyme reaction.
5. An understanding of factors affecting enzyme action is required for development of drugs. Many drugs act by decreasing rate of key metabolic reaction by blocking that particular enzyme. For example, AZT used in treatment of AIDS is an inhibitor of HIV virus enzyme. Lovastatin is used in treatment of atherosclerosis is an inhibitor of HMGCoA reductase, a cholesterol producing enzyme, captopril used in the treatment of hypertension is an inhibitor of angiotensin converting enzyme an enzyme of blood pressure regulation.
6. Some poisons work by abolishing (affecting) essential enzymatic reactions.

### 1. Enzyme concentration

The rate of enzyme catalyzed reaction is directly proportional to the concentration of enzyme.

### 2. Temperature

Like any chemical reaction, enzyme activity increases with increase in temperature initially. After a critical temperature, the enzyme activity decreases with increase in the temperature. When the effect of temperature on enzyme activity is plotted, cone-shaped curve is obtained. The figure indicates that there is an optimal temperature at which enzyme is optimally active. It is called as *optimum temperature*. For most of the enzymes, the optimum temperature is the temperature of the cell or body in which they occur. For example, human trypsin the optimum temperature in 37 °C which is the normal body temperature. The first half of the curve approaching the optimum temperature indicates that enzyme activity increased with increase in the temperature due to the increased kinetic energy of reacting molecules. The other half which corresponds to decreased catalytic activity with increased temperature is due to denaturation of enzyme.

Enzymes of plants and micro-organisms growing in hot climates or hot springs may exhibit optimal temperature close to the boiling point of water. Examples are enzymes of thermophilic bacteria, snake venom phospholipase and urease (55 °C).

### 3. Effect of pH or hydrogen ion concentration

Most of the enzymes are not maximally active throughout pH scale (1-14). Several enzymes has optimum activity between pH of 5 to 9. When enzyme activity measured at several pH values is plotted a bell shaped curve is obtained.

Since enzymes are proteins pH changes affects:

1. Charged state of catalytic site.
2. Conformation of enzyme molecules.

In addition low or high pH causes denaturation of enzymes. It accounts for the less activity of enzymes at acidic or alkaline pH. For most of the enzymes, optimum pH is the pH of body or cell in which they occur. However, for some enzymes optimum pH may not be in the neutral range.

In the case of oligomeric enzymes, optimum pH is required for the association of protomers. When the pH is altered, the protomers dissociate with loss of biological activity.

#### 4. Effect of substrate concentration

If the concentration of the substrate (S) is increased while other conditions are kept constant, the initial velocity  $v_0$  (velocity measured when little substrate is reacted) increases proportionately in the beginning. As the substrate concentration continues to increase, the increase in  $v_0$  slows down and reaches maximum  $V_{max}$  and no further. The plot of (S) versus  $v_0$  is rectangular hyperbola. It is called as *Michaelis plot*. To explain the reason for characteristic shape of the curve, Michaelis proposed that in an enzyme catalyzed reaction, the enzyme (E) combines with substrate (S) to form an enzyme-substrate (ES) complex which decomposes to form product (P) and free enzyme.

Based on this, reasons for the three phases of the curve can be interpreted.

1. In the first phase, substrate concentration is low and most of the enzyme molecules are free so they combine with the substrate molecules. Therefore, velocity is proportional to substrate concentration. At this state, enzymatic reaction shows first-order kinetics.
2. In the second phase, half of the enzyme molecules are bound to substrate, so the velocity is not proportional to substrate concentration. At this stage, enzymatic reaction shows mixed-order kinetics.
3. In the third phase, all the enzyme molecules are bound to substrate, so velocity remain unchanged because free enzyme is not available, although the substrate is in excess. At this stage enzymatic reaction shows zero-order kinetics.

The Michaelis plot is used to determine Michaelis constant a characteristics of enzyme and type of enzyme inhibition.

### **Michaelis Constant or $K_m$**

The substrate concentration that produces half the maximal velocity ( $V_{max}/2$ ) is known as Michaelis constant. Apart from graph  $K_m$  also can be determined from Michaelis-Menten equation. It is a simple equation and describes the dependence of initial velocity ( $v_0$ ) on the concentration of enzyme and substrate. It is the theoretical expression for rectangular hyperbola.

Significance of  $K_m$

1. It is an enzyme kinetic constant.
2. It indicates the substrate concentration required for the enzyme to work efficiently.

3. Low  $K_m$  indicates high affinity of enzyme towards substrate. High  $K_m$  indicates low affinity of enzyme towards substrate. Hence,  $K_m$  and affinity are inversely related. ( $K_m \propto 1/\text{affinity}$ ).

**Example:** Hexokinase and glucokinase both phosphorylates glucose. However, hexokinase can phosphorylate glucose 2000 times more efficiently than glucokinase because  $K_m$  of hexokinase is low ( $1 \times 10^{-5}$  M) whereas  $K_m$  of glucokinase is high ( $2.0 \times 10^{-2}$  M).

4.  $K_m$  is required when enzymes are used as drugs.

5. Use of enzymes in immunodiagnosics (ELISA) require  $K_m$  of the enzyme.

### Line Weaver–Burk Plot

1. Michaelis plot gives only approximate  $K_m$  and  $V_{max}$  values because proper  $V_{max}$  is difficult to obtain at very high substrate concentration.

2. By using Line Weaver-Burk Plot accurate  $K_m$  and  $V_{max}$  are obtained.

3. Line Weaver-Burk Plot is obtained by taking reciprocals of both sides of Michaelis-Menten equation

The equation represents  $Y = ax + b$  straight line equation with slope of  $K_m/V_{max}$ .

Further straight line is obtained by plotting  $1/S$  against  $1/V$ . Since  $1/S$  and  $1/V$  are reciprocals of  $S$  and  $V$ , respectively. This plot is known as “double reciprocal plot”.

4. The straight line intersects y-axis, which corresponds to  $V_{max}$  value. A line extended from point of intersection to x-axis of second quadrant provides  $K_m$  value (Fig. 4.6 c).

5. In addition to  $K_m$  and  $V_{max}$  values, type of inhibition is determined using this plot.

6. Inhibition constant ( $K_i$ ) of inhibitor is also determined using this plot.

Significance of  $K_i$

1.  $K_i$  indicates affinity of inhibitor towards enzyme. Like  $K_m$ ,  $K_i$  is inversely related to affinity.

2. Use of inhibitors as drugs requires knowledge of  $K_i$ . Since  $K_i$  and affinity are inversely related inhibitors of low  $K_i$  are highly potent drugs.

Direct Linear Plot

1. Determination of  $K_m$  and  $V_{max}$  values of enzymes, which are inhibited by substrate at high concentration is not feasible with Line Weaver-Burk plot.

2. In such cases, direct linear plot is used for  $K_m$  and  $V_{max}$  determination. They are read directly from plot without involving any calculation.

3. In this plot, each  $S$  and  $V$  are marked on  $X$  and  $Y$  axes, respectively. Then a straight line passing through two points and extending into first quadrant is drawn. When lines for all  $S$  and  $V$  values are drawn they intersect at common point which provides  $K_m$  and  $V_{max}$ .

## INHIBITORS

Substances that decrease the catalytic activity of enzymes are called as inhibitors. They may be protein or non-protein inhibitors. The decrease in enzyme activity is called as inhibition. More than two types of enzyme inhibition exist based on the mode of action of inhibitors.

### Competitive Inhibition

Competitive inhibition occurs at active site. Competitive inhibitor is structurally similar to that of substrate. Hence, it competes with substrate to bind at active site. Inhibition occurs when it binds at the active site of enzyme molecule. It is reversible. If the substrate concentration is increased then the competitive inhibition is relieved. Further, the rate of formation of product from (ES) complex is same as that of in the absence of inhibitor. So, velocity ( $V_{max}$ ) is not altered in competitive inhibition but  $K_m$  increases (affinity of enzyme towards substrate decreases) because of competition of substrate and inhibitor to bind at active site. The interaction of enzyme (E) substrate (S) and competitive inhibitor (I) is represented as equations below: In addition at high substrate concentration, the number of enzyme molecules available for the inhibitor are far less. So, the inhibition is masked. (becomes reversible.) Michaelis plot also indicates  $K_m$  alternation and unaffected  $V_{max}$  in the presence of competitive inhibitor. A classic example for reversible competitive inhibition is succinate dehydrogenase enzyme. Malonate competitively inhibits the enzyme because it is structurally similar to the substrate succinate.

### Competitive Inhibitors as Chemotherapeutic Agents

When used in clinical situations, the competitive inhibitors are called as *antagonists* or *anti-metabolites* of the substrate with which they compete. The use of anti-metabolites in the treatment of diseases is called as *chemotherapy*. Therefore, competitive inhibitors are useful chemotherapeutic agents. They are used

1. Antibiotics
2. Anti-cancer drugs
3. In the treatment of metabolic diseases like gout, atherosclerosis and hypertension.
  1. Sulfonamide antibiotics are used in the treatment of bacterial infections. Bacteria synthesize folic acid from p-aminobenzoic acid (PABA). Since these sulfonamide drugs contain sulfonilamide a structural analog of PABA, when used as chemotherapeutic agent, it blocks the synthesis of folic acid in bacteria. The lack of folic acid leads to death of bacteria. Sulfonamide act as competitive inhibitor for the enzyme involved in the formation of folic acid using PABA as substrate.  
Precursor Folic acid Bacterial growth arrest PABA blocked
  2. Competitive inhibitors used in the treatment of cancer are aminopterin and amethopterin (methotrexate). They are structural analog of folic acid. They are competitive inhibitors for the enzyme dihydrofolate reductase. They are used in the treatment of leukaemia, a type of cancer.

When used these drugs block formation of nucleic acids. For cell proliferation, nucleic acid are needed. So, lack of nucleic acids lead to arrest of tumour growth and advancement of cancer is prevented.

3. Allopurinol is a drug used in the treatment of gout. Gout is due to excessive production of uric acid. Xanthine oxidase is an enzyme involved in the formation of uric acid from hypoxanthine. Allopurinol is a structural analog of hypoxanthine and hence it is an antimetabolite of hypoxanthine. When it is used it blocks formation of uric acid by inhibiting the enzyme xanthine oxidase .

4. Lovastatin is a competitive inhibitor of enzyme HMG-CoA reductase, when used it blocks production of cholesterol. In atherosclerosis, cholesterol is more. Lovastatin reduces cholesterol formation thus arrest the advancement of atherosclerosis.

5. Competitive inhibitors used in the treatment of hypertension are captopril, lisinopril and enalapril. They competitively inhibit angiotensin converting enzyme, which is involved in regulation of blood pressure. When used they lower blood pressure by reducing activity of angiotensin converting enzyme.

### **Non-Competitive Inhibition**

In this type of enzyme inhibition no competition occurs between substrate and inhibitor to bind at active site of enzyme. Inhibitor is not structurally related to substrate. In addition inhibitor binds to some other site of enzyme which is far off from active site.

In non-competitive inhibition, the inhibitor can react with free enzyme as well as enzyme substrate complex, because its binding site is away from active site. In addition, the formation of product from enzyme substrate-inhibitor complex is not same as that of in absence of inhibitor. So, the  $V_{max}$  is decreased and  $K_m$  (affinity) remains same because no competition of substrate and inhibitor in non-competitive inhibition. Michaelis plot also indicates same in the presence of non-competitive inhibitor.

### **Examples for Non-competitive Inhibition**

Reversible non-competitive inhibitors are rare. Most of the known non-competitive inhibitors are irreversible. They are referred as enzyme poisons.

1. Iodoacetate blocks the formation of 1,3-bisphosphoglycerate from glyceraldehyde-3-phosphate by inhibiting enzyme glyceraldehyde-3-phosphate dehydrogenase

2. Fluoride blocks the action of enolase, which converts 2-phosphoglycerate to phosphoenol pyruvate.

3. Heavy metals like  $Hg^{2+}$ ,  $Ag^+$ ,  $Pb^{2+}$  and Arsenite are also enzyme poisons. They interact with  $-SH$  group of enzyme and activate it.

$Hg^{2+}$  inhibits  $-SH$  containing pyruvate dehydrogenase. Similarly, arsenite inhibits  $-SH$  containing  $\alpha$ -ketoglutarate dehydrogenase.

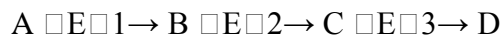
4. Some non-competitive inhibitors are used as pesticides. DDT, melathion and parathion are inhibitors of enzyme choline esterase that catalyzes hydrolysis of acetylcholine.

5. Di-isopropyl fluoro phosphate (DFP) is a non-competitive inhibitor used as nerve gas in World War II. It is an active site directed irreversible non-competitive inhibitor. It forms covalent linkage with –OH groups of serine residue of choline esterase. When used DFP causes constriction of larynx, pain in eyes and mental confusion.
6. CN<sup>-</sup> inhibits activity of cytochrome oxidase an enzyme of respiratory chain. Bitter almonds contain some cyanide.
7. Ethylene diaminetetra acetic acid (EDTA) inhibits metalloenzymes by forming complex with metal ion.
8. Tubers, bananas and beans contain inhibitors to trypsin, chymotrypsin and elastase.

### **FEEDBACK INHIBITION**

Inhibition of activity of enzyme of a biosynthetic pathway by the end product of that pathway is called as feedback inhibition.

For example, formation of a substance D from A is catalyzed by three enzymes E1, E2 and E3.



When enough D is formed it inhibits the activity of E1. By inhibiting E1, D regulates its own synthesis.

#### **Examples:**

1. Inhibition of aspartate trans carbamoylase by CTP.
2. Inhibition of HMG-CoA reductase by cholesterol.
3. Inhibition of ALA-synthase by heme.
4. Inhibition of anthranilate synthetase by tryptophan.

### **CO-FACTORS**

1. Cofactors are non-protein molecules required for activity of some enzymes. They may be involved in catalysis or in structure maintenance.
2. There are two types of cofactors:
  1. Organic cofactors, and
  2. Inorganic cofactors.

The organic cofactors are further subdivided into. 1. Prosthetic groups 2. Co-enzymes.

#### **1. Prosthetic Groups**

These organic molecules are covalently attached to the enzyme and they undergo change during catalysis but return to native state at the end of the reaction.

#### **2. Co-enzymes**

These organic molecules are loosely (non-covalent) attached to enzyme molecules. They undergo change during reaction. Since they undergo change along with substrate they are referred as co-substrates.

Apo-enzyme + Co-enzyme = Holo enzyme

2. Inorganic cofactors.

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(Protein) (Non-protein) (Active).

### Examples of Organic Co-factors

The major function of water soluble vitamins is to serve as co-factors, some of them as such serve as co-factor otherwise their derivatives serve as co-factors. They are divided on the basis of their function, in enzymatic reaction.

1. Co-enzymes of oxidation reduction reactions.

(a) Co-enzymes derived from niacin. They are NAD<sup>+</sup>, NADH + H<sup>+</sup> and NADP<sup>+</sup>, NADPH + H<sup>+</sup>. These co-enzymes are loosely bound to apo-enzymes.

(b) Co-enzymes derived from riboflavin. They are FMN, FMNH<sub>2</sub> and FAD, FADH<sub>2</sub>.

They are covalently linked to apo-enzymes. So, they are prosthetic groups.

2. Coenzymes of group transfer reactions.

(a) Co-enzyme of pantothenic acid. It is co-enzyme of A(CoA, CoASH). It is involved in CoA transfer reaction.

(b) Co-enzyme of thiamin. It is thiamnin pyro(di)phosphate (TPP, TDP). It is prosthetic group of several enzymes.

Pyruvate Pyruvate dehydrogenase Acetyl-CoA

TPP CoASH

(c) Co-enzyme of pyridoxine. It is pyridoxal phosphate (P-PO<sub>4</sub>). It is prosthetic group of enzymes involved in amino group transfer. Other reactions where it serve as coenzyme are decarboxylation, transulfuration etc.

(d) Co-enzymes of folic acid. It is tetrahydrofolate (FH<sub>4</sub>). It participates in one carbon transfer reaction.

(e) Biotin is the only water-soluble vitamin that function as coenzyme as such. It is the prosthetic group of carboxylases.

(f) Co-enzyme of vitamin B12 or cyanocobalamin. It is methylcobamide. It is involved in methyl transfer reactions.

3. Many nucleotides also function as co-enzymes. They are adenosine triphosphate (ATP),



cytidine diphosphate (CDP), uridine diphosphate (UDP), phosphoadenosine phosphosulfate (PAPS) and S-adenosyl methionine (SAM).

## **INORGANIC CO-FACTORS**

Many enzymes require metal ions. They are required for maintenance of protein (enzyme) conformation and catalysis. Metal ions participate in enzymatic reactions in three ways.

### **1. Metallo Enzymes**

Metal is tightly bound to enzyme molecule and it is an integral part of enzyme molecule. Metals are attached to enzyme through coordinate bonds. They participate in catalysis.

#### **Examples:**

- (a) **Iron (Fe<sup>2+</sup>):** It is required for cytochrome oxidase, catalase, xanthine oxidase, succinate dehydrogenase.
- (b) **Copper (Cu<sup>2+</sup>):** It is required for cytochrome oxidase, superoxidedismutase, lysyloxidase and ceruloplasmin.
- (c) **Zinc (Zn<sup>2+</sup>):** It is required for carbonic anhydrase, carboxy peptidase, alkaline phosphatase, alcohol dehydrogenase etc.

### **2. Metal-dependent Enzymes**

Metal is loosely associated with enzyme molecule or it may be required for enzyme substrate complex formation. In the absence of metal, enzyme may not interact with substrate molecule or with co-enzyme molecule.

#### **Examples:**

- (a) **Magnesium (Mg<sup>2+</sup>):** It is needed by enzymes using ATP. Formation of Mg: ATP complex is essential. They include hexokinase, galactokinase, pyruvate kinase etc.
- (b) **Calcium (Ca<sup>2+</sup>):** It is required for the activity of calpain, a calcium-dependent protease. Others are Na<sup>+</sup>/K<sup>+</sup>-ATPase and Ca<sup>2+</sup> ATPase.

### **3. Metal-activated Enzymes**

In presence of metals, some enzymes get activated *i.e.*, their activity increases many folds.

#### **Examples:**

- (a) **Chloride (Cl<sup>-</sup>):** It activates amylase and angiotensin converting enzyme.
- (b) **Calcium (Ca<sup>2+</sup>):** It activates trypsin.

## **ENZYME REGULATION**

Metabolic pathways are controlled by regulating enzyme activity. If enzyme activity is not regulated, it can harm cellular activities and may lead to the development of diseases. Alteration of enzyme regulation is one of the cause for cancer development. Over production of tyrosine kinase is associated with alteration of cell shape in tumour cells. Enzyme regulation can alter when drugs are used. Enzyme regulation can be altered by environmental toxins or pollutants.

Enzyme activity can be regulated by:

(a) changing catalytic efficiency.

(b) altering the amount or quantity of enzyme in cell or body.

**(a) Catalytic efficiency of enzymes can be regulated**

1. By subjecting enzyme to feedback inhibition
2. By allosteric regulation or inhibition
3. By covalent modification of enzyme molecule
4. By synthesizing enzyme in inactive form

### **Allosteric Inhibition**

Inhibition of activity of allosteric enzymes by allosteric inhibitor is called as allosteric inhibition.

Allosteric inhibition is seen in pathways that are subject to regulation. Allosteric inhibitors are not structurally similar to substrates of allosteric enzymes. They bind to enzyme at allosteric site which is different from active site. The activity of an allosteric enzyme is raised by allosteric activator. Most of the allosteric enzymes are oligomeric proteins *i.e.*, they consist of many subunits.

The most extensively studied allosteric enzyme is aspartate transcarbamoylase (Aspartate carbamoyltransferase). It catalyzes first reaction unique to pyrimidine nucleotide biosynthesis.

Carbamoylphosphate + Aspartic acid = Carbamoylaspartate + Pi

The enzyme consist of catalytic and regulatory subunits. It exists in less active form and high active form. Binding of CTP to regulatory subunit converts high active form to less active form. So CTP is called as negative effector or allosteric inhibitor. In contrast, binding of ATP to regulatory subunit convert less active form to high active form. So ATP is called as positive effector or allosteric activator.

Aspartate carbamoyl transferase Asparatate carbamoyl transferase

Less active form High active form

### **Kinetics of Allosteric Enzyme**

1. These enzymes does not exhibit Michaelis-Menten Kinetics. A plot of ( $v/S$ ) velocity versus substrate concentration is sigmoidal or S shaped curve rather than rectangular hyperbola
2. The sigmoidal curve indicates a rapid increase in velocity after a particular substrate concentration. It is due to phenomenon of co-operativity.
3. To explain co-operativity of allosteric enzymes 'T' and 'R' model was proposed. According to this model, the oligomer (allosteric enzyme) exist in two states. A tense (T) state and relaxed (R) state. Binding of substrate (ligand) to 'T' form which is intially slow causes a conformational change in subunits resulting in 'R' form. Further binding of ligand (substrate) to the subunits is rapid.
4. Allosteric inhibitor stabilizes the enzyme in 'T' form, so the enzyme is less active. In contrast, allosteric activator stabilizes the enzyme in 'R' form, so the enzyme is highly active.

### **Enzyme Regulation by Covalent Modification**

Enzyme activity is regulated by covalent attachment of a group to the enzyme molecule. Phosphate group is most commonly used to modify enzyme activity. Other group involved in regulation of enzyme activity by covalent modification is nucleotide. Enzymes which undergo regulation by covalent modification exist in two forms, a less active and a high active form. Depending on the enzyme, the phospho or dephospho enzyme may be less or more active, respectively. The phosphorylation (attachment of phosphate) and dephosphorylation are catalyzed by protein kinases and phosphatases, respectively. The –OH group of serine residue of the protein is the site of phosphorylation. ATP serve as donor of phosphate group. Many hormones influence the activities of proteinkinases and phosphatases.

#### **Examples:**

1. Phosphorylation of glycogen phosphorylase converts less active to high active form. Dephosphorylation converts high active to less active form.
2. Phosphorylation of glycogen synthase converts high active to less active form
3. HMG-CoA reductase, hormone sensitive lipase and acetyl-CoA carboxylase are also regulated by phosphorylation and de-phosphorylation.

### **Enzyme Regulation by Covalent Attachment of Nucleotide**

The activity of glutamine synthetase of E. Coli is regulated by covalent attachment of nucleotide to the enzyme molecule. The attachment of nucleotide to the –OH group of tyrosine residue of enzyme molecule converts more active enzyme to less active enzyme. Adenyl transferase catalyzes addition of nucleotide to enzyme molecule.

### **PRO-ENZYMES**

One way of regulating catalytic activity of an enzyme is synthesizing enzyme in inactive (precursor) form or pro-enzyme or zymogen. They are converted to active form later when need arises. The conversion of pro-enzyme to active enzyme involves limited proteolysis. Limited proteolysis removes few aminoacids from proenzyme which results in conversion of inactive enzyme to active enzyme. So, conversion of pro-enzyme to active enzyme accompanies decrease in molecular weight of pro-enzyme due to removal of amino acids (peptides). Most of the protein digesting enzymes of pancreas are synthesized in inactive forms to protect pancreatic cells from destructive action of proteases. Likewise, pepsin of stomach is also synthesized in pro-enzyme form to protect gastric mucosa from pepsin attack. Most of the blood clotting enzymes are also synthesized in inactive form. They are converted to active forms only at the time of blood coagulation.

#### **Pro-enzymes of Digestive Tract and their Conversion to Enzymes**

In the stomach pepsin is synthesized in inactive pepsinogen form. At the acidic pH of stomach pepsinogen undergo limited proteolysis, which results in the formation of pepsin. When once pepsin is formed it catalyzes its own formation from pepsinogen. This process is called as *autocatalysis*.

The protein splitting enzymes of pancreas are synthesized in inactive forms. They are trypsinogen, chymotrypsinogen, procarboxy peptidase and proelastase. A lipid digesting enzyme is also produced in pancreatic cells as a zymogen. It is phospholipase. The conversion of these pro-enzymes to active enzymes is initiated by enterokinase produced by mucosal cells of duodenum. Enterokinase removes a hexapeptide from trypsinogen by hydrolysing-Lys-Ile bond.

The removal of hexapeptide converts trypsinogen to trypsin. When once few molecules of trypsin are formed it further catalyzes not only formation from trypsinogen but also the conversion of other proenzymes to active enzymes . Since single molecule of trypsin can trigger the formation of battery of protein digesting enzymes, pancreas has another self protecting mechanism. It contains trypsin inhibitor in small amounts.

The formation of blood clot involves activation of (zymogens) blood clotting factors. Prothrombin is converted to active thrombin by factor X and V. Thrombin in turn converts fibrinogen to fibrin.

### **Medical Importance**

Though there are two in-built defensive mechanisms in the pancreas to avoid activation of pro-enzymes, in acute pancreatitis the pro-enzymes get activated and cause damage to pancreas and severe abdominal pain.

### **The quantity of enzyme in cell or body is regulated by**

1. Enzyme degradation
2. Enzyme induction and repression

### **Regulation of Enzyme Activity by Degradation**

Enzymes produced as a part of development or enzymes produced to overcome certain environmental conditions or enzymes produced to remove toxins are not needed any more later. Their continued presence may be harmful to the body. So, if enzymes were immortal, then it leads to creation of unwanted side effects in the body. Hence, enzymes undergo turnover. They are synthesized and degraded. Individual enzymes have life spans. Some enzymes may last few seconds or minutes in the cell. However, some enzymes may last few days in the body. There are specific mechanisms for degradation of enzymes. Enzymes that control key metabolic events are degraded very fast. Likewise if a defective enzyme is produced, it is degraded very rapidly because it is not useful any more to the body.

### **Enzyme Regulation by Induction and Repression**

The quantity of the enzyme can be increased by increasing its synthesis and quantity of the enzyme can be decreased by decreasing its synthesis. Depending on cell needs quantity of the enzyme increases or decreases. Enzymes which are regulated in this manner are called as *inducible enzymes*. It take place at nuclear level of the cell.

#### **Inducible Enzymes**

Normally these enzymes are present in small concentration but in presence of certain substance called as inducer their quantity increases.

#### **Induction**

Increased synthesis of an inducible enzyme in response to inducer is known as induction.

#### Constitutive Enzymes

These are present in fixed quantities. They are not inducible.

**Examples for enzyme induction:** When *E. Coli* is grown on medium containing lactose, they produce more of  $\beta$ -galactosidase or lactase required for lactose utilization. When the cells are transferred to medium free of lactose, formation of lactase decreases. Thus, lactose induces the synthesis of lactase. So, in this case lactose is inducer and lactase is an inducible enzyme.

#### Repression

Certain substances blocks their own synthesis by decreasing synthesis of enzymes, which are required for their formation. This process is called as repression. Substances are called repressors.

**Examples for repression:** When histidine is added to the *S. Typhi*. containing medium synthesis of all the enzymes required for histidine formation is blocked. In this case, histidine is repressor molecule.

In humans also, induction and repression of enzymes takes place. They are called as adaptable enzymes.

#### Examples:

1. Arginase, an enzyme of urea-cycle formation is more in starvation and on high protein diet.
2. Pyruvate carboxylase an enzyme of gluconeogenesis is induced by glucocorticoids and repressed by insulin.
3. Phenobarbitol and anti-convulsive drug induces alkaline phosphatase.

#### ISO-ENZYMES OR ISOZYMES

1. They are multiple forms of enzymes.
2. They catalyze same reaction but differ in physiochemical properties. They occur in same species or in same individual.
3. They are tissue specific or species specific.
4. They are present in serum and other biological fluids and tissues.
5. Iso-enzyme for dehydrogenases, transaminases and phosphotases have been reported.

#### Separation of Iso-enzymes

Most commonly used technique for the separation of iso-enzymes is electrophoresis. The serum lactate dehydrogenase (LDH) iso-enzyme pattern is obtained by subjecting serum to electrophoresis at pH 8.6. On electrophoresis, iso-enzymes of lactate dehydrogenase separates into five bands. Each band exhibits same catalytic activity . The five isoenzymes of LDH are LDH1, LDH2, LDH3, LDH4 and LDH5.

### **Structure of LDH Iso-enzymes**

Lactate dehydrogenase isoenzymes differ at the level of quaternary structure. The LDH consist of 4 subunits of two types. They are H and M subunits. The subunit composition of different LDH isoenzymes are shown below:

#### **Name of isoenzyme Subunit composition**

LDH1 HHHH or H<sub>4</sub>

LDH2 HHHM or H<sub>3</sub>M

LDH3 HHMM or H<sub>2</sub>M<sub>2</sub>

LDH4 HMMM or HM<sub>3</sub>

LDH5 MMMM or M<sub>4</sub>

The synthesis of two subunits H and M is controlled by different genes. H is acidic and M is basic in nature. The molecule weight of each subunit is 35,000.

#### **Alkaline Phosphatase Iso-enzymes**

Electrophoresis is used for the separation of isoenzymes of alkaline phosphatase in serum. On electrophoresis, iso-enzymes of alkaline phosphatase separates into four bands. The four iso-enzymes of alkaline phosphatase are tissue specific. They differ in their carbohydrate content. The four isoenzymes originate from bone, liver, placenta and intestine.

#### **Creatine Phosphokinase (CK) Iso-enzyme**

CK iso-enzymes can be separated by electrophoresis. CK has three isoenzymic forms. They are CK1, CK2 and CK3. They differ in subunit composition. CK is a dimer. It consist of two subunits M and B. The subunit composition of three isoenzymes of CK are BB, MB and MM for CK1, CK2 and CK3 respectively.

#### **Carbonic Anhydrase Iso-enzymes**

On electrophoresis carbonic anhydrase gives three bands. The three isoenzymes differ in amino acid composition.

### **CLINICAL ENZYMOLOGY**

1. It deals with quantitative estimation of enzymes in body fluids in normal and diseases conditions.
2. Depending on pathological conditions different body fluids are used for enzyme measurement.
3. Serum and plasma are most commonly used.
4. Other body fluids used for enzyme measurement are cerebrospinal fluid, amniotic fluid, pleural fluid, peritoneal fluid and synovial fluid.
5. Quantitative estimation of enzymes in serum is used to confirm the diagnosis which is made by observing clinical symptoms. Sometimes it is used to know the effectiveness of treatment *i.e.*, prognosis.
6. Hence measurement of serum enzyme levels is of both diagnostic and prognostic importance. Blood plasma contains several enzymes. Depending on their role they are divided into two groups.

### **A. Functional Enzymes**

They are present in plasma at higher level than in most of tissues and they perform functions in plasma. They include lipoprotein lipase, choline esterase and enzymes of blood coagulation etc.

### **B. Non-functional Enzymes**

They are present only at minimal concentrations in normal and have no known function in plasma. They mainly arises from normal destruction of various blood and tissues cells. So, they are mainly contributed by turnover of tissues. Increased concentration of these enzymes in plasma indicates increased tissue breakdown or damage to tissues due to disease or injury. If the plasma level of secretory enzyme is increased it indicates block in the secretory pathway. Further distribution of enzymes among tissues varies from one organ to another organ. If an organ is rich in an enzyme, injury or damage to that organ leads to release of the enzyme into plasma in significant amounts. Some diseases or cancers of that organ also causes release of the enzyme into plasma. Quantitative measurement of the enzymes in plasma under such conditions serve as good index of disease of that organ. Furthermore, the amount of enzyme released is proportional to the mass of the affected tissue.

Some of the clinically important enzymes which are routinely measured in clinical chemistry laboratory are:

#### 1. Transaminases

Aspartate amino transferase (AST) and alanine amino transferase (ALT) are two transaminases most frequently measured. Normal levels are 3-20 U/L for AST and 4-20 U/L for ALT (Units-U). The former enzyme is also referred as GOT (Glutamate oxalo acetate transaminase) and latter is referred as GPT (Glutamate Pyruvate Transminase). These two enzymes differ in distribution. Heart is rich in AST whereas liver contains both of them in equal amounts. Hence, AST estimation is most commonly done in diseases that affect heart. AST level increases in plasma following heart attack or myocardial infarction. Since liver contains more of ALT, its elevation in plasma is specific indicator of liver damage. Plasma ALT level is more in liver diseases like alcoholic cirrhosis, biliary obstruction, cancer and toxic hepatitis. Both the enzymes are elevated in acute infective hepatitis because liver contains both of them in significant amount. After the onset of viral hepatitis, the levels of these enzymes reaches peak rapidly and come back to normal reference level within a week. Since the skeletal muscle contains appreciate amounts of ALT, its level is increased in muscle damage as in severe trauma and in muscular dystrophy. Serum transaminases are also elevated in lung disease.

#### 2. Alkaline phosphatase

This enzyme catalyzes the hydrolysis of organic esters at alkaline pH 9.0, hence the name alkaline phosphatase. The normal level is 20-90 units/L. The level of the enzyme is elevated in rickets, obstructive jaundice, hyperparathyroidism, metastatic cancer, bone cancer and osteomalacia. In obstructive jaundice, its level is 10 times the normal level because its secretion is blocked due to obstruction. Its level also increases in some non-specific diseases

like leukemia, lung and kidney damages and congestive heart failure, Hodgkin's disease and intestinal disorders.

### 3. Acid phosphatase

This enzyme catalyzes the hydrolysis of organic esters at acidic pH (5.0) hence the name acid phosphatase. The normal level of the enzyme is 2.5-12.0 units/L. It is increased in prostate cancer. Small increases are seen in bone disease and breast cancer.

### 4. $\gamma$ -glutamyl trans peptidase (GGT)

It is involved in the degradation of glutathione. Its level is increased in alcoholic cirrhosis. The normal plasma level of GGT is less than 30 units/L. Since this enzyme is secreted into bile by liver, like alkaline phosphatase  $\gamma$ -glutamyl trans peptidase level increases in cholestatic or obstructive jaundice. It is also elevated in brain lesions.

### 5. Creatine phosphokinase (CK)

The normal level of this enzyme in plasma is 12-60 U/l. Since skeletal muscle is rich in CK serum CK level raises in disease affecting skeletal muscle. Its level is elevated in muscular dystrophy, polymyositis, severe muscle exercise, muscle injury, hypothyroidism, epileptic seizures and in tetanus.

CK level is also elevated in diseases affecting cardiac muscle because of its high content in it. CK level is elevated in myocardial infarction.

### 6. Lactate dehydrogenase (LDH)

The LDH normal level is 70-90 units/L. LDH levels are elevated in myocardial infarction. The serum LDH level raises within 24 hours after infarction, reaches peak level around 2-3 days and returns to normal in a week. Serum LDH level is also elevated in pernicious anemia, megaloblastic anemia, acute hepatitis, blood cancer and in progressive muscular dystrophy.

### 7. Isocitrate dehydrogenase

The normal level of this enzyme in plasma is 1-5 Units/L. Its level is elevated in inflammatory diseases of liver like infective hepatitis, toxic hepatitis. In obstructive jaundice, its level remains normal. This enzyme is found in cerebrospinal fluid. Measurement of enzyme in C.S.F. is a valuable diagnostic aid in the cases of meningitis and brain tumors. In meningitis the level is elevated more than that of in cerebral tumors.

### 8. Amylase

The normal range of this enzyme in plasma is 800-1800 Units/L. This enzyme is secreted by pancreas and salivary glands. So, its level is elevated mainly in acute pancreatitis and parotitis. Its level raised in other conditions like intestinal obstruction and in mumps.

### 9. Lipase

It is an enzyme produced by pancreas. It is secreted into duodenum through pancreatic duct. The normal level of this enzyme is up to 150 Units/L. It is mainly elevated in acute pancreatitis and pancreas cancer. It is also elevated in patients with abdominal lesions, perforated peptic ulcer, intestinal obstruction and in acute peritonitis.



## **ISOENZYMES IN CLINICAL MEDICINE**

1. In some cases, elevated serum enzyme level may not indicate severity and specific organ damaged, because the serum enzyme is derived from routine destruction of cells of various organs.
2. Since isoenzymes are organ specific, iso-enzyme determination gives an indication about the specific organ affected. Further, iso-enzyme distribution varies from one organ to other organ. Hence, if an organ rich in a isoenzyme is damaged or diseased, more of that iso-enzyme enters plasma.
3. By measuring that isoenzymes level in serum the specific organ diseased can be confirmed.
4. Therefore, iso-enzyme determination is useful in differential diagnosis.

### **(a) LDH Isoenzymes**

Serum LDH is the combination of five isoenzymes. Each iso-enzyme is derived from specific organ. LDH1 is derived from heart because heart is rich in LDH1. Similarly, LDH5 is derived from skeletal muscle because it is rich in LDH5. Liver also contain LDH2 to LDH5 isoenzymes in different amounts. LDH isoenzymes are present in different proportions. The proportions of LDH isoenzymes in normal serum are 25%, 35%, 27%, 8% and 5% for LDH1, LDH2, LDH3, LDH4 and LDH5, respectively.

When heart muscle is affected as in myocardial infarction, LDH1 level increases in plasma because of release of LDH1 from damaged heart muscle. So measurement of LDH isoenzyme in serum in myocardial infarction is more sensitive index of myocardial necrosis than the measurement of total LDH activity. Similarly, elevated levels of LDH5 is more specific of muscle lesions and liver inflammation of hepatitis.

### **(b) CK Isoenzymes**

The normal serum CK is composed of CK1, CK2 and CK3. In normal persons, CK2 accounts only 2% of total CK but it accounts for 20% of CK in a patient within 4 hours after heart attack.

### **(c) Alkaline Phosphatase Isoenzymes**

The normal serum alkaline phosphatase is composed of 4 isoenzymes. They are derived from bone, liver, placenta and intestine. Measurement of isoenzymes of alkaline phosphatase is used to distinguish liver lesions from bone lesions in metastatic carcinoma.

## **SERUM ENZYME PROFILES**

1. It involves estimations of different serum enzymes for few days following the onset of a disorder.
2. Multi enzyme determinations for a short span of time serve as good index of disorder. More over determination of more than one enzyme in a particular disease is more useful in prognosis.
3. Several serum enzymes serve as diagnostic indices of myocardial infarction. Serum AST level starts increasing by 6 hours after heart attack, reaches peak value around one to

second day and returns to normal by sixth day. CK level follows a pattern similar to AST. In contrast, LDH levels rise within 24 hours of heart attack, reach peak around 2-3 days and level remains increased even after a week.

Serum enzyme levels are also determined to detect inherited disorders associated with altered enzyme levels like galactosemia, glucose-6-phosphate dehydrogenase deficiency etc.

### **ENZYME-LINKED IMMUNO ADSORBENT ASSAY**

It is popularly known as ELISA technique. The technique combines enzymology with immunology and photometry. It is used for detection and estimation of substances which are either antigens or antibodies. It is based on immune complex formation. The immune complex consists of an antibody, antigen and second antibody with bound enzyme (antibody-antigen-antibody<sup>2</sup>-enzyme). Enzyme linked to second antibody has a crucial role in detection and estimation of antigen present in sample. When it reacts with substrate color is produced.

Intensity of the color is proportional to amount of antigen present in sample.

Steps of this technique are given below:

1. Antibodies specific to an antigen of interest are produced. They are fixed to support materials using coupling agent. The support materials are cellulose, plastic, polystyrene or glass. Plastic plates containing wells (depressions) which are coated with antibodies are commonly used.
2. Sample (serum) containing antigen is allowed to combine with antibody by placing sample in the well.
3. Unbound molecules of sample are removed by washing.
4. A second antibody linked to an enzyme is added. This also binds to antigen to form antibody-antigen-antibody<sup>2</sup>-enzyme complex. Thus, second antibody linked to enzyme is fixed to support material.
5. Unbound antibody<sup>2</sup>-enzyme complex is removed by washing.
6. In the final step, substrate is added. Enzyme linked to antibody<sup>2</sup> convert substrate to colored product which is measured.

### **Medical Importance**

1. Using this technique, antigens or antibodies that are present in very small amounts (picograms) in biological fluids are detected and estimated.
2. Several hormones like insulin, TSH, hCG, Calcitonin etc. are determined world wide using this technique.
3. Antibodies are detected using this technique by fixing antigen to support material.
4. Detection of highly infectious diseases like AIDS, Hepatitis, Malaria etc. World wide involves use of this technique.
5. Some tumor markers in biological fluids are detected and estimated using this technique.

## **CELL**

Cell is the universal functional unit of all forms of life. On the basis of differences in cell structure, all life forms are divided into two major classes. They are prokaryotes and eukaryotes. *Prokaryotes* are simple cells and in most cases, individual cell itself is the organism. They contain cell wall and cytosol is not divided into compartments. Examples for prokaryotes are bacteria, primitive green algae and archae bacteria. All other organisms are called *eukaryotes*. They are multicellular organisms. They are plants, animals, fungi, protozoa, uni-cellular yeast and true algae.

## **MEDICAL AND BIOLOGICAL IMPORTANCE**

1. All higher living organisms including humans are made up of cells.
2. Human body contains wide variety of cells that differ in structure and function.
3. Human cell contains subcellular structures like nucleus, mitochondria, lysosomes and peroxisomes etc.
4. Each subcellular structure has unique shape and functions.
5. Some diseases are due to a lack of subcellular structures.
6. Zellwegers syndrome is due to lack of peroxisomes.
7. Lysosomal enzymes are involved in spreading of cancer.
8. Lack of lysosomes or its enzymes results in lysosomal diseases.
9. Growth of cells requires cell divisions. Cell cycle encompasses all the events of cell division.
10. Cells are not immortal. They have finite life span. Because of this humans are not immortal.
11. Cell death is crucial for shaping of organs during development and for recovery from injuries.
12. Biochemistry explores molecular mechanisms of normal cellular processes as well as diseases.
13. Mitochondria is involved in apoptosis.
14. Endoplasmic reticulum, lysosomes and golgi complex are involved in the integration of pro-apoptotic signals.

## **MOLECULAR COMPOSITION OF CELL**

### **Water**

Water accounts for about 70-75% of the weight of the cell. Other cellular constituents are either dissolved or suspended in water.

### **Organic Compounds**

1. Organic compounds accounts for 25-30% of the cell weight.
2. They are nucleic acids, proteins, polysaccharides (carbohydrates) and lipids. Proteins accounts 10-20% of the weight of the cell. Nucleic acids account 7-10% of the cell weight. Polysaccharides usually account for 2-5% of the cell weight. About 3% of cell weight is

due to lipids. Lipids content may be higher in adipocytes or fat cells. Proteins may account more of cell weight in cells like erythrocytes.

3. Other low molecular weight organic compounds may account for 4% of cell weight. They are monosaccharides, aminoacids, fatty acids, purine and pyrimidine nucleotides, peptides, hormones, vitamins and coenzymes.

### **Inorganic Compounds**

1. Inorganic compounds account for the rest of the cell weight.

2. They are cations like sodium, potassium, calcium, magnesium, copper, iron and anions like chloride, phosphate, bicarbonate, sulfate, iodide and fluoride.

### **EUKARYOTIC CELL STRUCTURE AND FUNCTION**

In eukaryotes, cells aggregate to form tissues or organs and these are further organized to form whole organism. In humans, eukaryotic cells exist in large number of sizes and shapes to perform varieties of functions. For example, nerve cells differ from liver cell which differ from muscle cell and they differ in function also. Though the eukaryotic cells differ in sizes and shapes they have certain common structural features. Further, eukaryotes contain subcellular structures and well defined nucleus. Cells are surrounded by membranes. It separates the cells from surrounding and it is called as *plasma* or *cell membrane*. The other subcellular organelles are also composed in parts by membranes.

### **SUBCELLULAR STRUCTURES AND THEIR FUNCTIONS**

#### **Cell Membrane**

##### **Structure**

1. The outermost structure of the cell that decides its contour is the cell membrane.
2. It is a lipid bi-layer. It also consist of proteins and small amounts of carbohydrate

##### **Functions**

1. It is fluid and dynamic.
2. It is semi permeable, only selected compounds are allowed to pass through from outside. The selective permeability is responsible for the maintenance of internal environment of the cell and for creating potential difference across the membrane.
3. The modification of the cell membrane results in formation of specialized structures like axon of nerves, microvilli of intestinal epithelium and tail of spermatids.

#### **Nucleus**

##### **Structure**

1. Centre of the cell is nucleus.
2. It is surrounded by double-layer membrane of about 250-400 Å thick.
3. The two layers of nuclear membrane are an outer and inner membrane (layer). The two membranes fuse periodically to produce nuclear pores. Exchange of material between nucleus and rest of the cell occurs through nuclear pores.
4. The outer nuclear membrane continuous with other cytomembranes. In some eukaryotic cells, like erythrocyte nucleus is absent. In spermatozoa, nucleus accounts for 90% of

cell whereas in other cell's nucleus accounts for less than 10% of the cell. In prokaryotes, nucleus is not well defined.

#### Functions

1. Nucleus is the information centre of eukaryotic cell. More than 90% of the cellular DNA is present in the nucleus. It is mainly concentrated in the form of chromosomes.
2. Human cell contains 46 chromosomes. These chromosomes are composed of nucleoprotein chromatin, which consist of DNA and proteins histones. Some RNA may also present in the nucleus.
3. In prokaryotes, the DNA is present as thread in the cytosol.

#### **Nucleolus**

##### Structure and Function

These are small dense bodies present in the nucleus. Their number varies from cell to cell. There is no membrane surrounding them. They are continuous with nucleoplasm. Protein accounts for 80% of nucleolus remainder is DNA and RNA.

##### Nucleoplasm

It is also called as nuclear matrix. It contains enzymes involved in the synthesis of DNA and RNA.

#### **Cytosol, Cytoplasm or Cell Sap**

##### Structure

1. The extra nuclear cell content that possess both organelles and other material constitutes cytoplasm. Material other than subcellular components in the cytoplasm makes up the cytosol or cell sap.
2. Sometimes soluble portion of the cell is referred as cytosol. Cytoplasm accounts for 70-75% weight of the cytosol.

##### Functions

1. Numerous enzymes, proteins and many other solutes are found in cytosol.
2. Cytosol is the main site for glycolysis, HMP shunt, activation of amino acids and fatty acid synthesis.

#### **Mitochondria**

##### Structure

1. Are the second largest structures in the cell.
2. Generally mitochondria are ellipsoidal in shape and can assume variety of shapes.
3. The length of a mitochondrion is about 7 microns and has a diameter of 1 micron.
4. Mitochondria consist of outer and inner membranes. The outer membrane is composed of equal amount of protein and lipids.
5. The lipids are mainly phospholipids and cholesterol. The outer membrane functions as a limiting membrane and permeable to many compounds.
6. The inner membrane consists of 75% protein and remainder is lipid.
7. Cardiolipin is the important phospholipid of inner mitochondrial membrane.

8. The inner membrane is convoluted to form number of invaginations known as cristae extending to matrix.
9. These cristae are covered with knob like structures, which are composed of head piece, stalk and a base piece.

#### Functions

1. The number of mitochondria ranges from 1-100 per cell depending on type of cell and its function. Several factors influence the size and number of mitochondria in cells. In yeast, mitochondria is present in aerobic state and absent in anaerobic state. Exposure to cold increases mitochondria by 20-30% in liver cells.
2. In highly metabolically active cells mitochondria are more and large.
3. Location of mitochondria in cell also depends on types and functions of cell. In liver cell mitochondria are scattered. In muscles they are parallelly arranged. Mitochondria in liver cell may range up to 2000 whereas in kidney they may range up to 300.
4. Mitochondria is the *power house* of the cell. It is responsible for the production of energy in the form of ATP. The knob like structures function in electron transport and oxidative phosphorylation.
5. Mitochondria also contain other energy producing pathways like citric-acid cycle, fatty acid oxidation and ketone-body oxidation.
6. Some reactions of gluconeogenesis and urea cycle also occurs in mitochondria. Mitochondria is capable of synthesizing some of its proteins.
7. Mitochondria contains some DNA known as mitochondrial DNA and ribosomes.
8. Mitochondria which are essential for life because of their involvement in ATP production, also pay key role in programmed cell death of several types of cells. During apoptosis, mitochondrial membrane potential drops. This leads to permeabilization of mitochondrial membrane. Cytochrome-C or mitochondrial proteins are released into cytosol which activates death enzymes. Further alterations in mitochondrial morphology also occur during apoptosis.
9. In humans, mitochondria is derived from mother only. Hence, origin of mother of humans have been traced.
10. Outer and inner mitochondrial membranes contain translocase enzymes. They are involved in sorting of nuclear encoded proteins into mitochondrial sub-compartments as well as for their import into mitochondria. The inter mitochondrial membrane space is home for several lethal proteins like pro-death enzymes.

### **Lysosomes**

#### Structure

1. They are small vesicles present in cytoplasm.
2. They are surrounded by a membrane. Lysosomes are called as 'Suicidal bags' of the cell.

#### Functions

1. Lysosomes are rich in hydrolytic enzymes, which are active at acidic pH. The lysosomal enzymes digest the molecules brought into the cell by phagocytosis.

2. Macrophages are rich in lysosomes.

#### Medical Importance

1. Lysosomal enzymes are involved in bone remodelling and intracellular digestion.

2. Disease, shock or cell death causes rupture of lysosomes and release of enzymes. In some organisms, lysosomal enzymes are responsible for cell death of larval tissues.

3. Lack of one or more of lysosomal enzymes cause accumulation of materials in the cell resulting in lysosomal diseases.

4. In some disease like arthritis and muscular dystrophy, lysosomal enzymes are released to cause uncontrolled destruction of surrounding tissues. Lysosomal proteases cathepsins are involved in spreading of cancer (metastasis).

5. As the age advances in digestible material an age pigment 'lipofuscin' occurs in some cells.

6. Lysosomal cystine transporter cystinosin is defective in cystinosis, which is a lysosomal disease. Hence, cystine transport into cytosol from lysosome is blocked.

7. Lysosomes are involved in integration of pro-apoptotic signals.

### **Peroxisomes**

#### Structure

1. Are also small vesicles surrounded by a membrane. They are also called as *microbodies*.

#### Functions

1. They contain enzymes of  $H_2O_2$  metabolism. The concentration of protein in peroxisomes is very high and they may occur in crystalline form. The enzymes of  $H_2O_2$  catabolism present in peroxisomes are peroxidase and catalase.

2. Peroxisomes also contain other enzymes like D, L-amino acid oxidase, uric acid oxidase and L-hydroxy fatty acid oxidation that generates  $H_2O_2$ . Glycerophospholipids are also synthesized in peroxisomes.

#### Medical Importance

1. Lack of peroxisomes result in Zellwegers syndrome.

### **Cytomembranes**

There is an extensive network of membranes in the cytoplasm. These membranes are called as cytomembranes. They are divided into endoplasmic reticulum and golgi complex or apparatus. The endoplasmic reticulum is further subdivided into rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER).

## **Rough Endoplasmic Reticulum**

### Structure

1. It is continuous with outer nuclear membrane.
2. The cytoplasmic surface of rough endoplasmic reticulum is coated with ribosomes. Membrane enclosed channels of endoplasmic reticulum are called *cisternae*. The ribosomes are complexes of RNA and protein.

### Functions

1. Ribosomes and rough endoplasmic reticulum are involved in protein synthesis.
2. Protein synthesized, enters cisternae and later extruded.

## **Smooth Endoplasmic Reticulum**

### Structure

1. It is continuous with rough endoplasmic reticulum. It differs from RER by the absence of ribosomes. When isolated SER is called as microsomes.

### Functions

1. SER of intestinal cells is involved in formation of triglycerides.
2. In the adrenal cortex, SER is the site of steroid formation.
3. Cytochrome P450 dependent monooxygenases are present in liver cell SER.

## **Golgi Apparatus**

### Structure

1. It consist of cluster of paired cytomembranes. The margins of these cytomembranes are flattened.
2. It also contains several small vesicles, which are pinched off from the flattened margins of membranes.

### Functions

1. The golgi bodies are well developed in cells, which are involved in secretion. Material produced in the cell for export is processed by golgi body and is packaged as vesicle and is pinched off. The vesicles fuse with plasma membrane and their content is released to exterior by the process known as exocytosis. The digestive enzymes of pancreas and insulin are produced and released in this way.
2. Golgi apparatus helps in the formation of other subcellular organelles like lysosomes and peroxisomes.
3. Golgi apparatus is involved in protein targeting. It directs proteins to be incorporated into membranes of other subcellular structures. It is also involved in glycosylation and sulfation of proteins.
4. Golgi apparatus is involved in integration of proapoptotic signal. It generates preapoptotic mediator ganglioside GD3.

### Medical Importance

Some cases of diabetes are due to defective processing of insulin in golgi complex.



### **Intracellular Ion Channels**

Membrane of endoplasmic reticulum, golgi complex and nucleus has ion channels. They are involved in transport of ions between cytosol and these intracellular components. Calcium and chloride ion channels which are involved in their transport from these components into cytosol are known.

**Vacuoles.** Some animal cells contain vacuoles. They are membrane enclosed vesicles containing fluid. Mostly they contain nutrients.

**Cell Coat.** Some mammalian cells contain thin coat known as cell coat on the outer surface of the cell membrane. The cell coat is flexible and sticky. It is composed of mucopolysaccharides, glycolipids and glycoproteins. The adhesive properties of cell and organization of tissue is controlled by cell coat

### **Cytoskeletons**

These are filament like structures made up of proteins present in cytoplasm. Non-muscle cells perform mechanical work with these intracellular network of proteins.

(a) **Microfilaments.** They are actin like filaments. They form loose web beneath cell membrane.

(b) **Myosin Fibres.** Same as that of myosin of skeletal muscle.

(c) **Microtubules.** Tubulin is the building block of microtubules. Dendrites, axons of nerve cells and sperm cells contain microtubules. The sperm cell moves with the help of flagellum, a microtubule. These cyto skeletons are involved in the maintenance of cell shape, cell division, cell motility, phagocytosis, endocytosis and exocytosis.

(d) **Intermediate Filaments.** They are not involved in movement of cell. They are stable components of cytoskeleton. Neurofilament of neurons, glial filaments of glial cells and keratin of epithelial cells are some examples of intermediary filaments.

### **MEDICAL IMPORTANCE**

1. In all forms of life growth requires cell division.
2. However, some cells divide even after growth like erythrocytes and epithelial cells of intestine.

Sequence of events associated with cell division occur in cyclic manner. Hence, cell cycle consist of sequence of events, which occur in cyclic manner during cell division. There are four stages (phases) in cell cycle. They are

1. *S (Synthesis)-Phase*
2. *G1 (Gap 1)-Phase*
3. *G2 (Gap 2)-Phase*
4. *M (Mitosis)-Phase*

Sometimes, cell cycle is considered in two main events. They are mitosis and inter phase which consist of G1, G2 and S-phases.

1. *S (Synthesis)-Phase*: Division of a cell into two daughter cells requires duplication of DNA. During S-phase concentration of DNA precursors increases nearly 10-20 folds. In S-phase DNA synthesis occurs. Period of DNA synthesis is almost constant in all adult cells. (1 Hour)
2. *G1 and G2-Phases*: G1 and G2-phases are gaps or breaks in cell cycle. No special events occur during these phases except the size of the cell may increase. However, there may be many biochemical reactions taking place preparing the cell for division and checking that all appropriate steps are completed. The period of S1, G2 and M-Phases may range from 12-18 hours. But the period of G1-phase varies, it can be few hours to months or even years.
3. *Mitosis (M)-Phase*: Many events take place in this phase of cell cycle. At the end mitosis cell divides into two daughter cells. The daughter cells are in G1-phase.

#### Check Points in Cell Cycle

1. It is essential that during cell cycle, the synthesis of DNA, chromosomal segregation and cytoplasm division takes place in proper order. So, controls or check points within the cell cycle exist for all organisms.
2. During cell cycle, oscillation of cell from mitosis to interphase is controlled by many cellular proteins. Further check points exist at the G1/S and G2/M boundaries of cell cycle

#### **MEDICAL AND BIOLOGICAL IMPORTANCE**

1. Cells are not immortal i.e., they have finite life span. In the body, cells are formed and destroyed. So, cells are in dynamic state.
2. Cell division and cell death are two opposite processes required to maintain constant tissue volume (tissue homeostasis).
3. Further cell death plays an important role in shaping tissues and organs during development or during recovery from injuries.
4. Cell death may occur due to several external factors also.

There are three types of cell death.

1. *Necrosis*: It is also termed as cell murder. Cells undergo necrotic death if cell membrane is damaged or due to decreased oxygen supply and if energy (ATP) production is blocked.
2. *Apoptosis*: This type of cell death occurs in tissue turnover. Individual cells or groups of cells undergo this type of death. Aged cells in the body are removed by apoptosis. It is a genetically programmed cell death. In the initial stages of apoptosis, cell shrinks, followed by fragmentation and finally these fragments are eliminated by phagocytosis.
3. *Atrophy*: This type of cell death occurs in the absence of essential survival factors. Survival factors required by the cell are produced by other cells. Absence of nerve growth factor leads to atrophy of nerves. It is also genetically programmed cell death.

## **BIOCHEMISTRY, CELL AND DISEASE**

Biochemistry explains all cellular or biological events in chemical terms. The chemical reactions that occur in biological systems are called *biochemical reactions*. Biochemistry also explains how different sequences of biochemical reactions interact with each other for survival of cell (organism) under various conditions.

When all the biochemical events occur in proper order, the cell or body remains normal. Blocks in biochemical events manifest as disease. So, every known (to be known) disease must (may) be due to blocks in biochemical events. The goal of biochemistry is to explain all diseases in molecular terms. Therefore, biochemistry knowledge is required when one wishes to treat (cure) a disease. In addition, biochemistry suggests ways to manipulate life forms for the benefit of mankind.

### **CELLULAR BIOCHEMISTRY**

Cell is the universal functional unit of all forms of life. On the basis of differences in cell structure, all life forms are divided into two major classes. They are prokaryotes and eukaryotes. *Prokaryotes* are simple cells and in most cases, individual cell itself is the organism. They contain cell wall and cytosol is not divided into compartments. Examples for prokaryotes are bacteria, primitive green algae and archae bacteria. All other organisms are called *eukaryotes*. They are multicellular organisms. They are plants, animals, fungi, protozoa, uni-cellular yeast and true algae.

### **MEDICAL AND BIOLOGICAL IMPORTANCE**

1. All higher living organisms including humans are made up of cells.
2. Human body contains wide variety of cells that differ in structure and function.
3. Human cell contains subcellular structures like nucleus, mitochondria, lysosomes and peroxisomes etc.
4. Each subcellular structure has unique shape and function.
5. Some diseases are due to a lack of subcellular structures.
6. Zellwegers syndrome is due to lack of peroxisomes.
7. Lysosomal enzymes are involved in spreading of cancer.
8. Lack of lysosomes or its enzymes results in lysosomal diseases.
9. Growth of cells requires cell divisions. Cell cycle encompasses all the events of cell division.
10. Cells are not immortal. They have finite life span. Because of this humans are not immortal.
11. Cell death is crucial for shaping of organs during development and for recovery from injuries.
12. Biochemistry explores molecular mechanisms of normal cellular processes as well as diseases.
13. Mitochondria is involved in apoptosis.

14. Endoplasmic reticulum, lysosomes and golgi complex are involved in the integration of pro-apoptotic signals.

## **MOLECULAR COMPOSITION OF CELL**

### **Water**

Water accounts for about 70-75% of the weight of the cell. Other cellular constituents are either dissolved or suspended in water.

### **Organic Compounds**

1. Organic compounds accounts for 25-30% of the cell weight.
2. They are nucleic acids, proteins, polysaccharides (carbohydrates) and lipids. Proteins accounts 10-20% of the weight of the cell. Nucleic acids account 7-10% of the cell weight. Polysaccharides usually account for 2-5% of the cell weight. About 3% of cell weight is due to lipids. Lipids content may be higher in adipocytes or fat cells. Proteins may account more of cell weight in cells like erythrocytes.
3. Other low molecular weight organic compounds may account for 4% of cell weight. They are monosaccharides, aminoacids, fatty acids, purine and pyrimidine nucleotides, peptides, hormones, vitamins and coenzymes.

### **Inorganic Compounds**

1. Inorganic compounds account for the rest of the cell weight.
2. They are cations like sodium, potassium, calcium, magnesium, copper, iron and anions like chloride, phosphate, bicarbonate, sulfate, iodide and fluoride.

## **EUKARYOTIC CELL STRUCTURE AND FUNCTION**

In eukaryotes, cells aggregate to form tissues or organs and these are further organized to form whole organism. In humans, eukaryotic cells exist in large number of sizes and shapes to perform varieties of functions. For example, nerve cells differ from liver cell which differ from muscle cell and they differ in function also. Though the eukaryotic cells differ in sizes and shapes they have certain common structural features. Further, eukaryotes contain subcellular structures and well defined nucleus. Cells are surrounded by membranes. It separates the cells from surrounding and it is called as *plasma* or *cell membrane*. The other subcellular organelles are also composed in parts by membranes.

A typical eukaryotic cell is shown in Figure 1.1.

## **SUBCELLULAR STRUCTURES AND THEIR FUNCTIONS**

### **Cell Membrane**

#### **Structure**

1. The outermost structure of the cell that decides its contour is the cell membrane.
2. It is a lipid bi-layer. It also consist of proteins and small amounts of carbohydrates (Figure 1.1 a).

### **Cell 3**

**Fig. 1.1 (a)** Cell membrane

### Functions

1. It is fluid and dynamic.
2. It is semi permeable, only selected compounds are allowed to pass through from outside. The selective permeability is responsible for the maintenance of internal environment of the cell and for creating potential difference across the membrane.
3. The modification of the cell membrane results in formation of specialized structures like axon of nerves, microvilli of intestinal epithelium and tail of spermatids.

### **Nucleus**

#### Structure

1. Centre of the cell is nucleus.
2. It is surrounded by double-layer membrane of about 250-400 Å thick.
3. The two layers of nuclear membrane are an outer and inner membrane (layer). The two membranes fuse periodically to produce nuclear pores. Exchange of material between nucleus and rest of the cell occurs through nuclear pores.
4. The outer nuclear membrane continuous with other cytomembranes. In some eukaryotic cells, like erythrocyte nucleus is absent. In spermatozoa, nucleus accounts for 90% of cell whereas in other cells nucleus accounts for less than 10% of the cell. In prokaryotes, nucleus is not well defined.

#### Functions

1. Nucleus is the information centre of eukaryotic cell. More than 90% of the cellular DNA is present in the nucleus. It is mainly concentrated in the form of chromosomes.

### **4 Medical Biochemistry**

2. Human cell contains 46 chromosomes. These chromosomes are composed of nucleoprotein chromatin, which consist of DNA and proteins histones. Some RNA may also present in the nucleus.
3. In prokaryotes, the DNA is present as thread in the cytosol.

### **Nucleolus**

#### Structure and Function

These are small dense bodies present in the nucleus. Their number varies from cell to cell. There is no membrane surrounding them. They are continuous with nucleoplasm. Protein accounts for 80% of nucleolus remainder is DNA and RNA.

#### Nucleoplasm

It is also called as nuclear matrix. It contains enzymes involved in the synthesis of DNA and RNA.

### **Cytosol, Cytoplasm or Cell Sap**

#### Structure

1. The extra nuclear cell content that possess both organelles and other material constitutes cytoplasm. Material other than subcellular components in the cytoplasm makes up the cytosol or cell sap.

2. Sometimes soluble portion of the cell is referred as cytosol. Cytoplasm accounts for 70-75% weight of the cytosol.

#### Functions

1. Numerous enzymes, proteins and many other solutes are found in cytosol.
2. Cytosol is the main site for glycolysis, HMP shunt, activation of aminoacids and fattyacid synthesis.

### **Mitochondria**

#### Structure

1. Are the second largest structures in the cell.
2. Generally mitochondria are ellipsoidal in shape and can assume variety of shapes.
3. The length of a mitochondrion is about 7 microns and has a diameter of 1 micron.
4. Mitochondria consist of outer and inner membranes. The outer membrane is composed of equal amount of protein and lipids.
5. The lipids are mainly phospholipids and cholesterol. The outer membrane functions as a limiting membrane and permeable to many compounds.
6. The inner membrane consist of 75% protein and remainder is lipid.
7. Cardiolipin is the important phospholipid of inner mitochondrial membrane.
8. The inner membrane is convoluted to form number of invaginations known as cristae extending to matrix (Figure 1.1b).
9. These cristae are covered with knob like structures, which are composed of head piece, stalk and a base piece.

### **Cell 5**

#### Functions

1. The number of mitochondria ranges from 1-100 per cell depending on type of cell and its function. Several factors influence the size and number of mitochondria in cells. In yeast, mitochondria is present in aerobic state and absent in anaerobic state. Exposure to cold increases mitochondria by 20-30% in liver cells.
2. In highly metabolically active cells mitochondria are more and large.
3. Location of mitochondria in cell also depends on types and functions of cell. In liver cell mitochondria are scattered. In muscles they are parellely arranged. Mitochondria in liver cell may range up to 2000 whereas in kidney they may range up to 300.
4. Mitochondria is the *power house* of the cell. It is responsible for the production of energy in the form of ATP. The knob like structures function in electron transport and oxidative phosphorylation.
5. Mitochondria also contain other energy producing pathways like citric-acid cycle, fatty acid oxidation and ketone-body oxidation.
6. Some reactions of gluconeogenesis and urea cycle also occurs in mitochondria. Mitochondria is capable of synthesizing some of its proteins.
7. Mitochondria contains some DNA known as mitochondrial DNA and ribosomes.
8. Mitochondria which are essential for life because of their involvement in ATP production,

also play key role in programmed cell death of several types of cells. During apoptosis, mitochondrial membrane potential drops. This leads to permeabilization of mitochondrial membrane. Cytochrome-C or mitochondrial proteins are released into cytosol which activates death enzymes. Further alterations in mitochondrial morphology also occur during apoptosis.

9. In humans, mitochondria is derived from mother only. Hence, origin of mother of humans have been traced.

10. Outer and inner mitochondrial membranes contain translocase enzymes. They are involved in sorting of nuclear encoded proteins into mitochondrial sub-compartments as well as for their import into mitochondria. The inter mitochondrial membrane space is home for several lethal proteins like pro-death enzymes.

### **Lysosomes**

#### Structure

1. They are small vesicles present in cytoplasm.
2. They are surrounded by a membrane. Lysosomes are called as 'Suicidal bags' of the cell.

#### Functions

1. Lysosomes are rich in hydrolytic enzymes, which are active at acidic pH. The lysosomal enzymes digest the molecules brought into the cell by phagocytosis.
2. Macrophages are rich in lysosomes.

#### Medical Importance

1. Lysosomal enzymes are involved in bone remodelling and intracellular digestion.

### **6 Medical Biochemistry**

2. Disease, shock or cell death causes rupture of lysosomes and release of enzymes. In some organisms, lysosomal enzymes are responsible for cell death of larval tissues.
3. Lack of one or more of lysosomal enzymes cause accumulation of materials in the cell resulting in lysosomal diseases.
4. In some disease like arthritis and muscular dystrophy, lysosomal enzymes are released to cause uncontrolled destruction of surrounding tissues. Lysosomal proteases cathepsins are involved in spreading of cancer (metastasis).
5. As the age advances in digestible material an age pigment 'lipofuscin' occurs in some cells.
6. Lysosomal cystine transporter cystinosin is defective in cystinosis, which is a lysosomal disease. Hence, cystine transport into cytosol from lysosome is blocked.
7. Lysosomes are involved in integration of pro-apoptotic signals.

### **Peroxisomes**

#### Structure

1. Are also small vesicles surrounded by a membrane. They are also called as *microbodies*.

#### Functions

1. They contain enzymes of H<sub>2</sub>O<sub>2</sub> metabolism. The concentration of protein in peroxisomes is very high and they may occur in crystalline form. The enzymes of H<sub>2</sub>O<sub>2</sub> catabolism

present in peroxisomes are peroxidase and catalase.

2. Peroxisomes also contain other enzymes like D, L-amino acid oxidase, uric acid oxidase and L-hydroxy fatty acid oxidation that generates H<sub>2</sub>O<sub>2</sub>. Glycerophospholipids are also synthesized in peroxisomes.

Medical Importance

1. Lack of peroxisomes result in Zellwegers syndrome.

### **Cytomembranes**

There is an extensive network of membranes in the cytoplasm. These membranes are called as cytomembranes. They are divided into endoplasmic reticulum and golgi complex or apparatus. The endoplasmic reticulum is further subdivided into rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER).

### **Rough Endoplasmic Reticulum**

Structure

1. It is continuous with outer nuclear membrane.  
2. The cytoplasmic surface of rough endoplasmic reticulum is coated with ribosomes. Membrane enclosed channels of endoplasmic reticulum are called *cisternae*. The ribosomes are complexes of RNA and protein.

Functions

1. Ribosomes and rough endoplasmic reticulum are involved in protein synthesis.  
2. Protein synthesized, enters cisternae and later extruded.

### **Cell 7**

### **Smooth Endoplasmic Reticulum**

Structure

1. It is continuous with rough endoplasmic reticulum. It differs from RER by the absence of ribosomes. When isolated SER is called as microsomes.

Functions

1. SER of intestinal cells is involved in formation of triglycerides.  
2. In the adrenal cortex, SER is the site of steroid formation.  
3. Cytochrome P450 dependent monooxygenases are present in liver cell SER.

### **Golgi Apparatus**

Structure

1. It consist of cluster of paired cytomembranes. The margins of these cytomembranes are flattened.  
2. It also contains several small vesicles, which are pinched off from the flattened margins of membranes.



### Functions

1. The golgi bodies are well developed in cells, which are involved in secretion. Material produced in the cell for export is processed by golgi body and is packaged as vesicle and is pinched off. The vesicles fuse with plasma membrane and their content is released to exterior by the process known as exocytosis. The digestive enzymes of pancreas and insulin are produced and released in this way.
2. Golgi apparatus helps in the formation of other subcellular organelles like lysosomes and peroxisomes.
3. Golgi apparatus is involved in protein targeting. It directs proteins to be incorporated into membranes of other subcellular structures. It is also involved in glycosylation and sulfation of proteins.
4. Golgi apparatus is involved in integration of proapoptotic signal. It generates preapoptotic mediator ganglioside GD3.

### Medical Importance

Some cases of diabetes are due to defective processing of insulin in golgi complex.

### Intracellular Ion Channels

Membrane of endoplasmic reticulum, golgi complex and nucleus has ion channels. They are involved in transport of ions between cytosol and these intracellular components. Calcium and chloride ion channels which are involved in their transport from these components into cytosol are known.

**Vacuoles.** Some animal cells contain vacuoles. They are membrane enclosed vesicles containing fluid. Mostly they contain nutrients.

**Cell Coat.** Some mammalian cells contain thin coat known as cell coat on the outer surface of the cell membrane. The cell coat is flexible and sticky. It is composed of mucopolysaccharides, glycolipids and glycoproteins. The adhesive properties of cell and organization of tissue is controlled by cell coat.

## 8 Medical Biochemistry

### Cytoskeletons

These are filament like structures made up of proteins present in cytoplasm. Non-muscle cells perform mechanical work with these intracellular network of proteins.

(a) **Microfilaments.** They are actin like filaments. They form loose web beneath cell membrane.

(b) **Myosin Fibres.** Same as that of myosin of skeletal muscle.

(c) **Microtubules.** Tubulin is the building block of microtubules. Dendrites, axons of nerve cells and sperm cells contain microtubules. The sperm cell moves with the help of flagellum, a microtubule. These cyto skeletons are involved in the maintenance of cell shape, cell division, cell motility, phagocytosis, endocytosis and exocytosis.

(d) **Intermediate Filaments.** They are not involved in movement of cell. They are stable components of cytoskeleton. Neurofilament of neurons, glial filaments of glial cells and keratin of epithelial cells are some examples of intermediary filaments.

### **MEDICAL IMPORTANCE**

1. In all forms of life growth requires cell division.
2. However, some cells divide even after growth like erythrocytes and epithelial cells of intestine.

Sequence of events associated with cell division occur in cyclic manner. Hence, cell cycle consist of sequence of events, which occur in cyclic manner during cell division. There are four stages (phases) in cell cycle. They are

1. *S (Synthesis)-Phase*
2. *G1 (Gap 1)-Phase*
3. *G2 (Gap 2)-Phase*
4. *M (Mitosis)-Phase*

Sometimes, cell cycle is considered in two main events. They are mitosis and inter phase which consist of G1, G2 and S-phases.

1. *S (Synthesis)-Phase:* Division of a cell into two daughter cells requires duplication of DNA. During S-phase concentration of DNA precursors increases nearly 10-20 folds. In S-phase DNA synthesis occurs. Period of DNA synthesis is almost constant in all adult cells. (1 Hour)
2. *G1 and G2-Phases:* G1 and G2-phases are gaps or breaks in cell cycle. No special events occur during these phases except the size of the cell may increase. However, there may be many biochemical reactions taking place preparing the cell for division and checking that all appropriate steps are completed. The period of S1, G2 and M-Phases may range from 12-18 hours. But the period of G1-phase varies, it can be few hours to months or even years.
3. *Mitosis (M)-Phase:* Many events take place in this phase of cell cycle. At the end mitosis cell divides into two daughter cells. The daughter cells are in G1-phase.

### **Cell 9**

#### **Check Points in Cell Cycle**

1. It is essential that during cell cycle, the synthesis of DNA, chromosomal segregation and cytoplasm division takes place in proper order. So, controls or check points within the cell cycle exist for all organisms.
2. During cell cycle, oscillation of cell from mitosis to interphase is controlled by many cellular proteins. Further check points exist at the G1/S and G2/M boundaries of cell cycle. Cell cycle with check points is illustrated in Figure 1.2.

**Fig. 1.2** Four phases of cell cycle with check points

## **MEDICAL AND BIOLOGICAL IMPORTANCE**

1. Cells are not immortal i.e., they have finite life span. In the body, cells are formed and destroyed. So, cells are in dynamic state.
2. Cell division and cell death are two opposite processes required to maintain constant tissue volume (tissue homeostasis).
3. Further cell death plays an important role in shaping tissues and organs during development or during recovery from injuries.
4. Cell death may occur due to several external factors also.

There are three types of cell death.

1. *Necrosis*: It is also termed as cell murder. Cells undergo necrotic death if cell membrane is damaged or due to decreased oxygen supply and if energy (ATP) production is blocked.
2. *Apoptosis*: This type of cell death occurs in tissue turnover. Individual cells or groups of cells undergo this type of death. Aged cells in the body are removed by apoptosis. It is a genetically programmed cell death. In the initial stages of apoptosis, cell shrinks, followed by fragmentation and finally these fragments are eliminated by phagocytosis.

### **10. Medical Biochemistry**

3. *Atrophy*: This type of cell death occurs in the absence of essential survival factors. Survival factors required by the cell are produced by other cells. Absence of nerve growth factor leads to atrophy of nerves. It is also genetically programmed cell death.

Biochemistry explains all cellular or biological events in chemical terms. The chemical reactions that occur in biological systems are called *biochemical reactions*. Biochemistry also explains how different sequences of biochemical reactions interact with each other for survival of cell (organism) under various conditions.

When all the biochemical events occur in proper order, the cell or body remains normal. Blocks in biochemical events manifest as disease. So, every known (to be known) disease must (may) be due to blocks in biochemical events. The goal of biochemistry is to explain all diseases in molecular terms. Therefore, biochemistry knowledge is required when one wishes to treat (cure) a disease. In addition, biochemistry suggests ways to manipulate life forms for the benefit of mankind.

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