

Carbohydrates:

Carbohydrates are the most abundant organic compound widely distributed in animal and plant kingdom and have important structural and metabolic roles. Carbohydrates are primarily composed of carbon, hydrogen and oxygen. Carbohydrates are hydrates of carbon implying that there is same ration of H & O as in water with empirical formula $(C.H_2.O)_n$.

Chemically carbohydrates are the compounds with polyhydroxyaldehyde or polyhydroxyketones or the compounds that yields the same on hydrolysis. The term 'sugar' is applied to carbohydrates soluble in water and sweet in taste.

Major function of carbohydrates:

- Energy: carbohydrates are the principle source of energy supplying 60-80% of total calorie requirement of the body. (carlorific value os carbohydrates= 4 kilocalories/gram)
- Energy Storage: Glucose (Carbohydrate) is stored as glycogen in animal and starch in plants. Glucose is stored as glycogen during fed state and mobilized during fasting for the maintenance of blood glucose level.
- Carbohydrates acts as a precursor molecule for the synthesis of various compounds like amino acids, fats etc.
- The major constituent of synovial fluid is carbohydrates which help in lubrication of skeleton joints.
- Cellulose (carbohydrates) in diet acts as dietary fiber alleviating various medical problems as constipation.
- Heparin (carbohydrates) is natural anticoagulant.
- Carbohydrates are also involved in cell-cell recognition.

Classification:

The major classification of carbohydrates is based on the hydrolysis product and with this carbohydrates can be classified into monosaccharides, oligosaccharides and polysaccharides.

Monosaccharides: These are the simple carbohydrates which cannot be further hydrolysed to simpler carbohydrates. These are compound with polyhydroxyaldehydes or polyhydroxyketones. If monosaccharides have aldehyde as functional groups they are called aldoses: glucose, mannose, galactose, Ribose and if ketone is present as functional group they are called ketoses: Fructose, Ribulose. Monosacchardies are the basic structures which is found in higher structural form of carbohydrates.

Oligosaccharide: are the carbohydrates that yields 2-10 monosaccharides unit when hydrolysed. Based on the hydrolysis result or the no. of monosaccharides unit in the oligosaccharides they are named with the respective number saccharides. example. Disaccharides (two monosaccharide units), trisaccharides (3 units), tetrasaccharides (4 units) and so on.

Disaccharides:

Disaccharides are the most common oligosaccharides present in nature and is defined as the carbohydrates that yields two monosaccharides unit when hydrolysed or disaccharides are the carbohydrates with two monosaccharides unit (monosugars) linked together by glycosidic linkage. Most common example includes Maltose, Lactose, sucrose.

Maltose: is a disaccharide made of two α -D- glucose units linked together by glycosidic linkage. It is hydrolysed product of starch. Maltose is hydrolysed by maltase.

Lactose: is commonly known as milk sugar and is example of disaccharide composed of β - D-galactose and β - D- glucose linked by glycosidic linkage. Lactose is an important in nutrition of young mammals. Lactose must be hydrolysed to galactose and glucose to get absorbed from intestine and this is done by intestinal enzyme lactase. The enzyme lactase if not produced in significant amount will

lead that individual unable to digest lactose present in diet leading to lactose intolerance, characterized by abdominal pain, flatulence, nausea, watery diarrhea.

Sucrose: is a disaccharides composed of α -D- Glucose and β -D- fructose joined together with glycosidic linkage which is obtained commercially from sugarcane. The one which is used in our kitchen as sweetening agent.

Polysaccharides:

Polysaccharides are medium to high molecular weight carbohydrates with more than 10 monosaccharides unit linked together by glycosidic linkage. They are also called as glycans which differs in identity with the repeating monomeric unit. The structure of polysaccharide is either linear or branched. Based on that it is further classified as;

Homopolysaccharide: having same monomeric unit

Heteropolysaccharide: having at least two different monomeric unit.

Starch:::

Starch is the storable form of homopolysaccharide obtained from plant. Plant synthesizes glucose molecule and store it in the form of starch. Starch is one of the common carbohydrate in our daily diet. Structurally starch consists mixture of amylose and amylopectin structures made up of **α -D-Glucose**. Amylose is linear structure of **α -D** glucose linked together by $\alpha 1 \rightarrow 4$ glycosidic linkage and constitue a small fraction in starch. Amylopectin is highly branched structures with $\alpha 1 \rightarrow 6$ branch points. The branching generally occurs every 24-30 residues of glucose in the linear structure.

Glycogen:

Glycogen is the storable form of glucose in animal. Structurally it is similar to amylopectin but is highly branched in comparison to the amylopectin of starch. Here the branching occurs after 8-12 residues. The extensive branching of glycogen increases the surface area of the structure to be utilized by the enzymes (glycogen synthesis & degradation) for the rapid addition of glucose molecule during fed state and release of glucose molecule during fasting state.

Cellulose:

Cellulose is the most abundant polysaccharide found in nature. Cellulose is the structural polysaccharide which is composed of β -D glucose linked together by $\beta 1 \rightarrow 4$ glycosidic linkage. They are also regularly termed as natural fiber. Since we lack enzymes to hydrolyse the above bond it cannot be digested but is an essential element in our nutrition. Fiber slows the rate at which sugar is absorbed into the bloodstream. When you eat foods high in fiber such as beans and whole grains, the sugar in those foods is absorbed slower, which keeps your blood glucose levels from rising too fast.

Fiber helps you feel fuller for longer. Fiber absorbs water in your digestive tract which helps slow the movement of food down the tract, allowing for better absorption of nutrients. The result is that you fell fuller for a longer amount of time and end up snacking less in between meals as a result.

Fiber aids in digestion. It keeps movement within the intestine smooth preventing toxic buildup which can lead to several digestive problems.

Heteropolysaccharides:

Heteropolysaccharides are the polysaccharides that yields more than one type of monosaccharide or its' derivates on hydrolysis. Example: glycosaminoglycans.



Glycosaminoglycans:

Glycosaminoglycans, are a family of linear polymers composed of repeating disaccharide unit. One of 2 unit is always either N-Acetyl Glucosamine or N-Acetyl Galactosamine; the other in most cases a uronic acid usually D-glucuronic or L-iduronic acid In some glycosaminoglycans one or more of OH of the amino sugar are esterified with sulphate.

Extracellular space in the tissues of multicellular animals is filled with a gel like material known as extracellular matrix (ground substance) that holds the cells together & provides a porous pathway for diffusion of nutrients & O₂ to individual cells. Structurally extracellular matrix is composed of interlocking meshwork of heteropolysaccharides & fibrous protein as collagen, elastin, fibronectin & laminin. Sulphate & carboxylate groups of uronate gives a very high density -ve charge which gives the extended conformation to minimize the repulsive forces among neighboring charged groups. Sulphated & non-sulphated patterns provide specific recognition by variety of protein ligands that bind electrostatically to these molecules. Glycosaminoglycans are attached to extracellular proteins to form proteoglycans. Examples: Hyaluronate, Chondroitin sulphates, Heparin.

Hyaluronic acid: is a glycosaminoglycan composed of repeating disaccharide unit of D-glucuronic acid and N-acetylglucosamine. It forms clear highly viscous solution and serve as lubricants in synovial fluids of joints and jelly like consistency to vitreous humor of the vertebrate. Hyaluronate is essential component of extracellular matrix of cartilage and tendon that aids in tensile strength and elasticity of such structures.

Heparin: is the natural anticoagulant synthesized in mast cells. It has the highest negative charge density of any known biological macromolecules. It acts as anticoagulant by binding to antithrombin and activating it which causes the inhibition of thrombin.

Chondroitin sulphates: contributes to tensile strength of cartilage, tendons, ligaments, walls of aorta. Dermatan sulphate contributes to the pliability of skin.

Glycoconjugates:

Polysaccharide & oligosaccharides serve as destination labels for some proteins and as mediators of specific cell-cell interactions & interaction between cell & Extracellular matrix. Specific carbohydrate containing molecule act in cell-cell recognition & adhesion, cell migration during development, blood clotting, immune response, wound healing. In most of these cases the informational carbohydrate is covalently joined to a protein or a lipid to form a glycoconjugate (carbohydrate + conjugating molecule) which is biologically active molecule. Examples include proteoglycan, glycoprotein, glycolipids.

Proteoglycans: (carbohydrate + Protein)

Macromolecules of cell surface or Extracellular matrix in which one or more glycosaminoglycan chains are joined covalently to membrane protein or secreted protein. In proteoglycans greater fraction is glycosaminoglycan (carbohydrate) which is often the main site of biological activity. Proteoglycans are major components of connective tissue as: cartilage

Glycoproteins: (Protein + Carbohydrate)

One or several oligosaccharides of varying complexity joined covalently to a protein. Found on the outer surface of cell membrane, Extracellular matrix and in the blood. Oligosaccharide portion are less monotonous (variety of monosaccharide units). They are rich in information, forming highly specific sites for recognition and high affinity binding by other proteins.



Glycolipids: (Carbohydrate + Lipids)

Glycolipids are membrane lipids in which the hydrophilic head groups are oligosaccharides which as in glycoproteins, act as specific sites for recognition by carbohydrate-binding proteins.

Proteins:

The protein is derived from greek word proteios meaning first rank, for its primary position of any biological molecule in biological system important to life. Protein are the most abundant, high molecular weight nitrogen rich organic compounds with functional diversity in living system. Protein are the long polymeric chain of amino acid linked by peptide (amide) linkage. Virtually every life process depends on this class of molecule. The basic elemental composition of protein is Carbon, Hydrogen, Oxygen, Nitrogen and sulphur but besides these above it may also contain other elements as Phosphorus, iron, copper, Magnesium, zinc etc.

Major function of protein (Physiological Significance)

- **Structural functions:** Many structural protein like collagen, elastin, keratin are the major components in structures like bones, skin, ligament, hair, nair etc.
- **Catalytic functions:** One of the vital functions which protein impart. Life is in existence because of different set of biochemical reaction occurring in living system in well regulated and coordinated manner. Virtually all these reaction are catalyzed by enzymes and almost all the enzymes are protein in nature.
- **Transport:** Various proteins are involved in transport functions. Eg: Hemoglobin is involved in gaseous transport, transferring is involved in transport of Iron, albumin is involved in transport of drugs and other metabolites.
- **Regulatory:** To maintain a perfect balance & coordination between the various system there must be regulatory activities and one of this activities is performed by proteins. Eg: Peptide Hormones; Insulin, Glucagon, oxytocin...
- **Storage functions:** various proteins are involved in storage as ferritin stores iron.
- **Defensive function:** Antibodies (Immunoglobulins) are protein in nature that makes up our humoral Immune system.
- **Blood clotting factors** (most of which are again protein) maintains blood Hemostasis.
- **Albumin** (which is predominant protein) in blood help to maintain osmotic balance..

Amino Acids:

Amino acids are the organic acid with amino group. They are the monomeric or basic repeating structures in protein. There are more than 300 amino acid present in nature but most importantly the amino acids that makes up naturally occurring protein is composed of are just 20 in number and are L- α - in nature. These are known as standard amino acids which are arranged in the peptide according to the information present in the mRNA molecule which is read in a frame of triplet (3 nucleotide at a time) known as genetic code. The general structure of amino acid is as given in figure. Each amino acid has a carboxyl group, an amino group and distinctive side chain. At physiologic pH around 7.4, these groups are present in ionized form as carboxylate and ammonium ion. The nature of side chains ultimately imparts the role of an amino acid in a protein, therefore it is useful to classify them according to nature of side chain groups.

Classification of amino acids:

Classification based on polarity & Structure: Polar or non-polar side chains groups, type of group

- **Non-Polar amino acids:** Glycine, Alanine, Proline, Valine, Leucine, Isoleucine, methionine

- **Polar amino acid with no Charge on side chain (R) group:** Serine, Threonine, Cystine, Asparagine, Glutamine
- **Polar amino acid with positive R group:** Lysine, Arginine, Histidine
- **Polar amino acid with negative R group:** Aspartic acid, Glutamic acid.
- **Amino acid with Hydroxyl group:** Serine, Threonine, Tyrosine
- **Sulphur containing amino acid:** Cysteine, Methionine
- **Aromatic amino acid:** Phenylalanine, Tyrosine, Tryptophan
- **Imino acid:** Proline

Nutritional Classification of Amino acid: Based on Nutrition amino acid are either essential or non-essential.

- **Essential or Indispensable amino acids:** are those which cannot be synthesized by our body system and need to rely on exogenous source ie; diet. 10 amino acid namely Arginine, Valine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan. Out of 10 above 2 amino acid Histidine and Arginine is synthesis in grown ups (adults) so becomes non-essential at that time therefore these two are classified as semi-essential while the rest of the eight amino acids are absolute essential amino acid. (In case of infants all 10 amino acids are essential)
- **Non-essential amino acid:** are those amino acid which can be synthesized within the body. Glycine, Alanine, Serine, Cysteine, Aspartate, Asparagine, Glutamate, Glutamine, Tyrosine and Proline are non-essential amino acids.

Classification of Amino acids on Metabolic Fate: When amino acid are catabolized inside the body, beside being used for energy generation or various other products they can act as precursor for the synthesis of either glucose (glycogen) and Ketone bodies (Fats) or both.

Amino acid which can be used for the synthesis of glucose are **glucogenic or glucogenic** amino acids. Example: alanine, glutamate, aspartate, glycine, serine etc.

Amino acid which can be used for the synthesis of ketone bodies or fat are **ketogenic** amino acids. leucine, lysine are solely ketogenic

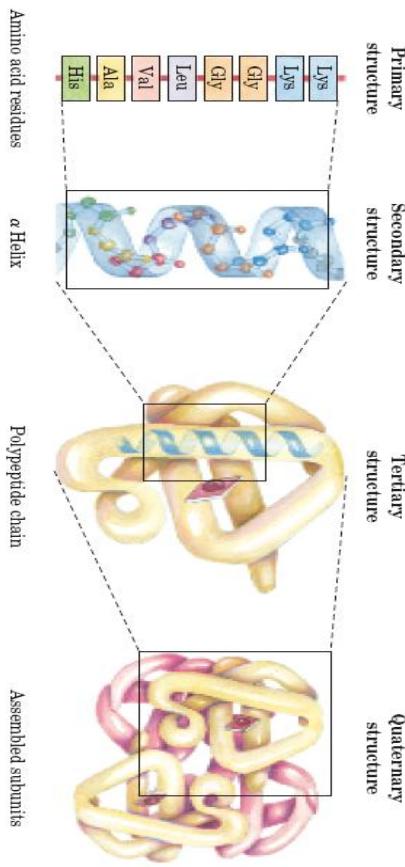
Amino acid from which both glucose or ketone bodies can be synthesized are **glucogenic & ketogenic** amino acids. 4 amino acid: tyrosine, phenylalanine, tryptophan, isolucine are ketogenic as well as glucogenic.

Protein Structure: Structural conformation of protein.

Proteins are the polymers of amino acid in which individual amino acid residues are linked together with peptide linkage. The linear sequence of the linked amino acid contains the information necessary to generate a protein molecule with a unique three dimensional shape. The complexity of protein structure is best analyzed by considering the molecule in terms of four organizational structural levels namely primary, secondary, tertiary and quaternary structures.

- **Primary structure:** The linear sequence of amino acids forming the backbone of proteins (polypeptides) linked together with peptide (amide linkage). The amino acid composition of protein in a definite sequence is presented in primary structure. It is the amino acid composition of protein that makes the higher structural conformation of protein.





- **Secondary structure:** The spatial arrangement of protein by twisting of the polypeptide chain making a coil like structures or making a sheet like structures because of intramolecular hydrogen bonding is secondary structures of protein. Most common examples include α -helix, β -pleated sheets, β -turns.
- **Tertiary structure:** The three dimensional structure of a functional protein because of various secondary structures that keeps the protein in stable conformation and biologically active is the tertiary structures.
- **Quaternary structure:** Quaternary structure is applied to the protein which is made up of more than one polypeptides. The spatial arrangement of 2 or more polypeptide chains, referred to as subunits gives the quaternary structure.

The protein term is generally used for a polypeptide >50 amino acids.

Classification of proteins:

Proteins can be broadly classified into 3 major groups on the basis of amino acid composition, structure.

- **Simple protein:** Protein with only amino acid residues are simple protein. Albumin, Globulin
- **Conjugated protein:** Besides amino acid residues, these contain non-protein moiety called conjugating group which could be Metal ions as in metaloproteins, phosphate as in phosphoprotein, Nucleic acid as in Nucleoprotein, heme as in Hemoglobin.
- **Derived proteins:** These are the denatured or degraded products of simple and conjugated proteins. Most of the proteins gets activated when they are derived from their nascent form (form in which they are naturally synthesized). Examples: Zymogens (Proenzyme) \rightarrow active enzyme by peptide cleavage, prohormone \rightarrow hormone, clotting factors \rightarrow activated clotting factors.

Based on the shape and solubility: Globular and Fibrous

- **Globular:** Globular proteins appear oval or spherical and are soluble in aqueous environment. Basically these are the protein involved in metabolic activities like catalysis, transports. Eg. Albumin, globulin, enzymes etc.
- **Fibrous:** Fibrous proteins have fiber like appearance in nature and are insoluble in aqueous environment. Basically these are structural proteins providing mechanical strength & elasticity to the tissues. Examples: elastin, collagen, keratin.

Based on Nutrition:

Complete proteins: Protein to be complete should have all 10 essential amino acid in required proportion to promote good growth and health. Example egg albumin, milk casein.

Partially incomplete protein: Protein in which one or more essential amino acid is not present in proper amount and can promote moderate growth are partially incomplete protein. Example: wheat and rice protein which is limiting in lysine and threonine.

Rfha

Incomplete protein: Protein which lacks one or more essential amino acids are Incomplete proteins. Example Gelatin lacks tryptophan, zein lacks tryptophan, lysine.

LIPIDS

Lipids are the class of biological molecule defined by low solubility in water and high solubility in nonpolar solvents like: chloroform, ether. Lipids are concentrated storage form of energy besides its role in cellular structure and biochemical functions. Lipids represent highly reduced form of carbon and upon oxidation yield large amount of energy and because of its hydrophobic nature is the molecule of choice for metabolic energy storage.

Biological functions of Lipids:

- They are the concentrated fuel reserve of the body and upon oxidation yields large amount of energy in comparison to carbohydrate, protein. (calorific value – 9kilocalories/gm)
- They are the chief constituent of biological membrane and regulate the permeability of those structures.
- Fat soluble vitamins are metabolized along with the metabolism of lipid (absorption, transport, storage) therefore act as a source of fat soluble vitamins.
- Various hormones like steroids and chemical messengers like prostaglandin, thromboxanes play important role in regulation of cellular metabolism.
- Warfarin is a lipid which is used as an anticoagulant.
- Lipid protects the internal organs acting as a padding material, provides insulation and too provides smooth appearance to the body.

Fatty acids:

Fatty acids are the key constituent of lipid. Chemically they are carboxylic acid (organic acid) with long hydrocarbon chain. Fatty acids occur in large amounts in biological system but are rarely free but are esterified as the major components of the various lipid. Most of the fatty acids found in nature have even number of carbon atom commonly with carbon form 14-24 carbons. Fatty acids with double bond are known as unsaturated fatty acids. If there is single double bound, it is monounsaturated fatty acid and if more than one double bond is present in fatty acid structure it is polyunsaturated fatty acid.

Essential fatty acids: Those fatty acids that cannot be synthesized by the body and have to rely totally on external sources are known as essential fatty acid. Generally Essential fatty acids are polyunsaturated fatty acid with the unsaturation beyond carbon no. 10. Example linoleic acid 18:2; 9, 12(ω -6 series) / Linolenic acid 18:3 9,12,15 (ω -3 series) / Arachidonic acid 20:4; 5, 8, 11, 14 (ω -6 series). Essential fatty acid are the key ingredients of membrane lipids and regulate it's function. It is involved in lipoprotein formation, lipid transport, prevention of fatty liver, synthesis of eicosanoids (Prostaglandin, thromboxane, lipoxins: locally acting hormones)

Classification: Lipids are broadly classified into

- **Simple lipids:** simple lipids are the esters of fatty acids with alcohol. Simple lipids is further classified into
 - **Fats and oils:** these represent esters of fatty acid with glycerol. *Triacylglycerol*.
 - **Waxes:** esters of fatty acid with high molecular weight monohydric alcohol.
- **Complex lipids:** ester of fatty acid with alcohols containing additional groups as phosphate, nitrogenous base, carbohydrate, protein..
 - **Phospholipids:** are the lipids with the phosphate and frequently a nitrogenous base like choline, ethanolamine. *Example: lecithin, cephalin, sphingomylein.*

- **Glycolipid:** are the lipid with fatty acid, carbohydrate, nitrogenous base attached to amino alcohol sphingosine. Example: *cerebroside, gangliosides*.
- **Lipoprotein:** are the macromolecular complex of lipid with protein and are involved in the transport of lipid. Examples: *chylomicron, very low density lipoprotein (VLDL) low density lipoprotein (LDL), High density lipoprotein (HDL)*.
- **Derived lipids:** are the lipids derived from the hydrolysis of simple and complex lipids having characteristics of lipid. Example monoacyl glycerol, diacylglycerol, lysolecithin..
- **Neutral lipids:** lipids having no charge are referred to neutral lipids. Example includes monacyl glycerol, diacylglycerol, cholesterol, cholesterol esters.
- **Miscellaneous:** includes large number of compounds possessing the characteristics of lipid. Examples: carotenoids, squalene, terpenes.

Nucleic Acid:

Nucleic acid are the long polymeric sequence of nucleotides linked together by phosphodiester linkage. Nucleic acids serve as repositories and transmitter of genetic information.

Nucleotide structures:

There are two class of nitrogen containing bases in nucleic acid one is purine and the other is pyrimidine. Adenine and guanine are the purine nitrogenous base and cytosine, uracil and thymine are pyrimidine nitrogenous bases. A nucleotide structure consist of pentose sugar (ribose/ deoxyribose), nitrogenous base (purine/ pyrimidine) and phosphate. On the basis of penose sugar present nucleotide may be ribonucleotide (if ribose sugar is present) and deoxyribonucleotide (if deoxyribose sugar is present).

Functions of Nucleotide:

- Nucleotides are the basic repeating structures in Nucleic acid. Deoxyribonucleotide in DNA and Ribonucleotide in RNA.
- Nucleotide acts as a carrier of chemical energy in biological system. Example: ATP, GTP
- Nucleotides serve as a structural component in various coenzymes. Example; Coenzyme A, Nicotinamide adenine dinucleotide (NAD+), Flavin Adenine dinucleotide (FAD)
- Nucleotide acts as chemical messenger to transduce the signal inside the cell. cAMP (cyclic Adenosine monophosphate)

Deoxyribonucleic Acid (DNA)

DNA is the polynucleotidic sequence of deoxyribonucleotide linked together by phosphodiester linkage. With few exception it is the genetic material that transfers the heredity character from generation to generation. The whole DNA content of an organismsm is known as its genome. DNA is regarded as the repository of genetic information as it has all the information to make up that organism. DNA is a double stranded twisted structure that runs antiparallel and by complexing with various protein assembled as Chromosomes in Eukaryotes. Human have 23 pair of chromosomes 22 of which are called autosomes and a pair is sex chromosomes.

Ribonucleic Acid (RNA)

RNA is polynucleotidic sequence of ribonucleotide linked together by phosphodiester linkage. Basically RNA is the expressive molecule which is used for the expression of genetic information contained in

DNA as well importantly involved in the process of translation. The ribonucleotide found in RNA molecule consist of 4 bases namely Adenine, Guanine, Cytosine and Uracil.

Types of RNA:

Depending upon the function of this molecule it has been classified into

Messenger RNA (mRNA): messenger RNA carries the ribonucleotidic sequence acquired from specific segment of DNA (Gene) and have the message that can be utilized for the synthesis of functional protein.

Transfer RNA (tRNA): transfer RNA are adapter molecules that faithfully translate the information in mRNA into a specific sequence of amino acids. It carries the amino acids for the protein synthesis (translation). Each amino acid has its specific tRNA molecule to be carried by. The Ribonucleotide sequence in mRNA is read as Triplet (Genetic code, 3 nucleotide at a time, non-overlapping) for which complementary base pairing is located at anticodon loop of tRNA. So, the specific triplet (genetic code) in the mRNA is decoded by the specific tRNA molecules.

Ribosomal RNA (rRNA): are the RNA that provides the structural framework for the ribosomes. The rRNA in ribosomal structure helps in the process of protein synthesis (translation).

Genes: a segment of DNA molecule that contains the information required for the synthesis of a functional biological product, whether protein or RNA, is referred to as gene. The storage and transmission of biological information are the only known functions of DNA. The flow of information from DNA to the form of proteins in biological system is known as central dogma of life. The information patches in DNA or genes are transcribed into mRNA which carries the information for specific protein, this mRNA is used for the protein synthesis which have structural and functional role.

Genetic code: the ribonucleotide sequence in mRNA molecule is used for the synthesis of protein. During this process the sequence is read 3 at a time (triplet) which specifies a specific amino acid to be inserted in the growing peptide chain during translation. The genetic code is universal i.e; with very few exception each genetic code codes the same amino from prokaryotes to eukaryotes.

AUG- codes methionine.

UUU- codes for phenylalanine

Enzymes:

Enzymes are one of the most important & vital molecule in biological system. Enzymes are the biocatalyst synthesized by the living cells that enhance the rate of biochemical reaction. Enzymes are very specific in their action and can process millions of substrate molecules per second. The most important part of enzyme is that it is subjected to regulation i.e.; the activity of enzyme gets controlled according to the requirement of the living system. Nearly all the reaction in the body is mediated by enzymes. They permit the reactions to go at conditions that the body can tolerate. Almost all the enzymes are protein in nature.

Some enzymes do not require other chemical groups for their activity other than the amino acid residues. Other enzymes require an additional chemical component called cofactor (inorganic ions) or coenzymes (complex organic or metallorganic molecule) to facilitate the enzyme catalysis. The functionally active enzyme is known as Holoenzyme which mostly have coenzyme or cofactor beside their apoprotein (protein only) part. Holoenzyme = apoenzyme + coenzyme/ cofactor or both.

Cofactors: cofactors are the inorganic ions usually divalent cations like Fe^{2+} , Mg^{2+} , Zn^{2+} that help the enzyme to catalyze the biochemical reaction.

Coenzymes:

Coenzymes are the small organic or metallorganic compound which helps the enzyme to catalyse the biochemical reaction. Coenzymes act as a transient carrier of various groups during catalysis and are regenerated when they transfer it to proper system in next set of reaction. So the requirement of the coenzymes is small as they are regenerated. Derivatives of water soluble vitamins B-complex & other molecules serve as the coenzymes in biological system. The need of coenzymes in enzyme based catalysis explains the essentiality of such vitamins in small amounts.

Examples: Coenzyme A, Flavin Adenine dinucleotide (FAD), Nicotinamide Adenine dinucleotide (NAD⁺), Pyridoxal phosphate (PLP), Tetrahydrofolate (THF), Methylcobalamin.etc...

Vitamin	Coenzyme	Reaction mediated
Thiamine (B ₁)	Thiamine pyrophosphate	Aldehyde transfer
Riboflavin (B ₂)	Flavin coenzymes	Oxidation-reduction
Niacin (B ₃)	Nicotinamide coenzymes	Oxidation-Reduction
Panthothenate (B ₅)	Coenzyme A	Acyl transfer
Pyridoxine (B ₆)	Pyridoxal phosphate	Amino group transfer
Biotin (B ₇)	Biocytin	Carboxylation
Folic acid (B ₉)	Tetrahydrofolate	One-carbon group transfer
Cobalamin (B ₁₂)	Cobalamin (B ₁₂) coenzymes	Alkylation

Classification of enzymes:

Enzymes are broadly classified into six major classes with a proper numeral assign to each class. Each number is representative of the enzyme class.

1. Oxido-reductase

The enzyme which are involved in carrying out oxidation reduction reaction falls under this class. Examples: Glyceraldehyde-3-phosphate dehydrogenase, Lactate dehydrogenase, amino acid oxidase, Ribonucleotide reductase.etc.

2. Transferase

The enzyme which are involved in carrying out transfer reaction are categorized in this second class. Examples: Hexokinase, Aminotransferase, protein kinase.

3. Hydrolase:

The enzymes in this class catalyses the hydrolysis reaction. Examples: Lactase, Maltase, Urease, Protease..

4. Lyases:

Enzymes of this class catalyses the breaking of various bonds (C-C, C-N, C-O). Examples: Aldolase, decarboxylase.

5. Isomerase:

The enzymes which are involved in carrying out the rearrangement reaction/ isomerism reaction are Isomerases. Examples: Isomerase, Racemase, Epimerase.

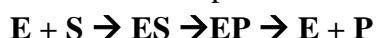
6. Ligase:

The enzyme which are involved in carrying out the bond formation/ joining reaction are ligases. Pyruvate carboxylase, glutamine synthetase, carbamoyl phosphate synthetase.

Enzyme Action:

The molecule which is catalysed by enzyme to form a product is substrate. Enzyme catalysed reaction are very faster than uncatalysed reaction. The enzyme speeds up the reaction by lowering the activation energy of reaction and this is achieved by the binding of substrate molecule at the enzymes's active site. Active site is a special pocket in enzyme structure where the substrate molecule bind and the groups

present in the active site facilitates the catalysis. So, in every reaction very first the enzyme binds with the substrate molecule forming enzyme substrate complex and after catalysis the substrate is turned to product which can be represented as below:



Enzyme activity: Enzyme activity is the amount of reaction that a certain amount of enzyme will produce in a specified period of time which is determined by measuring the amount of product formed or substrate that disappeared. Enzymes are never expressed in terms of concentration. Their activities are expressed. Enzyme activity is represented with various units like, International Unit (IU), katal, & others which is based on the method used for determining the activity of enzymes. The commonly used activity unit is International Unit which is the amount of enzyme necessary to produce 1 micromole of product (or the loss of 1 micromole of substrate) per minute under specified conditions. The activity of enzyme is affected by various parameters like: Enzyme concentration, Substrate concentration, Temperature, pH, Product concentration, Time.

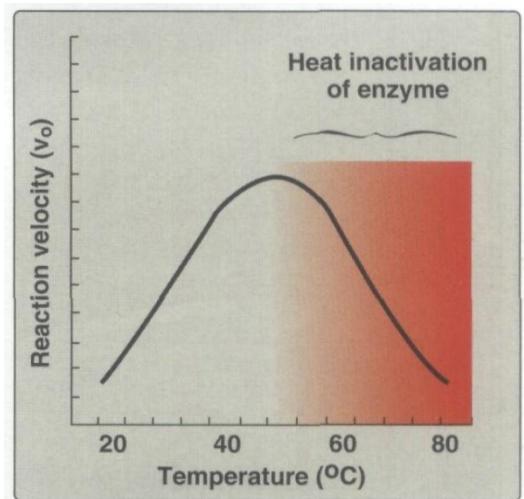
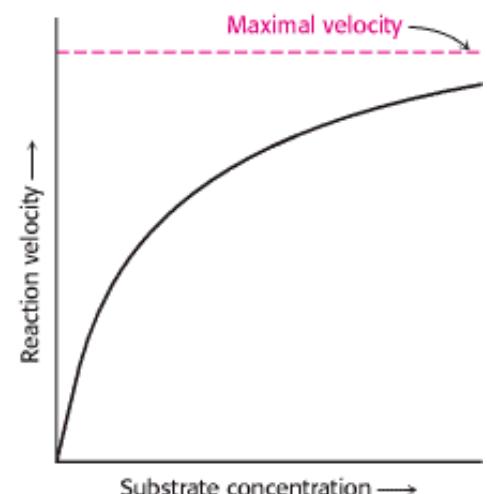
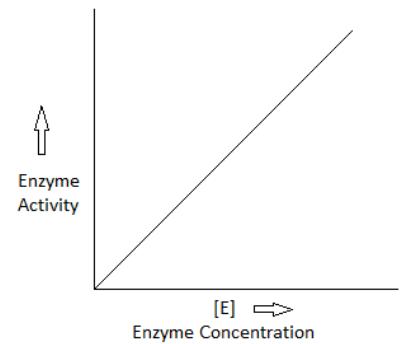
Enzyme concentration: The activity of enzyme is directly proportional to the concentration of enzyme.

Substrate concentration: Initially when the substrate concentration is increased there is increase in the activity of enzyme but after a certain concentration of substrate (when enzyme active site gets saturated) the activity remains constant. So a hyperbolic curve is obtained with mixed order kinetics

pH: Every enzyme has its own optimum pH which ensures its greater structure stability and biological active form. pH is one of the important factor for protein structure. Every enzymes can perform its best at specific pH which is its optimum pH where the activity of enzyme is highest, before or beyond that pH the activity of enzyme is found to be decreased. So a bell shaped curve is obtained when Activity Vs pH curve is plotted. At optimum pH the activity is found to be maximum and further increase or decrease in pH leads to decrease in activity. As in temperature relationship here we also get bell shaped curve.

Temperature: Every enzyme has its own optimum temperature where the activity of the enzyme is highest, before or beyond that temperature the activity of enzyme is found to be decreased. So a bell shaped curve is obtained when activity Vs Temperature curve is plotted. At optimum temperature the activity is found to be maximum and further increase in temperature leads to decrease in activity , and if we go on increasing so after certain temperature there will be no activity representing the loss of enzyme because of denaturation of enzyme due to high heat.

Time: The relationship of time with the enzyme activity is dependent on the various parameter talked above. If all the parameters above are set to optimum the time requirement for the enzyme catalysed reaction is minimum and highest activity is obtained in short time.



Clinical significance of enzymes

Enzymes do have lot of clinical application in the diagnosis as well as therapeutic agents.

- a. **Diagnostic importance:** In healthy individuals the level of intracellular enzymes are fairly constant in extracellular fluid (Blood) which is because of normal turnover of these cells. But when there is excessive damage to the cells, there is increase in the concentration of such enzymes in the blood and when measured one will obtain increased activity of such enzymes at that time representing that there has been tissue damage and the content being released in blood. The assessment of activity of specific enzyme can reflect the pathological condition relating it with condition or complaint produced by the patients. So, the right request of enzyme assay will be very meaningful in diagnosis disease/ condition. The activities of such enzymes are routinely determined for diagnostic purposes in disease of heart, liver, skeletal muscle & other tissues. Some of such examples includes:

In acute pancreatitis: activity of amylase is increased

In Hepatitis: activity of Alanine aminotransferase, Aspartate aminotransferase is increased

Bone & liver disease: Alkaline phosphatase

Myocardial infarction & liver disease: Aspartate amino transferase.

Isoenzymes:

Isoenzymes are the multiple forms of enzyme catalysing the same biochemical reaction but their physical and chemical properties are different like their amino acid composition, cumulative charge on them and separation under electric field (electrophoretic separation), their affinity towards substrate, their regulation, inhibition, site where they are present (compartment) etc.

Eg: *creatine phosphokinase* (CPK) is a dimeric enzyme with B & M type subunit (monomeric protein) and is responsible for reversible phosphate transfer from creatine-phosphate/ ATP to ADP/ Creatine. Creatine Phosphokinase is essentially important in various tissues for ATP generation by substrate level phosphorylation. The enzyme has 3 isoenzyme with BB, BM & MM type subunit and their presence in different tissues is different.

Isoenzyme of CPK	Subunit	Tissue of origin
CPK1	BB	Brain
CPK2	BM	Heart
CPK3	MM	Skeletal muscle

Type	Composition	Location
LDH ₁	HHHH	Heart and erythrocyte
LDH ₂	HHHM	Heart and erythrocyte
LDH ₃	HHMM	Brain and kidney
LDH ₄	HMMM	Skeletal muscle and liver
LDH ₅	MMMM	Skeletal muscle and liver

Lactate dehydrogenase is another example which is a tetrameric protein with H & M type of subunit and have 5 different isoenzymic forms HHHH, HHHM, HHMM, HMMM & HHHH. All these forms of LDH is distributed in different tissues.

Plasma levels of creatine kinase (creatine phosphokinase) & Lactate dehydrogenase are commonly determined in the diagnosis of myocardial infarction.

- b. **Enzyme as analytical reagent:** With the use of enzyme as an analytical reagent, it has been possible to accurately estimate the values of various biochemical parameters in blood. E.g. Glucose estimation using Glucose oxidase peroxidase method, Uric acid estimation using Uricase, Urea using Urease...
- c. The activity of enzyme also does reflect the prognostic value when monitored. E.g. In hepatitis there might be several fold increase in the activity of ALT. After the patient is treated he starts to recover which is reflected by the decrease in the activity of ALT before he normalizes. Such measurement reflects how well the individual is being recovered.
- d. **Enzymes as therapeutic agent:** Enzymes are used as therapeutic agent in alleviating various complications. Sreptokinase is therapeutically used for clearing blood clots, Asparaginase is used in treatment of leukemias. Digestive enzymes are used as the digestive supplements.

METABOLISM

Living cells are in very dynamic state in terms of its composition and activities. Various molecules are synthesized as well as breakdown continually in living system. Thousands of such reactions are occurring simultaneously in single cell. Thus the entire spectrum of such reaction is collectively metabolism. Based on the reaction type metabolism can be viewed as catabolism and Anabolism. Catabolism is defined as the reactions which are involved with the breakdown of complex molecules to simple ones and basically such reaction are involved in generation of energy or metabolites which can be utilized for the synthesis of complex molecules. E.g. Glycolysis, Fatty acid oxidation. Anabolism is defined as the reactions which are involved in synthesis of complex molecules from simpler ones. *E.g. Fatty acid synthesis, protein synthesis.*

In catabolism first the complex molecules are hydrolysed to their monomeric units (simple forms) where no useable form of energy is yield. Eg. Complex sugar to monosugars, protein to amino acids, lipids to glycerol & fatty acids.. secondly these monomeric units are break down to common intermediates like pyruvate, acetyl coA and finally these intermediates are oxidized to CO₂ and electrons are accomplished through central oxidative pathway (TCA cycle).

In anabolism common intermediates or simple compounds are utilized for the formation of monomeric unit and such simple units can be complexed to form more complex compounds and such reaction requires energy. E.g. formation of glucose from pyruvate, glycogen from glucose. Protein from amino acids..

Metabolic regulation:

There are thousands of metabolic reactions occurring in cells leading to formation of specific metabolite or energy. Therefore the pathways of metabolism must be coordinated so that the production of energy or the synthesis of end products meets the need of the cell and to avoid unnecessary production and underproduction of things required. Individual cells do not function isolate but are part of community of interacting tissues. Various regulatory signals are generated by body in response to specific stimulus that informs an individual cell of the metabolic state of the body as a whole include chemical messengers (neurotransmitter, hormones, cytokines) & electrical impulses.

Metabolism of carbohydrates

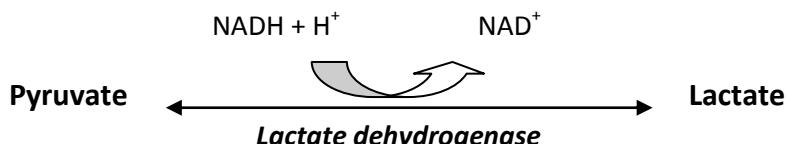
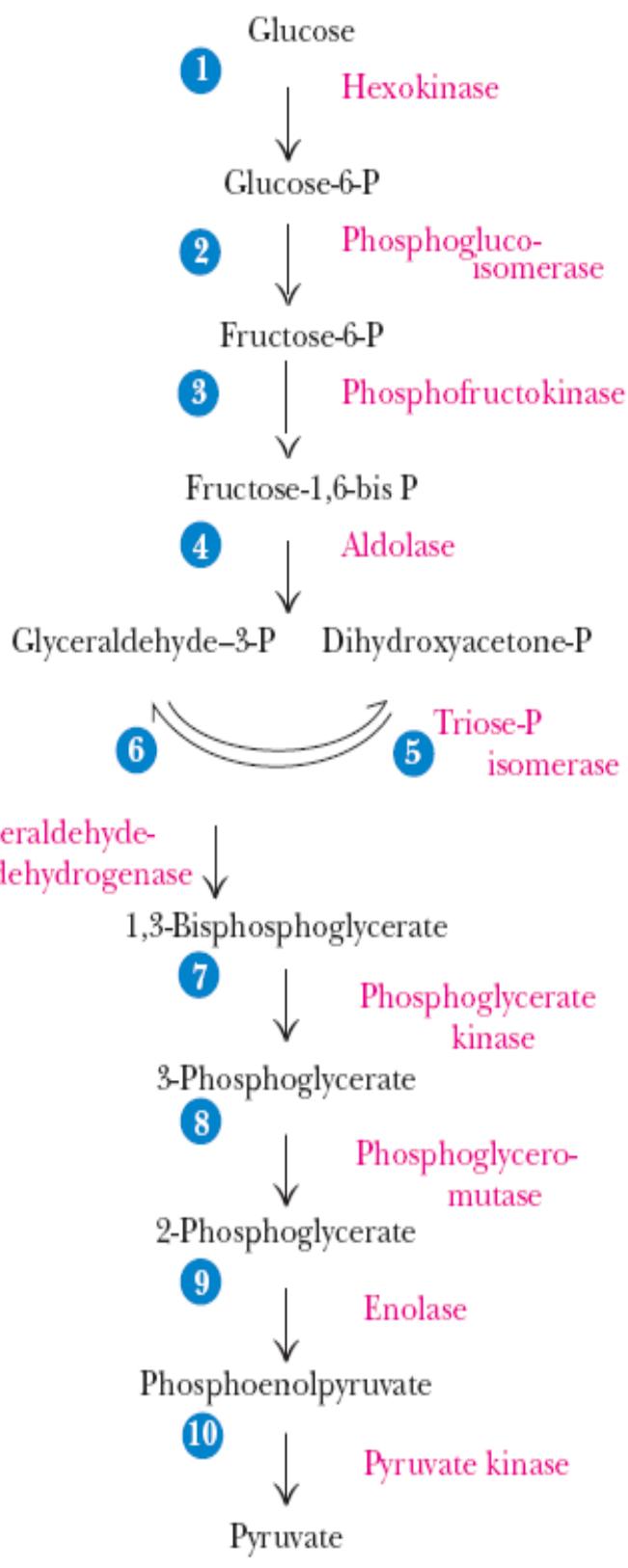
Carbohydrates is the principal source of energy. Beside being used as energy source carbohydrates are metabolized for the formation of various important compound in body like pentoses (ribose, deoxyribose), amino acids, fats. So, carbohydrate metabolism generally incorporates all the metabolic pathway which is associated with the mobilization of carbohydrates in the body. The principal carbohydrates in our diet is glucose or higher polymeric compounds of glucose (starch, glycogen). So carbohydrate metabolism can be viewed with reference to glucose metabolism as such. In carbohydrate metabolism glucose can be oxidized for the generation of energy (glycolysis → PDH reaction → TCA cycle), can be used to synthesize polymeric compound for storage (glycogen), conjugating compound (glucuronic acid), can be converted to amino acids & fats. All these metabolic events are tightly regulated to control the level of glucose in the body and satisfy the need.

Glycolysis:

Glycolysis (glucose = sweet, lysis = breakdown) simply is the breakdown of glucose molecule so that it could be further oxidized to generate energy or be transformed to other products. Depending upon oxygen availability in the cell and cell type glycolysis could be aerobic or anaerobic. Aerobic glycolysis occurs in the cell when there is presence of oxygen and the end product is pyruvate where as in absence of oxygen and in cell lacking mitochondria anaerobic glycolysis takes place with lactate as the end product.

Glycolysis alternatively known as EMP (Embden Meyerhof Pathway) and occurs in cytosol. Glycolysis is central in generating both energy and metabolic intermediates. Glycolysis constitutes 10 steps catalysed by 10 different enzymes of which 3 catalyses the irreversible steps. The overall aerobic glycolysis pathway follows as:

Glycolysis is the major energy generating pathway in tissue lacking mitochondria like red blood cells (RBC) where anaerobic glycolysis occurs so lactate is the end product. During anaerobic glycolysis conversion (reduction) of pyruvate to lactate regenerates the NAD^+ which is used by glyceraldehydes-3-phosphate dehydrogenase for the continuation of glycolysis. During oxygen deprivation NADH cannot be oxidized to NAD^+ and if NAD^+ is absent, the action of glyceraldehydes-3-phosphate dehydrogenase cannot progress and the glycolysis comes to halt. The conversion of pyruvate to lactate by lactate dehydrogenase generates NAD^+ by utilizing NADH to reduce the pyruvate and the glycolysis proceeds even in the absence of oxygen to supply ATP. The occurrence of uninterrupted glycolysis is very essential in skeletal muscle during strenuous exercise where oxygen supply is very limited. RBC generates energy from anaerobic glycolysis. Lactate thus generated in muscle, RBC is transported to liver where it is converted back to glucose molecule.



Energy yield from aerobic glycolysis:

ATP used during glycolysis = 2

ATP formed during glycolysis = 4

Net ATP generated from glycolysis = $(4-2) = 2$ ATP

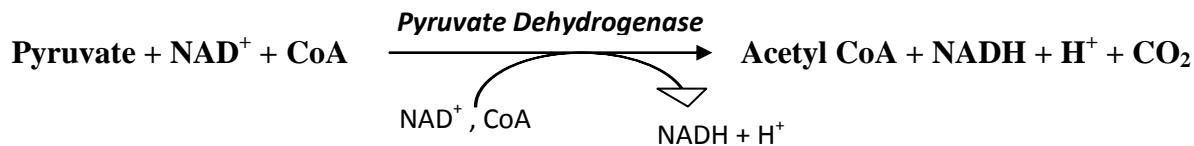
NADH generated during glycolysis = 2;

When condition is aerobic NADH is oxidized to NAD⁺ and this oxidation of NADH is equivalent to 3ATP, so, no. of ATP generated from the oxidation of NADH = 3ATP x 2 = 6 ATP
 Therefore, the total ATP generated in glycolysis is = 2+6 = 8 ATP

Regulation of Glycolysis:

Glycolysis is regulated by controlling the activity of 3 enzymes that catalyse the irreversible steps of glycolysis so are called rate limiting step catalyzing enzymes namely Hexokinase, Phosphofructokinase, and Pyruvate kinase. Insulin activates all the 3 enzymes while glucagon inactivates the enzymes, so with insulin glycolysis proceeds while in response to glucagon glycolysis is inhibited.

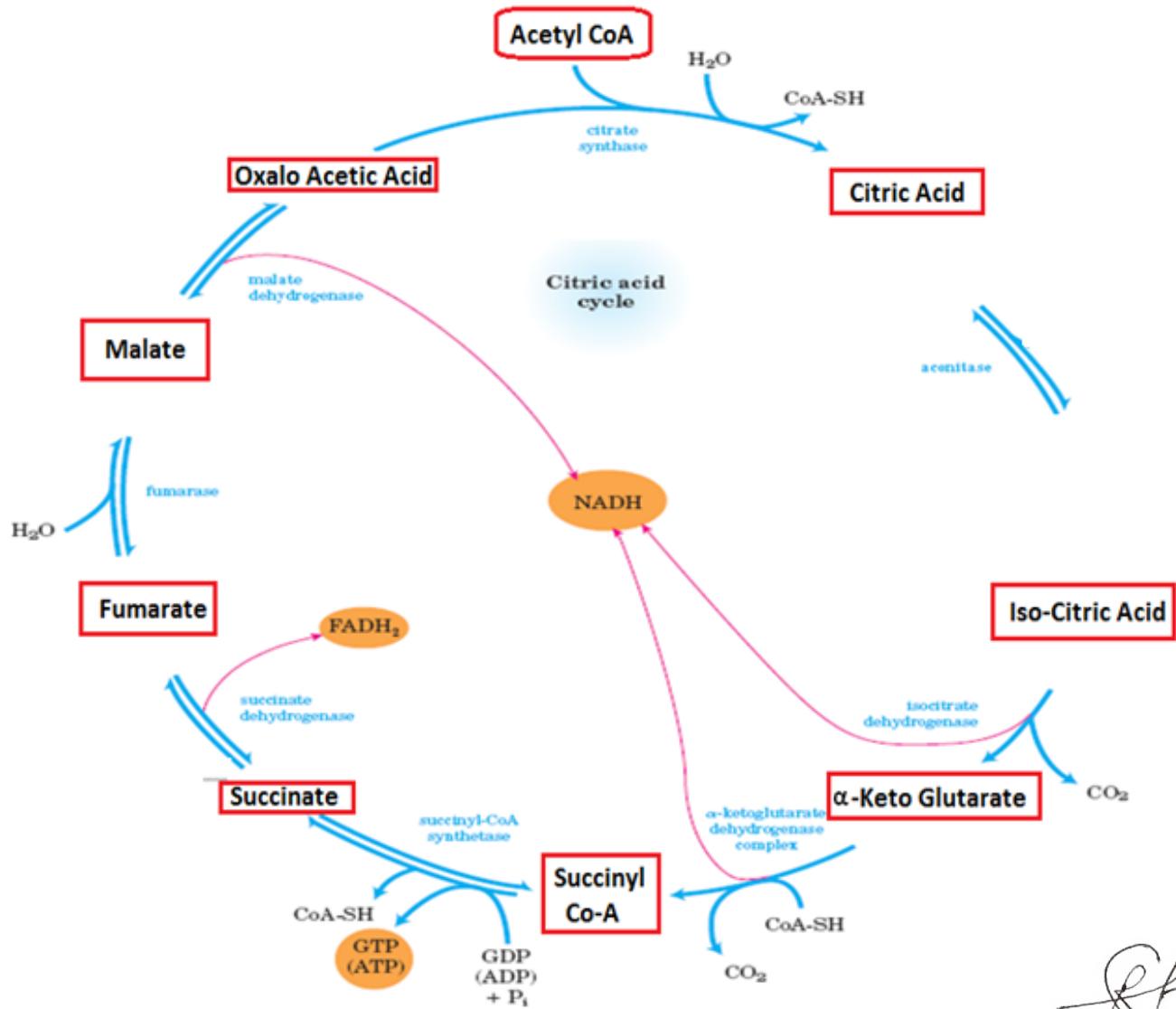
After the breakdown of glucose to pyruvate, very little energy is extracted (8ATP). Lots of energy is still contained within pyruvate which is released when it is oxidized to Carbon dioxide and water. For this pyruvate is transported to mitochondria where it undergoes Pyruvate dehydrogenase (PDH) reaction and gets converted to acetyl CoA with very first oxidation of glucose carbon as carbon dioxide.



TCA cycle: (occurs in mitochondria)

After formation of acetyl CoA, it enters TCA cycle where it is oxidized to carbondioxide and water.

The reactions of TCA cycle as follows:



Tricarboxylic Acid Cycle (TCA) Or Kreb cycle or Citric acid cycle is the most important metabolic pathway for energy supply to body. Complete oxidation of any fuel (carbohydrate, protein, lipid) molecule occurs through this cycle hence called metabolic furnace. When oxidizing such fuel molecules a lot of reducing equivalent are formed whose oxidation is linked with energy generation and requires about 2/3rd of oxygen we consumed.

TCA cycle is controlled by the cellular demand of ATP. If there is demand of ATP the pathway is active and the control/ regulation of the pathway is drawn by the 3 enzymes: citrate synthase, isocitrate dehydrogenase, α -kg dehydrogenase. The activity of these enzymes are turned off when the energy content of cell is high & turned on when the energy status/ content of cell is low.

TCA cycle is amphibolic in nature that it participates in both anabolic and catabolic reactions. So, the cycle is viewed as open cycle from which the intermediates can leave the cycle at any point and the intermediates can enter at any point in TCA cycle. TCA is therefore actively involved in gluconeogenesis, transamination and deamination.

Energetics of glucose oxidation:

When glucose is breakdown (glycolysis) and enters TCA cycle via PDH reaction, glucose can be completely oxidised to CO₂ & water and the yield of energy is maximum. The overall reaction for glucose oxidation is as follows:



Glucose when oxidised in biological system to CO₂ and water 38 ATP is generated which is equivalent to 1159 kJ.

Energy calculation:

From glycolysis glucose \rightarrow 2 Pyruvate = 8 ATP

PDH reaction 2 pyruvate \rightarrow 2 Acetyl CoA + 2CO₂ = 2 NADH generation = 6 ATP

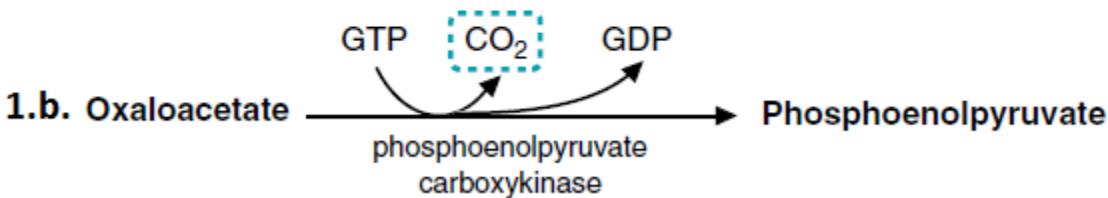
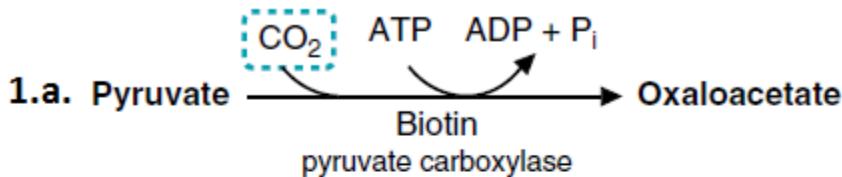
In TCA cycle 2 Acetyl CoA \rightarrow 4CO₂ = [6NADH = 18 ATP; 2FADH₂ = 4 ATP; 2 GTP = 2ATP]

Hence, when a glucose molecule is completely oxidised to 6CO₂ 38 ATP are formed.

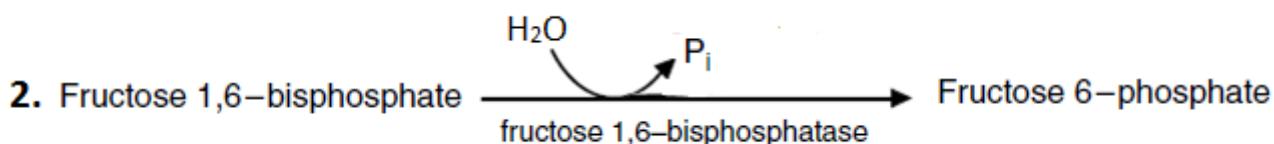
GLUCONEOGENESIS:

Glucose is the primary source of energy for all the tissues. Some tissues like brain, RBC, kidney medulla, lens... requires continuous supply of glucose as metabolic fuel. Even for some tissue which lacks mitochondria or have few no. of mitochondria or with reduced oxygen supply are mainly dependent on anaerobic glycolysis for energy generation. So, glucose should be available all the time and for this one of the mechanism is the synthesis of glucose from non-carbohydrates sources like pyruvate, lactate, glucogenic amino acid..gluconeogenesis occurs in cytosol and mostly takes place in liver.

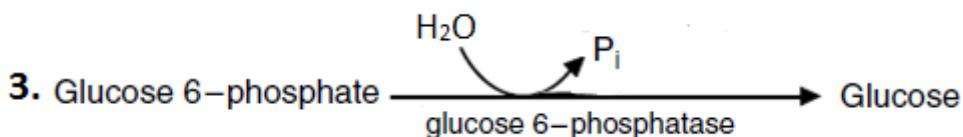
Gluconeogenesis is partial reversal of glycolysis. The 7 reversible reaction of glycolysis are shared by the gluconeogenesis in reverse direction. The 3 irreversible reaction of glycolysis at hexokinase, phosphofructokinase and pyruvate kinase need to be bypassed and this is done by the different set of enzyme at these steps. So gluconeogenesis can just be viewed regarding these reactions which need to be bypassed. So starting with the pyruvate, first reaction is conversion of pyruvate to phosphoenolpyruvate which is a two step process occurring in mitochondria and cytosol. First pyruvate is converted to oxaloacetic acid and then it is converted to Phosphoenol pyruvate by second reaction in which 2 high energy bonds (ATP) are hydrolysed to proceed the reaction and the reaction follows as:



From phosphoenolpyruvate, the reaction is same as glycolysis in reverse direction till formation of fructose 1,6 bisphosphate. Second bypassing reaction is the conversion of fructose 1,6 bisphosphate to fructose-6-phosphate which is catalysed by fructose 1,6-bisphosphatase



Fructose 6-phosphate can be isomerized to glucose 6-phosphate by the same enzyme of glycolysis and finally the third bypass reaction is the conversion of glucose 6-phosphate to glucose by the action of glucose 6-phosphatase.



The glucose so formed is used to maintain blood glucose level.

Regulation: Insulin inhibits gluconeogenesis while glucagon promotes gluconeogenesis by inhibition or activating the rate limiting enzyme of gluconeogenesis.

Glycogen Metabolism:

Glycogen is the storage polysaccharide of animals in Muscle and liver. During well fed state, glucose are stored as glycogen and mobilized when needed. During fasting liver glycogen is used as a reservoir to maintain adequate levels of glucose in blood. Muscle glycogen is used as a fuel for the supply of ATP during muscle contraction. Liver glycogen store increase in well fed state and depleted during fasting.

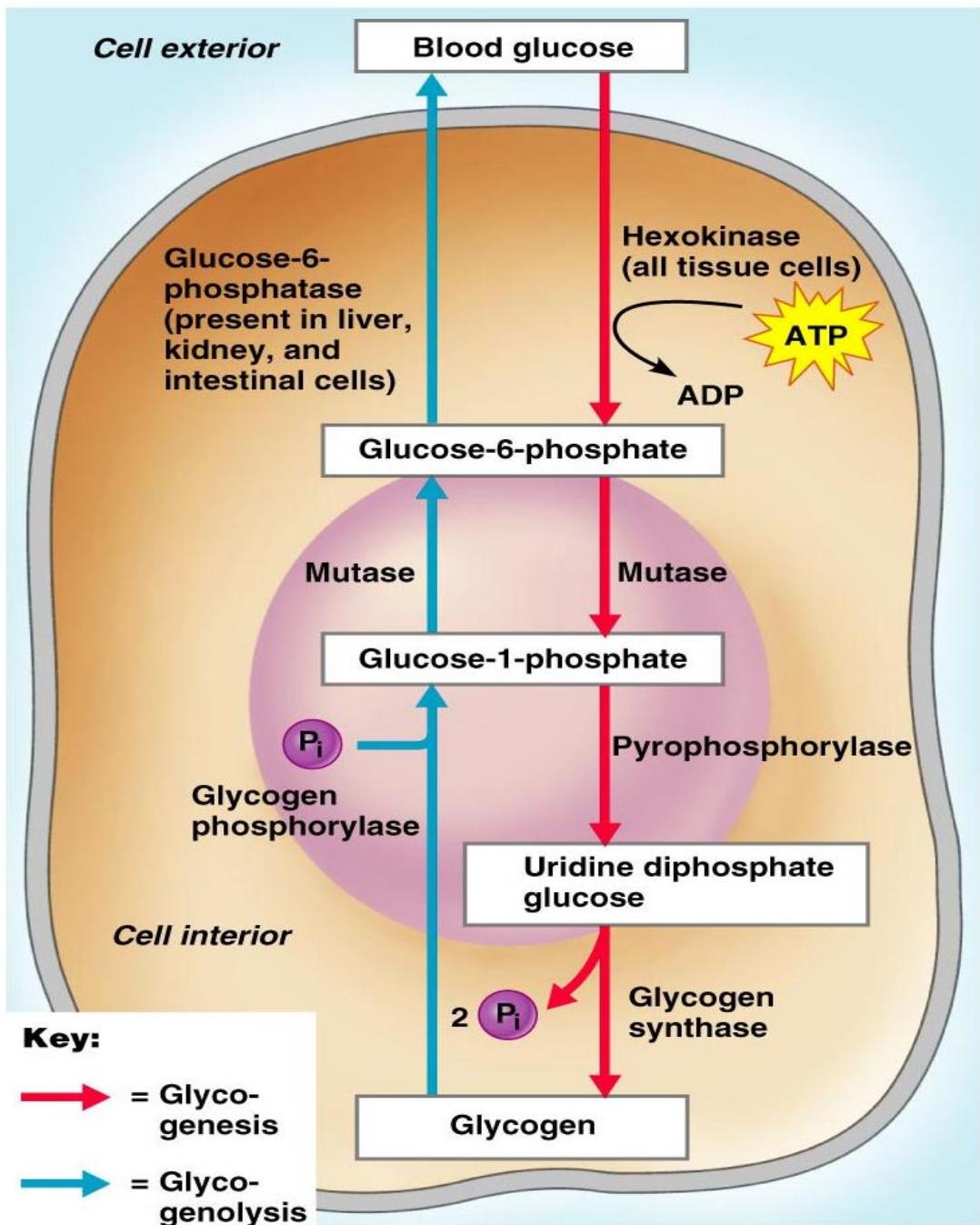
Glycogenesis (Glycogen Synthesis):

Glycogenesis is the process by which glycogen is synthesized from glucose to be stored in the liver or muscle. This process requires energy

Glycogenolysis:

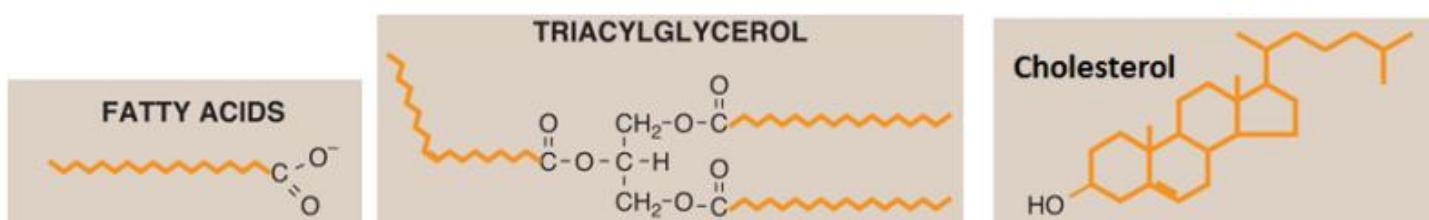
Glycogenolysis is the process by which glycogen is broken down into glucose-1-phosphate or glucose to be used for energy production or glucose homeostasis. The overall all pathway of glycogen metabolism can be viewed as:

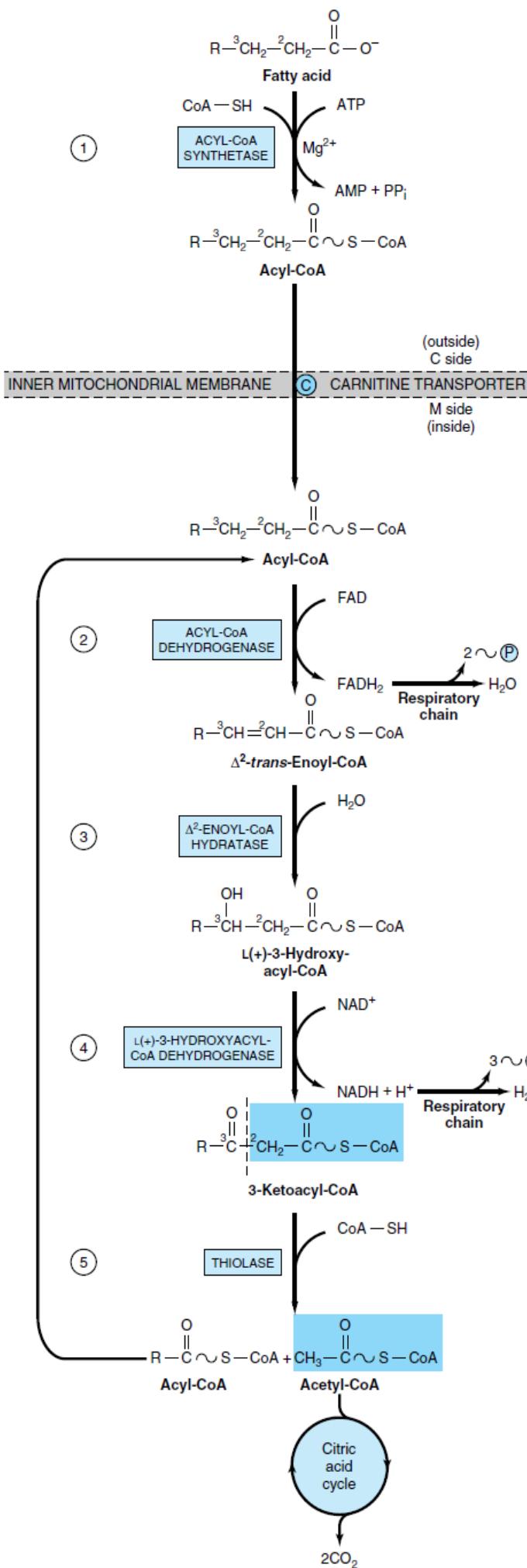
S. Shaha



Metabolism of Lipids:

Lipids are heterogeneous group of water insoluble organic compounds. Body lipids are generally found as membrane associated lipid or droplet of triacylglycerol in adipocytes or transported in plasma in association with lipoprotein particles. Lipids provide the hydrophobic barrier that permits the partitioning of the aqueous content of cells and subcellular structures. Imbalance of lipid metabolism can lead to some of the major clinical problems as atherosclerosis and obesity.





β-oxidation of Fatty acids.

The major pathway for the catabolism of saturated fatty acid is mitochondrial pathway in which 2 carbon fragment are successively removed as Acetyl CoA from the carboxyl end of the fatty acyl CoA producing NADH and FADH₂. The acetylCoA so produced can be further oxidized to water and CO₂ in TCA cycle and yields a huge number of ATPs in comparison to other fuel molecules. The outline of the pathway is given at left. During oxidation of fatty acid the produced FADH₂ and NADH can enter respiratory chain for generation of energy (electron transport chain coupled to phosphorylation: oxidative phosphorylation). The Acetyl CoA so produced can be further oxidized to CO₂ and H₂O leading to generation of NADH and FADH₂ which too enter respiratory chain for generation of energy. The fatty acid which is taken up by cells is converted to fatty acyl CoA which is then transported to mitochondria using carnitine transport system and the reaction goes as on left.

Energetics of Fatty acid (palmitic acid) oxidation:
Palmitic acid is 16 carbon saturated Fatty acid.

So, no. of **β-oxidation** $16/2 - 1 = 7$ and during this 8 Acetyl CoA are produced.

$$7\text{FADH}_2 = 7 \times 2\text{ATP} = 14\text{ATP}$$

$$7\text{NADH} = 7 \times 3\text{ATP} = 21\text{ATP}$$

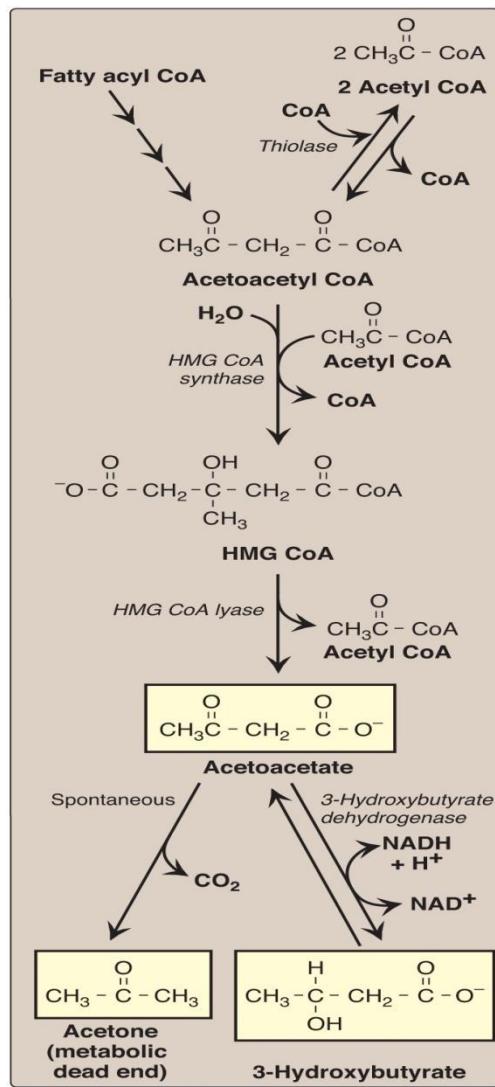
When a Acetyl CoA is completely oxidized 12ATP are generated.

$$8\text{Acetyl CoA} = 8 \times 12\text{ATP} = 96\text{ATP}$$

Total = 131 ATP, 2ATP is utilized for activation of fatty acid, therefore when a palmitic acid is completely oxidised 129 (131-2) ATP are generated.

Ketone Bodies:

The compounds categorized as ketone bodies are acetoacetate, β -hydroxybutyrate and acetone. Acetone is the only true ketone which is non-metabolizable side product and cannot be used by body as fuel while two are organic acid which is used by body tissues as fuel particularly heart and skeletal muscle. Liver mitochondria have the capacity to convert Acetyl CoA derived from fatty acid oxidation into ketone bodies and the process is known as ketogenesis. Ketone bodies are water soluble equivalent of fatty acid. Brain under normal circumstances glucose is the only fuel for brain but during starvation ketone bodies becomes the brains major fuel source as lipolysis is favoured during starvation leading to excess fatty acid release which is oxidized to generate Acetyl CoA, substrate for ketogenesis.



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Ketogenesis (Ketone Bodies synthesis):

The pathway for ketogenesis is outline on left side.

2 molecules of Acetyl CoA condense in presence of thiolase enzyme to form acetoacetyl CoA. A molecule of acetyl CoA is further added to form HMG CoA (Hydroxy methyl Glutaryl CoA) by HMG CoA synthase. HMG CoA is cleaved to Acetoacetate with the release of Acetyl CoA. Acetoacetate can be reduced to β -hydroxy butyrate by dehydrogenase enzyme system. Acetoacetate spontaneously is decarboxylated to form acetone which is a true ketone and dead end metabolite exhaled out of lungs. Acetoacetate and β -hydroxybutyrate are the ketone bodies (which actually are organic acid) which are utilized by the body as fuel.

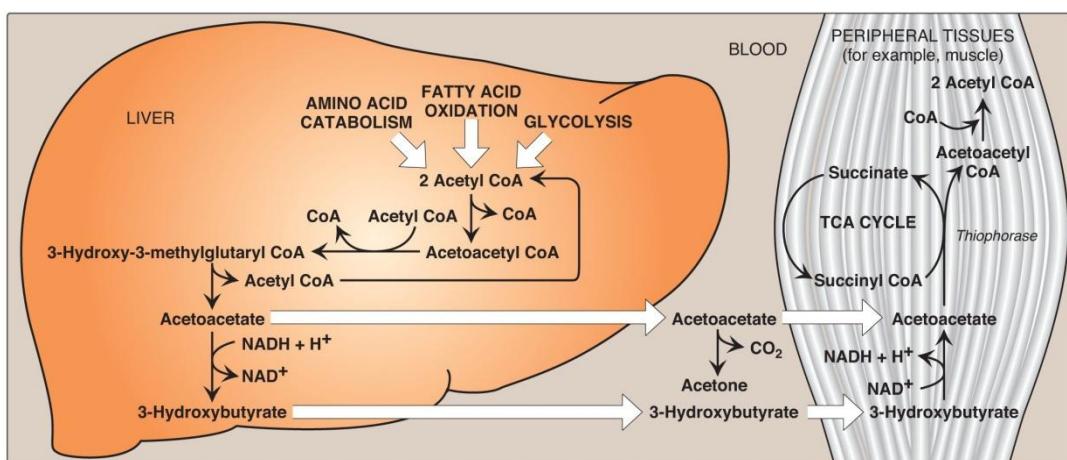
Excessive lipolysis leads to ketonebody formation and that occurs during fasting and starvation. Therefore Ketone body is one of the major fuels for body at that particular condition.

Utilization of ketone body by peripheral Tissues:

(Figure below pictureizes ketone body utilization by peripheral tissues)

Ketone body is synthesized in liver and transported to peripheral tissues where it is activated to acetoacetyl CoA by Thiophorase enzyme and ultimately to 2 molecules of Acetyl CoA which is oxidized in TCA for energy generation.

Although liver is involved in synthesis of ketone bodies it cannot utilize it as it lacks thiophorase the enzyme that is responsible for activation of ketone body.



Note: ketone bodies synthesis is increased in condition like starvation and diabetes mellitus. Since these are organic acid (β -hydroxy butyric acid, aceto acetic acid), their excessive production can lead to ketoacidosis and ketonuria.

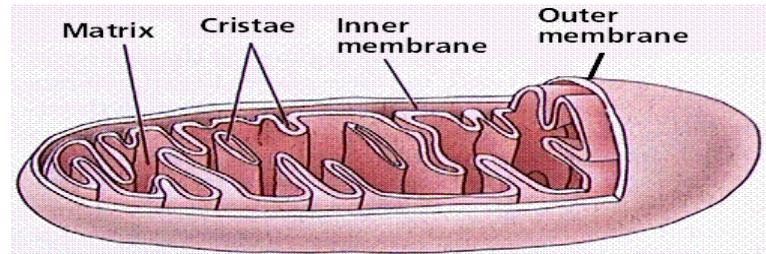
Electron Transport chain:

Living system continuously require energy to perform various biochemical reaction which include anabolic reaction (synthesis of various biomolecules), maintenance of concentration gradient, muscle movement. Therefore it's a fundamental property of living organism to harness energy and to channel it into biological work. For this considerable portion of the cellular biochemical system must therefore be devoted to formation and utilization of energy. Living system (cell) is composed of complex, intricately regulated system of energy producing and energy utilizing system. ATP links energy producing and energy utilizing systems.

Energy rich molecules as glucose, lipid (Fatty acid), amino acids are metabolized by a series of reaction and completely oxidized to CO_2 and water. During oxidation there is reduction of specific coenzymes NAD^+ and FAD i.e. these coenzymes accept the electron during above process and get reduced. Reduced coenzyme in turn can donate a pair of electron (e^-) to specialized set of e^- carriers organized in inner mitochondrial membrane, collectively called electron transport chain (ETC). As e^- pass along the ETC, they lose much of their free energy which can be captured by living system in the form of energy. This process of ATP formation (Phosphorylation of ADP to ATP) which is because of oxidation of such reduced coenzymes in proper system (ETC) is known as oxidative phosphorylation.

Mitochondria:

Mitochondria are one of the important organelle in eukaryotic system principally involved in energy generation. Mitochondria is a double membrane structure; Inner and outer mitochondrial membrane. Depending on energy demands of cell the number of mitochondria vary between cells of different tissues. The space between these membranes is called inner membrane space. Inner membrane contains metabolite transporters and the organized electron transport chain. ETC is operative in mitochondria and supplies major energy demand of cell therefore mitochondria are known as power house of cells. Beside its involvement in energy generation mitochondria is also involved in formation of various metabolic intermediates (urea, heme), ketone bodies in hepatocytes mitochondria and oxidation of fuel molecules (TCA cycle). Mitochondrion is essential for cell death regulation either programmed cell death (apoptosis) or unprogrammed cell death (necrosis).

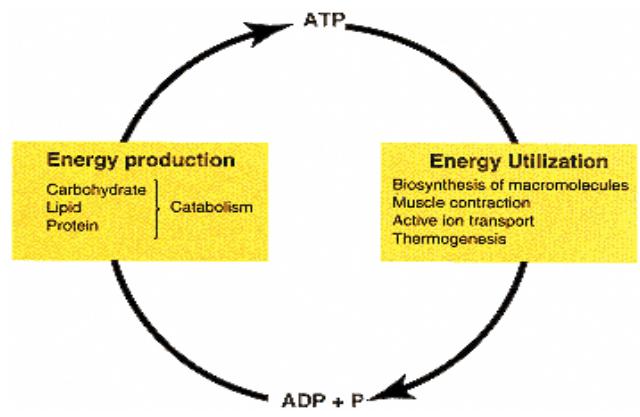


Organization of Electron transport chain:

The inner mitochondrial membrane comprises five separate enzyme complexes as I, II, III, IV and V required for ATP synthesis (oxidative phosphorylation). Complex form I-IV are the complexes of ETC where as the V complex catalyses the ATP synthesis. Electron from the reduced coenzymes NADH , FADH_2 are passed through the complexes of ETC. These reducing equivalents (NADH , FADH_2) carries electrons from various fuel oxidation (glycolysis, TCA cycle, Fatty acid oxidation, amino acid oxidation..) and channel them to electron transport chain. Such channelling or transferring of electron in ETC is accompanied by free energy release which is used for proton pumping from mitochondrial matrix to inner membrane space and a proton gradient is established which is utilized by V complex (ATP synthase) to generate ATP by phosphorylating ADP. So, overall pathway for ATP generation is actually the event of oxidation of reduced coenzymes in ETC and phosphorylation of ADP to form ATP by complex V. Hence, the name is Oxidative phosphorylation which is a coupled process of oxidation and phosphorylation.

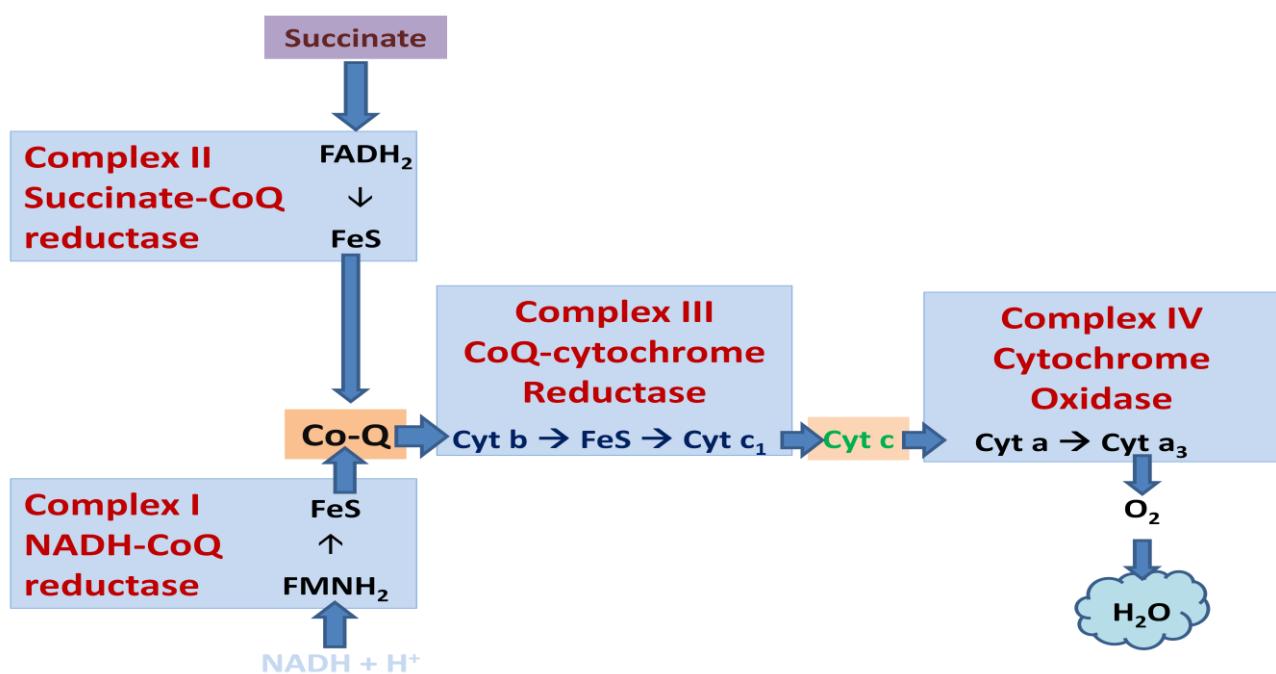
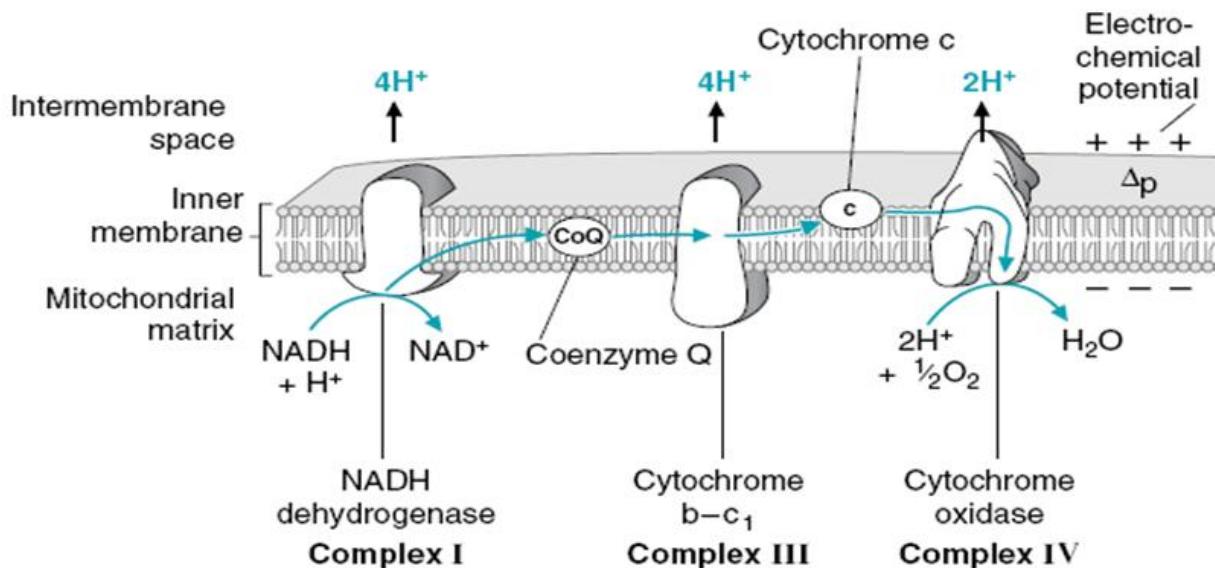
The electron flow in the ETC occurs in two ways

1. $\text{NADH} \rightarrow \text{Complex I} \rightarrow \text{Complex III} \rightarrow \text{Complex IV} \rightarrow \text{O}_2$
2. $\text{FADH}_2 \rightarrow \text{Complex II} \rightarrow \text{Complex III} \rightarrow \text{Complex IV} \rightarrow \text{O}_2$



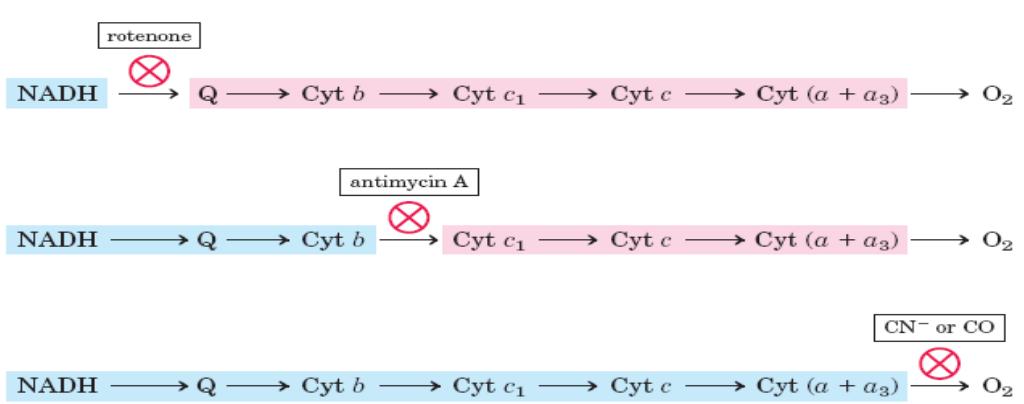
Rfha

There is a specific mobile carrier that carries electron form one complex to another complex in inner mitochondrial membrane. These molecules are lipid soluble. The carrier system between complex I & III is called coenzyme Q or ubiquinone and the carrier system between complex III & IV is called cytochrome C. following figure shows the organization and flow of electron in the complexes.



Inhibitor of Electron Transport chain:

Inhibitor molecules are those that bind with the system (protein) and inhibit them to do their routine what they are meant to and the process is known as inhibition. Three sites of action are identified for the various inhibitor molecules that inhibit the transfer of electron in ETC which is listed in table & figure below.



Inhibition of Electron Transfer	
Compound	Target/ mode of action
Amytal	Prevent electron transfer from Fe-S center to ubiquinone
Piericidin A	(Complex I)
Rotenone	
Antimycin A	Blocks electron transfer from cytochrome b to cytochrome c1
Myxothiazol	(Complex III)
Cyanide	Inhibits cytochrome oxidase
Carbon monoxide	(complex IV)

Uncouplers: Inhibitors of oxidative phosphorylation.

Oxidative phosphorylation (ATP synthesis in mitochondria) is a coupled process of oxidation of NADH/ FADH₂ and phosphorylation by ATP. Any compound that removes this connection (uncouples) are uncouplers. The uncouplers allow the oxidation of substrates with ATP formation. The uncouplers increase the permeability of inner mitochondrial membrane to protons disrupting the proton gradient which is the basic requirement in the synthesis of ATP by complex V and hence these two processes get uncoupled. Uncouplers could be chemical or physiological (synthesized by body)

Chemical uncouplers:

These are lipid soluble compounds that can bind protons. They bind with protons in the inner mitochondrial membrane space and transport them to the matrix. E.g. 2, 4 DNP, dinitrocresol.

Physiological uncouplers:

These are highly regulated proteins (uncoupling proteins/ thermogenin) synthesized by the body that form the channel through which protons are transported to the matrix side disrupting the protein gradient which uncouples ETC from Phosphorylation by complex V.

Inhibitor of oxidative phosphorylation:

Oligomycin: is an antibiotic which binds with ATP synthase (complex V) and inhibits ATP synthesis.

Attractyloside: is a plant toxin which inhibits the transporter molecule which transports ADP inside the mitochondria and is a basic requirement in ATP synthesis. Complex V adds Phosphate to ADP forming ATP. So, if ADP is lacking ATP cannot be synthesized.

Energy production in Biological System:

The synthesis of ATP is adding a phosphate to ADP i.e. phosphorylation process. In biological systems there are two processes for synthesizing ATP.

1. Oxidative phosphorylation (discussed above, major pathway for ATP)
2. Substrate level phosphorylation.

Substrate level Phosphorylation: ATP is directly synthesized during conversion of high energy substrate to low energy product during substrate level phosphorylation for ATP synthesis. Generally these high energy substrates (energy rich compounds) are phosphorylated intermediates of metabolic pathways. E.g. 1,3 bisphosphoglycerate, creatine phosphate, phosphoenol pyruvate, succinyl CoA..



Nucleic Acid Metabolism: Genetics (Replication, Transcription and Translation)

Nucleic acids are the long polymeric form of nucleotides. Depending upon type of nucleotide, nucleic acid is either the long chain of deoxyribonucleotide called deoxyribonucleic acid (DNA) and the long chain of ribonucleotide called (RNA). DNA is the genetic repository molecule that have all the information required for the formation and maintenance of living organism (structural as well as functional). For the continuation of the race the information should be efficiently transferred from parents to their offspring and for this the genetic information should be replicated and transferred. The information stored in DNA molecule should be effectively used for the formation of various functional molecule; includes RNA and Protein for the continuation of life. This information flow in biological system is called as Central Dogma of life. For the change in the information there need a change in deoxynucleotide sequence in DNA which could a single nucleotide change or frame change leading to positive or negative consequences for the organism and is called mutation. Mutation could make the organism more adaptive to its living environment in us as in sickle cell anemia or it could disorient the organism with various complications; in us leading to various genetic defects. So, in following segment we will discuss the central dogma of life: Replication, Transcription & translation.

DNA Replication:

When a cell divides, it carries the exact genetic information from the parental cells. For this the cell first must copy its DNA and the process is DNA replication. DNA replication is a reaction in which daughter DNA are synthesized using parental DNA as the Template. i.e. The replicating system copy the exact information (complete nucleotide sequence in the DNA of parents). During replication specific deoxyribonucleotide are joined making 3'-5' phosphodiester linkage complementary to parental DNA strand. This is done by complementary base pairing. Following are the requirements for DNA replication.

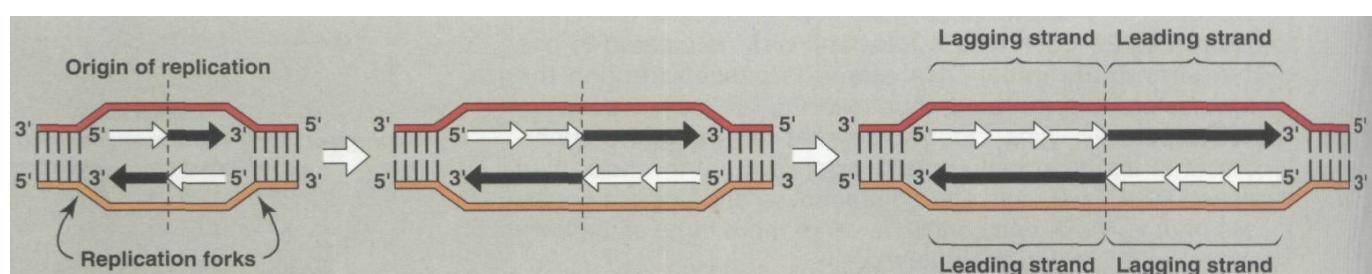
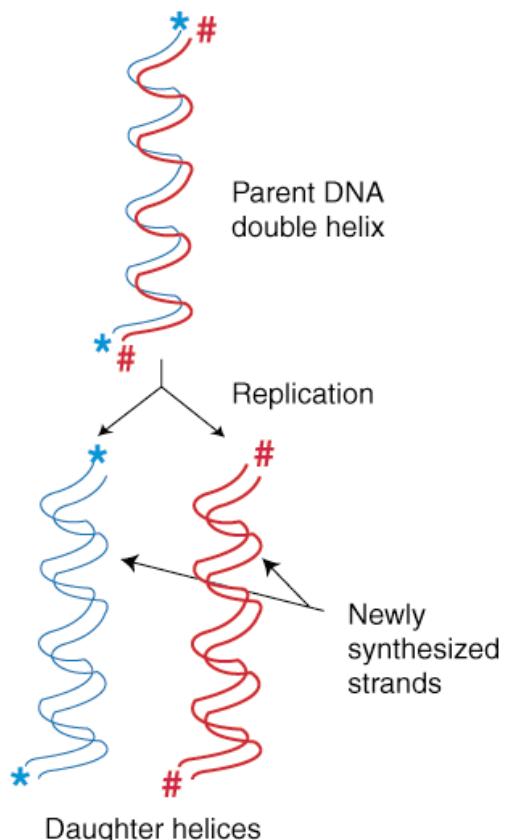
Template: Parental Double Stranded DNA

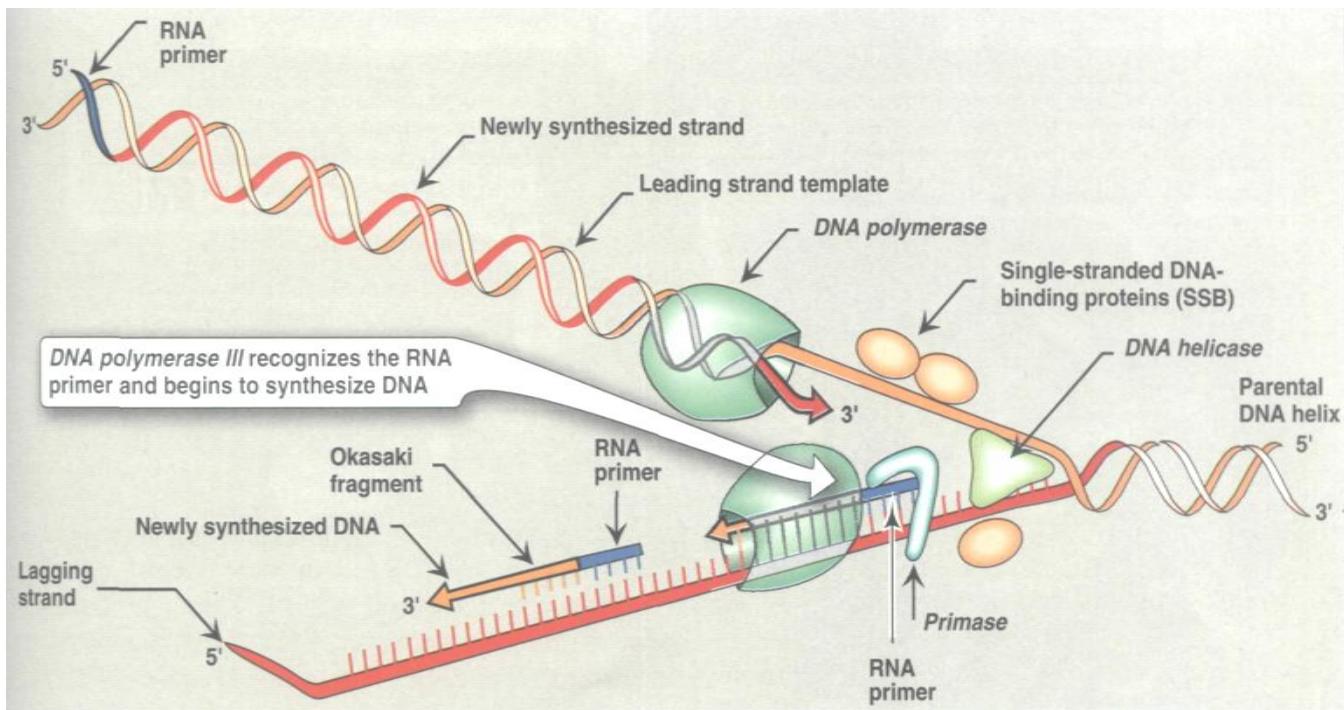
Substrate: deoxyribonucleotides (dNTPs i.e. dATP, dGTP, dCTP, dTTP)

Primer: Short RNA fragment complementary to DNA strand for initiation of replication

Enzymes: DNA dependent DNA Polymerase, other enzymes (Primase, DNA gyrase, Helicase), Protein factors..

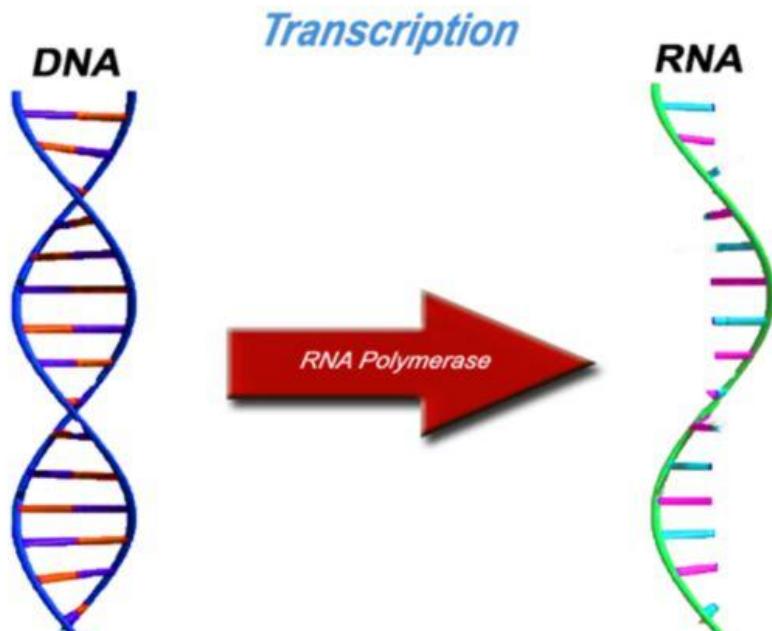
DNA replication is semi-conservative process with high fidelity progressing in bidirection and the nature is semicontinuous. DNA polymerase is the enzyme which reads the template DNA strand in 3'-5' direction and that synthesizes the DNA in 5'-3' direction. DNA replication starts at the site known as origin where DNA double stranded DNA is denatured to single strand. Primase synthesizes the primer to which DNA polymerase adds the nucleotide complementary to the base of template. One of the strand is synthesized continuously and is called leading strand whereas next is synthesized in fragments called Okazaki fragments. After the replication is over the RNA primer is replaced with DNA and finally sealed.





Transcription:

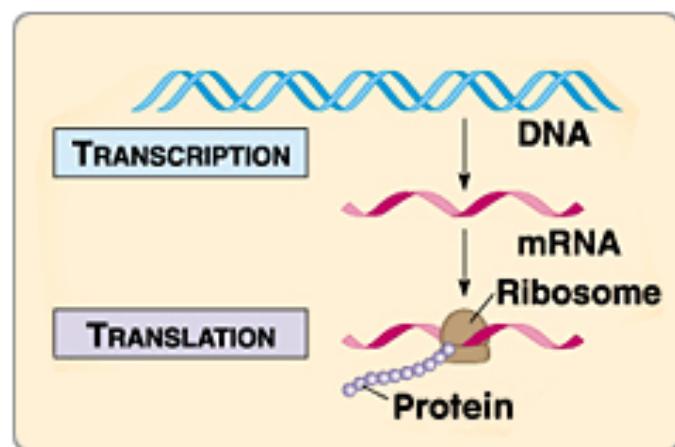
Transcription is the process in which RNA molecules are synthesized using DNA strands as the templates. The whole information of organism is contained in DNA ie. Genetic master plan is retained in DNA. The information is turned to action by the RNA molecules. So, simply RNAs are the working copies of DNA which either could be structural (rRNA), Functional (tRNA) or carries information to make up a certain protein (mRNA). In response to the development requirement, physiological need and environmental changes small portion of genome is transcribed. DNA regions that can be transcribed into RNA are called structural genes. Transcription requires single stranded DNA as the template and progress in 3' → 5' direction catalyzed by DNA dependent RNA polymerase which joins the ribonucleotide complementary to the template strand. RNA polymerase does not require primer unlike DNA replication.



Translation:

Genetic code: (see above)

Translation is the process which translates the information in Nucleic Acid (mRNA) into language of amino acid (proteins). The information in mRNA in the form of nucleotide sequences is transformed into the



amino acid sequences. In translation the nucleotide sequences is read 3 at a time known as genetic code which represents an amino acid to be inserted in growing peptide chain. Ribosome is the structure where translation occurs. The amino acids are carried by specific t-RNA molecules.

Nervous system:

Cerebro spinal Fluid (CSF):

Cerebrospinal fluid (CSF) is the liquid surrounding the brain and spinal cord which provides physical support and protection for the nervous system. It provides a controlled chemical environment, nutrient supply and waste removal from the nervous system along with its involvement in intra and extra cerebral transport.

Indications for laboratory investigation of CSF:

In normal healthy condition the proper chemical environment (glucose, protein, electrolytes) of CSF is maintained in healthy range where as in various conditions like trauma, cerebral injury, infection, CNS malignancy, demyelinating disease these analytes alters. So, CSF examination could reveal the secret behind the present problems.

Collection:

CSF specimen is obtained by lumbar puncture generally at the interspace between L₃-L₄ or lower using aseptic technique by skillful hand. Therefore it is precious sample and should be collected very carefully. While collecting it is collected in at least 3 aliquots and send immediately for analysis in

- Biochemistry & serology
- For microbiology
- For cell count.

Soon, after the sample approaches biochemistry lab, it is analysed for various analyte mainly glucose, protein, pH. The composition of CSF in healthy individuals is tabulated below:

Color & appearance:	Clear, colorless, no coagulum & deposit.
Specific gravity:	1.006-1.007
Cells:	0-4 mononuclear cells/mm³
pH:	7.3
Protein content:	10-45 mg/dl
Glucose:	50-100 mg/dl
Chlorides:	700-760 mg/dl (NaCl)
Urea:	20-40 mg/dl
Calcium:	5.5-6 mg/dl

Abnormal findings:

Appearance:

Normal CSF is clear and colourless and gives no coagulum or sediment on standing, if it is not contaminated. Abnormalities in appearance may arise in regard to color, turbidity, coagulum.

A red tinge to CSF indicates the presence of blood. Presence of blood is the main cause which could be as a result of trauma, haemorrhage. Color differences can occur with hyperbilirubinemia, melanoma or elevated proteins. A cloudy appearance may indicate an increase in the white blood cell count or protein

pH:

pH of CSF collected anaerobically is 7.31 approx and is mainly dependent on the pCO₂ content. The pH may be a major regulator of the activity of the respiratory center, while CO₂ equilibrates between plasma and CSF relatively quickly, changes in HCO₃⁻ concentration are slower. Therefore too rapid replacement of HCO₃⁻ deficit in plasma may adversely affect the CSF pH.



Glucose:

Glucose is measured in CSF using GOD-POD (glucose oxidase-peroxidase) method as done for blood glucose. Glucose concentration varies from 50-80 mg/dL, though a range of 45-100 mg/dL is often allowed. CSF glucose level is slightly lower than blood glucose. CSF glucose is dependent on blood glucose level.

Increased levels: small increase in blood glucose level is seen in some cases of encephalitis, poliomyelitis. Considerable increase are seen to occur in diabetic hyperglycemia but still lower than that of blood glucose.

Decreased level of glucose in the CSF is the most important pathological change seen. In coccidioidomycosis (meningococci, staphylococci, pneumococci), tubercular meningitis, viral meningitis low levels of glucose is seen. Also low level of glucose is associated with hypoglycemia and decreased permeability of glucose.

Protein:

The protein content of normal CSF ranges from 15-45 mg/dL. Protein content is almost entirely albumin, with small amounts of globulins.

Increase in the total protein is the commonest abnormality seen. The protein in such cases is a mixture of albumin and globulins. Albumin is solely synthesized in the liver, whose increased CSF concentration must occur through blood-brain barrier. CSF immunoglobulins especially IgG can arise from plasma cells within the CSF and from the blood through blood-brain barrier. Increased CSF immunoglobulin without increase in CSF albumin is suggestive of local production of immunoglobulin. The increase in proteins results from a breakdown of 'blood-CSF and Brain-CSF barriers' which may be due to inflammatory reaction, occasionally may be due to obstruction.

Marked increase in CSF protein is seen in various forms of acute meningitis. Also marked increase is seen in polyneuritis and in presence of tumors as acoustic neuroma and meningomas. In chronic condition as multiple sclerosis and general paresis there may be slight rise in CSF protein which is principally due to increase in γ -globulins. Very high CSF protein upto 10g/l are found in CSF below spinal block (usually due to tumor) and is mainly due to albumin leaked from the plasma.

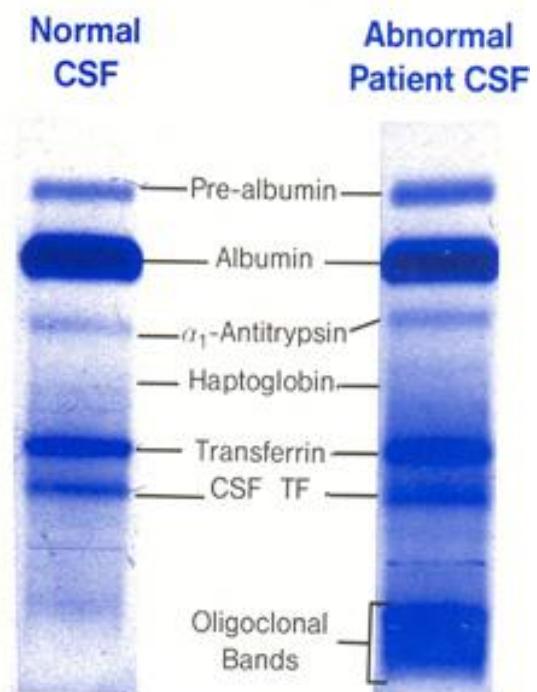
Chlorides:

The chloride content of normal CSF lies between 700-760mg NaCl/dL, higher than the plasma chloride. CSF chloride is affected by the plasma concentration and the difference between CSF and plasma becomes less if the meninges are inflamed.

A decrease in CSF chloride is seen in meningitis. The reduction is generally more marked in tubercular meningitis as compared to coccidioidomycosis. An increase is sometimes found in the hyperchloraemic acidosis in chronic renal failure.

CSF electrophoresis: oligoclonal bands

Electrophoresis of CSF shows the presence of many different globulins. When electrophoresis is done on normal CSF, the proteins are separated into different fractions. γ -globulins increase are disproportionately greater in multiple sclerosis and in neurosyphilis. In multiple sclerosis there is a predominant increase in IgG and "oligoclonal" γ -globulin band derived from plasma cells and lymphocytes are often present.



Besides these parameter urea, uric acid, glutamine, calcium, copper, phenylalanine, enzyme can also measured in CSF.

Neurotransmitters:

Neurotransmitter are chemical messengers (chemical mediator) released by presynaptic cells that is capable of transmitting an electrical impulse. Structurally neurotransmitters are classified into 2 categories:

Small nitrogen containing molecules: examples of which include; Glutamate, GABA (gamma amino butyric acid), glycine, acetylcholine, dopamine, histamine, epinephrine, nitric oxide, serotonin.

Neuropeptides: are the peptides synthesized and processed in CNS. Some of these have target within CNS (eg. Endorphin: which bind to opioid receptor and block pain signals) where as others are released in circulation to bind receptors.

Functions of Neurotransmitters:

Neurotransmitters are secreted form the neuron in response to an electrical stimulus called action potential. Neurotransmitter diffuse across synapse to another excitable cell, bind to receptor and elicit the response. Various neurotransmitters have different roles as:

Acetylcholine: Neurotransmitter at neuromuscular junction to stimulate muscular contraction.

Serotonin: is a powerful vasoconstrictor & contracts smooth muscle in bronchioles and arterioles. Controls behavioural patter, evokes release of peptide hormone from GIT.

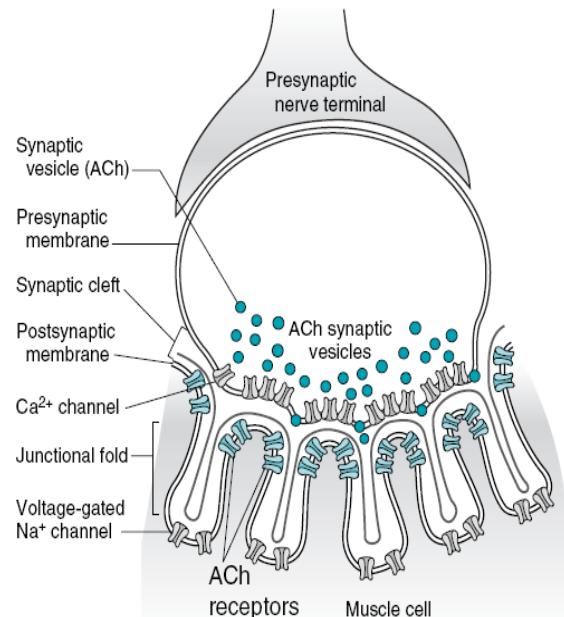
Histamine: causes smooth muscle contraction, increases vascular permeability and secretion of nasal and bronchial glands, stimulates gastric secretion.

Glutamate: excitatory function within CNS (excite the neurons)

GABA (γ -amino butyric acid) is the major inhibitory neurotransmitter.

Nitric oxide: vasodilatation & relaxation of smooth muscle, inhibitor of platelet aggregation and adhesion, bactericidal action.

Glycine: major inhibitory neurotransmitter in spinal cord.



Fuels of brain:

The principal fuel for brain is glucose. But during prolonged fasting (48hrs, starvation) brain can rely on alternative fuel sources as ketone bodies (3-hydroxy butyrate, acetoacetate), lactate, acetate, pyruvate.

Special sensor and integumentary system:

Vitamins:

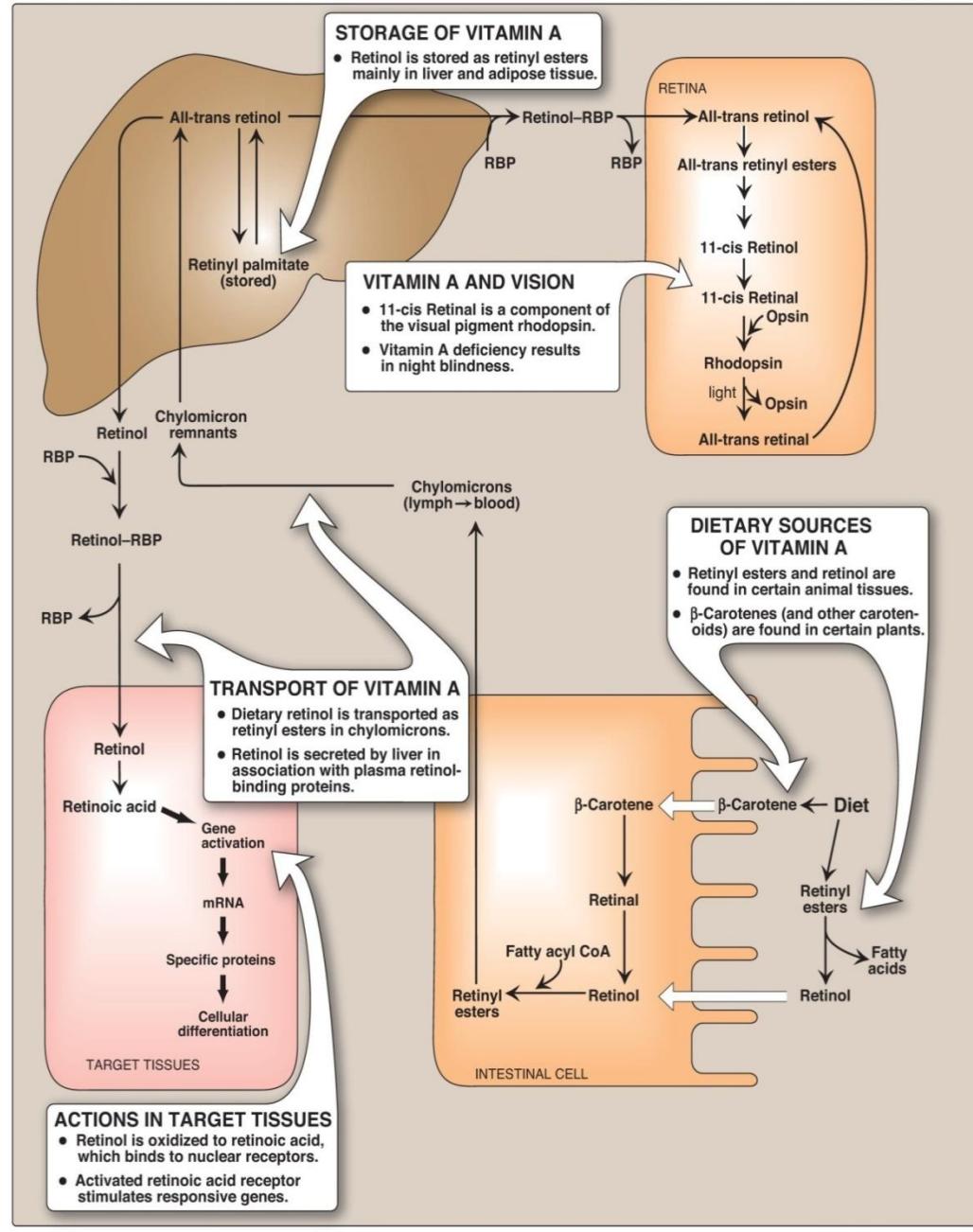
Vitamins may be defined as organic compounds occurring in small quantities in the different natural foods and necessary for growth and maintenance of good health in human beings and other experimental animals. Vitamin is a general term for a number of unrelated organic compounds that occur in many foods and are required in trace ($\mu\text{g}/\text{mg}$) for the normal metabolic functioning of the body. Vitamins are chemically unrelated organic compounds that cannot be synthesized by humans therefore must be supplied by the diet. Vitamins are required to perform specific cellular functions. *E.g. many of water soluble vitamins serve as coenzymes helping enzymes to catalyze the*

specific reactions. Vitamer is the term used to describe any of a number of compounds that possess a given vitamin activity.

Vitamin A: Vitamin A is fat soluble vitamin. Vitamin A serves many important functions in the body with its role in vision being particularly significant. They are retinoids with various functional groups. The alcohol, aldehyde and acid form of vitamin A is called Retinol, Retinal and Retinoic acid respectively. All these form of vitamin A are called vitamers of Vitamin A. β -Carotene is a provitamin which can be oxidatively cleaved in the intestine to yield 2 molecules of retinal.

Absorption, Transport and storage:

Vitamin A as such is present in animal sources whereas provitamin A is present in plant. They occur as fatty acid esters. The digestion and absorption of fat soluble vitamin takes place along with the fat and follows as dietary lipids. Dietary retinyl esters are hydrolysed by hydrolases. Emulsification of vitamin-A & provitamin-A forms to the micellar level by bile salts enhances their uptake by mucosal cells of small intestine. Intestinal cell absorption of free retinol is followed by reesterification with long chain fatty acid, predominantly palmitic & stearic within mucosal cell. Those carotenoids capable of being cleaved by the cellular dioxygenase system to retinal is reduced to retinol. Retinyl esters are



then packed in chylomicrons which then pass via the lymphatic system to the liver, where uptake by parenchymal cells again involve hydrolysis and stored. As when needed Vitamin A is released from the liver as free retinol. Retinol is transported in circulation by the plasma retinol binding protein (RBP) in association with pre-albumin. The retinol-RBP complex bind to specific receptors on the cell membrane of peripheral tissue and enters the cell. **(Above figure depicts absorption, distribution, storage and biochemical functions of Vitamin A)**

Many cells of target tissue contain cellular retinol-binding protein that carries retinol to the nucleus and bind to the chromatin. Most of the aldehyde is reduced reversibly to retinol, lesser amounts are oxidized to retinoic acids in liver, kidney, intestine. Retinoic acid is not reduced significantly to retinal but is metabolized rapidly in tissue, to yield more polar catabolites which is excreted.

Sources: Liver, kidney, cream, butter, egg yolk, yellow & dark green vegetables.

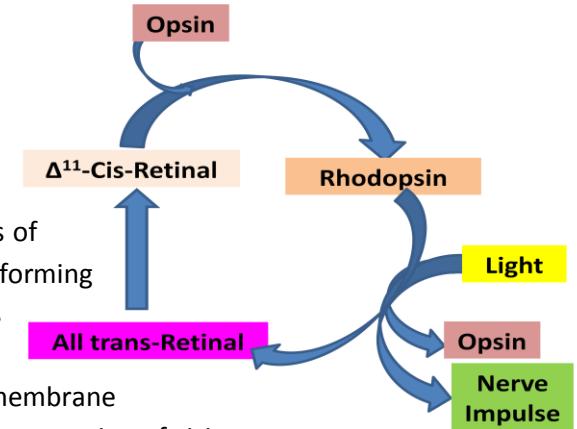
RDA (WHO & ICMR expert group)

Men : 1000 µg/day

Women: 800 µg/day

Biochemical functions:

Vitamin A is essential vision. Vitamin A undergoes several changes in rods of eye. Rods are used for seeing at low intensities of light. 11-cis retinal (vitamer of Vitamin A) is complexed with opsin forming Rhodopsin which is a vision complex. When light strikes opsin is dissociated from and 11-cis retinal is converted to all trans retinal through series of reaction. And this change brings the change in membrane potential by opening and closing of various ion channels leading to perception of vision.



Color vision: Color sensitive retinal-opsin complexes are bleached in different proportions results in the perception of different colors by brain.

Retinol & retinoic acid functions as steroid hormones: They regulate protein synthesis & are involved in Cell growth & differentiation.

Vitamin A is essential for normal reproduction. Vitamin A prevent keratin synthesis thus helps to maintain healthy epithelial tissue. It is essential for the maintenance of proper immune system. It is Involved in synthesis of transferrin, which is iron transporting protein.

Increased consumption of β-carotene is associated with decrease incidence of heart attack & lung cancer

Deficiency Disease:

The deficiency manifestations are related to eyes, skin and growth

Night Blindness (Nyctalopia): subject cannot see object in dimlight

Xerophthalmia: dryness in conjunctiva & cornea due to keratization of epithelial cells. If Xerophthalmia persists for long time progress towards Keratomalacia, destruction of cornea. Reproductive system adversely affected. Skin becomes rough & dry

Hypervitaminosis A : Excess of vitamin A lead to toxicity and could result in Dermatitis (drying & redness of skin)
Enlargement of liver, Skeletal decalcification, Hair loss, Joint pain, Associated with formation of urinary stones

Musculoskeletal System:

Minerals are the inorganic ions which are required in small quantities for the proper maintenance of health. Minerals constitute only small proportion of body weight. Though they are present in small quantities they perform several vital functions which is essential for the very existence of the organism: bone formation, blood coagulation, neuromuscular irritability, acid-base balance, water balance, osmotic regulation. Some of the minerals are the integral components of biologically important compounds like hemoglobin (iron), thyroxine (iodine), Insulin (Zinc), Vitamin B₁₂ (Cobalamin). Among the requirements some of the minerals are required daily above 100mg and are called principal minerals which include calcium, magnesium, sodium, chlorine, potassium, phosphorous, sulphur and those whose requirement is below 100mg are regarded as trace element, includes zinc, iodine, cobalt, manganese, copper etc.

Calcium:

Calcium is the 5th most common minerals in the body constituting about 1-1.5 kg (in adult) of which 99% is present in bones and teeth. Rest of the calcium is distributed throughout the body fluids and soft tissues. The normal concentration of serum calcium 9-11mg/dl of which 50% calcium is present as free (ionized) calcium, 40% protein bound and rest of calcium is complexed with various other anions. Ionized form is functionally the most active and usually in laboratory determination of calcium all three fraction of calcium is measured together.

Functions:

Calcium is required for the development of bones and teeth. Calcium along with other minerals and phosphates mineralizes the bone. Calcium is required for muscle contraction and is important for the transmission of nerve impulse. Calcium (clotting factor IV) is important for blood clotting and hence is involved in homeostasis. Calcium acts as cofactor for various enzymes (pancreatic lipase, ATPase). Calcium acts as secondary messenger in various signaling cascade and involved in various secretory process.

Phosphorous:

Phosphorous is found in every cells of the body. Phosphorous in the form of inorganic or organic phosphate is an important and widely distributed element in the human body. About 85% of the phosphate is found in bones, 15% in soft tissues and <0.1% in extracellular fluids. Plasma contains approxly 2.5-4.5mg/dL of inorganic phosphate, the fraction measured by clinical laboratories. Approxly 10% of phosphate present in serum is protein bound, 35% forms complex with sodium, calcium and magnesium and the remainder is free.

Functions:

Inorganic phosphates is a major component of hydroxyapatite in bone, thereby playing an important role in structural and support of the body (formation of bones and teeth) and acts as phosphate reservoir for the extracellular and intracellular pool of phosphate. It has a critical role as high energy phosphate bond (ATP, GTP, UTP, Creatine Phosphate) and a constituent of cyclic adenine (cAMP) and guanine (cGMP) nucleotides as well as in structures like NADP. Phosphate is also an essential element in phospholipid, nucleic acids, phosphoproteins. Phosphate play important role in regulation of several important enzyme system. Phosphate is the component of phosphate buffer.

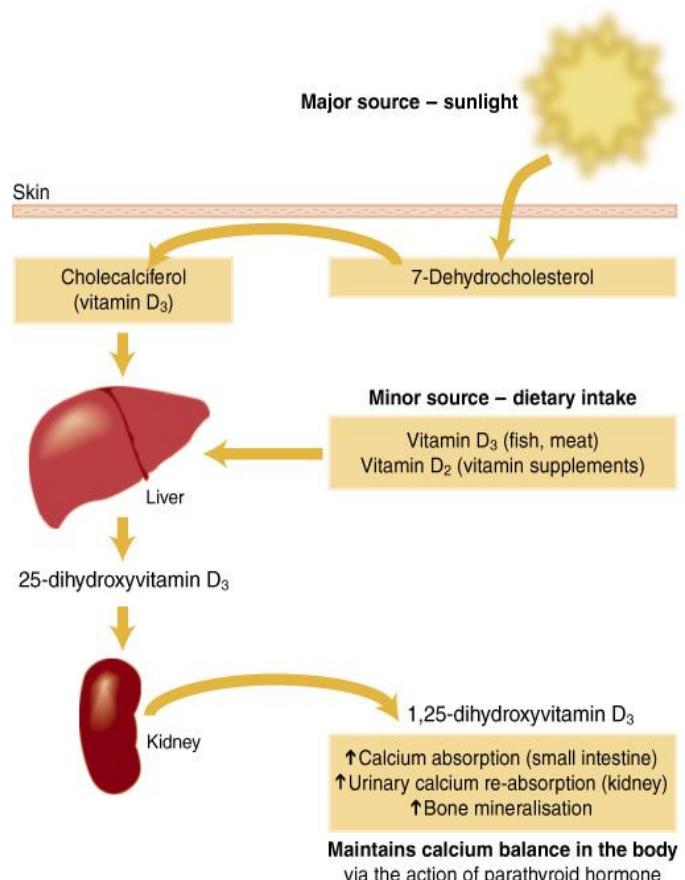
Hormonal regulation:

The hormones mainly parathyroid hormone, calcitriol and calcitonin works in coordination to regulate the plasma calcium within a narrow range. Parathyroid and calcitriol hormone is involved in increasing the blood calcium level while calcitonin in decreasing blood calcium level.

Parathyroid hormone: parathyroid hormone is a peptide hormone secreted from parathyroid gland. Secretion of parathyroid hormone is increased in response to low blood calcium level and does so by increasing the synthesis of calcitriol (1,25-dihydroxycholecalciferol).

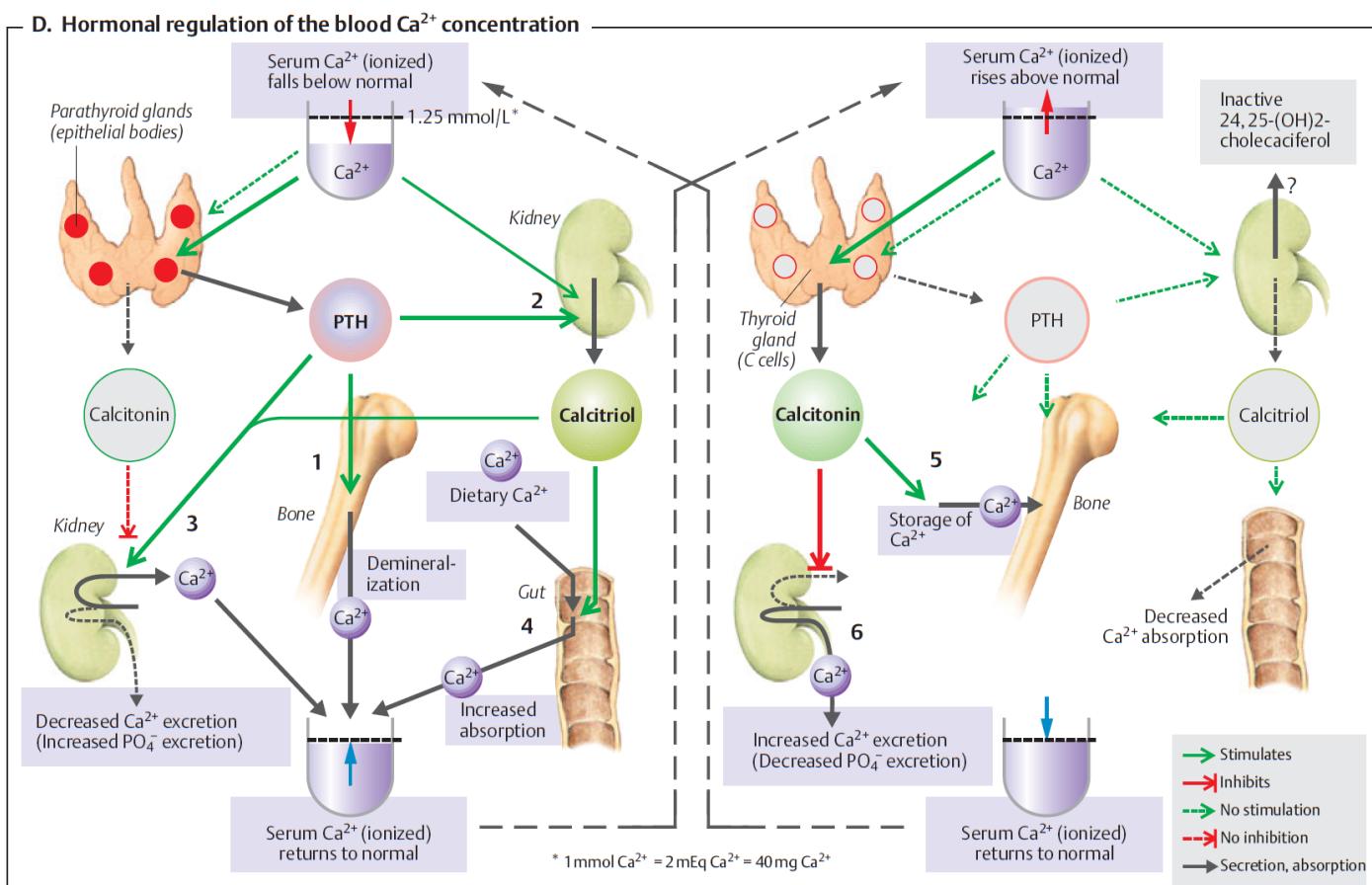
Calcitriol: (1,25 dihydroxy-Vitamin D; 1,25-dihydroxycholecalciferol)

Calcitriol is the active form of vitamin D which is involved in increasing blood calcium level. In response to parathyroid hormone signaling the conversion of vitamin D to calcitriol is increased. During this coversion vitamin D is first hydroxylated at 25 position to form 25-hydroxy vitamin D in liver which is then hydroxylated at 1 position



to form 1,25-dihydroxy vitamin D which is principal regulator of calcium and phosphate. Calcitriol enhances the intestinal absorption of calcium and phosphorous, promotes bone mineralization, mobilizes calcium from bone as needed (along with PTH) and promotes renal calcium retention and phosphate excretion.

Calcitonin: is a peptide hormone secreted by parafollicular cells of thyroid gland. Calcitonin secretion is elevated in response to high calcium level and decreases blood calcium level by increasing bone mineralization (increasing activity of osteoblast) decreasing bone resorption (inhibiting osteoclastic activity) and increasing the excretion of calcium in urine. The following figure illustrates the regulation of Calcium in body.



Causes of calcium imbalance: (Hypercalcaemia or Hypocalcaemia)

Calcium level blood should be within 9-11 mg/dl fine range, above or below of which is termed as hyper or hypocalcaemia.

Hypercalcemia:

When blood/serum level exceeds 11mg/dl, it is called hypercalcemia which may be due to hyperparathyroidism, malignancies, hyperthyroidism, acromegaly, acute adrenal insufficiency, tuberculosis, sarcoidosis, Hypervitaminosis A and D, drugs like: thiazide diuretics, spiro lactone, renal failure (acute-diuretic phase/ chronic). Hypercalcemia leads to lethargy, muscle weakness, appetite loss, constipation, nausea and susceptibility to fractures etc.

Hypocalcaemia:

Hypocalcaemia is said to exist when serum calcium is less than 8.5mg/dl as determined by standard methods. The commonest cause of hypocalcaemia is hypoalbuminaemia closely followed by renal failure. The other most important cause of hypocalcaemia is surgically induced hypoparathyroidism. Hypocalcaemia is more serious and life threatening causing tetany.

Sfha

Fuels of Muscle:

Muscle cells use stored glycogen and circulating glucose, fatty acids and amino acids as energy sources. Muscle stores high energy phosphates as creatine phosphate and generates ATP from substrate level phosphorylation. During excess of ketone body formation muscle too can rely on ketone bodies as fuel.

(The heart primarily uses fatty acids (60–80%), lactate, and glucose (20–40%) as its energy sources. Ninety-eight percent of cardiac ATP is generated by oxidative means; 2% is derived from glycolysis. The lactate used by the heart is taken up by a monocarboxylate transporter in the cell membrane that is also used for the transport of ketone bodies. However, ketone bodies are not a preferred fuel for the heart, because the heart prefers to use fatty acids)

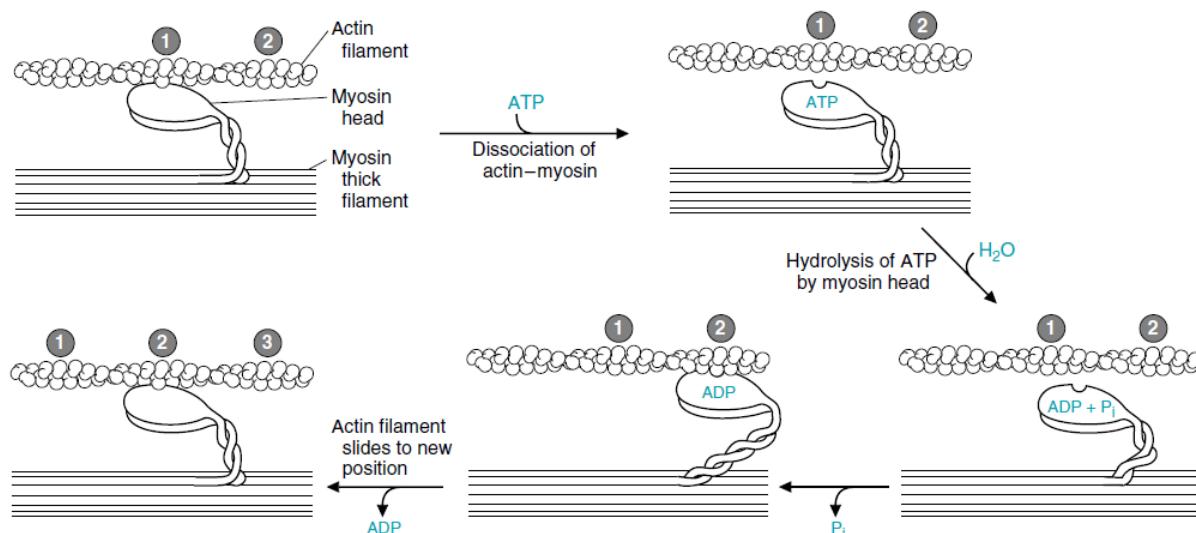
Molecular basis of Muscle contraction:

Sliding filament model explain the basics of muscle contraction where contractile protein actin slides over myosin with a series of biochemical changes. The contractile unit of muscle contains arranged actin, myosin filament and regulatory protein troponin.

Actin filaments form a network controlling the shape of the cell and movement of the cell surface. Actin polymers form the thin filaments (also called microfilaments) in the cells that are organized into compact ordered bundles or loose network arrays by crosslinking proteins.

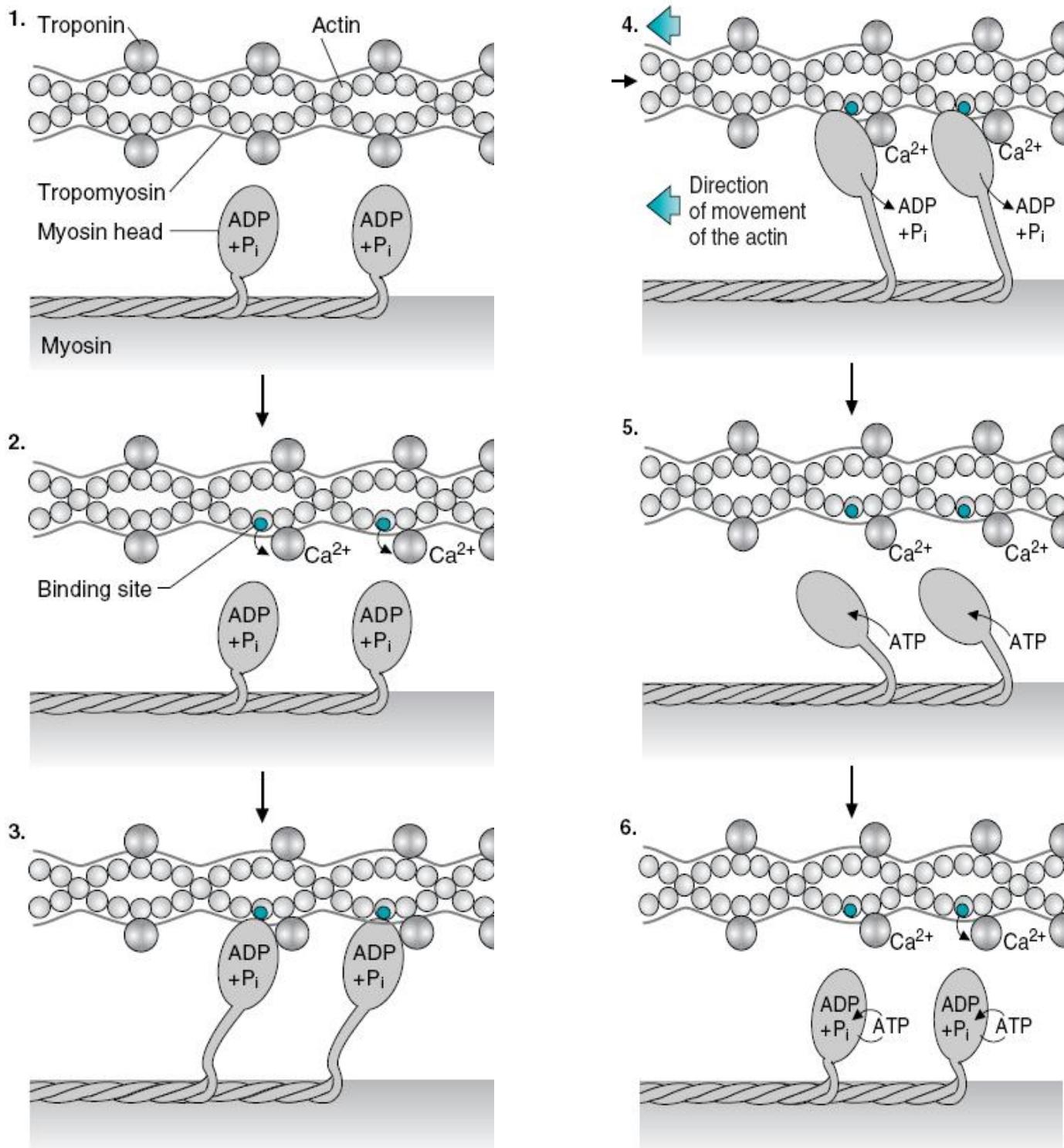
Troponin is a heterotrimeric protein involved in the regulation of striated and cardiac muscle contraction. Most troponin in the cell is bound to the actin–tropomyosin complex in the muscle fibril.

The contractile unit in action can simply be explained with the following diagram.



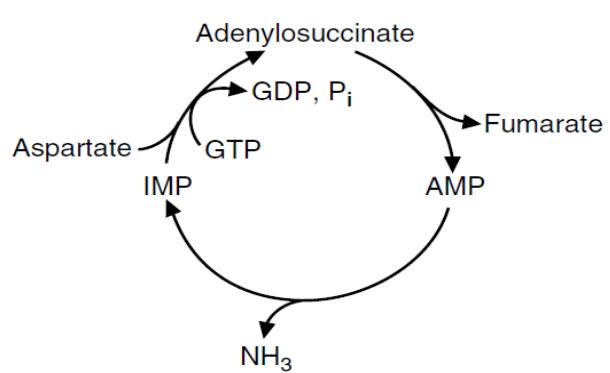
The release of neurotransmitter at neuromuscular junction in response to stimuli ultimately releases the calcium in the muscle cell increasing the intracellular calcium level. The calcium ion binds to troponin, resulting in a conformational change in the troponin–tropomyosin complexes such that they move away from the myosin-binding sites on the actin. When the binding site becomes available, the myosin head attaches to the myosin-binding site on the actin. The binding is followed by a conformational change in the myosin head, which shortens the sarcomere.

After the pivoting, ATP binds the myosin head, which detaches from the actin and is available to bind another myosin-binding site on the actin. As long as calcium ion and ATP remain available, the myosin heads will repeat this cycle of attachment, pivoting, and detachment. This movement requires ATP, and when ATP levels are low (such as occurs during ischemia), the ability of the muscle to relax or contract is compromised. As the calcium release channel closes, the calcium is pumped back into the sarcoplasmic reticulum against its concentration gradient by use of the energy-requiring protein SERCA (sarcoplasmic reticulum Ca²⁺ ATPase), and contraction stops. This is the basic process occurs in all muscle cell types, with some slight variations between cell types.



Purine Nucleotide cycle:

Purine Nucleotide cycle is very important in muscle cells. Using a combination of biosynthetic and salvage enzymes there is conversion of aspartate to fumarate and ammonia. The cycle is very important in muscle as it generates the fumarate which is TCA intermediate and helps to maintain the concentration of TCA intermediate along with ammonia which is used in buffering the proton generated from lactate in muscle cells during heavy exercise. Exercise increases the activity of the purine nucleotide cycle.



Respiratory system:

pH is the negative log of Hydrogen ion concentration { $\text{pH} = -\log[\text{H}^+]$ }. Acid are the compounds that can release proton $[\text{H}^+]$ and the base are the compounds that can accept proton. pH is a universal and essential activity of living organism. pH should be maintained fairly constant with in a fine normal range in living organism in different compartments. Blood pH is maintained fairly constant at 7.35-7.45 and condition in which pH drop below 7.35 is Acidosis or elevation above 7.45 is alkalosis. Proteins structures are greatly affected by the pH, so the slight variation in pH greatly affect its structure and function leading it to non-functional. This inactivation of protein is very dangerous as protein do have very important role: catalytic (enzymes), transport (albumin, transferring) in which all the biological reaction and processes are dependent. Hence pH needs to maintain fairly at constant level within a fine range.

Our body is acid producing factory as in various metabolic process we generate huge amount of CO_2 , organic acids (lactic acid, ketone bodies) which in turn fluctuates the pH. So, there should be proper systems and co-ordination between these systems to maintain pH homeostasis. For this buffer system, respiratory system and renal system are involved.

Buffers:

Buffer (pH buffers) is a solution that resist the change in pH when small amount of acid or base is added to it. The buffers maintain pH by accepting or releasing proton so, that the pH remains unaffected. Buffer is important pH regulating system that absorbs the pH shock and don't let the pH change instantly. Simply buffer is the mixture of weak acid and its conjugate base or weak base and its conjugate acid. For examples carbonic acid and its conjugate base bicarbonate form the bicarbonate buffer system. So, buffer system can furnish or use the proton

Buffer Action: (Mechanism of Buffering)

Lets consider HA is a weak acid and A^- its conjugate base furnished from its respective salt XA .



In buffer system above reaction is in equilibrium

When Acid $[\text{H}^+]$ is added the reaction shifts towards left forming HA, so that added H^+ is not free and hence no effect in pH change. When Base is added proton are removed from the system & pH may rise but the reaction shifts towards right, dissociating more & more HA to furnish H^+ . Hence pH remains unaffected.

The above reaction is the representative buffer action of all the buffer system. For bicarbonate buffer HA is carbonic Acid (H_2CO_3) and A^- is bicarbonate (HCO_3^-). The capacity of buffer to resist the pH change is known as buffering capacity and is dependent on the concentration of its component of mixture and their ratio.

Buffer system in body fluids:

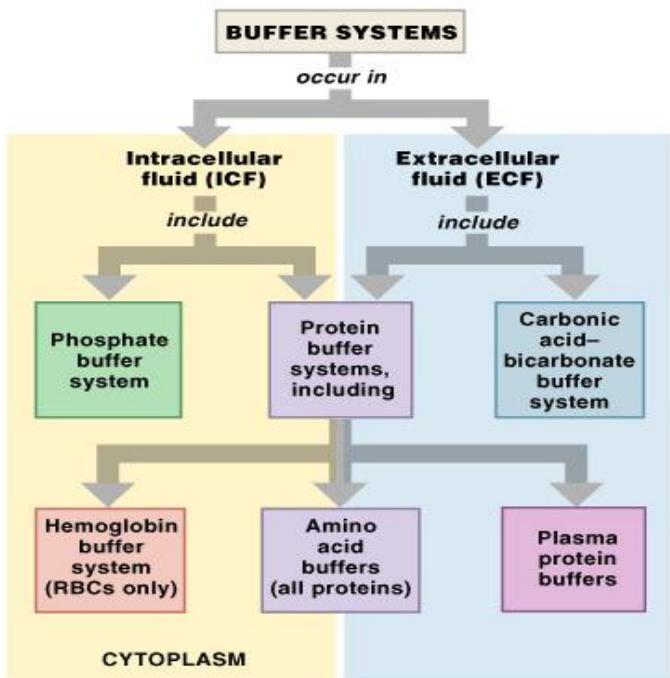
Buffers are present in body fluids either inside or outside and hence are intracellular or extracellular buffers.

Intracellular buffer:

- Phosphate buffer system
- Protein buffer system
 - Hemoglobin buffer (RBC)
 - Amino acid buffer (All proteins)

Extracellular buffer system: buffers of blood

- Bicarbonate buffer system
- Protein buffer (plasma proteins)



Phosphate buffer:

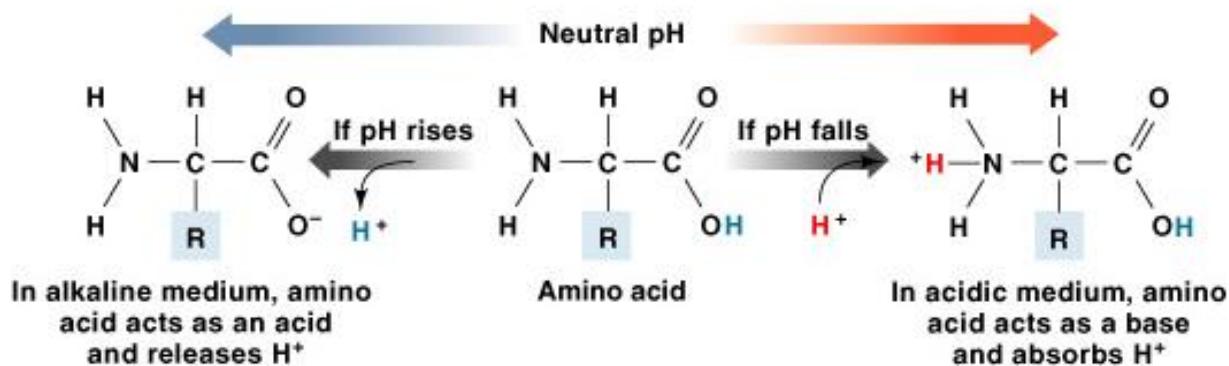
Consists of weak acid-conjugate base pair $[H_2PO_4^- / HPO_4^{2-}]$; dissociation occurs as



Phosphate buffer is important buffer in intracellular fluids where its concentration is 75mEq/L (high) and in blood 4mEq/L (low). As its concentration is high in intracellular fluid it is the major buffer system inside cell. pH of cell fluid is approx 7.2 (6.9-7.4), an equimolar mixture of $H_2PO_4^-$ and HPO_4^{2-} is typically present. Buffering action of phosphate buffer is similar to above reaction where conjugate base (HPO_4^{2-}) combines to form acid $H_2PO_4^-$ when acid (proton) is added and $H_2PO_4^-$ dissociates to furnish proton when base is added.

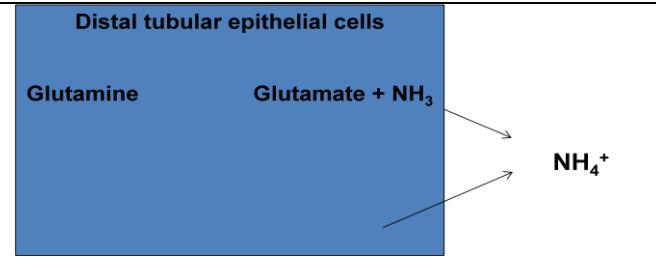
Protein buffer:

Proteins are present in significant concentration intracellularly and extracellularly. Hence proteins are the body's most plentiful and powerful buffers. The several types of ionizable groups which can accept or donate proton are responsible for the buffering action of protein. Free amino acid can act as buffer as its amino group and carboxylic group are dissociable and can donate or accept proton as shown in following figure:



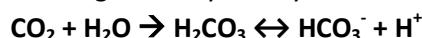
Ammonia buffer:

Very important in distal tubular cells and muscle cells. During excessive muscle contraction there is excess production of lactic acid and at this time purine nucleotide cycle is active forming fumarate and ammonia. Ammonia binds with proton and buffers the proton (acid). In distal tubular cells NH_3 (ammonia) is generated from deamination, passed to tubular lumen where it binds with the excreted proton and helps to excrete proton.



Bicarbonate buffer: ($\text{H}_2\text{CO}_3 / \text{HCO}_3^-$):

Bicarbonate buffer system is one of the most important as the buffer component can simply be adjusted by our body system. Carbonic anhydrase is the enzyme helping us for this. CO_2 concentration can be regulated by the rate of respiration while bicarbonate level can be regulated by kidneys.



If bicarbonate (alkali) concentration decreases, kidney removes H^+ from blood, shifting equilibrium to right, increasing HCO_3^- and when excess of HCO_3^- is produced it is excreted by kidneys.

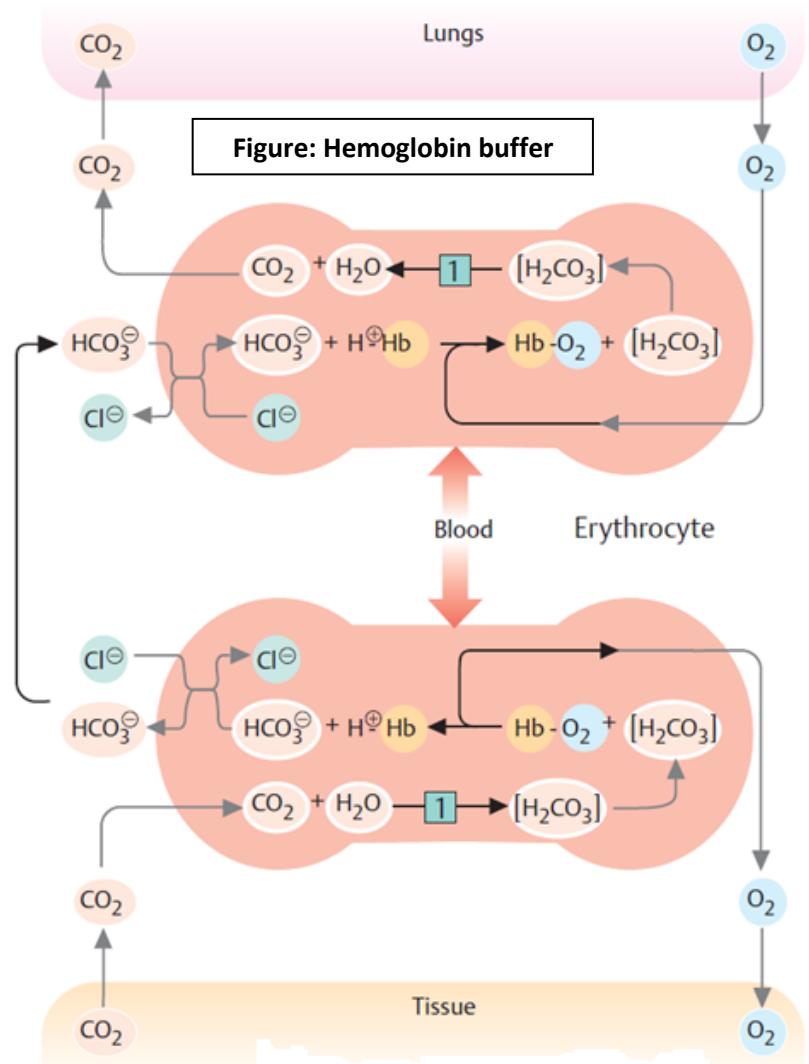
Hemoglobin buffer:

Hemoglobin is the intracellular buffer in RBC. In peripheral tissues where pCO_2 is high, CO_2 diffuses across RBC membrane and condenses with H_2O to form carbonic acid by carbonic anhydrase. The acid so formed dissociates into H^+ and HCO_3^- . The bicarbonate is exchanged with extracellular Cl^- and the process is chloride shift. The H^+ is buffered by hemoglobin as it binds with hemoglobin to form protonated hemoglobin. Hemoglobin is the only intracellular buffer system which has an immediate effect on pH of ECF.

ACID-BASE Balance:

The pH of the body fluid from different compartment should be maintained at the same pH (within fine range) because the protein gets adversely affected with the change in pH and if the pH get fluctuates below or above this range the conditions could be life threatening. The pH of blood is maintained at 7.4 ± 0.05 . Buffer system, respiratory system and Renal system all work in coordination for the maintenance of pH in body fluids. The instant change in pH as acid or base is added to fluids in our body is overcome by the buffering action of various buffers in these fluids which is then slowly taken up by respiratory system followed by renal system. The condition in which blood pH drops below 7.35 is called acidosis (acidemia) and above 7.45 is called alkalosis (alkalemia). The pH imbalance could be either respiratory or metabolic.

Respiratory Acidosis: Respiratory acidosis is also called primary carbonic acid [H_2CO_3] excess, which is due to increase in carbonic acid in blood. It is followed by decreased elimination of CO_2 (retention) in the pulmonary alveoli which may result from:
Breathing air containing abnormally high percentage of CO_2 and conditions in which elimination of CO_2 from lungs is reduced.



Causes:

- Conditions in which there is depression or suppression of respiration: damage to CNS, loss of ventilator functions.
- Conditions that cause impaired diffusion of CO_2 : emphysema, pulmonary oedema,
- Conditions in which there is an obstacle to the escape of CO_2 from alveoli: laryngeal obstruction, Asthma
- Conditions in which pulmonary blood flow is insufficient: certain congenital heart disease.

Respiratory Alkalosis: Respiratory acidosis is also called primary carbonic acid [H_2CO_3] deficit, which is due to decrease in carbonic acid in blood with no corresponding change in HCO_3^- . It is followed by increased elimination of CO_2 out of blood by hyperventilation.

Causes:

- Stimulation of respiratory center: CNS diseases (meningitis, encephalitis), salicylate poisoning, hyperpyrexia.
- Other causes: hysteria, high altitude effects, injudicious use of respirators.

Arterial blood gas Analysis:

Blood gas analysis, also called arterial blood gas (ABG) analysis, is a test done to measure the amount of oxygen and carbon dioxide in the blood as well as the acidity (pH) of the blood. An ABG analysis evaluates how effectively the lungs are delivering oxygen to the blood and how efficiently they are eliminating carbon dioxide from it. The test also indicates how well the lungs and kidneys are interacting to maintain normal pH (acid base balance). Blood gas analysis are usually done to assess respiratory disease and other conditions that affect the lungs, and to manage patients receiving oxygen therapy (respiratory therapy).

An Arterial blood gas analysis is typically requested to determine the pH of the blood and the partial pressure of carbondioxide and the partial pressure of oxygen within it, to access the effectiveness of gaseous exchange and ventilation whether spontaneous or mechanical. The ABG allows to access the metabolic status of the patients too, giving an indication of how they are coping with their illness.

Therefore Clinical management of respiratory and metabolic disorders often depends on rapid, accurate measurement of the partial pressure of oxygen (pO_2) and partial pressure of cabondioxide (pCO_2). ABG analysis helps to preserve and support life in individuals with cardiopulmonary impairment which depend largely on assisted ventilation through use of gaseous mixture that are tailored in response to laboratory blood gas analysis and acid-base results.

Sample:

Sample of arterial blood is collected from radial artery in the forearm or from the femoral artery (less commonly) by skillful and responsible hands (phlebotomist, nurse, respiratory therapist, doctor) in a heparinized syringe and immediately taken for analysis. If the sample cannot be processed immediately, it is chilled in an ice bath in glass syringe. The biochemical profile done in ABG analysis include following in the given table.

Reference ranges of arterial blood gas analysis	
Parameter	Reference value
$[H^+]$	35-43 mmol/L
pH	7.35-7.45
pCO_2	4.5-6 kPa
pO_2	10.5-13.5 kPa
HCO_3^- (bicarbonate)	24-30 mmol/L



After a pulse is found, a blood sample is taken from the artery



Information provided by an arterial blood gas analysis:

pH: The pH measures the hydrogen ion concentration $[H^+]$ in the blood. The pH of blood is maintained within 7.35–7.45.

pCO_2 : This is the partial pressure of carbon dioxide dissolved within the arterial blood. The value is used to access the effectiveness of ventilation. A high pCO_2 (respiratory acidosis) indicates underventilation while low pCO_2 (respiratory alkalosis) indicates hyperventilation. In chronic pulmonary disease it may be considerably higher and still normal for that patient.

pO_2 : This is partial pressure of oxygen dissolved within the arterial blood and will determine oxygen binding to hemoglobin. Low values of it normally indicate hypoxaemia.

HCO_3^- : Bicarbonate is one of the component of bicarbonate buffer system and the level of it in blood indicates whether a metabolic problem is present (such as ketoacidosis). Low bicarbonate level indicates acidosis and a high bicarbonate level indicates alkalosis. Bicarbonate levels can also become abnormal when the kidneys are working to compensate for a respiratory issue so as to normalize blood pH.

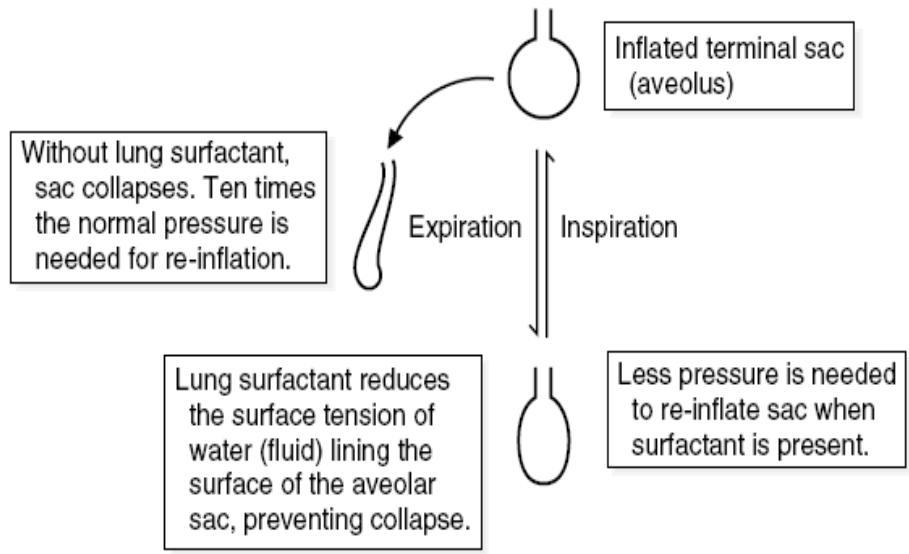
Neonatal Hyaline Disease:

Infant respiratory distress syndrome (IRDS), also called neonatal respiratory distress syndrome, respiratory distress syndrome of newborn, or increasingly surfactant deficiency disorder (SDD) and previously called hyaline membrane disease (HMD) is a syndrome in premature infants caused by developmental insufficiency of surfactant production and structural immaturity in the lungs.

Surfactants are the compounds that lower the surface tension (or interfacial tension) between two liquids or between liquid and a solid. Pulmonary surfactant is a surface active lipoprotein complex formed by alveolar cells.

By adsorbing to air-water interface of alveoli the main lipid component dipalmitoylphosphatidylcholine (phospholipid) reduces surface tension. Surfactant in lungs increases pulmonary compliance, prevent collapse of lung (atelectasis) at the end of expiration and facilitate recruitment of collapsed airways. Simply, surfactant is very essential requirement in respiration.

Infant respiratory distress syndrome can also result from genetic problem with the production of surfactant associated proteins. The incidence decreases with advancing gestational age.



The effect of lung surfactant

CARDIOVASCULAR SYSTEM

Cardiac markers:

Cardiac markers are proteins (regulatory, functional, and catalytic) which are drawn to blood when cardiac tissues are damaged and at that time their presence is high in blood. By estimating the concentration and activity of these proteins in affected individuals, a clear picture of diagnosis can be done. The cardiac marker includes Myoglobin (functional protein), Cardiac specific troponins (regulatory protein) and isoenzymes of heart (creatinine kinase (CK-MB), Lactate dehydrogenase (LDH), Aspartate aminotransferase (AST) [catalytic protein].

Myoglobin: Normal findings- < 90 mcg/L ($\mu\text{g}/\text{L}$)

Myoglobin is an oxygen binding protein found in cardiac and skeletal muscle. Measurement of myoglobin provides an early index of damage to the myocardium in myocardial infarction (MI) or reinfarction. Increased levels, which indicate cardiac muscle injury or death, occur in about 3 hours. The test is non-specific as injury related to skeletal muscle too show elevated levels of myoglobin.

Abnormal findings: Increased levels of myoglobin is found in Myocardial infarction, skeletal muscle inflammation, muscular dystrophy, skeletal muscle ischemia, skeletal muscle trauma, rhabdomyolysis.

Troponins:

Normal findings:

Cardiac specific troponin T (cTnT): <0.2 ng/ml

Cardiac specific troponin I (cTnI): <0.03 ng/ml

Cardiac troponins are promising biochemical markers for cardiac disease. This test is used to assist in the evaluation of patients with suspected acute coronary ischemic syndromes. In addition to improving the diagnosis of acute ischemic disorders, troponins are also valuable for early risk stratification in patients with unstable angina.

Troponins are proteins that exist in skeletal and cardiac muscle that regulate the calcium dependent interaction of myosin with actin for muscle contraction. Cardiac troponin can be specifically determined. Because of their extraordinary high specificity for myocardial cell injury, cardiac troponins are very helpful in the evaluation of patients with chest pain. Unlike other markers which could be elevated with skeletal muscle injury, brain or lung

injury or renal failure; cardiac troponins are more specific towards cardiac muscle injury. Cardiac troponins will nearly always be normal in noncardiac muscle diseases. Cardiac troponins becomes elevated sooner (as early as 3 hours after myocardial injury) and remains elevated longer (7 to 14 days). Therefore this test is more preferable to other measurement like: CK-MB, LDH, Myoglobin.

Abnormal findings: Myocardial injury, Myocardial infarction.

Cardiac enzymes:

Normally very low concentration of intracellular proteins are present in blood. But these protein concentration gets elevated in blood when the contents get drained in blood due to damage to tissues. Among these proteins enzyme activity can be selectively determined and can be used for the diagnosis.

Isoenzymes are the enzymes that catalyse the same biochemical reaction but their physical and chemical properties are different like structure, electrophoretic mobility, Km, Vmax, pH optimum, relative susceptibility to inhibitors and degree of denaturation. Different organs frequently contain characteristic proportions of different isoenzymes. The pattern of isoenzyme found in plasma may therefore serve as a means of identifying the site of tissue damage and aid in the diagnosis of problem.

Some of enzymes which is routinely investigated for cardiac tissue injury include:

Creatine kinase (creatine phosphokinase; CK-MB)

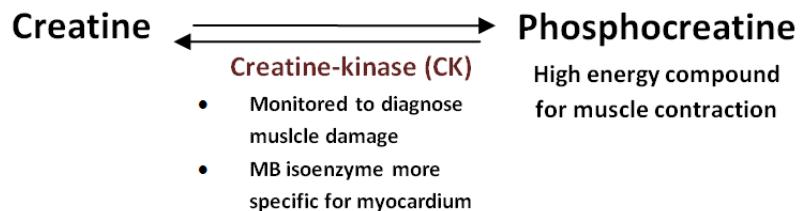
Lactate dehydrogenase

Aspartate aminotransferase

Creatine kinase:

Creatine kinase (CPK) catalyses the reversible conversion of creatine to creatine-phosphate. Creatine phosphate is the form in which the high energy is stored and which is utilized to generate the ATP by substrate level phosphorylation. CK is found predominantly in the heart muscle, skeletal muscle and brain. Serum CK levels are elevated whenever injury occurs to these muscle or nerve cells.

Creatine kinase is a dimeric protein with B & M type subunit. Creatine kinase has 3 isoenzymes namely CK1 (CK-BB), CK2 (CK-MB) and CK3 (CK-MM). CK2 or CK-MB isozyme is the enzyme of cardiac tissue which appears in blood after damage to these tissues. The CK-MB level is helpful in both quantifying the degree of myocardial infarction and timing the onset of infarction.

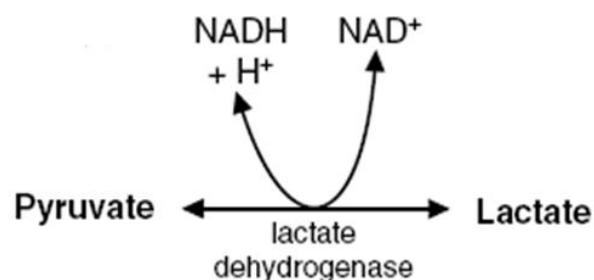


Following an acute myocardial infarction, CK-MB appears approxly 4-8 hours following onset of chest pain and if the damage is not persistent, reaches a peak of activity at about in 24 hours and returns to normal in 2-3 days.

Increased levels of CK-MB is seen in acute myocardial infarction, cardiac aneurysm surgery, cardiac defibrillation, myocarditis, ventricular arrhythmias, cardiac ischemia.

Lactate dehydrogenase:

Lactate dehydrogenase catalyses the reversible conversion of pyruvate to lactate or lactate to pyruvate as given in following reaction.



Lactate dehydrogenase (LDH) is found in the cells of many body tissues, especially the heart, liver, RBC,

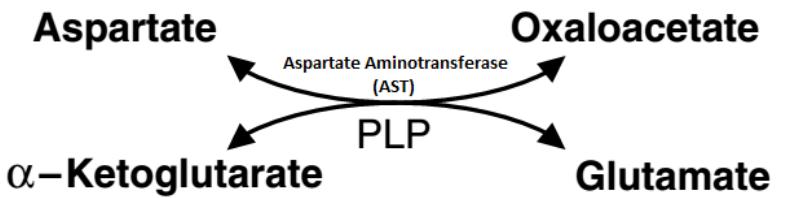
kidneys, skeletal muscle, brain and lungs. Because LDH is widely distributed through the body, the total LDH level is not a specific indicator of any one disease affecting any one organ. LDH is a tetrameric protein with 2 different subunit labeled as H & M. LDH have five isoenzymic forms that make up the total LDH. In general LDH1 comes mainly from heart, LDH2 comes primarily from the reticuloendothelial system, LDH3 comes from the lungs and

other tissues, LDH4 comes from the kidney, placenta and pancreas and LDH5 comes mainly from liver and skeletal muscle.

With myocardial injury, the serum LDH level rises within 24-48 hours after a myocardial infarction, peaks in 2-3 days and returns to normal in approxly 5-10 days. LDH activity is thus, of diagnostic value in patients admitted more than 48 hours after the infarction. Newer cardiac marker (cardiac specific troponin) have replaced the indication for LDH in the MI patients.

Aspartate aminotransferase (AST):

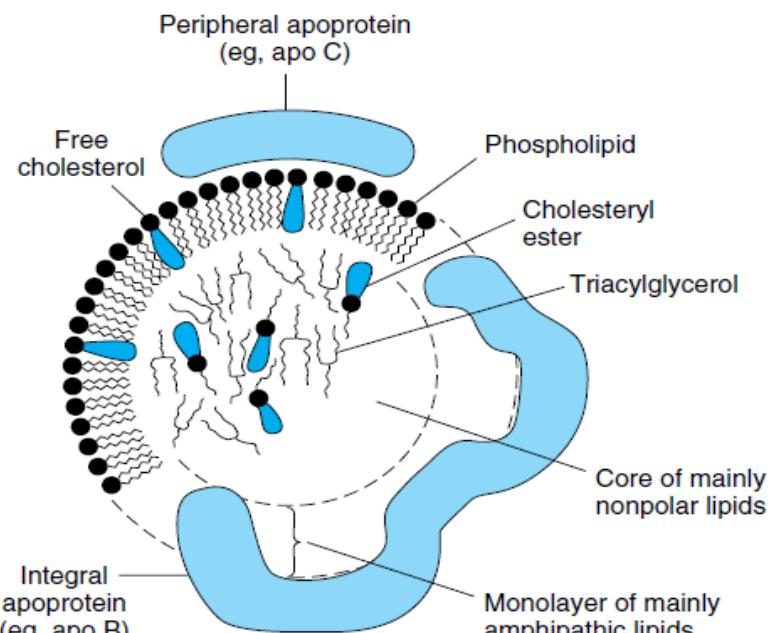
AST which was formerly called serum glutamic-oxaloacetic transaminase (SGOT) catalyses the following transamination reaction. This test is used in the evaluation of suspected coronary occlusive heart disease or suspected hepatocellular diseases. The AST has been replaced by newer cardiac markers.



Lipoprotein Metabolism:

Lipoprotein is a association of non-polar (water insoluble) lipids like triacylglycerol, cholesterol esters with amphipathic lipids (phospholipids) and proteins.

It consists of non-polar core (mainly triacylglycerol and cholesterol ester surrounded by a single surface layer of amphipathic lipids and cholesterol along with the protein. Protein present in lipoprotein are called apoprotein or apolipoprotein which could be either peripheral or integral. Depending upon the type of protein the lipid and protein content vary. The basic purpose of this structure formation is for the transport of various water insoluble lipid molecules throughout the body either synthesized in body or absorbed from diet. Lipoproteins are water soluble structure carrying water insoluble lipids within it. The general structure of lipoprotein is as above. There are various types of lipoproteins generated from various tissues with varying lipid composition.

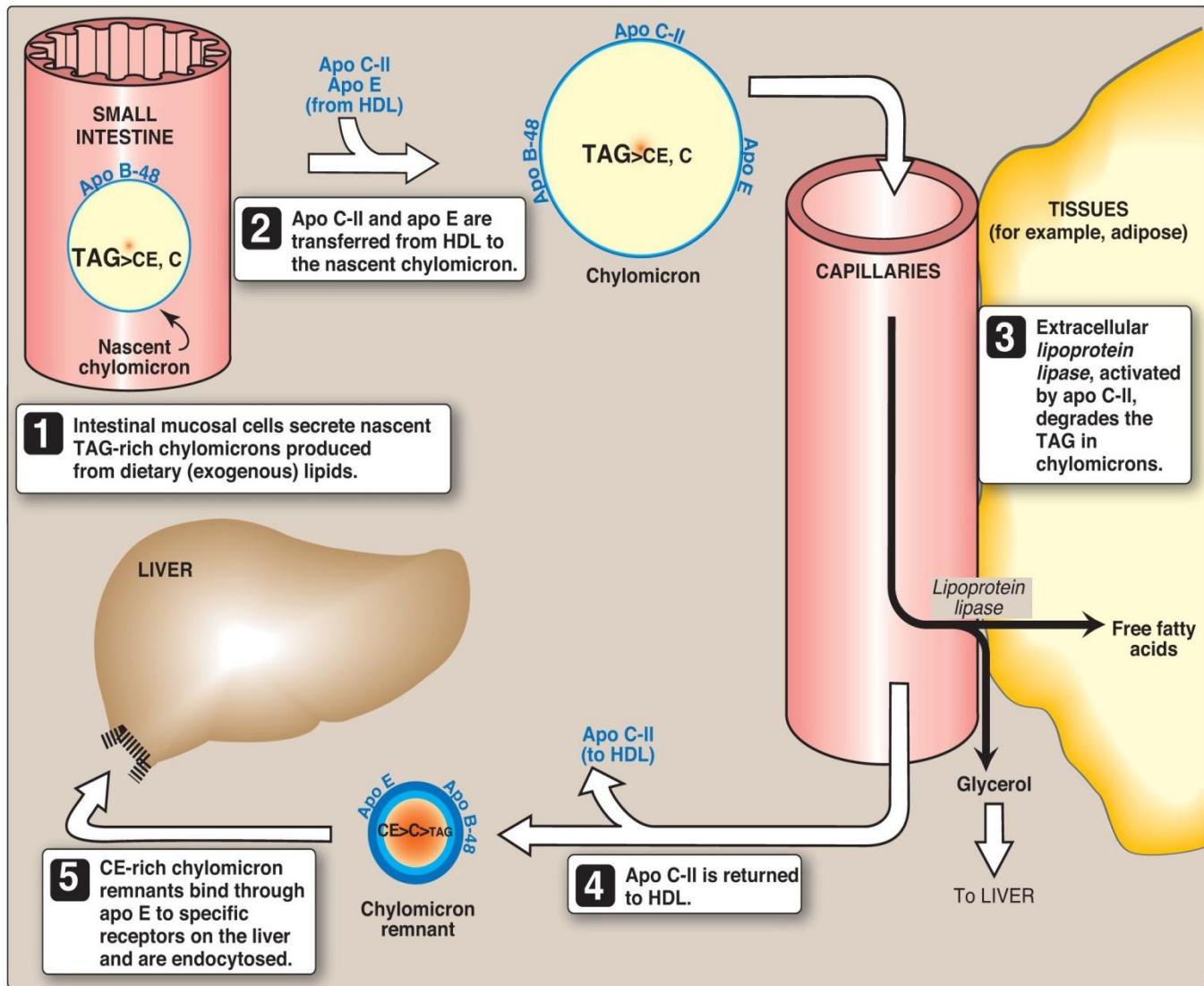


1. Chylomicron [Intestine]
2. Very low density lipoprotein (VLDL) [Liver]
3. Low density lipoprotein (LDL) [from VLDL]
4. High density lipoprotein (HDL) [Liver, intestine]

Proteins in the lipoprotein structures are called apoprotein or apolipoprotein and have following functions:

- Apoprotein forms the part of the lipoprotein structure. E.g. apo B-48 is characteristic protein of chylomicron, apo B-100 in VLDL.
- Apoprotein in lipoprotein activates enzymes in its metabolism. E.g. Apo C-II activates lipoprotein lipase, Apo A-I activates LCAT.
- Apoprotein are enzyme inhibitors. E.g. apo A-II and apo C-III inhibits the activity of lipoprotein lipase.
- Apoprotein in lipoprotein structure acts as ligands for the interaction with lipoprotein receptors. E.g. apo B-100 and apo E for LDL receptor.

Chylomicron metabolism:



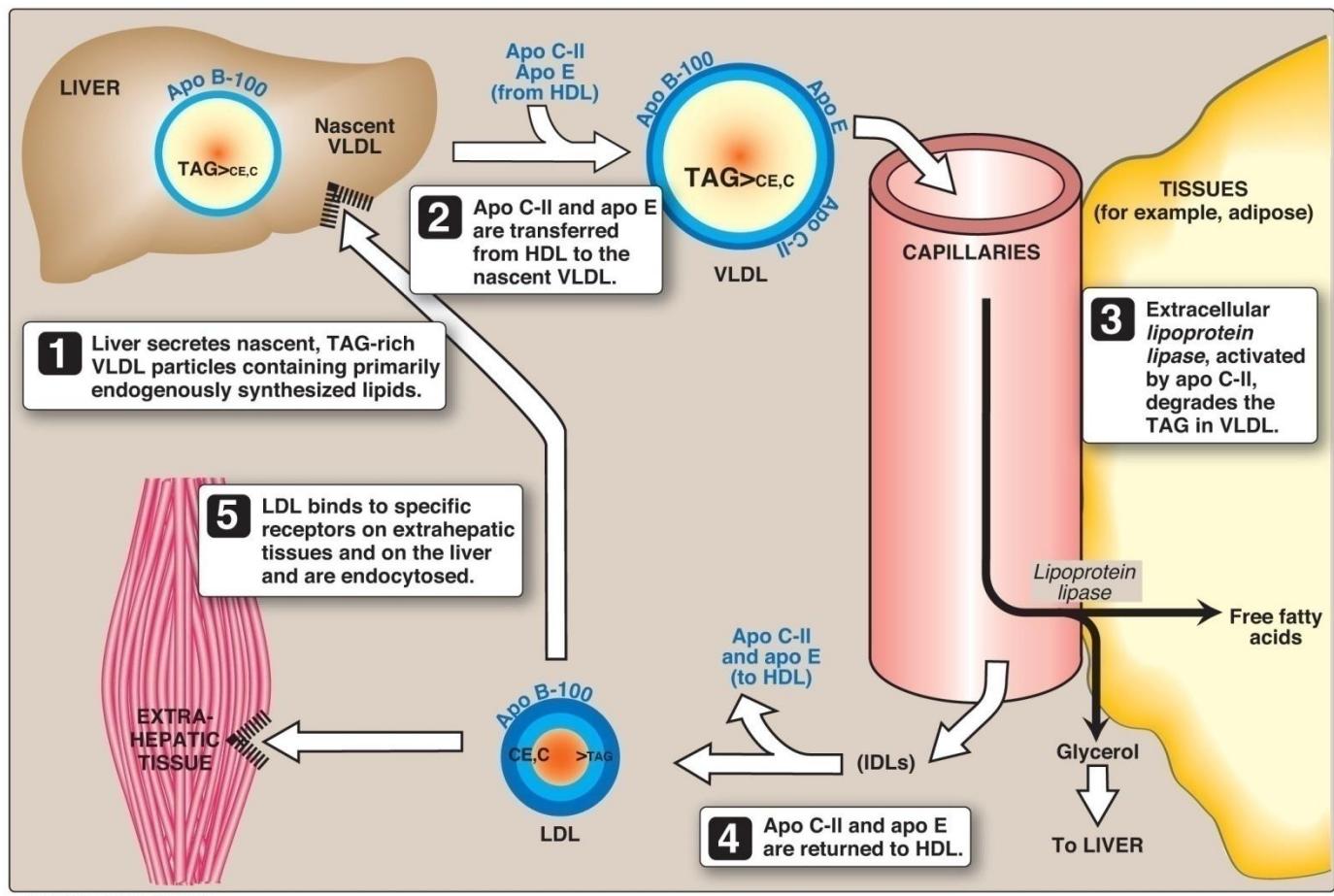
The clear picture of chylomicron metabolism is self explained by above figure. The lipids in diets which is absorbed in intestine are packed in a lipoprotein particle called chylomicron. This particle constitutes dietary triacylglycerol, cholesterol ester and cholesterol in its core among which triacylglycerol is highest. Apo B-48 is the characteristic protein of chylomicron. The chylomicron secreted in blood stream and is called nascent chylomicron which acquires apo C-II and apo-E from HDL in circulation and converted to chylomicron. Lipoprotein lipase in peripheral tissue membrane hydrolyses the triacylglycerol and take up fatty acid. The triacylglycerol content is uptaken by peripheral tissues in such manner and is reduced. The apo C-II is transferred to HDL and the chylomicron is converted to chylomicron remnant having cholesterol and it's ester in its core, which is uptaken by liver through receptor mediated endocytosis.

VLDL & LDL metabolism:

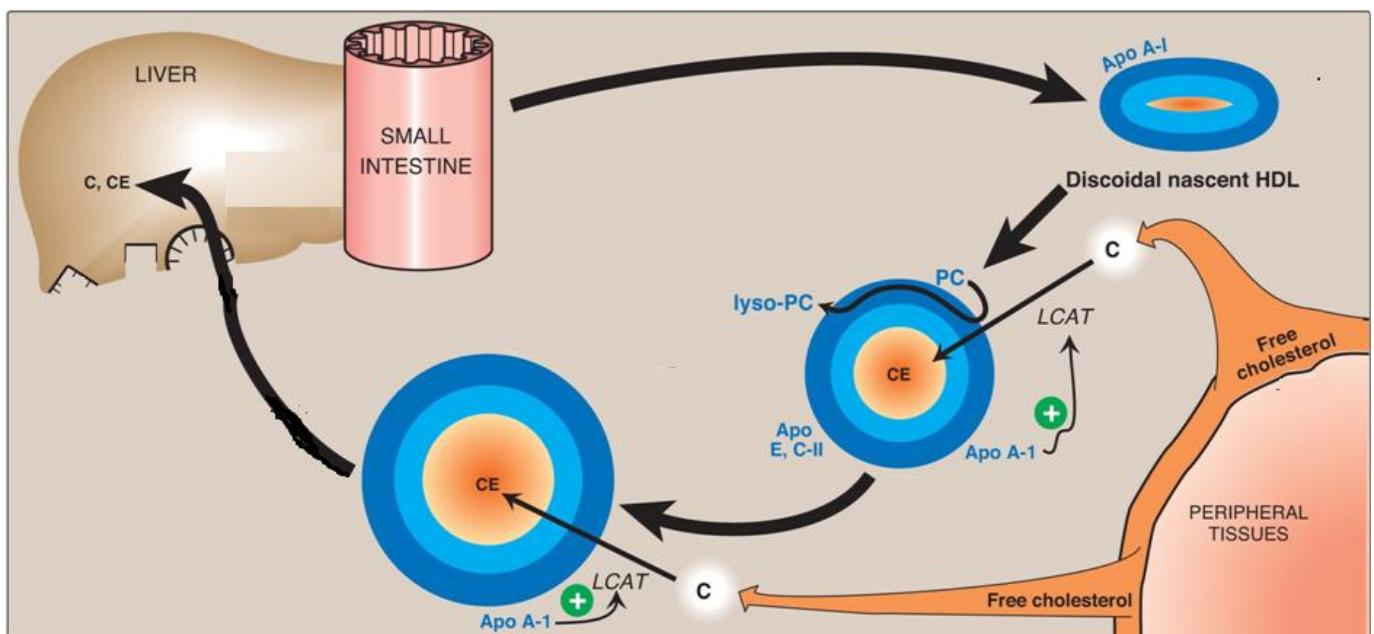
The figure below clearly depicts the metabolism of very low density lipoprotein and low density lipoprotein. The lipid synthesized in liver is packed and secreted in the form of lipoprotein called nascent VLDL with apo B-100. Nascent VLDL acquires apo C-II and apo-E from circulating HDL. Peripheral tissues mobilize the triacylglycerol from circulating VLDL by membrane bound lipoprotein lipase. The triacylglycerol content gradually decreases and now the lipoprotein contains mostly cholesterol and its esters. Apo C-II and apo-E are returned back to HDL and now this remnant is called low density lipoprotein with high content of cholesterol and cholesterol esters. LDL binds to specific receptor on extrahepatic and hepatic tissues for endocytosis. LDL is generated from VLDL and supplies cholesterol to extrahepatic tissues.

Rfha

The LDL cholesterol is regarded as bad cholesterol because increase in the LDL cholesterol have increase incidence of coronary artery disease as a result of its deposition in blood vessels and narrowing the blood vessels by forming the plaque obstructing the flow of blood.



Metabolism of HDL:



High density lipoprotein is synthesized in liver and intestine. The nascent HDL is discoidal in shape with little core lipids but highly densed particle as have numerous proteins. The nascent HDL in circulation accepts/ collects the cholesterol from extrahepatic tissues which is esterified with fatty acid by LCAT and converts cholesterol to cholesterol esters. As cholesterol content gradually increases the shape changes to spherical and finally it is uptaken by liver by receptor mediated endocytosis. Here, the excess extrahepatic cholesterol is collected and brought to liver by HDL cholesterol, which is the site for the manipulation and excretion of cholesterol. In this way

HDL is involved in reverse cholesterol transport (tissues to liver), the cholesterol content in HDL or HDL-cholesterol is regarded as good cholesterol.

Hyperlipidemia:

Hyperlipidemia refers to the increased serum lipoproteins levels. In lab, instead of lipoproteins, lipid such as triacylglycerol, cholesterol, HDL-cholesterol, LDL-cholesterol are measured which is collectively known as lipid profile. Lipid profile is the test done to check the circulating levels of lipids for various risk assessment. The test is done with 12 hours fast serum sample with patient in regular diet and following measurements are done.

- Total cholesterol (total cholesterol content)
- LDL cholesterol (cholesterol content in LDL)
- HDL cholesterol (cholesterol content in HDL)
- Triacylglycerol

BLOOD, LYMPHATICS AND IMMUNE SYSTEM

Iron is trace mineral. Total content of iron varies from 3-5 grams. Most of the iron about 70% is present in RBC (hemoglobin) and 5% in myoglobin and rest of the associated with various other compounds like hemoproteins (cytochromes, catalase etc) and non-heme proteins (transferrin, ferritin, hemosiderin). The daily requirement of iron in adult man is around 10mg/day, for menstruating women around 18mg/day and during pregnancy and lactation the requirement is increased up to 40mg/day.

The rich sources of iron are organ meat, good sources include: green leafy vegetables, pulses, cereals, fish, apples, dried fruits.

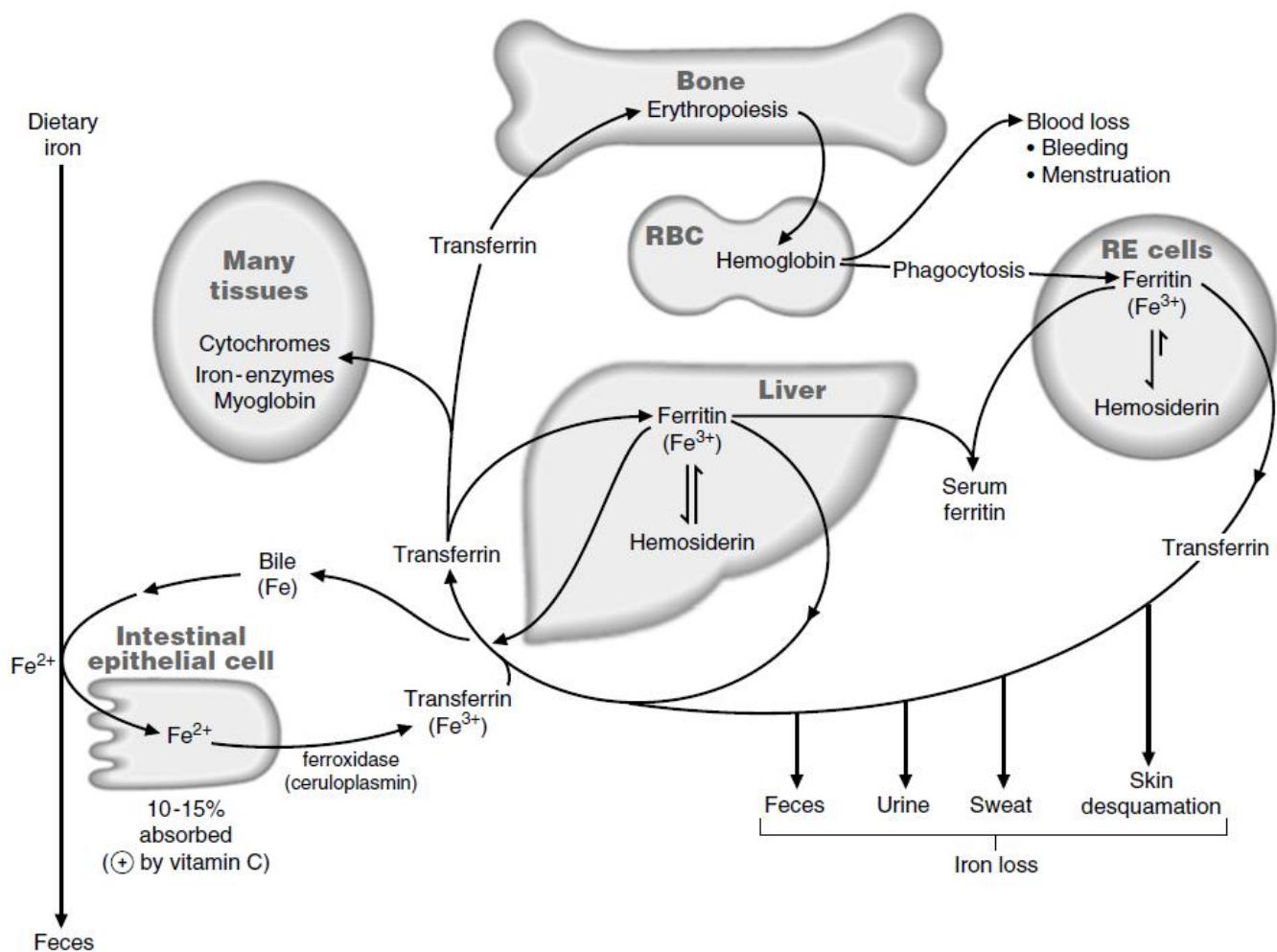
Iron mainly shows its functions through the compounds in which it is present. The main functions of iron includes:

- Iron in hemoglobin, myoglobin is important in gaseous transport.
- Cytochromes and certain non-heme proteins (all iron containing) are integral part of electron transport chain and oxidative phosphorylation. This whole system is devoted to energy generation in biological system.
- Peroxidase is iron containing lysosomal enzyme.
- Iron is associated with effective immunocompetence of the body.

Absorption, transport and storage:

The absorption of iron occurs principally in duodenum. Normally 10% of dietary iron is absorbed. In iron deficient and growing children where the iron requirements are high, higher proportion of dietary iron is absorbed i.e. increases with the body demands. The iron in food is found in Fe^{3+} from bound to protein or organic acids which is released in acidic medium. The free iron is reduced to Fe^{2+} in presence of ascorbic acid which is soluble and readily absorbed. The iron in meats is in the form of heme, which is readily absorbed.

Iron is absorbed in the ferrous (Fe^{2+}) state, but is oxidized to the ferric state by a ferroxidase known as ceruloplasmin (a copper-containing enzyme) for transport through the body. Because free iron is toxic, it is usually found in the body bound to proteins. Iron is carried in the blood (as Fe^{3+}) by the protein apotransferrin, with which it forms a complex known as transferrin. Storage of iron occurs in most cells but especially those of the liver, spleen, and bone marrow. In these cells, the storage protein, apoferitin, forms a complex with iron (Fe^{3+}) known as ferritin. Normally, little ferritin is present in the blood. This amount increases, however, as iron stores increase. Therefore, the amount of ferritin in the blood is the most sensitive indicator of the amount of iron in the body's stores. Iron can be drawn from ferritin stores, transported in the blood as transferrin, and taken up via receptor-mediated endocytosis by cells that require iron (e.g., by reticulocytes that are synthesizing hemoglobin). When excess iron is absorbed from the diet, it is stored as hemosiderin, a form of ferritin complexed with additional iron that cannot be readily mobilized.



Iron is lost from the body with bleeding and sloughed-off cells, sweat, urine, and feces. Hemosiderin is the protein in which excess iron is stored. Small amounts of ferritin enter the blood and can be used to measure the adequacy of iron stores. Deficiency is very common in menstruating women.

Iron deficiency anemia:

Anemia which is because of inadequate iron in the body is iron deficiency anemia which could result from insufficient iron intake, inadequate absorption of iron, increased requirement (children, late pregnancy), or due to blood loss. Iron deficiency results in decreased production of hemoglobin, which in turn results in small, pale red blood cells (microcytic, hypochromic).

Diagnosis of Iron deficiency anemia:

Various biochemical tests are performed for the diagnosis of iron deficiency anemia which includes, serum iron level, total iron binding capacity (TIBC), Ferritin. A decreased serum iron level, elevated TIBC and low ferritin value are characteristics of iron deficiency anemia.

Serum level of Iron:

Normal findings:

- **Male:** 80-180 $\mu\text{g}/\text{dl}$ or 14-32 $\mu\text{mol}/\text{L}$
- **Female:** 60-160 $\mu\text{g}/\text{dl}$ or 11-29 $\mu\text{mol}/\text{L}$
- **New Born:** 100-250 $\mu\text{g}/\text{dl}$
- **Child:** 50-120 $\mu\text{g}/\text{dl}$

The serum iron determination is a measurement of the quantity of iron bound to transferring. The low level of serum iron is indicative of iron deficiency anemia while high levels are seen in hemochromatosis.

Total Iron binding capacity (TIBC):

Normal findings: 250-460 µg/dl or 45-82 µmol/L

TIBC is a measurement of all proteins available for binding mobile iron. Transferring represents the largest quantity of iron binding protein. Therefore TIBC is an indirect yet accurate measurement of transferrin. Ferritin is not included in TIBC because it binds only stored iron. Increased levels of TIBC is found in individuals with iron deficiency anemia.

Ferritin:

Normal findings:

- Male: 12-300ng/ml
- Female: 10-150ng/ml
- Children
 - Newborn: 25-200ng/ml
 - 1 month: 200-600ng/ml
 - 2-5 months: 50-200ng/ml
 - 6months-15 years : 7-142 ng/ml

The serum ferritin study is a good indicator of available iron stores in the body. Ferritin is the major iron storage protein and is normally present in the serum in concentration directly related to iron storage. Decreased ferritin level indicates a decrease in iron storage associated with iron deficiency anemia. Ferritin level less than 10 ng/dl is diagnostic of iron deficiency anemia.

A decreased serum iron level, elevated TIBC & low ferritin value are characteristic of iron deficiency anemia.

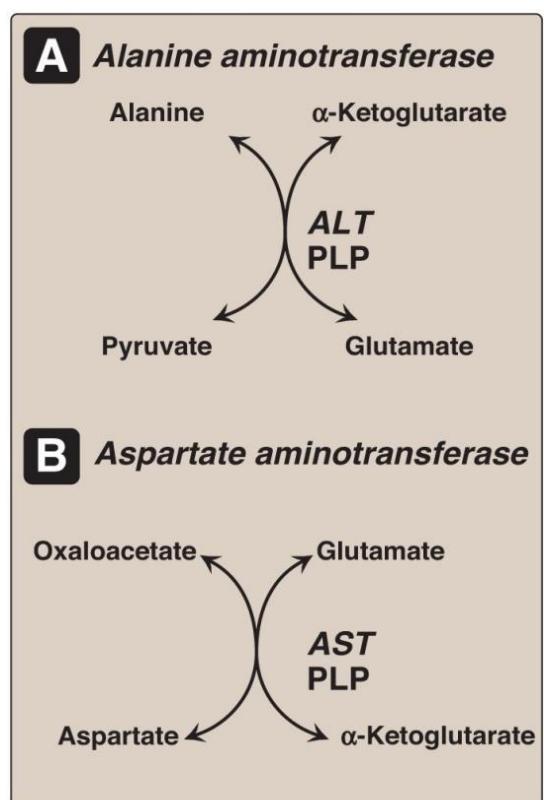
GASTROINTESTINAL SYSTEM:

Amino acid in excess are catabolized for energy generation or conversion to fat, glucose. The amino acid cannot be processed unless and until the α - amino group is removed. Transamination and deamination reaction facilitates the removal of amino group and resulting carbon skeleton of amino acid structure is then easily processed.

Transamination:

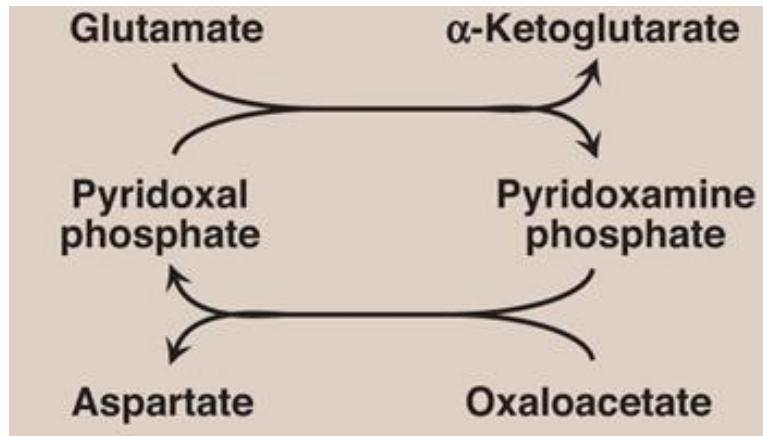
Transamination reaction is transfer of an amino group from an amino acid to a keto acid converting amino acid to its respective keto acid form and keto acid to its respective amino acid form. The main feature of this reaction is that the amino group is transferred in bound form. The reaction is catalysed by enzymes called aminotransferase which requires pyridoxal phosphate (vitamin B6 derivative) as a coenzyme. In transamination reaction a pair is required one of which is amino acid and next is α -keto acid. α -ketoglutarate plays a unique role in amino acid metabolism by accepting the amino groups from other amino acid and gets converted to glutamate. Although transamination reaction is reversible, generally the α -keto acid is α -ketoglutarate collecting amino group from various amino acids. Thus transamination reaction funnels the amino groups of various amino acids to glutamate. Nearly all amino acids undergo transamination reaction at some point of their catabolism.

Examples: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT)



Mechanism of transamination:

Transamination reaction is catalyzed by aminotransferase and is dependent on coenzyme pyridoxal phosphate (PLP). During transamination reaction the amino group is not liberated as ammonia rather it is transferred in bound form. For this transfer the coenzyme is very important. The pyridoxal phosphate accepts the amino group from amino acid converting it to respective keto acid and gets converted to pyridoxalamine phosphate and this pyridoxalamine phosphate transfers the amino group to keto acid converting it to respective amino acid. In figure the reaction catalysed by aspartate aminotransferase is shown.



Deamination:

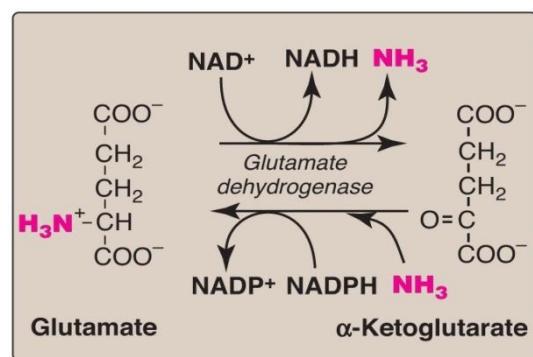
Deamination is another reaction by which amino group is removed from amino acid. In deamination the amino group from amino acid is removed as ammonia (NH_3). Deamination is either oxidative or non-oxidative.

Oxidative deamination:

Oxidative deamination mostly occurs in liver and kidney. In this reaction ammonia is liberated along with the oxidation. The purpose of this reaction is the liberation of ammonia for the synthesis of urea and keto acids for a variety of reaction, including energy generation. Examples: glutamate dehydrogenase, amino acid oxidase.

Glutamate dehydrogenase:

Glutamate dehydrogenase catalyses the oxidative conversion of glutamate to α -ketoglutarate and ammonia along with the reduction of NAD^+ to NADH and H^+ .



Amino acid oxidase:

Amino acid oxidase catalyses the oxidative deamination converting amino acid into ammonia and respective keto acid form.

L-Amino acid oxidase is FMN linked enzyme while D-amino acid oxidase is FAD linked enzymes. Activity of D-amino acid oxidase is high in tissues (mostly in liver and kidney) and play a significant role for the conversion of unnatural D-amino acid to L-amino acid in the body.

Non-oxidative Deamination:

Some amino acid undergo deamination without oxidation. Examples include: Histidase, desulphhydrase.

Metabolism of ammonia and urea synthesis:

Ammonia is constantly liberated from the metabolism of amino acid and other nitrogenous compounds. Ammonia is toxic as such, therefore ammonia is channeled into a single excretory non-toxic product known as urea which is excreted through urine. The metabolic pathway for the synthesis of urea is urea cycle and occurs in liver. Urea is

the major nitrogenous waste present in urine constituting about 80-90% of nitrogen waste product present in urine. Urea molecule constitutes 2 amino groups, one is derived from ammonia and the other from the aspartate.

Urea cycle:

Urea cycle is a five step cyclic process with 5 distinct enzymes and occurs in liver in two different compartments. The first two enzymes are present in mitochondria and rest of the 3 enzymes are present in cytosol. The very first reaction of urea cycle is the condensation of ammonia with carbondioxide to form carbamoyl phosphate catalysed by the enzyme carbamoyl phosphate synthetase (CPS-I). The reaction requires 2 ATP. In second reaction carbamoyl phosphate is condensed with ornithine to form citrulline catalysed by ornithine transcarbamoylase. These two reactions occur in the mitochondria of the liver cells.

Citrulline so produced is transported to cytosol where it is condensed with aspartate to form arginosuccinate by enzyme arginosuccinate synthetase. Arginosuccinate is lysed to arginine and fumarate in fourth reaction catalysed by arginosuccinate lyase. The fumarate so produced link urea cycle with the TCA cycle. In fifth final reaction the arginine is cleaved to urea and ornithine by enzyme arginase. The ornithine is transported back to mitochondria and take part in other round of urea cycle.

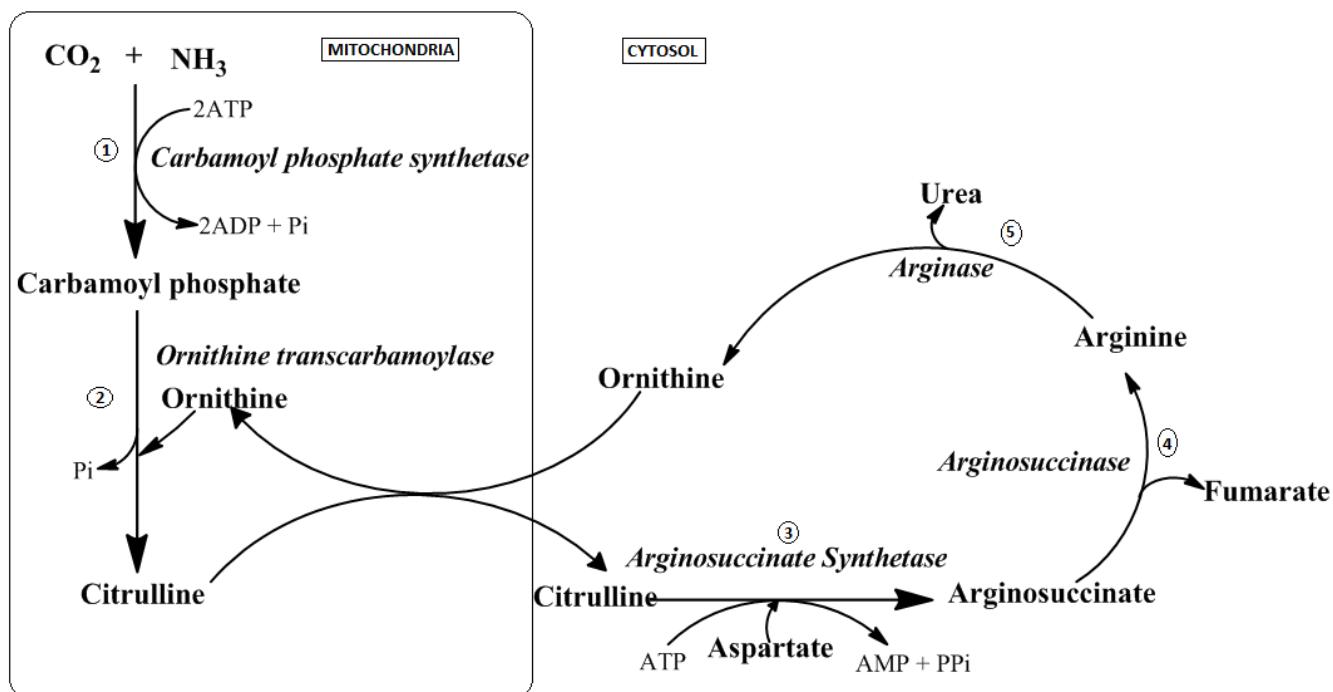
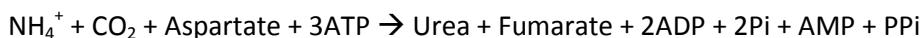


Figure:: Reactions of Urea cycle

Overall reaction and energetic



Although there is utilization of 3 ATP, 1 of it is hydrolysed to AMP, therefore 4 ATP are actually utilized.

Importance of Urea cycle:

During amino acid metabolism and metabolism of numerous nitrogen containing substances, lots of ammonia is generated which hampers the energy generating metabolic pathway and proves fatal. The toxic effect of ammonia is neutralized by converting the ammonia to urea which is non toxic and easily excreted out via urine.

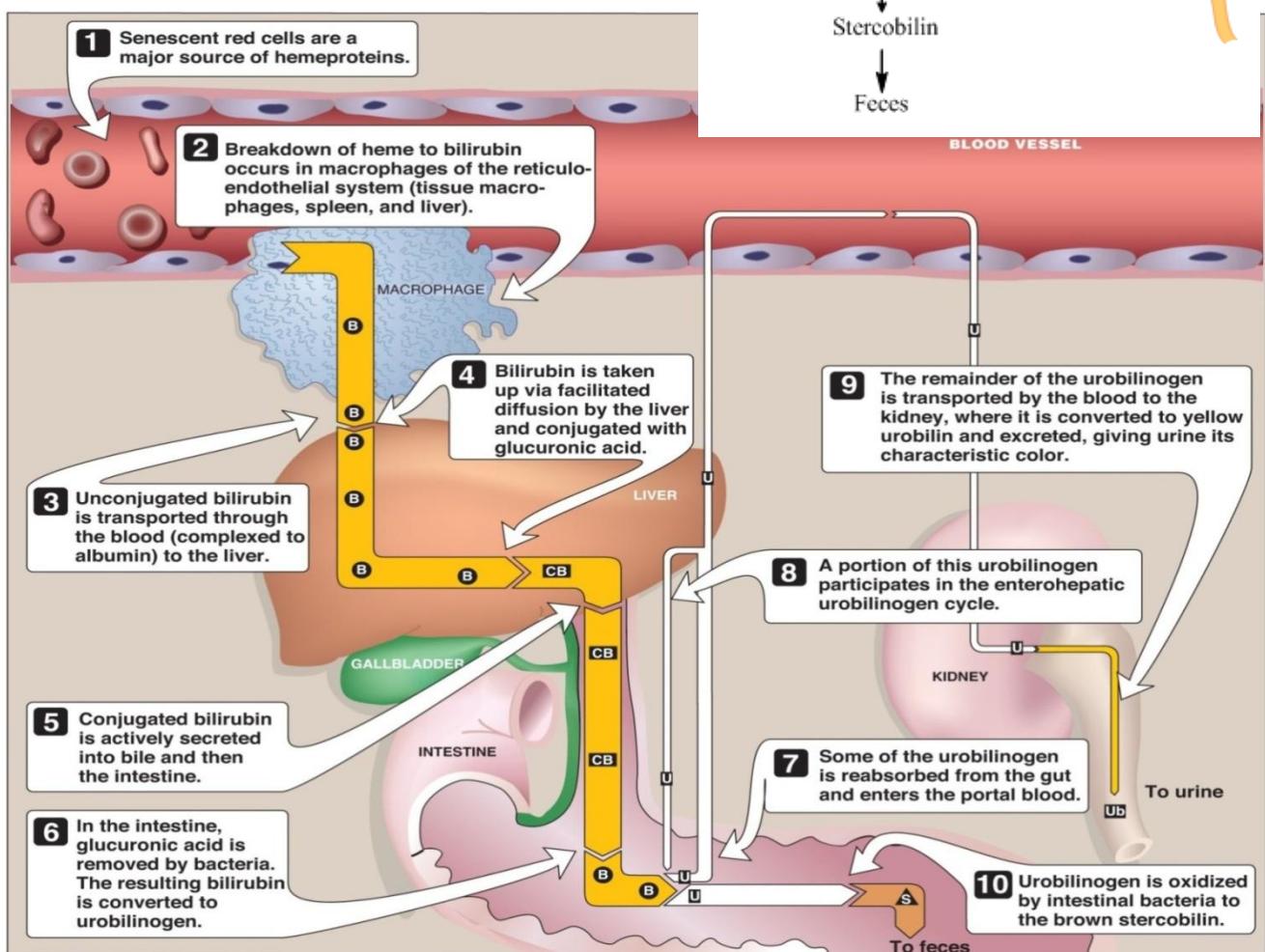
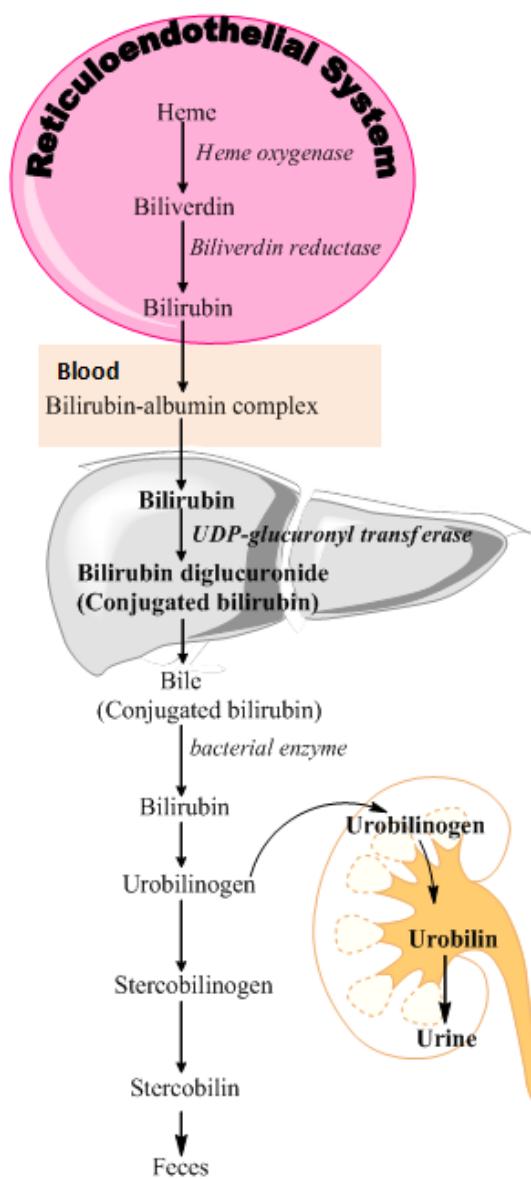
Catabolism of Heme (Bilirubin synthesis):::

Bilirubin is the bile pigment that imparts color to the bile and is the catabolic product of heme degradation. Heme containing compounds primarily heme of RBC is the major source of bilirubin. RBC have half life of 120 days, after which they are destroyed and heme is released which is converted to bilirubin in reticuloendothelial system. The

bilirubin so formed is transported in circulation to liver, bound to albumin. Bilirubin is taken up by liver and conjugated with glucuronic acid and is called conjugated bilirubin (bilirubin diglucuronide) which is actively secreted into bile and then intestine. In the intestine bacteria removes the glucuronic acid from bilirubin and is converted to urobilinogen. Some of the urobilinogen is reabsorbed from the gut and enters the portal circulation, a portion of this participates in the enterohepatic urobilinogen cycle. The remaining urobilinogen is transported to kidney, where it is converted to yellow urobilin and excreted, giving urine its characteristic color. Urobilinogen is oxidized by intestinal bacteria to the brown stercobilin and excreted imparting characteristic color to feces.

Functions of liver:

Liver is metabolically active organ where nearly all the metabolic reaction do occur. Generally hydrophobic compounds to be excreted is excreted from liver as bile pigment, bile salts, cholesterol. Liver is the major site for xenobiotic metabolism (detoxification). Liver synthesizes certain blood coagulation factors as prothrombin, factor V, VII, X, fibrinogen so is involved in hemostasis. Albumin is solely synthesized in liver Vitamin A, vitamin B12 & glycogen is stored in liver.



Liver function tests:

Liver function tests are the biochemical investigation to access the capacity of liver to carry out any of the functions it performs.

Major LFT may be classified as follows:

- **Test based on excretory function:** measurement of bile pigments, bile salts, bromosulphthalein
- **Test based on serum enzymes derived from liver:** aminotransferases, ALP, γ-GT..
- **Test based on metabolic capacity:** galactose tolerance, antipyrine clearance.
- **Test based on synthetic functions:** prothrombin time, serum albumin
- **Test based on detoxification:** Hippuric acid synthesis.

The commonly performed LFT is the following

- **Bile pigment; Bilirubin [0.2-1mg/dL]**
 - Conjugated (direct) [0.2-0.4 mg/dL]
 - Unconjugated (indirect) [0.2-0.6 mg/dL]
- **Activity of Serum enzymes derived from liver:**
 - ALT (SGPT) [alanine aminotransferase]
 - AST (SGOT) [aspartate aminotransferase]
 - ALP [alkaline phosphatase]

Hyperbilirubinemia:

Interferences at any one of the points of bilirubin processing in bilirubin metabolism can lead to an increased bilirubin level in blood known as hyperbilirubinemia. In general, If hyperbilirubinemia persists and exceeds 2mg/dL, it starts getting deposited in skin, scleras, nail beds, mucous membranes imparting yellow coloration; a condition known as Jaundice. So, Jaundice is a condition rather than a disease. The cause could be increased bilirubin production (excessive hemolysis), reduced bilirubin uptake by hepatic cells, disrupted intracellular conjugation, disrupted secretion of bilirubin into bile canaliculi or intra-extra hepatic bile duct obstruction.

Classification of Jaundice:

Hemolytic jaundice (pre-hepatic jaundice)

There is excessive production of bilirubin due to increased hemolysis of RBC. This could happen during incompatible blood transfusion, malaria, sickle cell anemia. The load of bilirubin is high to be taken up and processed by liver and there is elevated levels of serum unconjugated bilirubin. There is increased excretion of urobilinogen in urine, dark brown color of feces due to high content of stercobilinogen.

Hepatic Jaundice:

Jaundice due to dysfunction of liver due to damage of liver cells which could be attributed to viral infections (viral hepatitis, exposure to poison & toxins, liver cirrhosis). There is increased levels of conjugated and unconjugated bilirubin. Individuals pass dark color urine due to presence of bilirubin and urobilinogen. Pale, clay colored feces due to absence of stercobilin. The activity of alanine aminotransferase & aspartate aminotransferase is elevated.

Obstructive Jaundice:

Due to obstruction in bile flow, such conditions are seen. The conjugated bilirubin enters the circulation and the levels are seen elevated. Serum alkaline phosphatase activity is elevated. Dark color urine due to elevated excretion of bilirubin and clay colored feces due to absence of stercobilin. As bile flow is obstructed, lipid digestion is compromised and feces contain excess fat.



Neonatal-Physiologic Jaundice:

This condition is seen in neonates which is because of immature hepatic system for the uptake, conjugation and secretion of bilirubin. The activity of conjugating enzyme UDP-glucuronyl transferase is low in newborns as well as the conjugating substrate UDP-glucuronate is limited. By phototherapy bilirubin is converted to non-toxic isomer lumirubin, which is easily excreted by kidneys in unconjugated form.

Biochemical investigations are very important in the differential diagnosis of jaundice. The result can be managed to differentiate the various jaundice. A simple clear picture is tabulated below.

Parameter	Hemolytic jaundice	Obstructive jaundice	Hepatic jaundice
Serum bilirubin	Unconjugated bilirubin ↑	Conjugated bilirubin↑	Both ↑
Serum enzymes	ALT, AST & ALP →	ALP↑↑, ALT & AST marginal ↑	ALT & AST ↑↑, ALP marginal ↑
Bilirubin in urine	Not excreted	Excreted	Excreted
Urobilinogen in urine	Excretion ↑	→ or ↓	→ or ↓
	↑--elevated	→- Normal	↓- decreased

RENAL ELECTROLYTE SYSTEM:

Non-electrolytes such as glucose, urea etc. do not dissociate in solution. While substances like NaCl, KCl in solution dissociate into sodium (Na^+), potassium (K^+) and chloride (Cl^-) ions, they are called as electrolytes. The positively charged ions are called cations and negatively charged ions are called anions. The electrolytes are distributed unevenly between fluid compartments [extracellular fluid (ECF) and intracellular fluid (ICF)]. Fluids in body compartment will contain equal number of +ve charges and -ve charges making these compartment electrically neutrality.

Sodium, potassium (+vely charged) and chloride (-vely charged) are the major electrolytes in the body fluid. Na^+ and Cl^- are the major electrolytes in the ECF while K^+ and phosphates are the major electrolytes intracellularly. This distribution is maintained principally by energy requiring transporters that pump Na^+ out of cell in exchange for K^+ . They establish ion gradient across membranes, maintain water balance and neutralize positive and negative charges on protein and other molecules.

Electrolyte composition of body fluids

Extracellular Fluid			
Cations mEq/L	Anions mEq/L	Cations mEq/L	Anions mEq/L
Plasma: $\text{Na}^+ = 143$ $\text{K}^+ = 5$ $\text{Ca}^{++} = 5$ $\text{Mg}^{++} = 2$ $\text{Total} = 155$	 $\text{Cl}^- = 103$ $\text{HCO}_3^- = 27$ $\text{HPO}_4^{--} = 2$ $\text{SO}_4^{--} = 1$ $\text{Proteins} = 16$ $\text{Organic acids} = 6$ $\text{Total} = 155$	Interstitial tissue fluid: $\text{Na}^+ = 145$ $\text{K}^+ = 5$ $\text{Ca}^{++} = 3$ $\text{Mg}^{++} = 2$ $\text{Total} = 155$	 $\text{Cl}^- = 116$ $\text{HCO}_3^- = 27$ $\text{HPO}_4^{--} = 3$ $\text{SO}_4^{--} = 2$ $\text{Proteins} = 1$ $\text{Organic acids} = 6$ $\text{Total} = 155$
Plasma & TF electrolyte composition is similar except that Cl^- largely replaces protein as anion in TF			

Intracellular Fluid:

Cations mEq/L	Anions mEq/L
$\text{Na}^+ = 150$	$\text{HPO}_4^{2-} = 110$
$\text{K}^+ = 40$	$\text{Proteins} = 50$
$\text{Mg}^{++} = 5$	$\text{SO}_4^{2-} = 20$
	$\text{HCO}_3^- = 10$
	$\text{Cl}^- = 5$
Total = 195	Total = 195

Under normal conditions in health, the relative volumes of water in above three compartments is kept constant.

Water can pass freely through the membrane which divides plasma from tissue fluid and tissue fluid from intracellular fluid. But the distribution of water is controlled by the osmotic pressure exerted by substances in each compartments i.e. mainly electrolytes and proteins. Normally there is osmotic equilibrium between ICF and tissue fluid, but if this is disturbed, water is drawn from the compartment with lower osmotic pressure to higher osmotic pressure until equilibrium is restored. The osmotic imbalance between these two compartments results in water being either sucked out of cells (producing cellular dehydration) or water is drawn into cells (producing cellular oedema).

Urine:

Water and water-soluble compounds are excreted with the urine. The volume and composition of urine are subject to wide variation and depend on food intake, bodyweight, age, sex, and living conditions such as temperature, humidity, physical activity, and health status. As there is a marked circadian rhythm in urine excretion, the amount of urine and its composition are usually given relative to a 24-hour period.

A human adult produces 0.5–2.0 L urine per day, around 95% of which consists of water. The urine usually has a slightly acidic pH value (around 5.8). However, the pH value of urine is strongly affected by metabolic status. After ingestion of large amounts of plant food, it can increase to over 7.

Organic components:

Nitrogen-containing compounds are among the most important organic components of urine. **Urea**, which is mainly synthesized in the liver, is the form in which nitrogen atoms from amino acids are excreted. Breakdown of pyrimidine bases also produces a certain amount of urea. When the nitrogen balance is constant, as much nitrogen is excreted as is taken up, and the amount of urea in the urine therefore reflects protein degradation: 70 g protein in food yields approximately 30 g urea in the urine.

Uric acid is the end product of the purine metabolism. When uric acid excretion via the kidneys is disturbed, gout can develop. **Creatinine** is derived from the muscle metabolism, where it arises spontaneously and irreversibly by cyclization of creatine and creatine phosphate. Since the amount of creatinine an individual excretes per day is constant (it is directly proportional to muscle mass), creatinine as an endogenous substance can be used to measure the glomerular filtration rate. The amount of amino acids excreted in free form is strongly dependent on the diet and on the efficiency of liver function. Amino acid derivatives are also found in the urine (e.g., hippurate, a detoxification product of benzoic acid).

Inorganic constituents:

The main inorganic components of the urine are the cations Na^+ , K^+ , Ca^{2+} , Mg^{2+} , and NH_4^+ and the anions Cl^- , SO_4^{2-} , and HPO_4^{2-} , as well as traces of other ions. In total, Na^+ and Cl^- represent about two-thirds of all the electrolytes in the final urine. Calcium and magnesium occur in the feces in even larger quantities. The amounts of the various inorganic components of the urine also depend on the composition of the diet. For example, in acidosis there can be a marked increase in the excretion of ammonia. Excretion of Na^+ , K^+ , Ca^{2+} , and phosphate via the kidneys is subject to hormonal regulation.

Modified amino acids, which occur in special proteins such as hydroxyproline in collagen and 3-methylhistidine in actin and myosin, can be used as indicators of the degradation of these proteins. Other components of the urine are conjugates with sulfuric acid, glucuronic acid, glycine, and other polar compounds that are synthesized in the liver by biotransformation. In addition, metabolites of many hormones (catecholamines, steroids, serotonin) also appear in the urine and can provide information about hormone production. The proteohormone chorionic gonadotropin (hCG, mass ca. 36 kDa), which is formed at the onset of pregnancy, appears in the urine due to its relatively small size. Evidence of hCG in the urine provides the basis for an immunological pregnancy test.

The yellow color of urine is due to urochromes, which are related to the bile pigments produced by hemoglobin degradation. If urine is left to stand long enough, oxidation of the urochromes may lead to a darkening in color.

<i>Urine composition in general</i>	
Organic substances:	Inorganic minerals
Urea	Chlorides
Uric acid	Bicarbonate
Creatinine	Calcium
	Magnesium
	Sulphates
	Ammonium
	Sodium
	Potassium

Urine analysis provides valuable information and should be examined soon after being passed/ collected. For random examination, a random specimen is satisfactory but for certain investigation urine passed at a particular time of the day is valuable e.g. to detect glycosuria, urine excreted 2 hours after meal is examined.

Abnormal constituent in urine:

Portein: (Proteinuria)

Evaluation of protein is a sensitive indicator of kidney function. Normally protein is not present in the urine because the spaces in the normal glomerular filtrate membrane are too small to allow its passage. If the glomerular membrane is injured, as in glomeronephritis, the spaces become much larger and protein seeps out into the filtrate and in the urine. If this persists at a significant rate, the patient can become hypoproteinemic because of severe protein loss through the kidneys. Proteinuria is probably the most important indicator of renal disease. In addition to screening for nephritic syndrome, urinary protein also screens for complications of diabetes mellitus, glomerulonephritis, amyloidosis and multiple myeloma.

Glucose: (glucosuria)

This screening test for the presence of glucose within the urine may indicate the likelihood of diabetes mellitus or other causes of glucose intolerance.

Ketone bodies: (ketonuria)

Normally no ketones are present in the urine; however a patient with poorly controlled diabetes who is hyperglycemic may have massive fatty acid catabolism. This test is also important in evaluating ketoacidosis associated with alcoholism, fasting, starvation, high protein diets and isopropyl ingestion.

Nitrites:

The nitrite test is a screening test for the identification of urinary tract infections. This test is based on the principle that many bacteria produce an enzyme called reductase, which can reduce urinary nitrates to nitrites. A positive result would indicate the need for a urine culture.

RBC: Hematuria; Hematuria is seen in Haemorrhage of urinary tract.

Bile pigments:

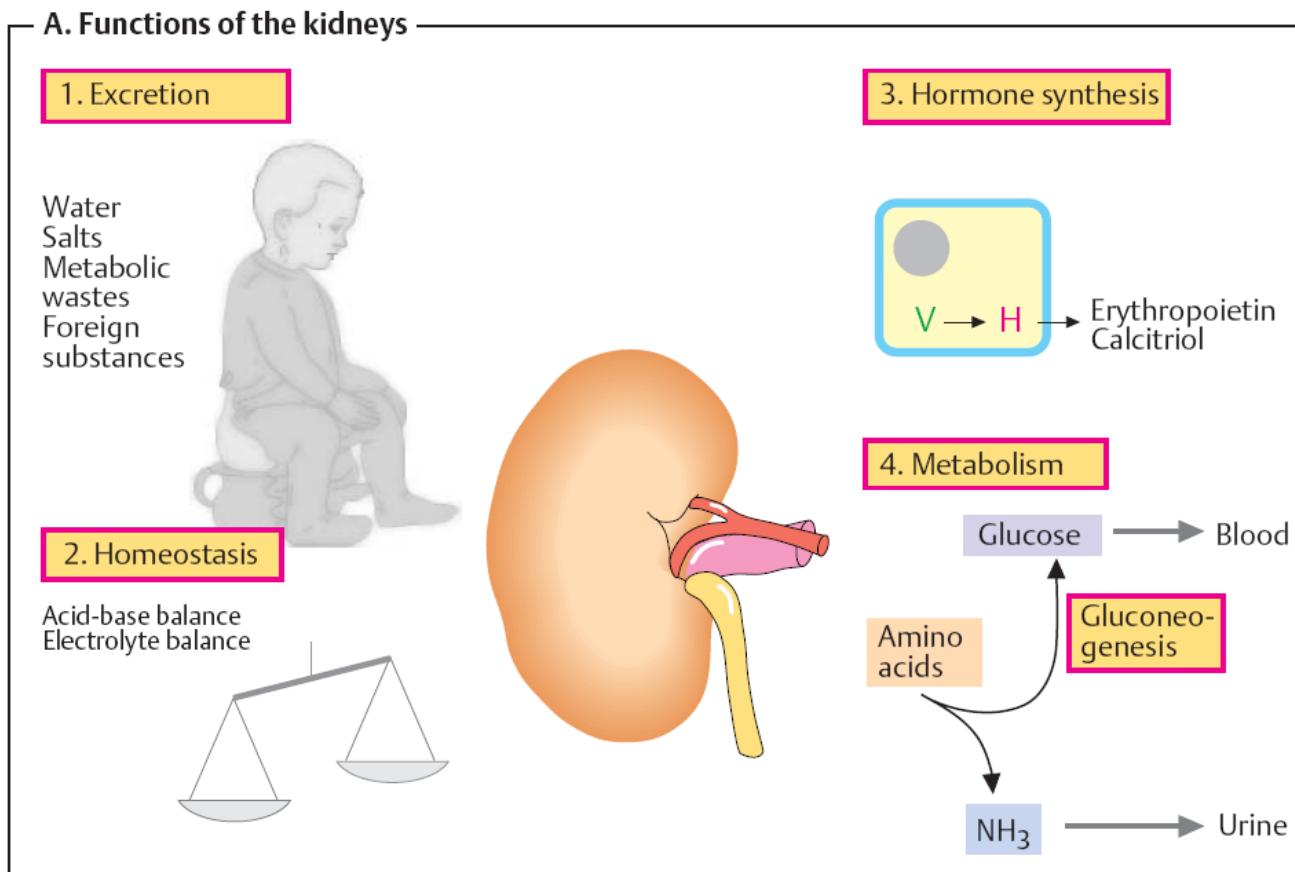
Urobilinogen: Reported increased in hemolytic jaundice and toxic jaundice.

Specific gravity:

The specific gravity is a measure of concentration of particles, including wastes and electrolytes in the urine. A high specific gravity indicates a concentrated urine; a low specific gravity indicates dilute urine. The specific gravity is used to evaluate the concentrating and excretory power of the kidney. Renal disease tends to diminish the concentrating capability of the kidney. The specific gravity is also measurement of the hydration status of the patient.

Major functions of kidney:

Kidney filter out the metabolic wastes to be excreted from body. It maintains salt, water and acid-base balance in the body.



Renal function test: (RFT)

Renal function test are the biochemical investigation done for accessing function of the kidney. Following are the test done usually done in RFT.

Blood (serum) Urea

Blood (serum) Creatinine

Blood (serum) electrolytes ($\text{Na}^+ - \text{K}^+$)

Blood (serum) uric acid

Creatinine Clearance test

S. Ha

Urea

Urea is formed in the liver as the end product of protein metabolism. During ingestion, protein is broken down into amino acids. In the liver these amino acids are catabolized, and free ammonia is formed. The ammonia is combined to form urea, which is then deposited into the blood and transported to the kidneys for excretion. Therefore blood urea is directly related to the metabolic function of liver and the excretory function of the kidney. It serves as an index of the function of these organs. Nearly all renal diseases cause an inadequate excretion of urea which causes the blood concentration to rise above normal.

Creatinine:

Creatinine is a catabolic product of creatine phosphate, which is used in skeletal muscle contraction. Creatinine is excreted entirely by the kidneys and therefore is directly proportional to renal excretory function. Only renal disorders such as glomerulonephritis, pyelonephritis, acute tubular necrosis and urinary obstruction will cause an abnormal elevation in creatinine.

Serum electrolytes (sodium and potassium)

Sodium: sodium is principal extracellular cation. Sodium level is depleted (hyponatremia) with diarrhea, vomiting adrenocortical insufficiency, overhydration and chronic renal diseases.

Potassium: Potassium is principal intracellular cation. High or low levels are dangerous since potassium effects the contractility of heart muscle. High levels of potassium (hyperkalemia) is associated with renal failure, adrenocortical insufficiency and dehydration.

Uric acid:

Uric acid is a nitrogenous compound that is a product of purine catabolism. Uric acid is excreted to a large degree by the kidney and to a smaller degree by intestinal tract. When uric acid levels are elevated (hyperuricemia), the patient may have gout. Causes of hyperuricemia can be overproduction or decreased excretion of uric acid (e.g. kidney failure).

Clearance Test (Creatinine):

Creatinine is entirely excreted by the kidneys and therefore is directly proportional to the glomerular filtration rate (GFR). The volume of plasma that would be completely cleared of creatinine per minute is creatinine clearance. The creatinine clearance requires a 24 hour urine collection and a serum creatinine level. Creatinine clearance is then calculated using the following formula:

$$\text{Creatinine clearance} = \frac{U V}{P}$$

Where,

U = concentration of creatinine in 24 hour urine (mg/dL)

V = volume of urine in milliliters per minute

P = Concentration of creatinine in serum (mg/dL)

A decrease in creatinine clearance value (<75%) serves as sensitive indicator of decreased GFR, due to renal damage.

METABOLIC ACIDOSIS-ALKALOSIS

Metabolic acidosis:

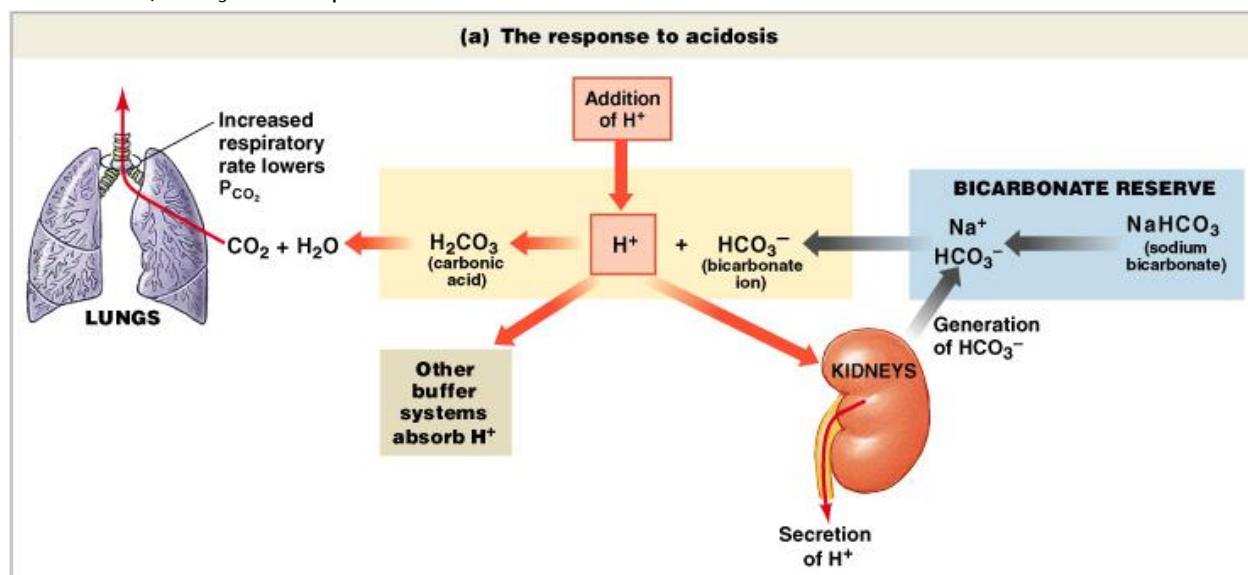
Also called primary alkali deficit. It is the commonest disturbance of acid-base balance observed clinically. It is caused when there is a reduction in the plasma $\text{HCO}_3^- \downarrow$ (bicarbonate).

Causes:

- Abnormal increase in anions other than HCO_3^- resulting from endogenous production of acid, ingestion of acidifying salts (acetyl salicylic acid, phosphoric acid, HCl, NH_4Cl etc.), utilization in buffering H^+ , renal insufficiency.
- Abnormal loss of bicarbonate (HCO_3^-): excessive loss of intestinal secretions (diarrhea, small bowel fistulae)

Compensation:

Primary compensation is done by stimulating respiratory center by acidosis causing deep and rapid breathing. Secondary compensatory mechanism is the renal mechanism and does so by conserving cations, NH_3^+ formation, H^+ excretion, HCO_3^- reabsorption.



Metabolic alkalosis:

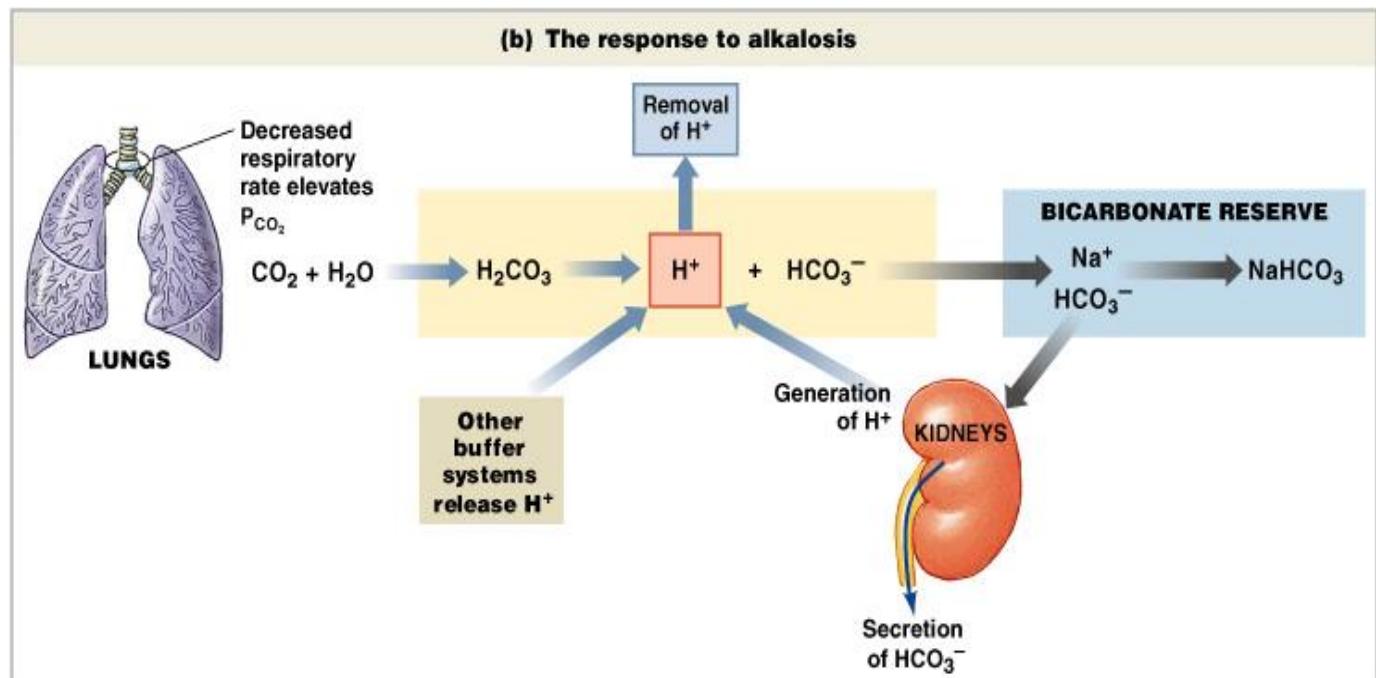
Also called as primary alkali excess. The condition results from an absolute or relative increase in $[\text{HCO}_3^-]$.

Causes:

- Excessive loss of HCl as seen in Pyloric obstruction, high intestinal obstruction.
- Alkali ingestion and alkali administration.
- Excessive loss of K^+ leading to K^+ deficiency.

Compensation:

Primary compensation is done by depressing respiratory centre and hypoventilation leading to retention of CO_2 . Secondary compensation is done by conserving H^+ , decreasing NH_3^+ formation, decreasing bicarbonate reabsorption, increasing K^+ excretion and retaining Chlorides.



Rfha

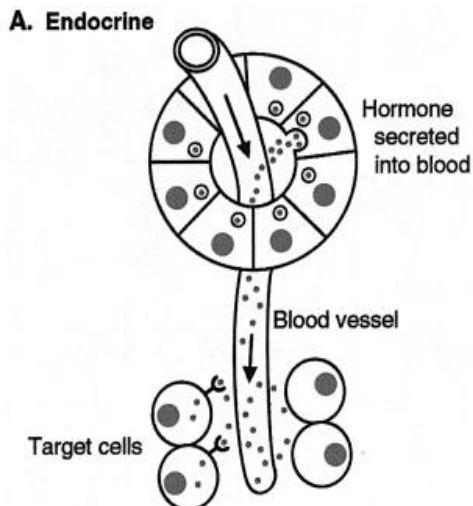
ENDOCRINE SYSTEM:

The living body possesses Remarkable communication system to coordinate its biological functions. It is achieved by 2 distinct organized functional system.

- Nervous system: transmission of electrochemical impulses.
- Endocrine system: wide range of chemical messengers; hormones.

HORMONES:

Organic substances, produced in small amounts by specific tissues (endocrine glands), secreted into the blood stream to control the metabolic and biological activities in the target cells. These are chemical messengers (signaling molecules) that transmit messages between cells. They are secreted from one cell in response to a specific stimulus and travel to target cell where they bind to a specific receptor and elicit a response. Major endocrine organ in human body include pineal, pituitary, thyroid, pancreas, adrenal, testis, ovary....



Classification:

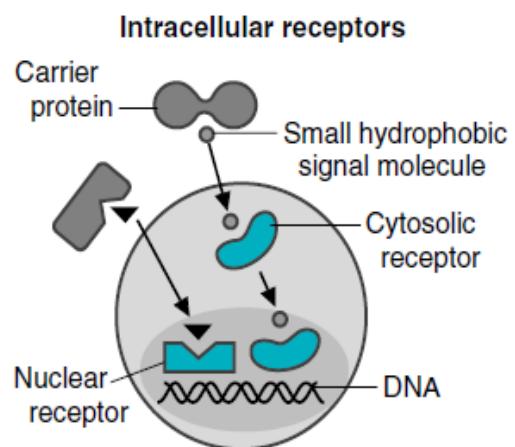
Hormones can be classified based on their chemical nature or on the basis of their mechanism of action.

- **Based on chemical nature:**
 - Protein or peptide hormone: Insulin, glucagon
 - Steroid hormone: glucocorticoids, sex hormones
 - Amino acid derivatives: epinephrine, thyroxine,
- **Based on Mechanism of action:**

• Group I hormones:

Hormones under this class binds with intracellular receptors to form receptor hormone complex (intracellular msn). The hormone receptor complex binds to specific regions on the DNA called Hormone Responsive Element (HRE) and causes specific expression of gene. These are lipophilic in nature & mostly derivatives of cholesterol (exception T₃ & T₄{Thyroid hormones})

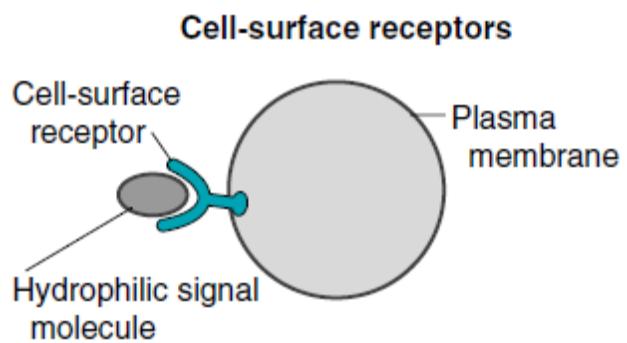
Eg: estrogens, glucocorticoids, progesterone, calcitriol, thyroid hormones



• Group II Hormones:

Hormones under this class binds to cell surface receptors and stimulate release of certain molecules 2nd messenger, which perform the biochemical functions. All the peptide hormones, amino acid derivatives impart their by binding to extracellular receptor and generating 2nd messenger intracellularly. These molecules are hydrophilic in nature.

- cAMP as 2nd msn: ACTH, FSH, LH, glucagon, PTH, calcitonin.
- Phosphatidyl inositol/Ca⁺⁺: TRH, GnRH, gastrin, CCK



Contrasting properties of hydrophilic and lipophilic hormones:

Properties	Hydrophilic hormones	Lipophilic hormones
Transport in blood	Free	Transport protein involved
Half-life	Short	Long
Receptor site	Plasma membrane	Nucleus
Extraglandular activation	Rare	Common
Mechanism of action	Second messenger	Transcription factor
Examples	Protein & peptides, catecholamines	Limited water solubility, all steroid hormones & thyroid hormones

Some of the hormones in system regulation:

System	Hormone involved
Ionic homeostasis Control of Na ⁺ /K ⁺ ratios in blood Control of Ca ⁺⁺ in blood Control of water balance	Aldosterone Parathyroid hormone, calcitonin, calcitriol Antidiuretic hormone
Fuel homeostasis Control of blood glucose level	Insulin in fed state Glucagon, epinephrine, cortisol, growth hormone in fast & exercise
Basal metabolic rate	Thyroid hormones
Regulation of growth Growth of cartilage Growth & development	Growth hormone Growth hormone, thyroid hormones
Regulation of reproduction, sexual differentiation and lactation Control of gonadal function Control of lactation Pregnancy	Luteinising hormone, FSH, testosterone, Oestradiol Prolactin, oxytocin hCG, hCS (somatomammotrophic), progesterone, Oestradiol
Gastrointestinal activity Pepsin & acid production Bicarbonate release from pancreas Release of bile from gallbladder Enzyme release from pancreas	Gastrin Secretin Cholecystokinin Cholecystokinin

Thyroid hormone synthesis:

Iodine is essential for the synthesis of thyroid hormone. Uptake of iodine by thyroid gland occurs by active transport which is controlled by thyroid stimulating hormone (TSH). Iodine (I⁻) is converted to active iodine (I⁺) in the thyroid gland and this active iodine is required for iodination. Thyroglobulin is a glycoprotein synthesized in thyroid gland with large number of tyrosine residues. The tyrosine residue of this protein is iodinated for the production of thyroid hormones. Tyrosine residue is first iodinated at 3-position to form monoiodotyrosine (MIT) and then at 5-position to form diiodotyrosine (DIT). After this there is large number of MIT & DIT residues on this protein. Two DIT couple to form thyroxine (T₄). One MIT when couples with one DIT, Triiodothyronine (T₃) are formed. After completion of iodination, proteolysis occurs and frees the bound hormones and released into the

blood. Thyroglobulin containing T₃ & T₄ can be stored for several months (1-3) in thyroid glands which when required digested by proteolytic enzymes in the thyroid gland.

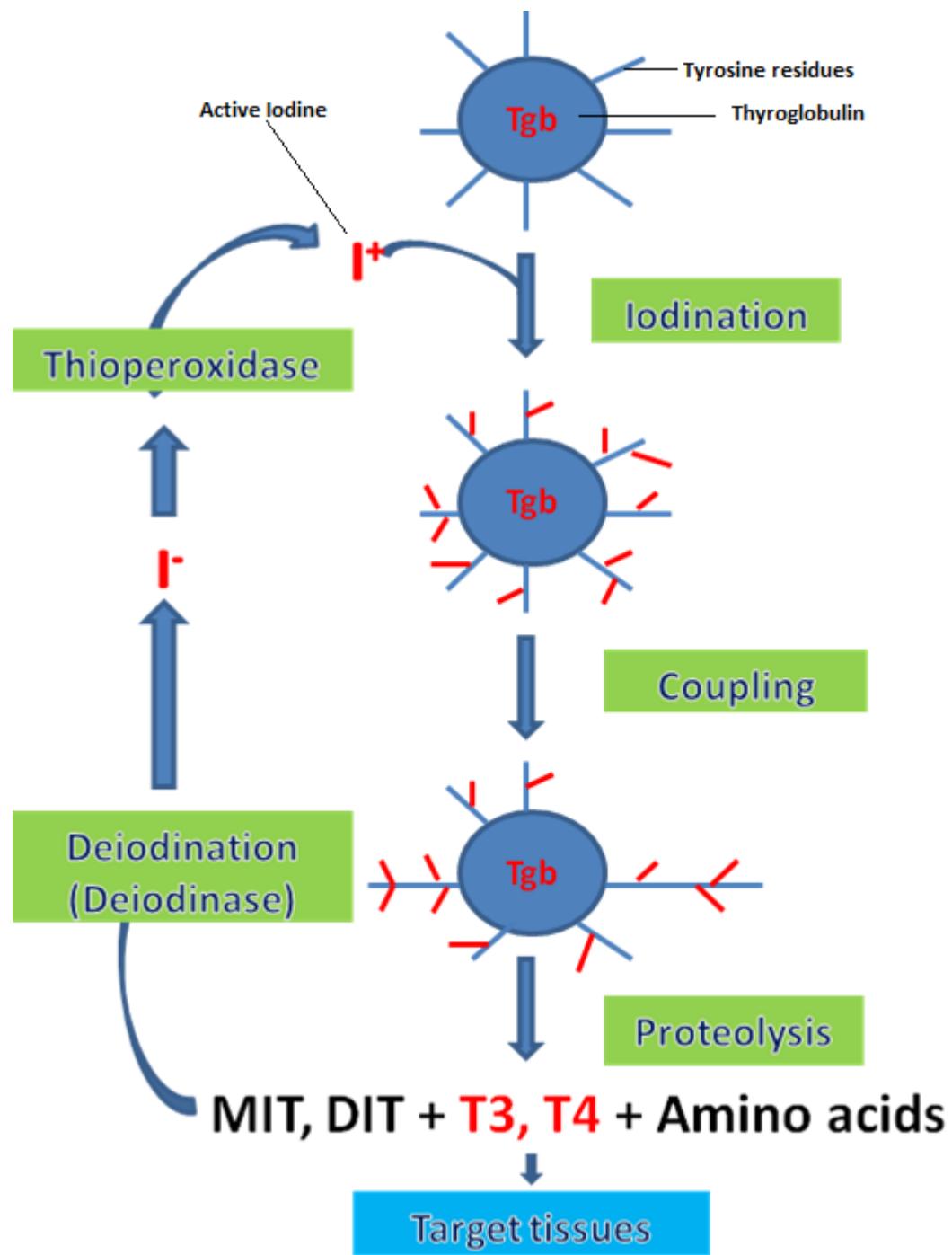


Figure: Thyroid hormone synthesis

S. Shaha