Chapter-1 | Introduction to biochemistry

Syllabus

Introduction to biochemistry: Scope of biochemistry in pharmacy; Cell and its biochemical organization

Introduction to biochemistry:

- Biochemistry is the study of the chemical processes and substances that occur within living organisms.
- It combines the principles of chemistry, biology, and physics to understand the complex chemical reactions and structures that make up living systems.
- Biochemists study the structure and function of biomolecules such as proteins, nucleic acids, carbohydrates, and lipids, as well as the enzymes and metabolic pathways that drive these reactions.
- They also investigate the genetic and molecular mechanisms that regulate these processes. Biochemistry is a fundamental field in the life sciences, with applications in medicine, agriculture, and biotechnology.

Scope of biochemistry in pharmacy:

The scope of biochemistry in pharmacy is quite broad, as it encompasses many different aspects of drug discovery, development, and delivery. Some of the key areas where biochemistry plays a role in pharmacy include:

1. **Drug design and development:** Biochemistry is used to understand the structure and function of biomolecules such as enzymes and receptors, which are important targets for drug development. Biochemists use this knowledge to design and develop new drugs that can bind to these targets and modulate their activity.

2. **Drug metabolism and pharmacokinetics:** Biochemistry is also used to understand how drugs are metabolized in the body, which can affect their efficacy and safety. Biochemists use this knowledge to optimize the pharmacokinetics of drugs, making them more effective and less toxic.

3. **Natural products:** Biochemistry is used to identify and isolate natural compounds from plants, microbes, and other sources, which can be used as drugs or drug leads. Biochemists use techniques such as chromatography and mass spectrometry to purify and analyze these compounds.

4. **Biotechnology:** Biochemistry is used to develop biotechnology products such as vaccines, diagnostic tests, and gene therapies. Biochemists use techniques such as recombinant DNA technology and protein engineering to create these products.

5. **Pharmaceutical formulation:** Biochemistry is used to develop dosage forms of drugs such as tablets, capsules, and injectable solutions. Biochemists use this knowledge to optimize the stability, solubility, and bioavailability of drugs in these dosage forms.

Overall, biochemistry plays a critical role in many different aspects of pharmacy, from drug development to formulation to delivery.

Cell and its biochemical organization:

The cell is the basic unit of life and is composed of a variety of different biochemical compounds and structures. The main components of a cell include:

1. **The cell membrane:** The cell membrane is a selectively permeable barrier that surrounds the cell, it composed of a lipid bilayer that controls the movement of molecules in and out of the cell.
2. **The cytoplasm:** The cytoplasm is the gel-like substance that fills the cell and contains all of the cell's organelles and other structures.
3. **The nucleoplasm:** The nucleoplasm is the gel-like substance that fills the nucleus of the cell, it contains the genetic material of the cell (DNA) which is responsible for the cell's growth and reproduction.
4. **The mitochondria:** The mitochondria are structures within the cytoplasm that are responsible for generating the energy needed by the cell through the process of cellular respiration.
5. **The ribosomes:** The ribosomes are small structures within the cytoplasm that are responsible for synthesizing proteins, they are made up of RNA and proteins
6. **The endoplasmic reticulum and Golgi apparatus:** The endoplasmic reticulum and Golgi apparatus are structures that help to transport and modify molecules within the cell, they are essential for the cell's internal organization and communication.
7. **The lysosomes:** The lysosomes are small structures within the cytoplasm that are responsible for breaking down and recycling waste materials within the cell.

All of these different structures and compounds work together to maintain the integrity and function of the cell, and to ensure that the cell can carry out all of its necessary functions such as growth, reproduction, and metabolism.

Chapter-2 | Biochemistry | Carbohydrates

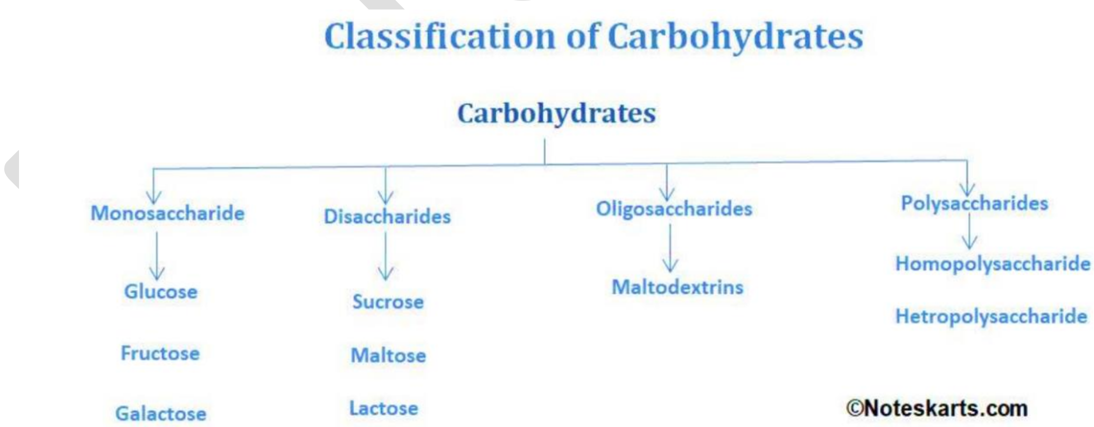
- **Definition, classification with examples, chemical properties**
- **Monosaccharides - Structure of glucose, fructose and galactose**
- **Disaccharides - structure of maltose, lactose and sucrose**
- **Polysaccharides - chemical nature of starch and glycogen**
- **Qualitative tests and biological role of carbohydrates**

Carbohydrates:

Definition:

- Carbohydrates are polyhydroxy aldehydes or ketones or compounds derived from their hydrolysis.
- Carbohydrates are the most abundant organic constituents of plants.
- They are the major source of chemical energy for living organism (e.g. Sugars & Starch).
- It compassed of carbon, Hydrogen and Oxygen.
- The general molecular formula of carbohydrates is c(H2O). Starch and cellulose are two common carbohydrates.

Classification of Carbohydrates



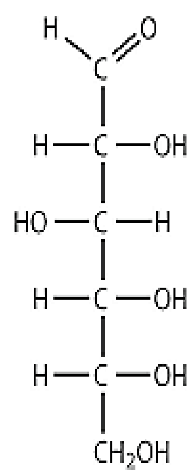
Monosaccharaides:

- Carbohydrates that cannot be hydrolyzed to simpler compound are called Monosaccharide.
- Monosaccharaides have the one sugar molecule.

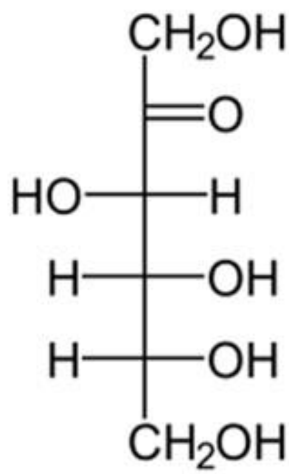
Classification of Monosaccharaides based on the number of Carbon Atoms:

Number of Atoms	Name	Example
3	Triose	Dihydroxy acetone, Glyceraldehyde
4	Tetrose	Erythrose
5	Pentose	Ribulose, Xylulose, Ribose
6	Hexose	Glucose, Fructose, Mannose, Galactose
7	Heptose	Glucoheptose, Galactoheptose, Sedoheptose

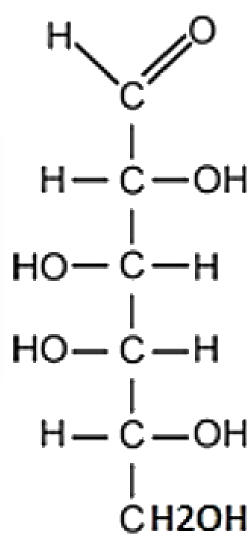
Structure of Glucose:



Structure of Fructose



Structure of Galactose



Disaccharides

Carbohydrates that give two monomeric units on hydrolysis are called Disaccharides.

Eg. Maltose, Sucrose, Lactose.

Classification of Disaccharides

They have two sugar molecules.

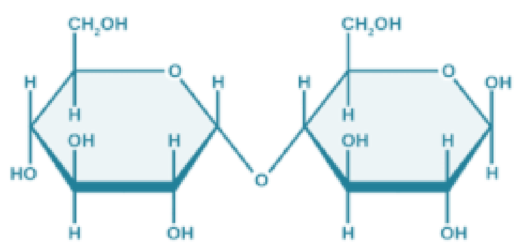
1. **Oligosaccharides :-**

- Carbohydrates that gives There to (10) ten monosaccharide on hydrolysis are called oligosaccharides. **eg: Reffinose, Maltotriose.**
- They have two or ten sugar molecules.

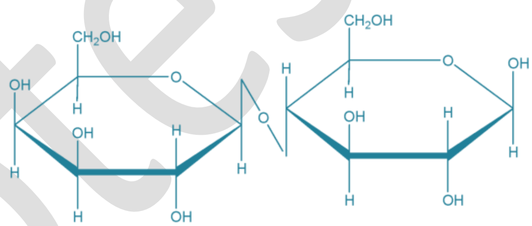
2. **Polysaccharides:**

- Carbohydrates that give many monosaccharide on hydrolysis are called polysaccharides.
- They have ten or more sugar molecules.
- Polysaccharides are made-up of one or different types of sugars.

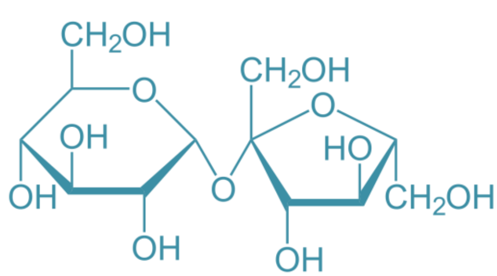
Structure of Maltose



Structure of Lactose



Structure of Sucrose



Polysaccharides

- Polysaccharides are major classes of biomolecules.

- They are long chains of carbohydrate molecules, composed of several smaller monosaccharides.
- These complex bio-macromolecules functions as an important source of energy in animal cell and form a structural component of a plant cell.

Classification of Polysaccharides

1. **Homopolysaccharides:**
   - The monomeric units are arranged in the form of long chain either unbranched or branched.
   - Eg: Starch, Glycogen, Cellulose etc.

2. **Hetrropolysaccharides:**
- Hemicellulose is a polymer containing D-xylose, L-arabinoc, D-Galactose, LRhamnose, D-Monnose and D-Glucoronic acid
- Eg. : Heparin

Chemical nature of Starch

- It is formed by the condensation of amylose and amylopectin. It is found largely in plants, fruits, seeds, etc.
- The chemical properties of starch are dependent on the reactivity of starch which is a function of the polyhydroxyl functional groups in the constituent glucose monomers.
- The hydroxyl groups at position C-2, C-3 and C-6 which are free from the glycosidic bond linkages and pyranose ring formation, are usually free for substitution reactions involving either the attached hydrogen or the entire hydroxyl group.

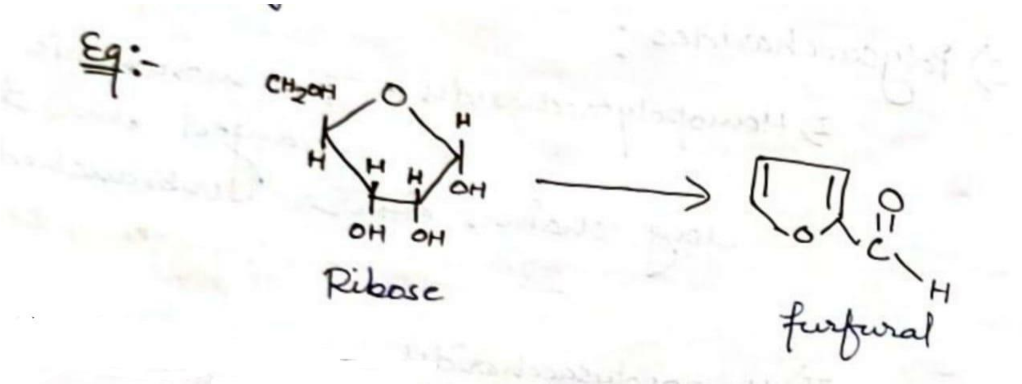
Chemical nature of Glycogen

- It is made up of a large chain of molecules. It is found in animals and fungi.
- It can be obtained by decomposition through the action of water.
- It is a major fuel store in plants, but is absent from animals where the equivalent is glycogen.

Chemical reactions and Qualitative teste for Carbohydrates:-

1. Dehydration:-

- Carbohydrates on dehydration give for fural or its derivative.
- Concentrated sulphuric acid is used as a dehydrating agent.



Molisch test :

It is general test for ***Carbohydrates*** identification

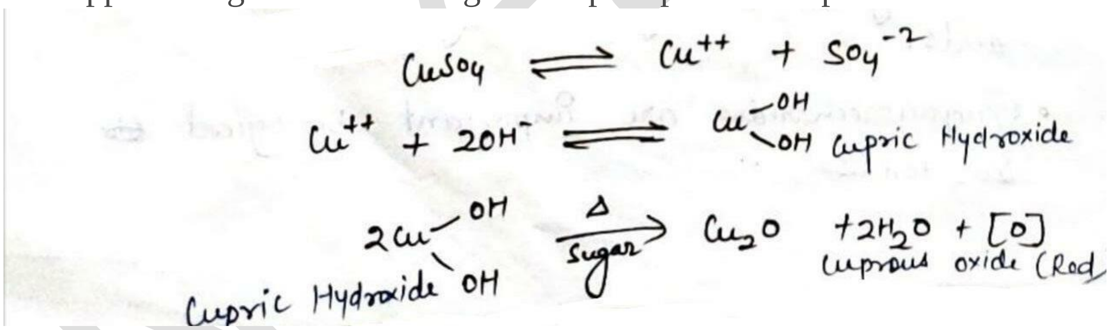
Furfural or its derivative formed during dehydration, react with α-nepenthes to giveviolet colour.

- In this test concentrated sulphuric acid is used as a dehydrating agent.

2. **Reactions of carbonyl group:**

1) Benedict's test –

- Carbohydrate is wanted with alkaline copper sulphates.
- Copper ions get reduced and give red precipitate of cuprous oxide.



Note:- All reducing Sugars give this test positive while sugar like sucrose does notgive this test positive.

2) **Barfoed's test:**

- This test's used for the identification of reducing mono-saccharidies.
- In presence of weak acidic condition only mono-saccharidies can

reduce copper ionto give red precipitate.

3) Fehling's Test :-

- Reducing sugar reduces the copper ions present in the Fehling solution so as to givered precipitate.

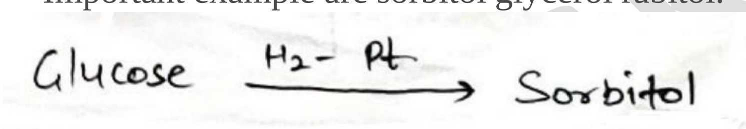
4) Formation of asazone:

- When reducing Sugar is heated with phenyldyrazine yellow crystalline compoundcalled osazones are formed.
- e.g.

D-Glucose +*Phenylhydrazine* →Glucose (Yellow crystal) Phenyldyrazine + H2O

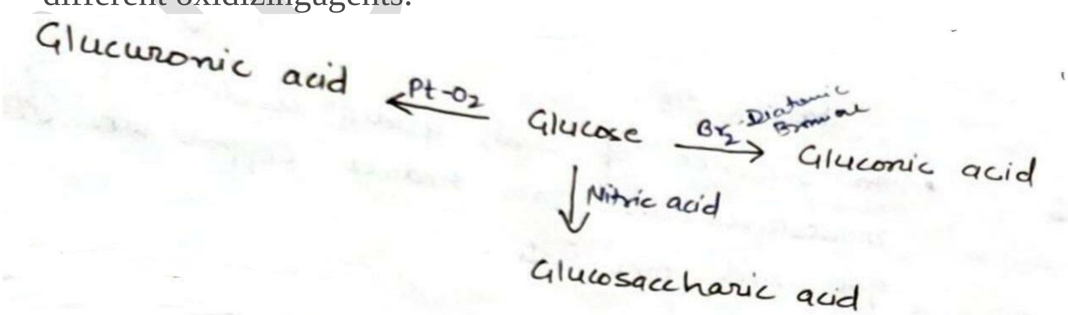
3. Reduction:-

- The carbonyl group of sugar can be reduced by variety of reagents suchas ***Hydrogen*** and ***platinum*** to an alcohol.
- Such <u>Carbohydrate</u> derivatives are called alditols.
- Important example are sorbitol glycerol rabitol.



4. Oxidation:

- Sugar are oxidation gives acid. The oxidation product depends upon oxidizingagent used in the reaction.
- g. Glucose is oxidising to different products with the help of different oxidizingagents.



5. Mucic Acid test:

- This is used for identification of galactose and lactose.
- Galactose or Lactose on oxidation in presence of conc.

Nitric acid givesgalactosaccharid acid (Mucic Acid).

6. Iodine test:

- Iodine reacts with starch dextrin and Glycogen to form a coloured complex.

Type of Polysaccharide		Colour with Iodine
1	Starch	Blue
2	Dextrin	Brown

3	**Glycogen**	**Pink**
4	**Amylose**	**Deep Blue**
5	**Amylopect in**	**Purple**

Disease related to Carbohydrate metabolism:-

- Various disorders have been reported due to abnormal metabolism ofCarbohydrates.

A) Diabetes mellitus:-

- It is a group of metabolic disorders with a common characteristics feature ofhyperglycemia.
- Hyperglycemia in diabetes mellitus due to defect in insulin action insulin secretionor both.
- Diabetes comes from the Greek words "Siphon" and Implies that a lot of urine ismade.
- The second term Mellitus come from the Latin word "Mel" which means Honey.

Diabetes mellitus is broadly classified into 2 categories.

1. **a) Type 1 Diabetes**
2. **b) Type 2 Diabetes**

a) Type 1 Diabetes:-

- It is characterized by absolute deficiency of insulin due to distruction of B-cell ofpancreas.
- A chronic condition in which the pancreas produce little or no insulin.

Symptoms:-

- Increase Thrust
- Frequent Urination
- Hunger
- Fatigue

b) Type 2 Diabetes:-

- This this type of diabetes a due to inadequate secretion of insulin by B- cells ofpancreas.
- In this type of diabetes the body either does not produce enough insulin.

Symptoms:-

- Increase thirst
- Frequent Urination hanger
- Fatigue
- Blurred vision.

Note:- Normal range- (70-120mg/dl)
High range – (←200mg/dl)

B)Glycosuria :-

- when Glucose is excreted in urine the condition is called as glycosuria.
- Glycosuria occurs due to elevated blood glucose level.
- Glycosuria more common during pregnancy.

C) Galactosemia:-

- Due to deficiency of enzyme galactose 1-phosphate luridly transferees andGalactosemia.
- Galactose cannot be converted in glucose which leads to a condition called asGalactosemia.
- It is characterized by increased galactose level in circulation and urine.
- Accumulated galactose into galactic which responsible for development of cataract.

Symptoms:-

- Jaundice
- Hepatospicenmegaly
- Mental retardation, etc.

D) Fructose intolerance:-

- One of the very normal hexose sugars of fruits (i.e. Fructose) gets normallymetabolised to give energy and CO2 but defective metabolism of fructose developed in blood, disorder know as fructose in tolerance.
- This disease occurs when calls on the surface of the intestine are not able to breakdown fructose efficiently.

E) Glycogen storage disease:-

- The metabolic abnormalities related with glycogen synthesis is termed as glycogenstorage disease.
- Glycogen is main source of energy when an enzyme is missing glycogen can buildup in the liver or glycogen may not form properly.

Symptoms:-

- Not growing fast enough
- Low blood sugar
- Weak muscles.

Biochemical importance of Carbohydrates:-

1. Carbohydrates are important constituents of the cell structures in the form ofglycolipid, glycoprotein, heparin, cellulose, starch, Glycogen.
2. Carbohydrates serie as an important source and store of energy.

- Carbohydrates are important basic material for many organic compounds like-Amino acids, Nucleic acid, Lipids.

1. Carbohydrates are important row material for the industrial production of productslike – Glucose, maltose, alcohol, Acids etc.

Chapter-3 | Proteins | Biochemistry and Clinical Pathology

Topic In this Notes

Proteins

- Definition, classification of proteins based on composition and solubility with examples
- Definition, classification of amino acids based on chemical nature and nutritional requirements with examples
- Structure of proteins (four levels of organization of protein structure)
- Qualitative tests and biological role of proteins and amino acids
- Diseases related to malnutrition of proteins

Definition.

- **Proteins** are naturally occurring polymers made up of amino acids.
- Almost everything that occurs in the cells involves one or more Proteins.
- Proteins provide structure, cellular reaction and carried out the tasks.
- 20 amino acids are found in protein and they are called standard amino acid. These amino acids contain the carboxyl group and the amino group attached to α carbon.

Role of protein.

- Protein performs difference role in the living system.
- Proteins which catalyse by your chemical reactions are called enzymes.
- Proteins are responsible for transportation of metabolites fructose, Glucose or Gases (like Oxygen, Carbon dioxide) are called transport proteins.
- Protein which are responsible for to protect from infection and other toxic

- substances are called antibiotics or defense proteins.
- Proteins which are required to give strength to cells or tissue are called structural proteins.
- Proteins which are required to carry out mechanical work are called muscle proteins.

Classification of proteins based on composition.

1. Complete Proteins: – Proteins which contain all the essential amino acids in required quantity are called complete Proteins.

2. Incomplete Proteins: - Proteins not containing all the essential amino acid are called incomplete protein.

Also classified as-

1. Simple Proteins   2. Conjugated Proteins   3. Derived Proteins

1. Simple Proteins: - Simple protein contain only amino acid reduce and other intimately bound material. Ex- Albumins, globulins, glutelin etc.

2. Conjugated Proteins: – Conjugated proteins contain in addition to polypeptide chain others substance or groups which impart characteristics properties. Ex- Nucleoproteins, Glycoproteins, Haemoglobin, Phosphoproteins etc.

3. Derived Proteins: - Derived protein are derived from partial to complete

hydrolysis from the simple or conjugated proteins by the action of acids, alkalis or enzymes. Ex- Peptones, peptides, proteoses etc.

Classification of protein based on solubility.

It is divided into two types-

a. Globular protein— It is made by the irregular amino acid sequence and forms the rounded or spherical structure.
    - It is generally soluble in water.
    - It is also called as functional protein and it is more sensitive towards PH and temperature change.
b. Fibrous protein— It is made by same type of amino acid sequence and forms the long narrow structure.

- It is generally Insoluble in water.
- It is also called as structural protein and it is less sensitive towards PH and temperature change.

AMINO ACIDS

Definition

- Amino acids are building blocks of protein.
- Amino acids are compound that contain and amino group and carboxyl group and the amino group attached to alpha carbon.
- The key elements of an amino acid are Carbon (C) Hydrogen (H) Oxygen (O) and nitrogen (N).
- There are there are 20 amino acids at present in our body in which 9 Essential and 2 Non-essential amino acids.

Properties of Amino Acids

A. Physical properties of the Amino acids.

1. **Solubility.**
   - All amino acids are Soluble in water but their Solubility various to a great extent.
   - Solubility depends on the nature of R- group. i.e., polarity of the amino acid.
   - Polar amino acids are highly Soluble in water.
   - Non-polar amino acids are highly Soluble in organic solvents like chloroform, ether etc.
2. **Acid and base behaviours.**
   - Amino acids contain the acidic carboxyl group (-COOH) and the basis group amino (-NH2) Hence amino acids are called as amphoteric molecules or ampholytes (i.e., Amphoteric electrolytes).
3. **Optical activity.**
   - All standard amino acid except glycine has an asymmetric carbon atom due to these amino acids are optically active.

B. Chemical properties of the Amino acids.

1. Ninhydrin reaction:

Amino acid + Ninhydrin ⟶ Purple/Blue Pigment + Aldehyde

2. Reaction with Dansyl Chloride:

Amino acid + Dansyl Chloride ⟶ Fluorescent Dansyl derivative Of amino acids.

3. Reaction due to COOH Group: -

- Salt formation with alkalis.
- Ester formation with alcohols.
- Amide formation with amines and decarboxylation .

Classification of amino acids based on chemical nature.

On the basis of chemical nature amino acids are divided as-

a. Acidic amino acid- Ex- Aspartic acid, Glutamic acid.
b. Basic amino acid- Ex- Histidine, Lysine, Arginine.
c. Hydrophilic amino acid- Ex- Asparagine, Glutamine.
d. Hydrophobic amino acid-Ex- Leucine, isoleucine, valine.
e. Sulphur containing amino acid- Ex- cystine, Methionine.
f. Aromatic amino acid- Ex- Phenylalanine, Tyrosine.

Classification of amino acids based on nutritional requirements

1. Essential Amino Acids.

- The amino acids which cannot be synthesized in the body but are required for normal function of body are called as essential amino acids.
- These amino acids should be supplied through diet.

2. Non-Essential Amino Acids.

- The amino acids which are synthesized in the body are called as non-essential amino acids.

S. N	Essential Amino acids	Non-Essential Amino acids
1.	Valine	Alanine
2.	Histidine	Asparagine
3.	Leucine	Aspartic acid
4.	Phenylalanine	Cysteine
5.	Tryptophan	Glutamic acid
6.	Lysine	Glutamine
7.	Alginine	Glycine
8.	Methionine	Cysteine
9.	Threonine	Proline
10.	Isoleucine	Serine

Structure of proteins (four levels of organization of protein structure).

Due to arrangement of the amino acids in the protein, we find the four level of organisation/modification in the proteins.

1. **Primary protein:** - It is the simple/basic/polypeptide chain like proteineous structure formed by the joining of amino acid by the help of covalent or peptide bond. Its biological activity mainly depends on the, types of amino acids. Many of genetic disorders are occurs due to changes in the primary protein structure.
2. **Secondary protein: -** Secondary structure forms by the interaction between the polypeptide chain of primary protein amino group and carboxyl group. It mainly presents two structural forms-
   - α secondary protein (Helix Form).
   - β secondary protein (Sheet/Plate form).
3. **Tertiary protein: -** Tertiary protein form by the further chemical modification between the secondary protein. It is stabilized due to presence of the H-bond, electrostatic forces, disulphides bonds, and Vander walls forces.
4. **Quaternary protein: -** The quaternary structure of a protein is the association of several protein chains or subunits into a closely packed arrangement.

Qualitative tests for Proteins and Amino acids.

1. Heat test: -

- When protein solution is heated in boiling water both the protein gets coagulated and lose their biological activity.
- This is called thermal denaturation of proteins.
- e.g. – Boiling the eggs.

2. Test with trichloroacetic acid (TCA)-

- TCA is normally used to precipitate proteins from their solution. TCA denatures the proteins.

3. Biuret Test: -

- Biuret reagents consists of copper sulphate in an alkaline medium when proteins are treated with Biuret reagent it's shows a violet colour.

4. Hydrolysis Test: -

- Proteins on hydrolysis gives free amino acids Hydrolysis can be carried out by acids like HCL, H2SO4, etc. Or Alkalis like – NaOH, KOH etc.

5. Xanthoproteic test: -

- Nitration of aromatic amino acids of protein give yellow colour concentrated nitric acid is used for Nitration.

6. Millon's Test: -

- Phenolic group of tyrosine of proteins react with mercuric sulphate in the presence of sodium nitrate and sulphuric acid to give red colour.

7. Precipitation test: -

- Protein are Precipitated by using different agents the common precipitation agents are salt, Organic solvent heavy metal ion, acids etc.
  - Salt – Ammonium sulphate, Sodium chloride.
  - Acids – Trichloroacetic acid (TCA), Acetic acid, Hydrochloric acid.
  - Organic solvents – Acetone alcohol
  - Heavy metal ions – Ammonium molybdate, Copper or Mercury salts.

Biological role of proteins and amino acids.

- Proteins give amino acids on hydrolysis during digestion and amino acids are the building blocks required for a cell to synthesis for proteins.
- Proteins are the structural component of protoplasm cell and tissues.

- Enzymes and few hormones are Proteins in nature antibiotics, haemoglobin are also Proteins.
- Protein is one of the important components of diet it is required to maintain growth and healthy functioning of the body.
- In the cell, cell membrane is also made up by the protein, protein play the role in the transporting the cellular and outer material through the active or passive transport.
- In our body some amount of protein stored, for the starvation, critical condition for energy.
- During the clinical condition protein also play the major role (protein excrete in urine, during blood examination)

Diseases related to malnutrition of proteins.

A. Kwashiorkor: -

- The symptoms of the diseases slow down the growth, oedema and change in skin, hair pigmentation and texture.
- Frequently there is liver enlightenment there is vomiting and diarrhoea and stools contain much undigested food.
- The course of this disease due to large family size, poor mental health, poor environmental conditions and delayed supplementary feeding.

Note: - This disease appears most commonly in children between the ages of 1 to 4 year.

B. Nutritional oedema: -

- It results from long contained loss of protein and usually occurs in famine areas. The Proteins deficiency in adults is very rare.
- The deficiency symptoms include loss of weight reduced fat, infections, frequent loose stools delay in healing of wounds and Oedema.
- Use of soybean, milk and eggs and other nutritious diet can cure the Protein deficiency syndrome in adults.

C. Marasmus: -

- It is a disease of infants below one year of age.
- Its cause is Proteins and carbohydrate or other nutritional factor deficiencies.
- Proteins and energy deficiency disease is also known as **Marasmus kwashiorkor**. Marasmus is more likely to occur in poor people.

Chapter-4 | Lipids | Biochemistry and Clinical Pathology

Lipids

- Definition, classification with examples
- Structure and properties of triglycerides (oils and fats)
- Fatty acid classification - Based on chemical and nutritional requirements with examples
- Structure and functions of cholesterol in the body
- Lipoproteins - types, composition and functions in the body
- Qualitative tests and functions of lipids

Lipids

Definition—The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, that are related more by their physical than by their chemical properties. They have the common property of being **(1) relatively insoluble in water and (2) soluble in nonpolar solvents such as ether and chloroform.**

They are important dietary constituents not only because of the high energy value of fats, but also because essential fatty acids and fat-soluble vitamins and other lipophilic micronutrients are contained in the fat of natural foods. So, we consider lipids, (lipo-, fat) a third major class of biomolecules.

Classification of lipid with examples—

Lipid are classified as mainly three classes as-

1. **Simple lipids—** The simplest lipids are the fatty acids that have the general formula R— COOH, where R represents a hydrocarbon chain composed of various lengths of —CH2-(methylene) units. It includes fats and waxes which are esters of fatty acids with various alcohols.
   a. **Fats—** Esters of fatty acids with glycerol. Oils are fats in the liquid state.

b. **Waxes—** Esters of fatty acids with higher molecular weight monohydric alcohols.

2. **Complex Lipids—**Complex lipids are esters of fatty acids containing groups in addition to an alcohol and one or more fatty acids.
    a. **Phospholipids—** Lipids containing, in addition to fatty acids and an alcohol, a phosphoric acid residue. They frequently have nitrogen-containing bases (eg, choline) and other substituents. In many phospholipids the alcohol is glycerol (glycerophospholipids), but in sphingophospholipids it is sphingosine, which contains an amino group.
    b. **Glycolipids (Glycosphingolipids)—** Lipids containing a fatty acid, sphingosine, and carbohydrate.
    c. **Other complex lipids—** Lipids such as sulfolipids and amino lipids. Lipoproteins may also be placed in this category.
3. **Derived/precursor lipid—** These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, ketone bodies, hydrocarbons, lipid-soluble vitamins and micronutrients, and hormones.

NOTE— Acylglycerols (glycerides), cholesterol, and cholesteryl esters are termed neutral lipids because they are uncharged.

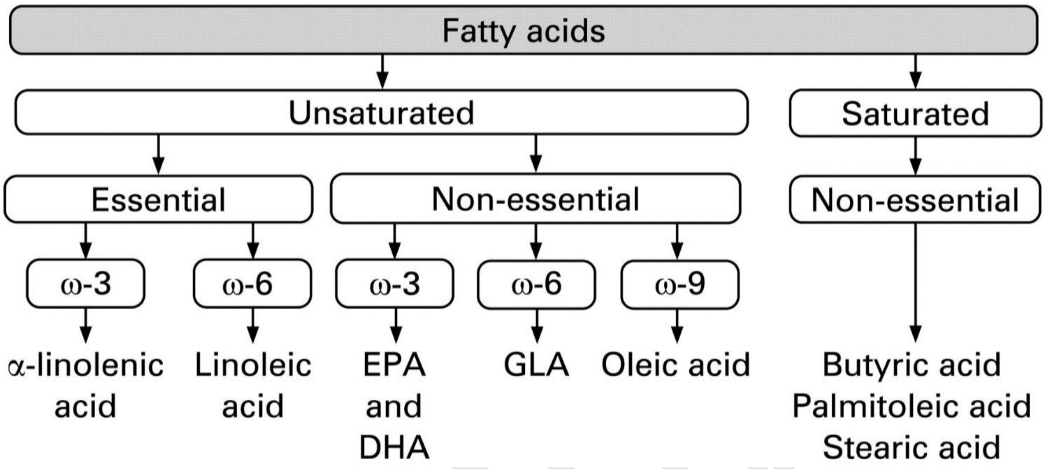
Fatty Acids

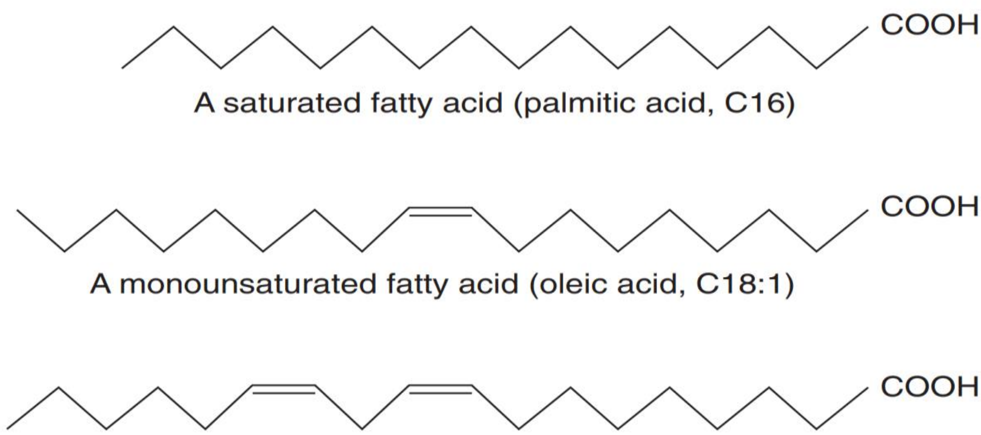
In higher plants and animals, the predominant fatty acid residues are those of the C16 and C18 species— palmitic, oleic, linoleic, and stearic acids.

Fatty acids that occur in natural fats usually contain an even number of carbon atoms. The chain may be saturated (containing no double bonds) or unsaturated (containing one or more double bonds).

1. **Saturated fatty acids—** Saturated fatty acids may be envisaged as based on acetic acid (CH3 —COOH) as the first member of the series in which— CH2 — is progressively added between the terminal CH3 — and —COOH groups. Ex- Palmitic acid.
2. **Unsaturated fatty acids—** Fatty acids contain one or more Double bonds.

a. Monounsaturated— containing one double bond. Ex-oleic acid.
b. Polyunsaturated— containing two or more than two double bonds. Ex- linoleic acid.
c. Eicosanoids— These compounds, derived from eicosa (20-carbon) polyenoic fatty acids. Ex- Prostaglandins, thromboxanes.





A saturated fatty acid (palmitic acid, C16)

A monounsaturated fatty acid (oleic acid, C18:1)

A polyunsaturated fatty acid (linoleic acid, C18:2)

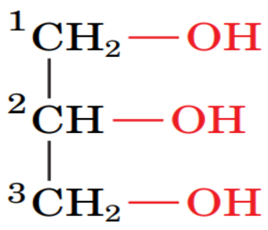
3. **Omega 3(ω3) –** Long chain ω3 fatty acids such as α-linolenic (ALA) (found in plant oils), eicosapentaenoic (EPA) (found in fish oil) and docosahexaenoic (DHA) (found in fish and algal oils) have anti-inflammatory effects, perhaps due to their effects in promoting the synthesis of less inflammatory prostaglandins and leukotrienes as compared to ω6 fatty acids.

Triglycerides

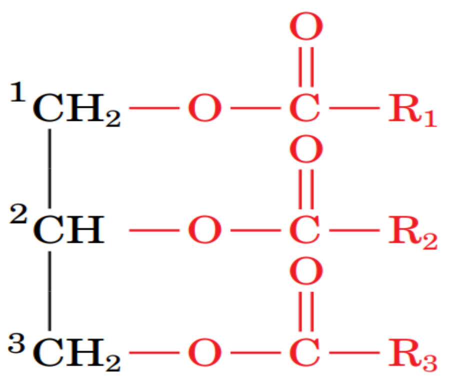
Triacylglycerols (formerly triglycerides) are the esters of glycerol with fatty acids. The fats and oils that are widely distributed in both plants and animals are chemically triacylglycerols. They are insoluble in water and non-polar in character and commonly known as neutral fats.

Fats (solid at room temperature) and **oils** (liquid at room temperature) are complex mixtures of triacylglycerols whose fatty acid compositions vary with the organism that produced them. Plant oils are usually richer in unsaturated fatty acid residues than animal fats, as the lower melting.

Structural representation—



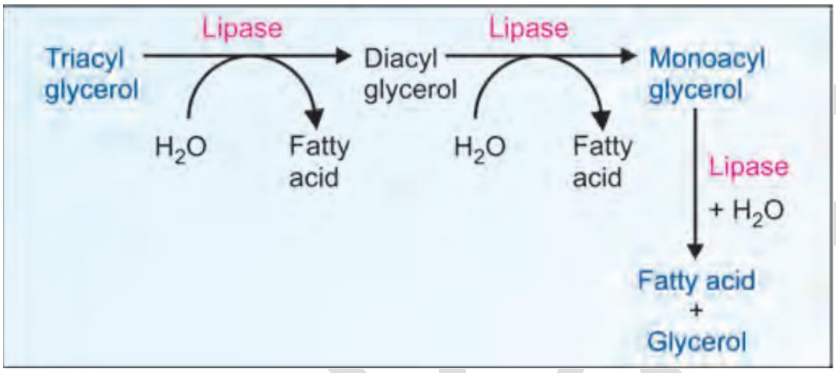
Glycerol



Triacylglycerol

Properties of triglycerides—Triacylglycerols are less oxidized than carbohydrates or proteins and hence yield significantly more energy per unit mass on complete oxidation.

1. **Hydrolysis**— Triacylglycerols undergo stepwise enzymatic hydrolysis to finally liberate free fatty acids and glycerol. The process of hydrolysis, catalysed by lipases is important for digestion of fat in the gastrointestinal tract and fat mobilization from the adipose tissues.



2. **Saponification**— The hydrolysis of triacylglycerols by alkali to produce glycerol and soaps is known as saponification.
   Triacylglycerol + 3 NaOH ⟶ Glycerol + 3 R-COONa (soaps).

3. **Rancidity**— Rancidity is the term used to represent the deterioration of fats and oils resulting in an unpleasant taste. Fats containing unsaturated fatty acids are more susceptible to rancidity. Rancidity occurs when fats and oils are exposed to air, moisture, light, bacteria etc.
4. **Lipid peroxidation**— In the living cells, lipids undergo oxidation to produce peroxides and free radicals which can damage the tissue. The free radicals are believed to cause inflammatory diseases, ageing, cancer, atherosclerosis etc

Lipoproteins

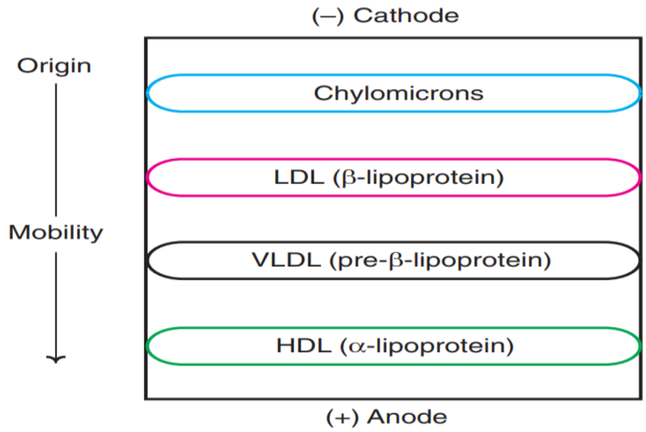
Definition and compositions— Lipoproteins are molecular complexes of lipids with proteins. They are the transport vehicles for lipids in the circulation.

Classification of lipoprotein— Based on their separation by electrophoresis is classified in five major class.

1. **Chylomicrons (CM)**— They are synthesized in the intestine and transport exogenous (dietary) triacylglycerol to various tissues. They consist of highest (99%) quantity of lipid and lowest (1%) concentration

of protein. The chylomicrons are the least in density and the largest in size, among the lipoproteins.

2. **Very low-density lipoprotein (VLDL)**— They are produced in liver and intestine and are responsible for the transport of endogenously synthesized triacylglycerols.
3. **Low density lipoprotein (LDL)**— They are formed from VLDL in the blood circulation. They transport cholesterol from liver to other tissues.
4. **High density lipoprotein (HDL)**— They are mostly synthesized in liver. Three different fractions of HDL (1, 2 and 3) can be identified by ultracentrifugation. HDL particles transport cholesterol from peripheral tissues to liver (reverse cholesterol transport).
5. **Free fatty acids—Albumin**— Free fatty acids in the circulation are in a bound form to albumin. Each molecule of albumin can hold about 20-30 molecules of free fatty acids. This lipoprotein cannot be separated by electrophoresis



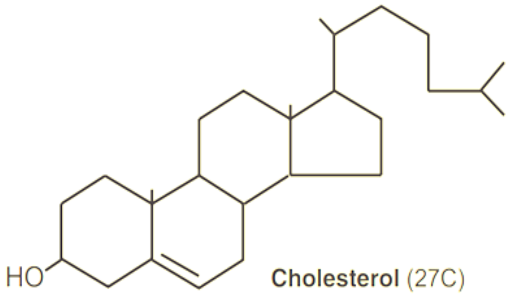
Electrophoresis of plasma lipoprotein Chart

Functions of Lipoprotein—

- Lipoproteins deliver the lipid components (cholesterol, triacylglycerol etc.) to various tissues for utilization.
- They function as transport vehicles for lipids in blood plasma.

Cholesterol

Definition and structures— Cholesterol is found exclusively in animals; hence it is often called as animal sterol. The total body content of cholesterol in an adult man weighing 70 kg is about 140 g i.e., around 2 g/kg body weight. Cholesterol is amphipathic in nature, since it possesses both hydrophilic and hydrophobic regions in the structure. In healthy individuals, the total plasma cholesterol is in the range of 150-200 mg/dl.



Cholesterol (27C)

Biosynthesis of cholesterol— About 1 g of cholesterol is synthesized per day in adults. Almost all the tissues of the body participate in cholesterol biosynthesis. The largest contribution is made by liver (50%), intestine (15%), skin, adrenal cortex, reproductive tissue etc.

The enzymes involved in cholesterol synthesis are found in the cytosol and microsomal fractions of the cell. Acetate of acetyl CoA provides all the carbon atoms in cholesterol.

Cholesterol was first isolated from bile. Cholesterol literally means 'solid alcohol from bile.'

Functions of Cholesterol—

- It is the structural components of the cell, which maintain the stability and the metabolism of the cells.
- Cholesterol is the precursor for the synthesis of all other steroids in the body. These include steroid hormones, vitamin D and bile acids.
- It is an essential ingredient in the structure of lipoproteins in which form the lipids in the body are transported.
- Fatty acids are transported to liver as cholesteryl esters for oxidation.

Qualitative tests of lipids

1. **Solubility—**
   - Solubility of Lipid in organic solvents depends on length of hydro-carbon chain of the fatty acids attached to glyceride.
   - Lipids are soluble in solvents like- Chloroform, ether, alcohol, hexane etc.

2. **Formation of translucent spot-on paper/Spotting effects—**
   - All the lipids are greasy in nature therefore the test may be taken as group test for lipids. So, take 3ml of ether in a test tube and dissolve 5 drops of oil in test tube.
   - The put a drop of the solution on the filter/ normal paper and let it dry.
   - A translucent spot on the filter paper observed and this indicates the greasy character of lipids.
3. **Emulsification—**
   - When oil or liquid fat is shaken with water. It is finely divided and is displayed in the water to form what is known as emulsion.
   - Shake a drop of oil with little water in a test tube. The oil becomes finally divided forming an emulsion.

4. **Iodine absorption test.**
   - This test is for unsaturated fatty acids for fat. A drop of iodine is added to fat (Fat some is prepared in chloroform) and shaken.
   - This solution will decolorize if unsaturated fatty acid is present.

Importance/functions of lipids

- It is the dietary constituents and provide the energy after metabolism, so we consider lipids a third major class of biomolecules.
- Lipids serve additional functions in the body, for example, some fat-soluble vitamins have regulatory or coenzyme functions, and the prostaglandins and steroid hormones play major roles in the control of the body's homeostasis.
- Lipid also provide the hydrophobic barrier that permits partitioning of the aqueous contents of cells and subcellular structures.
- Fat is stored in adipose tissue, where it also serves as a thermal insulator in the subcutaneous tissues and around certain organs. Nonpolar lipids act as electrical insulators, allowing rapid propagation of depolarization waves along myelinated nerves.
- Lipids have essential roles in nutrition and health and knowledge of lipid biochemistry is necessary for the understanding of many important biomedical conditions, including obesity, diabetes mellitus, and atherosclerosis.

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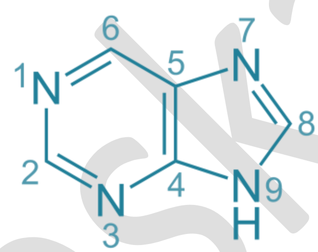
Nucleic acids

- Definition, purine and pyrimidine bases
- Components of nucleosides and nucleotides with examples
- Structure of DNA (Watson and Crick model), RNA and their functions

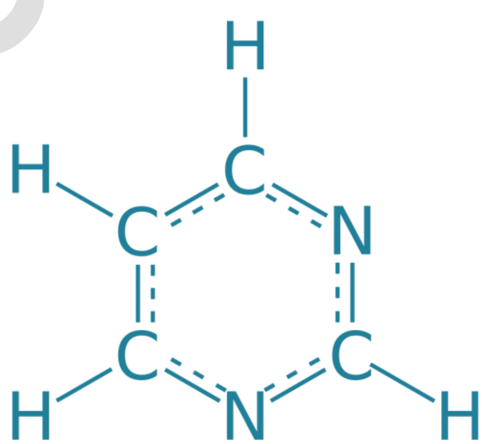
Purine and Pyrimidine

- Purines and pyrimidine are both organic compounds that take part in the synthesis of DNA and RNA, therefore they are called as the building blocks of the genetic material – DNA and RNA.
- They are nitrogenous bases that make up the two different nucleotides in DNA and RNA.



- Purine is a heterocyclic aromatic organic compound composed of a pyrimidine ring fused with imidazole ring.It comprises adenine and guanine as nucleases.
- It consists of two hydrogen-carbon rings and four nitrogen atoms.
- The melting point of purine is 214 °C .Catabolism results in the production of uric acid

Example-Purines (adenine and guanine) are two-carbon nitrogen ring base.



- Pyrimidine is a heterocyclic aromatic organic compound that is composed of carbon and hydrogen.
- It comprises cytosine, thymine, uracil as nucleobases.
- It consists of one hydrogen-carbon ring and two nitrogen atoms.
- The melting point of pyrimidine is 20-22 °C. Catabolism produces carbon dioxide, beta-amino acids and ammonia

Example - pyrimidines (cytosine and thymine) are one-carbon nitrogen ring bases.

Nucleoside and Nucleotide

1. Nitrogenous Base: They comprise pyrimidine or purine base. DNA contains adenine (A), guanine (G), cytosine (C) and thymine (T) whereas RNA contains adenine, guanine, cytosine and uracil (U).

2. Sugar: A nucleotide comprises a pentose sugar. DNA (Deoxyribonucleic acid) contains deoxyribose sugar and RNA (Ribonucleic acid) contains a ribose sugar.

- A Nitrogenous base attached with the sugar is called "Nucleoside".

3. Phosphate: Phosphate is associated with the sugar of nucleoside by an ester bond with the 5thC hydroxyl group. Nucleotides at least contain one phosphate group.

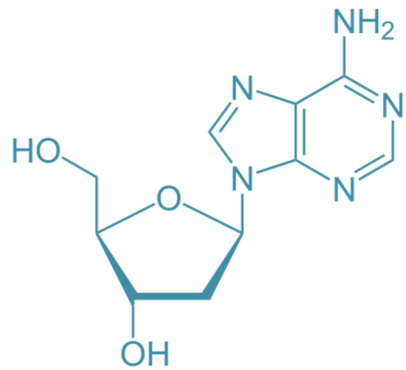
- A nucleoside is a purine or a pyrimidine nucleobase with a pentose sugar component, which is either ribose or deoxyribose.

Therefore:

nucleoside = nucleobase + ribose or deoxyribose

- A nucleoside is a glycoside formed from the hydrolysis of nucleic acid.
- In a nucleoside, the anomeric carbon is attached to the N9 of a purine (or to the N1 of a pyrimidine) by a glycosidic bond.

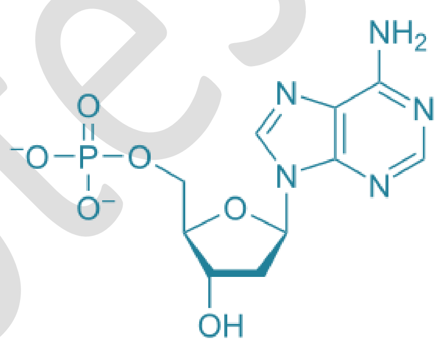
Nucleoside structure:



- Nucleotides are organic molecules consisting of a nucleoside and a phosphate.
- They serve as monomeric units of the nucleic acid ***polymers – deoxyribonucleic acid*** and **ribonucleic acid**, both of which are essential biomolecules within all life-forms on Earth.
- A molecule consisting of a nitrogen-containing base (adenine, guanine, thymine, or cytosine in DNA; adenine, guanine, uracil, or cytosine in RNA), a phosphate group, and a sugar (deoxyribose in DNA; ribose in RNA).

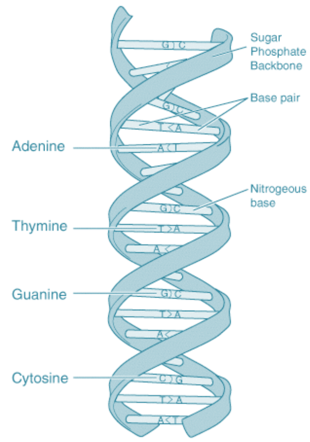
nucleotide = nucleoside + phosphate group

Nucleotide structure:



Watson and Crick model of DNA

- DNA as an acidic substance present in the nucleus was first identified by Frederich Meischer in 1869. He named it as 'nucleon'. Due to technical limitations in isolating such a long polymer intact the elucidation of structure of DNA remained elusive for a long period of time.
- It was only in 1953 that James Watson and Francis Crick proposed the very simple but famous double helix model for the structure of DNA.
- The main opposition was base pairing between the two strands of polynucleotide chains.



Structure of DNA double helix

The salient features of double helix structure of DNA are as follows:

- It is made up of two polynucleotide chains.
- The two chains have antiparallel polarity if one has polarities and the second chain must have polarity.
- The base into strands is paired through hydrogen bond forming base pairs. Adenine forms to hydrogen bonds with thymine from opposite strands and vice versa.
- Similarly guanine forms three H bonds with cytosine. As a result, purine comes opposite to pyrimidine.
- Because of this approximate a uniform distance between the two strengths of The Helix occurs.
- The two chains are called in a right-handed fashion. Pitch of the helix is and there are roughly 10bp in each turn.
- The plane of one base pair is stacked over the other in a double helix. This confirms stability of the helical structure.

Note: The proposition of a double helix structure for DNA and its simplicity in explaining the genetic implication become revolutionary.

Function of DNA and RNA:

RNA:

- You can think of an RNA molecule, as a disposable copy of a segment of DNA, a working copy of a single gene.
- RNA has many functions, but most RNA molecules are involved in protein synthesis only.
- RNA controls the assembly of amino acids into proteins.

- Each type of RNA molecule specializes in a different aspect of this job. The three main types of RNA are messenger RNA, ribosomal RNA, and transfer RNA.

DNA has two functions:

1. **Hold information on how to make proteins**
2. **Make more DNA**

The main function of DNA is to store genetic information.

- The information that DNA stores is how, when, and where to make protein.
- The second function of DNA is simply to make more DNA; this is called replication.

Enzymes

- **Definition, properties and IUB and MB classification**
- **Factors affecting enzyme activity**
- **Mechanism of action of enzymes, Enzyme inhibitors**
- **Therapeutic and pharmaceutical importance of enzymes**

Enzymes:

An enzyme is a biomolecule that can be synthesized biologically (naturally occurring) or through other processes (synthetically). Its main function is to act as a catalyst to speed up a reaction without itself being changed in the process.

Properties:

- Enzymes are specific in action.
- Enzymatic activity decreases with increase in temperature.
- They show maximum activity at an optimum pH of 6 – 8.
- Enzymes are complex macromolecules with high molecular weight.
- They catalyze biochemical reactions in a cell. They help in the breakdown of large molecules into smaller molecules or bring together two smaller molecules to form a larger molecule.
- Enzymes do not start a reaction. However, they help in accelerating it.
- Enzymes affect the rate of biochemical reaction and not the direction. Most of the enzymes have a high turnover number. Turnover number of an enzyme is the number of molecules of a substance that is acted upon by an enzyme per minute. High turnover number of enzymes increases the efficiency of the reaction.

Classification of Enzymes:

Classification of Enzymes by IUB System

- Enzymes are classified by complex system, suggested by commission on enzymes of International Union of Biochemistry (IUB). Based on their action they are divided into 6 major classes.

1. **Oxido-Reductases:**
   Enzymes in this class are involved in Oxidation-Reduction reactions.
   Example: Alcohol Dehydrogenase.
2. **Transferases:**
   Enzymes that catalyze transfer of Functional groups are called as Transferases.
   Example: Phosphorylases

3. **Hydrolases:**
   These are enzymes that bring about hydrolysis of various compounds.
   Example: Lipase
4. **Lyases:**
   Enzymes specialized in addition or removal of water.
   Example: Aldolase
5. **Isomerases:**
   The Isomerases enzymes catalyze the structural shifts present in a molecule, thus causing the change in the shape of the molecule.
   - Enzymes involved in all isomerization reactions.
     Example: Phosphotriose Isomerase.
6. **Ligases:**
   The Ligases enzymes are known to charge the catalysis of a ligation process.

Enzymes catalyzing synthetic reactions where two molecules are joined together and ATP are used.
Example: Succinate thiokinase

Factors affecting enzyme activity

Enzyme activity can be affected by several factors, including:

1. **Temperature:** Enzymes have optimal temperatures at which they function best. An increase in temperature can increase enzyme activity, but excessive heat can denature the enzyme, rendering it inactive.
2. **pH:** Enzymes have optimal pH levels at which they function best. A change in pH can affect the shape of the enzyme, making it unable to bind to its substrate, and therefore less active.
3. **Substrate concentration**: As the concentration of substrate increases, the rate of enzyme-catalyzed reactions also increases, up to a point. Beyond that point, the enzymes become saturated and the reaction rate levels off.
4. **Enzyme concentration**: Increasing the amount of enzyme present will increase the rate of the reaction, up to a point. Beyond that point, the reaction rate levels off.
5. **Inhibitors:** Compounds that bind to enzymes and prevent them from functioning properly are called inhibitors. They can be competitive or non-competitive, and can be reversible or irreversible.
6. **Co-factors:** Many enzymes require small, non-protein molecules, called cofactors, to function. Cofactors can be metal ions or organic molecules, and if they are not present, the enzyme will not function.

The mechanism of action of enzymes can be broken down into several steps:

1. **Binding:** The enzyme and substrate bind to form an enzyme-substrate complex. The binding process is specific, with the enzyme's active site (a specific region on the enzyme's surface) fitting the substrate like a lock and key.
2. **Catalysis:** Once the enzyme and substrate are bound, the enzyme catalyzes a chemical reaction that converts the substrate into a product. The enzyme does this by lowering the activation energy required for the reaction to occur.
3. **Release:** After the reaction is complete, the product is released from the enzyme's active site. The enzyme is then free to bind to another substrate and repeat the process.
    - It's important to note that enzymes are not consumed or changed in the reaction and can be used again.

Mechanism of action of enzymes:

- Enzymes catalyze reactions by lowering the activation energy required for the reaction to occur. Enzymes do this by binding the substrate at the active site, a specific region on the enzyme where the substrate binds, and bringing the substrate into a favorable conformation for the reaction to occur.

Enzyme inhibitor

- Enzyme inhibitors are molecules that bind to enzymes and decrease their activity. There are several types of enzyme inhibitors, including competitive inhibitors, non-competitive inhibitors, and irreversible inhibitors.
- Competitive inhibitors bind to the active site of an enzyme and prevent substrate molecules from binding, while non-competitive inhibitors bind to a different site on the enzyme and alter its conformation, making it less active.
- Irreversible inhibitors covalently bind to the enzyme and permanently inactivate it.
- Enzyme inhibitors are commonly used in medicine to target specific enzymes involved in disease processes and in the development of pesticides.

Therapeutic and pharmaceutical importance of enzymes

Enzymes play a critical role in many biological processes and are therefore important targets for the development of therapeutics and pharmaceuticals.

- **Metabolic disorders:** Enzymes involved in metabolic pathways, such as those involved in the breakdown of sugars and lipids, can be targeted to treat metabolic disorders such as diabetes and hyperlipidemia.
- **Cancer:** Many enzymes are involved in the growth and proliferation of cancer cells, and targeting these enzymes can be an effective strategy for cancer treatment. For example, the enzyme EGFR is often overexpressed in certain types of cancer, and drugs that target EGFR have been developed to treat these cancers.

- **Inflammation:** Enzymes involved in the inflammation process can be targeted to reduce inflammation and treat inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease.
- **Synthesis of drugs:** Enzymes are used in the synthesis of many drugs, including antibiotics, hormones, and vaccines. For example, enzymes such as penicillin amylase and recombinant DNA technology are used to produce many drugs.
- **Pesticides:** Enzymes involved in the growth and survival of pests can be targeted for the development of pesticides. For example, enzymes such as chitin synthase and acetyl cholinesterase are targeted for the development of pesticides.
- **Antibiotics:** Enzymes play a critical role in bacterial growth and survival, and targeting these enzymes can be an effective strategy for the development of antibiotics.
- **Enzyme replacement therapy:** Some diseases are caused by the lack of a specific enzyme, such as Gaucher disease, Fabry disease and Pompe disease, these disorders can be treated by administering the missing enzyme.

Overall, enzymes are important targets for the development of therapeutics and pharmaceuticals because they are involved in a wide range of biological processes and their inhibition can have a significant impact on disease processes.

Chapter-7

Vitamins

- Definition and classification with examples
- Sources, chemical nature, functions, coenzyme form, recommended dietary requirements, deficiency diseases of fat- and water-soluble vitamins

Vitamins.

Definition of vitamins—

- The word "Vitamin" comes from the Latin word "Vita" means "life".
- Vitamins are organic component in food that is needed in very small amount for growth and for maintaining good health.
- Vitamins are chemicals found in very small amounts in many different foods.
- They required to the body through diet because they cannot be synthesized by the body.
- Water soluble vitamins cannot be stored in human's tissues. Their excess is exerted with urine.
- Fat soluble vitamins can be stored in adipose tissue and the liver.

Common functions of vitamins—

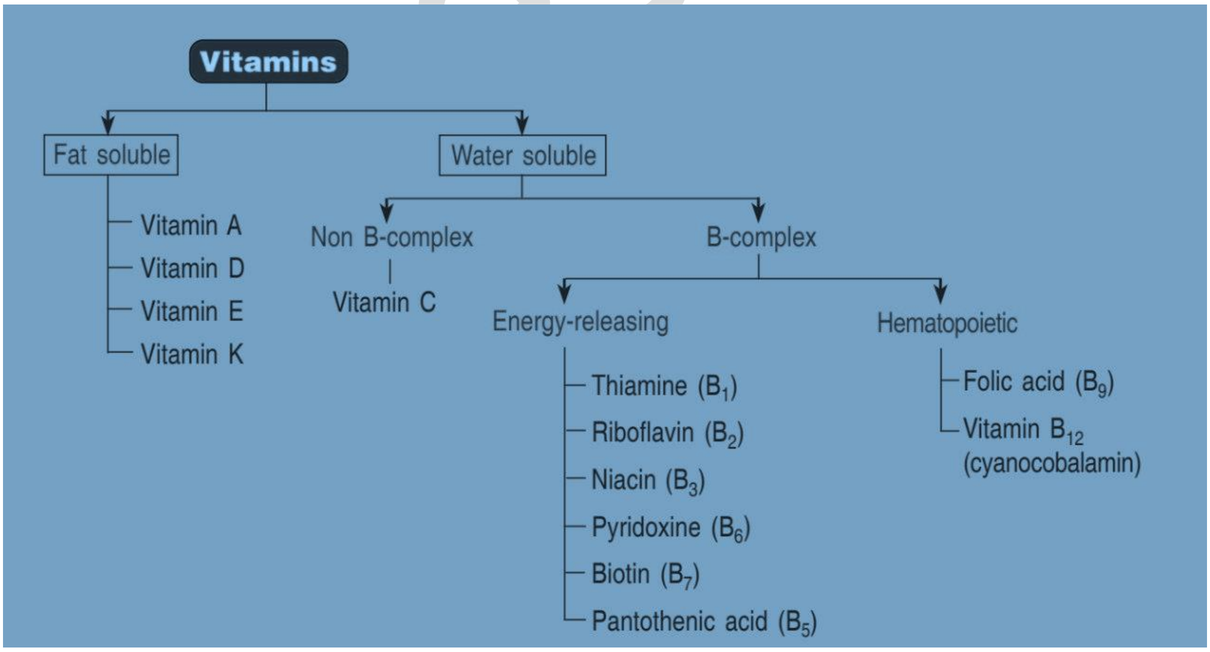
- They build up the resistance of the body against disease.
- Prevent and cure various disease caused by deficiency.
- Help the Digestion and Utilization of Minerals salts and carbohydrates in the in the body.
- Stimulate and give strength to digestive & Nervous system.
- Help Maintenance of Proper Health & normal Growth.

Classification of Vitamins with examples—

On the basis of solubility vitamins are mainly 2 types.

1. **Fat Soluble Vitamins:** Vitamins that dissolve in fat because fat is easily stored in our body, Fat soluble vitamins can be stored within out fat. This means they can accumulate and be saved for later use.
   - Vitamins A
   - Vitamins D
   - Vitamins E
   - Vitamins K
2. **Water Soluble Vitamins:** Water Soluble Vitamins that dissolve in water because our body is a watery environment. These vitamins can move through our body pretty easily & they can also be flushed out by the kidneys.
   A. B- complex.
   - **Energy-releasing—** Thiamine (B_1), Riboflavin (B_2), Niacin (B_3), Pantothenic acid (B_5), Pyridoxine (B_6), Biotin (B_7).
   - **Hematopoietic—** Folic acid (B_9), Cyanocobalamin(B_{12})

   B. **Non-B-complex. Example**— Vitamin C



Sources, chemical nature, functions, coenzyme form, recommended dietary requirements, deficiency diseases of fat-and water-soluble vitamins.

Vitamin A

- Vitamin A is also known as "Retinol" Dehydroretinol.
- It is a Colourless Vitamin insoluble in water & soluble in fat.
- Destroyed when exposure to sun light.

Sources.

- Carrot
- Chesses
- Yellow corn
- Papaya
- Fish Liver oil
- Mangoes

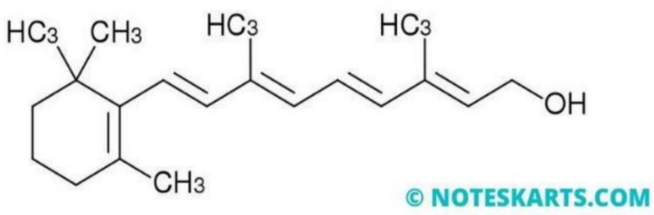


Fig: Retinol

Chemical nature.

Dietary requirements.

- For adult men— 900 micrograms (mcg).
- For adult women— 700 micrograms (mcg).
- For pregnant and lactating women have higher requirements.

Functions.

- Provide the defence against illness and infections.
- Helping for the vision
- Keeping skin and lining of some parts of the body healthy.

Coenzyme form.

- The active form of vitamin A is retinal, which converted from retinol by the action of retinol dehydrogenase enzyme and finally transported throughout the body.

- Retinoic acid (hormone like substance and is involved in the cell growth and differentiation).

Deficiency disease.

- Xerophthalmia – (Blindness in Childhood)
- Keratomalacia – (Whole Eye ball may shrink)

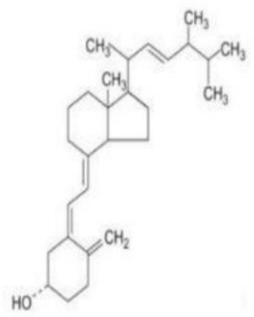
- Phrynoderma – (Skin lesion – Follicular Hyperkeratosis).

Vitamin D.

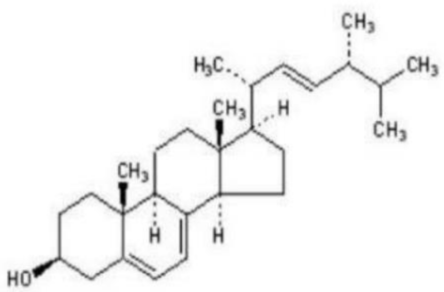
- Vitamin – D also known as "Calciferol" sunshine vitamin antirachitic factors.
- It is white crystalline substance
- Fairly heat resistance
- Soluble in fat & fat solvents.

Sources.

- Milk
- Sun light exposure
- Mushrooms
- Cod liver oil
- Egg Yolk
- Butter, etc.



Ergosterol structure



Ergocalciferol structure

Chemical nature

Dietary requirements.

- According to the institute of medicine (IOM), the daily intake for adults is 600-800 international units (IU).

Functions.

- Vitamin d helps to regulate the immune system and cell growth and differentiation.
- It is essential for the maintaining healthy bones and teeth.

Coenzyme form.

- Calcitriol is an active form of vitamin D and acts as a coenzyme.

Deficiency disease.

- Child – Rickets – Disease of Growing Bones

- Adult – Osteomalacia – Similar as rickets (Bones become softer).
- Osteoporosis and others auto immune disorders.

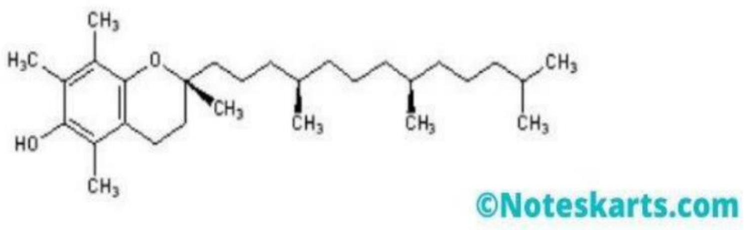
Vitamin E.

- It is also known as "Tocopherol" anti-sterility factor.
- Vitamin E is light yellow oil.
- Slowly oxidized and destroyed by UV- Rays.

Sources.

- Corn
- Egg
- Milk
- Soybean
- Meat
- Rice, etc.

Tocopherol structure



Chemical nature.

Dietary requirements— According to the national institutes of health (NIH), the recommended daily intake for adult men and women is 15 milligrams per day.

Functions.

- It helps to maintain the health of the cells in the retina and necessary for the proper function of the visual cycle.
- Plays an important role in the immunity functions.
- It acts as an antioxidant and prevent the body against any damage.
- Helps in cell growth and differentiation.

Coenzyme— Tocopherols and Tocotrienols.

Deficiency disease.

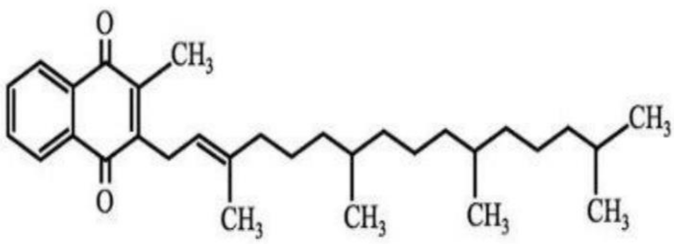
- Very low birth weight infants
- Neurological Problem
- Impairment of Immune Response
- Neuromuscular Problem etc.

Vitamin K

- It is also known as “Phylloquinone” Antihemorrhagic factors.
- It is Yellow viscid oil.
- Sensitive to light.

Sources.

- Spinach
- Coriander leaf
- Cabbage
- Broccoli
- Guava & Other Fruits.



Phylloquinone structure

Chemical nature.

Dietary requirements— The recommended intake for the adult men and women is 120 micrograms per day.

Functions.

- Vitamin K2 is important for blood clotting and maintaining healthy bones, it is produced by bacteria in the gut and is also found in fermented foods such as cheese and natto.

Coenzyme form

- Phylloquinone – Vitamin-K1
- Menaquinone – Vitamin-K2
- Menadione – Vitamin-K3

Deficiency diseases.

- Cause loss of Blood – Clotting Power
- In Infants haemorrhage should form.

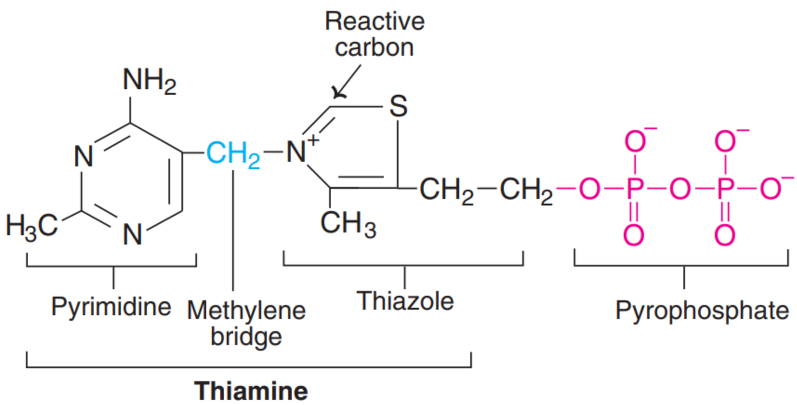
Vitamin (B_1).

- Thiamine is water soluble. it has a specific in the carbohydrate metabolism (also called anti-Beri-Beri or antineurotic).

Sources.

- Cereals.
- Pulses.
- Seeds oil.
- Nuts.
- Yeast, animal liver, kidney, heart etc.

Chemical nature.



Dietary requirements.

- Dietary supply for adults 1-1.5 mg/day.
- For children 0.7-1.2 mg/day.
- Pregnancy and lactation 2 mg/day.

Functions.

- It helps in the metabolism of the carbohydrates into the energy.
- It also plays a role in muscle contraction and conduction of nerve signals.

Coenzyme form— Thiamine pyrophosphate

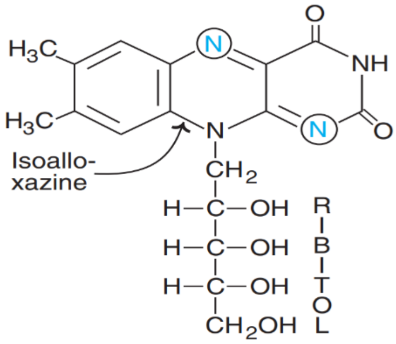
Deficiency disease.

- Beri-Beri is most common.
- Loss of appetite
- Peripheral neuropathy.

Vitamin (B_2)

Sources.

- Milk and milk products.
- Meat.
- Eggs.
- Liver,kidney.



Riboflavin

Chemical nature.

Dietary requirements—Daily requirement for adults 1.2-1.7 mg.

Functions.

- Riboflavin through its coenzyme takes part in a variety of cellular oxidation-reduction reactions.
- It helps in the blood cell production and body growth.

Coenzyme form.

- Flavin mononucleotide (FMN).
- Flavin adenine dinucleotide (FAD).

Deficiency disease.

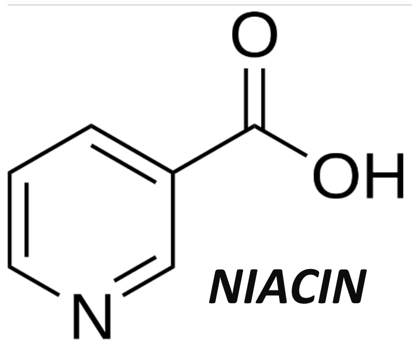
- Cheilosis.
- Glossitis.
- Dermatitis.

Vitamin (B_3)

- Niacin or nicotinic acid is also known as pellagra preventive factor.

Sources.

- Liver.
- Yeast.
- Whole grains, cereals.
- Pulses like beans, peanuts.



Chemical nature.

Dietary requirements— Daily requirements for adults is 15-20 mg.

Functions.

- Therapeutically it shows the many biochemical effects on body than vitamin.
- There is tendency for the increased levels of glucose and uric acid in the circulation.

Coenzyme form.

- Nicotinamide adenine dinucleotide (NAD+).
- Nicotinamide adenine dinucleotide phosphate (NADP+).

Deficiency disease.

- Pellagra.
- Dermatitis.

Vitamin (B_5)

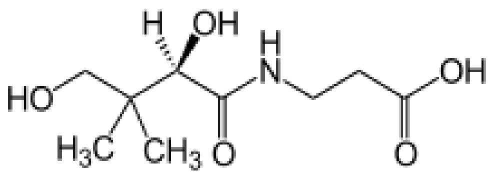
- It is also known as chick anti-dermatitis factor is widely distributed in nature.

Sources.

- Eggs.
- Liver.
- Meat.
- Yeast.
- Milk.

Chemical nature.

Dietary requirements— Daily intake for adult is 5-10 mg.



Pantothenic acid

Functions.

- The functions of pantothenic acid are exerted through coenzyme A. it plays a unique role in integrating various metabolic pathways. More than 70 enzymes that depends on coenzyme A are known.

Coenzyme form— Coenzyme A

Deficiency disease.

- Burning feet syndrome (pain and numbness in the toes, sleeplessness, fatigue etc).

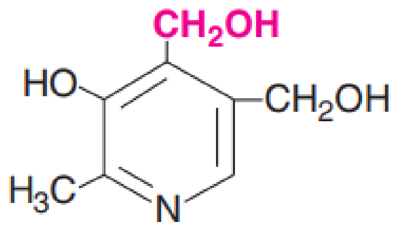
Vitamin (B_6).

- Vitamin B_6 is used to collectively represent the three compounds namely pyridoxine, pyridoxal, and pyridoxamine.

Sources.

- Egg yolk.
- Milk.
- Meat.
- Cabbage.
- Roots and tubers.

Chemical nature.



Pyridoxine

Dietary requirements—Daily requirements about- 2-2.2 mg/day. During pregnancy/lactation 2.5 mg/day.

Functions.

- It helps in the amino acid metabolism and helps in the secretion of serotonin, histamine etc.
- It helps in the antibody's synthesis.

Coenzyme form— Pyridoxal phosphate (PLP).

Deficiency disease.

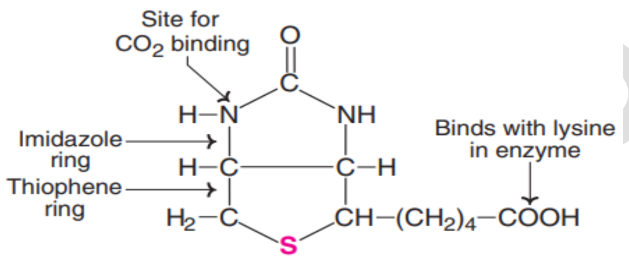
- Neurological disorder like- depression, mental confusion.
- Decrease secretion of amines like histamine.
- Decreased in haemoglobin level.

Vitamin (B_7)

- Biotin or vitamin H is a sulphur containing B complex vitamin.

Sources.

- Liver.
- Kidney.
- Egg yolk.
- Milk.
- Tomatoes.
- Grains.



Biotin with binding sites.

Chemical nature.

Dietary requirements— Daily requirement for adult is 100-300 mg.

Functions.

- Biologically it helps in the synthesis of fatty acids, gluconeogenesis, citric acid etc.
- Biotin used as the treatment for hair loss and to promote healthy hair, skin and nails.

Coenzyme form— Biocytin is coenzyme form.

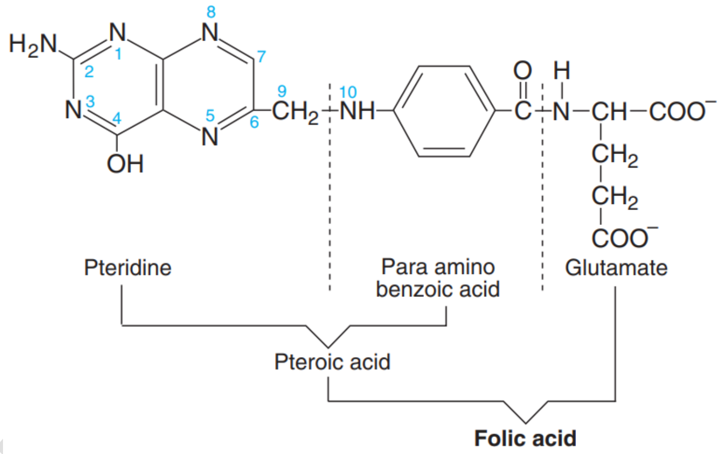
Deficiency disease.

- The symptoms of biotin deficiency include anaemia, loss of appetite, nausea, dermatitis.
- May also cause depression, hallucinations etc.

Vitamin (B_9)

Sources.

- Green leafy vegetables.
- Whole grains.
- Cereals.
- Liver
- Kidney.
- Eggs.



Chemical nature.

Dietary requirements.

- Daily requirements in adults are 200 µg.
- During pregnancy 400 µg.
- During lactation 300 µg.

Functions.

- It helps in the production of DNA and RNA, the genetic materials.
- It is important when cells and tissues are growing rapidly, such as in infancy, adolescence, and pregnancy.
- Helps in the iron metabolism and maintain the iron level in body

Coenzyme form— Tetrahydrofolate (THF or FH_4).

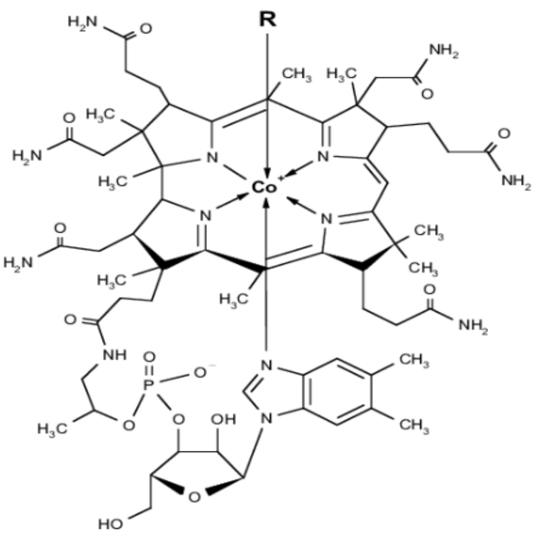
Deficiency disease.

- Macrocytic anaemia.
- Neural defects in the foetus.

Vitamin (B_{12}).

Cobalamin is also known as anti- pernicious anaemia vitamin. It is the unique vitamin, synthesized by only microorganisms and not by animals and plants.

Vitamin B_{12}



Sources.

- Liver, kidney
- Milk.
- Fish.
- Curd.
- Chicken etc.

Chemical nature.

Dietary requirements.

- Daily requirement for adult is 3µg.
- For children 0.5-1.5 µg/day.
- During pregnancy/lactation 4µg

Functions.

- It is the necessary substance for the red cells production and the DNA.
- It is also for functioning and development of brain and nerve cells.

Coenzyme form—

- Methylcobalamin (MeCbl).
- Adenosylcobalamin(AdoCbl).

Deficiency disease.

- Pernicious anaemia.
- Neuronal degeneration.

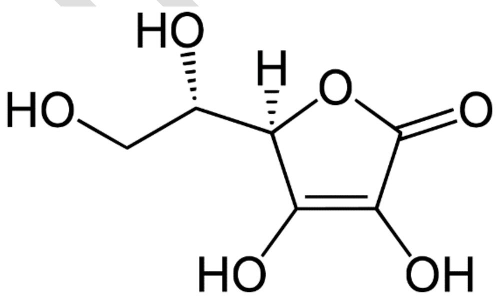
Vitamin C

- Vitamin C is a water-soluble versatile vitamin. The acidic property of vitamin C is due to the enolic hydroxyl groups.

Sources.

- Citrus fruits.
- Goose berry.
- Guava.
- Green vegetables.
- Tomatoes.
- Adrenal gland and gonads.

Chemical nature



Ascorbic acid

Dietary requirements— Daily requirements for the adult is 60-70 mg.

Functions.

- Maintaining the healthy conditions of skin, blood vessels, bone and cartilage.
- Helps in the wound or infections healing.
- Helps in growth of hairs.

Coenzyme form— Ascorbic acid.

Deficiency disease

- Scurvy.
- Sore gums, loose teeth, anaemia.
- Decreased immunocompetence, delayed wound healing.

Chapter-8

Metabolism (Study of cycle/pathways without chemical structures)

(BIOCHEMISTRY & CLINICAL PATHOLOGY)

Metabolism (Study of cycle/pathways without chemical structures)

Unit-1

- **Metabolism of Carbohydrates: Glycolysis, TCA cycle and glycogen metabolism, regulation of blood glucose level. Diseases related to abnormal metabolism of Carbohydrates.**

Unit-2

- **Metabolism of lipids:** Lipolysis, β-oxidation of Fatty acid (Palmitic acid) ketogenesis and ketolysis. Diseases related to abnormal metabolism of lipids such as Ketoacidosis, Fatty liver, Hypercholesterolemia

Unit-3

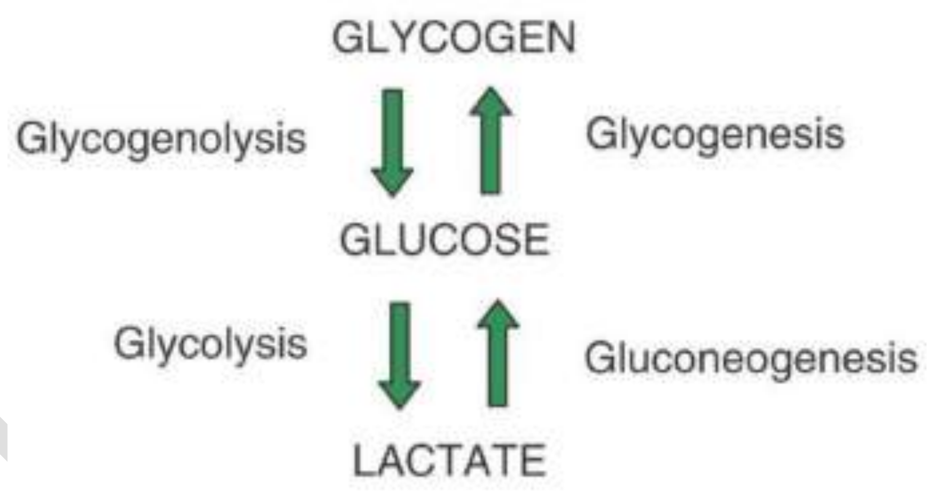
- **Metabolism of Amino acids (Proteins):** General reactions of amino acids and its significance– Transamination, deamination, Urea cycle and decarboxylation. Diseases related to abnormal metabolism of amino acids, Disorders of ammonia metabolism, phenylketonuria, alkaptonuria and Jaundice.
- **Biological oxidation:** Electron transport chain and Oxidative phosphorylation

Unit-1

Metabolism of carbohydrates.

Introduction—The major pathways of carbohydrate metabolism either begin or end with glucose. An understanding of the pathways and their regulation is necessary because of the important role played by glucose in the body. Glucose is the major form in which carbohydrate absorbed from the intestinal tract is presented to cells of the body. Glucose is the only fuel used to any significant extent by a few specialized cells and the major fuel used by the brain. Indeed, glucose is so important to these specialized cells and the brain that several of the major tissues of the body work together to ensure a continuous supply of this essential substrate.

Relationship of glucose to the major pathways of carbohydrate metabolism.

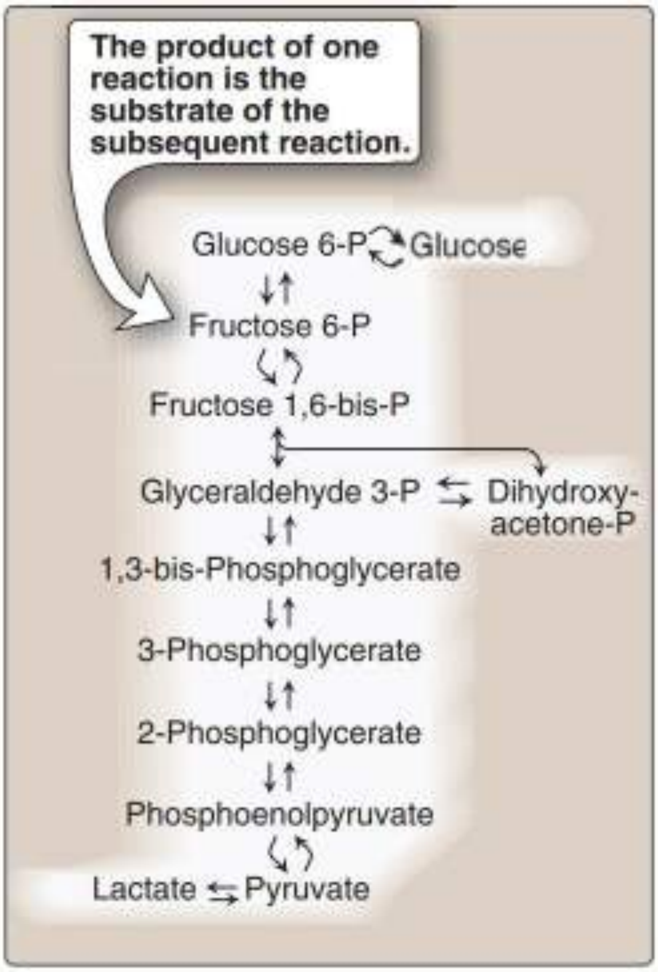


Glycolysis

- Glycolysis, a pathway used by all cells of the body to extract part of the chemical energy inherent in the glucose molecule. This pathway also converts glucose to pyruvate and sets the stage for complete oxidation of glucose to CO_2 and H_2O.

- In contrast to glycolysis, which produces ATP, gluconeogenesis requires ATP and is therefore an energy requiring process.

Metabolic pathway of glycolysis.



Reactions of glycolysis.

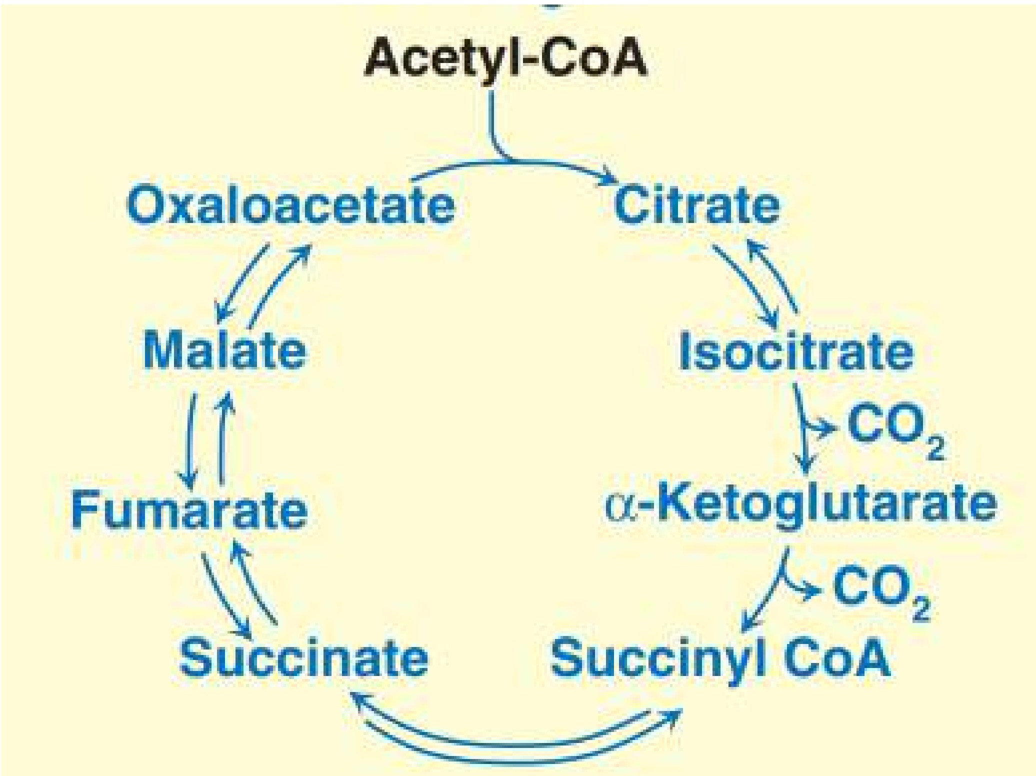
The conversion of glucose to pyruvate occurs in two stages. The first five reactions of glycolysis correspond to an energy investment phase in which the phosphorylated forms of intermediates are synthesized at the expense of ATP. The subsequent reactions of glycolysis constitute an energy generation phase in which a net of two molecules of ATP are formed by substrate-level phosphorylation per glucose molecule metabolized.

1. **Phosphorylation of glucose**— Phosphorylated sugar molecules do not readily penetrate cell membranes, because there are no specific transmembrane carriers for these compounds, and because they are too polar to diffuse through the lipid core of membranes. Mammals have several isozymes of the enzyme hexokinase that catalyse the phosphorylation of glucose to glucose 6-phosphate.
2. **Isomerization of glucose 6-phosphate**—The isomerization of glucose 6-phosphate to fructose 6-phosphate is catalysed by phosphoglucose isomerase.
3. **Phosphorylation of fructose 6-phosphate**— The irreversible phosphorylation reaction catalysed by phosphor fructokinase-1 (PFK-1) is the most important control point and the rate-limiting and committed step of glycolysis.
4. **Cleavage of fructose 1,6-bisphosphate**—Aldolase cleaves fructose 1,6-bisphosphate to dihydroxy acetone phosphate and glyceraldehyde 3-phosphate.
5. **Isomerization of dihydroxyacetone phosphate**—Triose phosphate isomerase interconverts dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (see Figure 8.16). Dihydroxy -acetone phosphate must be isomerized to glyceraldehyde 3-phosphate for further metabolism by the glycolytic pathway. This isomerization results in the net production of two molecules of glyceraldehyde 3-phosphate from the cleavage products of fructose 1,6-bisphosphate
6. **Oxidation of glyceraldehyde 3-phosphate**—The conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate by glyceraldehyde 3-phosphate dehydrogenase is the first oxidation-reduction reaction of glycolysis.
7. **Synthesis of 3-phosphoglycerate producing ATP—**When 1,3-BPG is converted to 3-phosphoglycerate, the high-energy phosphate group of 1,3-BPG is used to synthesize ATP from ADP. This reaction is catalysed by phosphoglycerate kinase.
8. **Shift of the phosphate group from carbon 3 to carbon 2**—The shift of the phosphate group from carbon 3 to carbon 2 of phosphoglycerate-by-phosphoglycerate mutase is freely reversible
9. **Dehydration of 2-phosphoglycerate**— The dehydration of 2-phosphoglycerate by enolase redistributes the energy within the 2-phosphoglycerate molecule, resulting in the formation of phosphoenolpyruvate (PEP), which contains a high energy enol phosphate

10. **Formation of pyruvate producing ATP—**The conversion of PEP to pyruvate is catalysed by pyruvate kinase, the third irreversible reaction of glycolysis. The equilibrium of the pyruvate kinase reaction favours the formation of ATP
11. **Reduction of pyruvate to lactate—**Lactate, formed by the action of lactate dehydrogenase, is the final product of anaerobic glycolysis in eukaryotic cells.
12. **Energy yield from glycolysis—**Despite the production of some ATP during glycolysis, the end products, pyruvate or lactate, still contain most of the energy originally contained in glucose. The TCA cycle is required to release that energy completely
    - Anaerobic glycolysis— Two molecules of ATP are generated for each molecule of glucose converted to two molecules of lactate. There is no net production or consumption of NADH
    - Aerobic glycolysis— The direct consumption and formation of ATP is the same as in anaerobic glycolysis—that is, a net gain of two ATP per molecule of glucose. Two molecules of NADH are also produced per molecule of glucose. Ongoing aerobic glycolysis requires the oxidation of most of this NADH by the electron transport chain, producing approximately three ATP for each NADH molecule entering the chain.

TCA cycle

- The tricarboxylic acid cycle (TCA cycle, also called the Krebs cycle or the citric acid cycle) plays several roles in metabolism. It is the final pathway where the oxidative metabolism of carbohydrates, amino acids, and fatty acids converge, their carbon skeletons being converted to CO_2. This oxidation provides energy for the production of the majority of ATP in most animals, including humans.
- The cycle occurs totally in the mitochondria and is, therefore, in close proximity to the reactions of electron transport which oxidize the reduced coenzymes produced by the cycle.
- The TCA cycle is an aerobic pathway, because O_2 is required as the final electron acceptor. Reactions such as the catabolism of some amino acids generates intermediates of the cycle and are called anaplerotic reactions.



Reaction of TCA cycle.

1. **Oxidative decarboxylation of pyruvate—**Pyruvate, the end product of aerobic glycolysis, must be transported into the mitochondrion before it can enter the TCA cycle. This is accomplished by a specific pyruvate transporter that helps pyruvate cross the inner mitochondrial membrane. Once in the matrix, pyruvate is converted to acetyl CoA by the pyruvate dehydrogenase complex, which is a multienzyme complex. Strictly speaking, the pyruvate dehydrogenase complex is not part of the TCA cycle proper, but is a major source of acetyl CoA—the two-carbon substrate for the cycle.
2. **Synthesis of citrate from acetyl CoA and oxaloacetate—**The condensation of acetyl CoA and oxaloacetate to form citrate (a tricarboxylic acid) is catalysed by citrate synthase. This aldol condensation has an equilibrium far in the direction of citrate synthesis
3. **Isomerization of citrate—** Citrate is isomerized to isocitrate by aconitase, an Fe-S protein.
4. **Oxidation and decarboxylation of isocitrate—**Isocitrate dehydrogenase catalyses the irreversible oxidative decarboxylation of isocitrate yielding

the first of three NADH molecules produced by the cycle, and the first release of CO_2

5. **Oxidative decarboxylation of α-ketoglutarate**—The conversion of α-ketoglutarate to succinyl CoA is catalysed by the α-ketoglutarate dehydrogenase complex, a multimolecular aggregate of three enzymes. The mechanism of this oxidative decarboxylation is very similar to that used for the conversion of pyruvate to acetyl CoA by the PDH complex. The reaction releases the second CO_2 and produces the second NADH of the cycle. The coenzymes required are thiamine pyrophosphate, lipoic acid, FAD, NAD^+, and CoA.
6. **Cleavage of succinyl CoA**— Succinate thiokinase (also called succinyl CoA synthetase—named for the reverse reaction) cleaves the high-energy thioester bond of succinyl. This reaction is coupled to phosphorylation of guanosine diphosphate (GDP) to guanosine triphosphate (GTP). GTP and ATP are energetically interconvertible by the nucleoside diphosphate kinase reaction
7. **Oxidation of succinate**—Succinate is oxidized to fumarate by succinate dehydrogenase, as FAD (its coenzyme) is reduced to $FADH_2$. Succinate dehydrogenase is the only enzyme of the TCA cycle that is embedded in the inner mitochondrial membrane.
8. **Hydration of fumarate**—Fumarate is hydrated to malate in a freely reversible reaction catalysed by fumarase (also called fumarate hydratase).
9. **Oxidation of malate**—Malate is oxidized to oxaloacetate by malate dehydrogenase. This reaction produces the third and final NADH of the cycle.

Glycogen metabolism.

- Glycogen metabolism refers to the process by which glycogen is synthesized, stored, and broken down in the body. Glycogen is a complex carbohydrate made up of glucose molecules that is primarily stored in the liver and muscle tissue.
- The synthesis of glycogen, also known as glycogenesis, occurs when glucose molecules are joined together through a process called glycosylation. This process is catalysed by the enzyme glycogen synthase and requires the presence of a primer molecule called glycogenin.

- Glycogen is broken down through a process called glycogenolysis, which occurs when the body needs energy. The breakdown of glycogen is catalysed by the enzyme glycogen phosphorylase, which cleaves off individual glucose molecules from the glycogen chain. These glucose molecules are then converted into glucose-6-phosphate and can be further metabolized to produce energy.
- Control of glycogen metabolism—The regulation of glycogen metabolism is tightly controlled by hormones such as insulin and glucagon. Insulin promotes the synthesis of glycogen, while glucagon stimulates the breakdown of glycogen to release glucose into the bloodstream. The regulation of glycogen metabolism is critical for maintaining blood glucose levels and providing energy to the body.

Regulation of blood glucose level

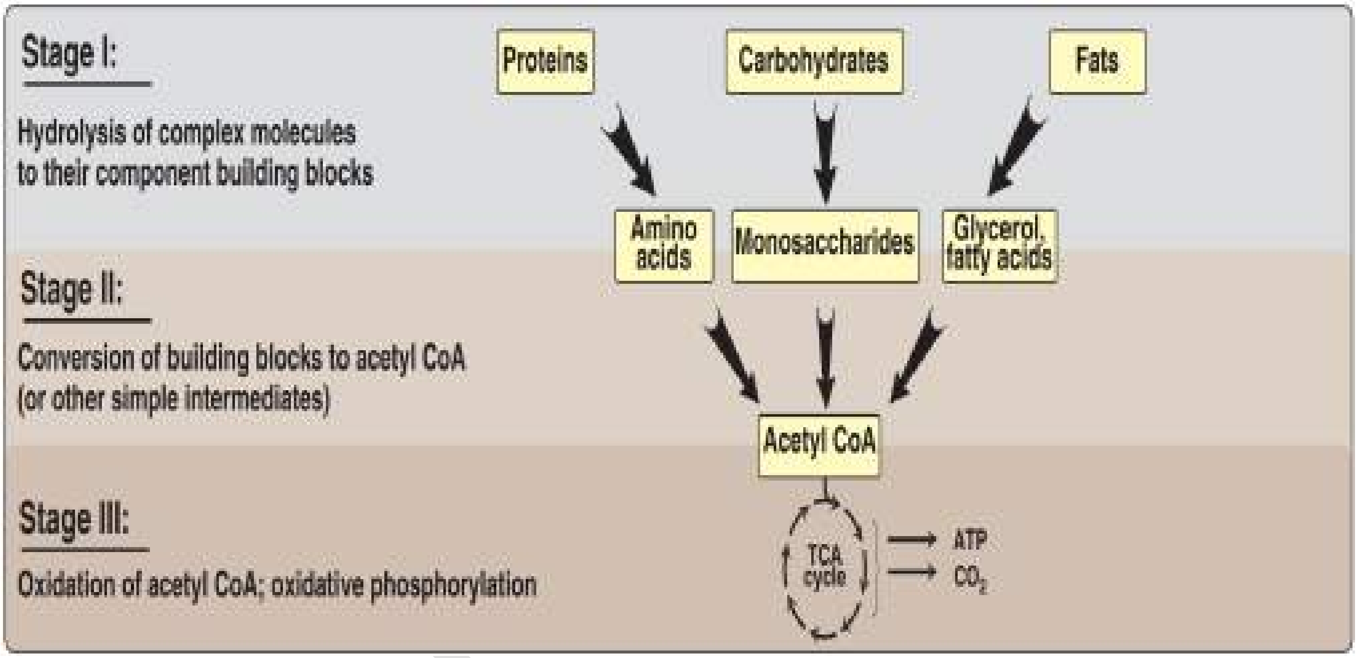
The regulation of blood glucose level is a complex process involving various hormones and physiological mechanisms. The main hormones involved in this process are insulin, glucagon, and epinephrine.

- **Insulin—**Insulin is a hormone produced by the pancreas that helps regulate glucose levels in the blood. It stimulates the uptake of glucose from the blood into cells, where it can be used for energy or stored as glycogen. Insulin also promotes the conversion of excess glucose into fat for storage.
- **Glucagon**—Glucagon, another hormone produced by the pancreas, has the opposite effect of insulin. It stimulates the liver to break down glycogen into glucose and release it into the bloodstream, thereby increasing blood glucose levels.
- **Epinephrine—**Epinephrine, also known as adrenaline, is a hormone produced by the adrenal glands in response to stress or exercise. It increases blood glucose levels by stimulating the liver to release glucose and by reducing the uptake of glucose by muscles.

Diseases related to abnormal metabolism of Carbohydrates.

- Glucose metabolism is defective in two very common metabolic diseases, obesity and diabetes, which contribute in development of a number of major medical problems, including
- Atherosclerosis,
- Hypertension,
- Small vessel disease,
- Kidney disease, and blindness etc.

NOTE—Three stages of catabolism of substrate.



Chapter-8

Metabolism (Study of cycle/pathways without chemical structures)

(BIOCHEMISTRY & CLINICAL PATHOLOGY)

Metabolism (Study of cycle/pathways without chemical structures)

Unit-1

- **Metabolism of Carbohydrates:** Glycolysis, TCA cycle and glycogen metabolism, regulation of blood glucose level. Diseases related to abnormal metabolism of Carbohydrates.

Unit-2

- **Metabolism of lipids: Lipolysis, β-oxidation of Fatty acid (Palmitic acid) ketogenesis and ketolysis. Diseases related to abnormal metabolism of lipids such as Ketoacidosis, Fatty liver, Hypercholesterolemia**

Unit-3

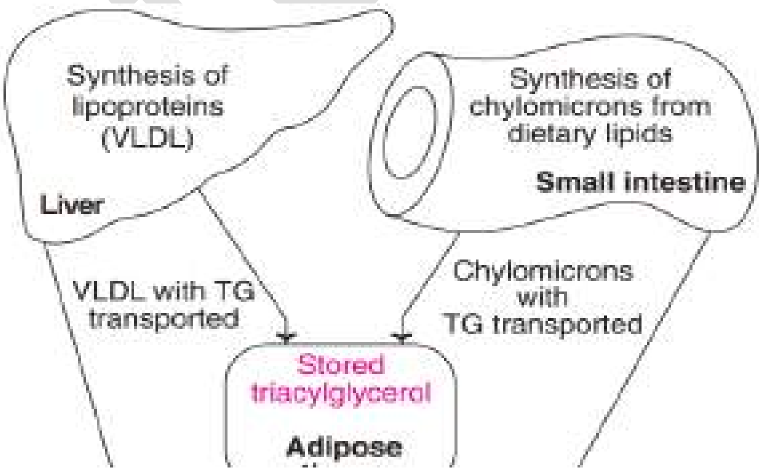
- **Metabolism of Amino acids (Proteins):** General reactions of amino acids and its significance– Transamination, deamination, Urea cycle and decarboxylation. Diseases related to abnormal metabolism of amino acids, Disorders of ammonia metabolism, phenylketonuria, alkaptonuria and Jaundice.
- **Biological oxidation:** Electron transport chain and Oxidative phosphorylation

Unit-2

Metabolism of lipid.

Lipids constitute about 15-20% of the body weight in humans. Triacylglycerols (formerly triglycerides) are the most abundant lipids comprising 85-90% of body lipids. Most of the triacylglycerols are stored in the adipose tissue and serve as energy reserve of the body. Triacylglycerols (TG) are highly concentrated form of energy, yielding 9 Cal/g, in contrast to carbohydrates and proteins that produce only 4 Cal/g. This is because fatty acids found in TG are in the reduced form. Fat also acts as an insulating material for maintaining the body temperature of animals.

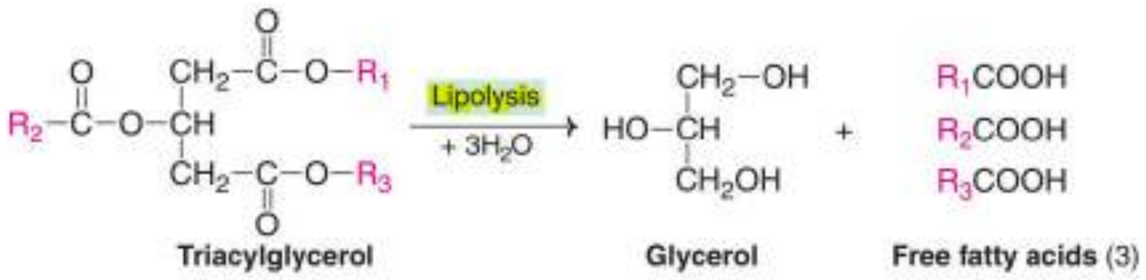
Transport of lipid— The insoluble lipids are solubilized in association with proteins to form lipoproteins in which form lipids are transported in the blood stream. Free lipids are undetectable in blood. Chylomicrons, very low-density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL) and albumin-free fatty acids are the different lipoprotein complexes that transport lipids in the blood stream.



Overview of lipid metabolism

Lipolysis

- Triacylglycerol (TG) is the stored fat in the adipose tissue. The enzyme, namely hormone sensitive triacylglycerol lipase, removes the fatty acid either from carbon 1 or 3 of the triacylglycerol to form diacylglycerol. The other two fatty acids of TG are cleaved by additional lipases specific for diacylglycerol and monoacylglycerol. The complete degradation of triacylglycerol to glycerol and free acids is known as lipolysis.
- Hormone-sensitive TG-lipase is so named because its activity is mostly controlled by hormones. Lipase is present in an inactive form 'b' and is activated (phosphorylated) by a cAMP dependent protein kinase to lipase 'a'. Several hormones—such as epinephrine (most effective), norepinephrine, glucagon, thyroxine, ACTH etc.— enhance the activity of



Complete hydrolysis (lipolysis) of triacylglycerol.

adenylate cyclase and, thus, increase lipolysis.

- **Fate of glycerol—** The adipose tissue lacks the enzyme glycerol kinase, hence glycerol produced in lipolysis cannot be phosphorylated here. It is transported to liver where it is activated to glycerol 3-phosphate. The latter may be used for the synthesis of triacylglycerols and phospholipids. Glycerol 3-phosphate may also enter glycolysis by getting converted to dihydroxyacetone phosphate.
- **Fate of free fatty acids—**The fatty acids released in the adipocytes enter the circulation and are transported in a bound form to albumin. The free fatty acids enter various tissues and are utilized for the energy. About 95% of the energy obtained from fat comes from the oxidation of fatty

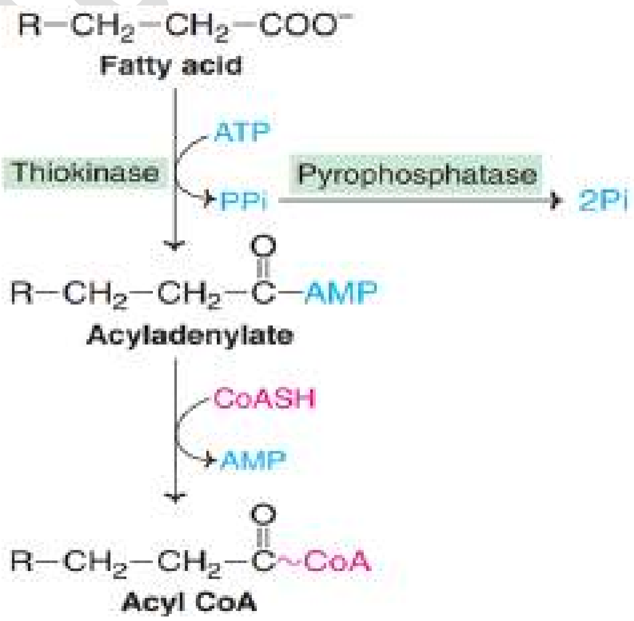
acids. Certain tissues, however, cannot oxidize fatty acids, e.g., brain, erythrocytes.

β-oxidation of Fatty acid (Palmitic acid).

Introduction—This process is known as beta-oxidation, because the oxidation and splitting of two carbon units occur at the beta-carbon atom. The oxidation of the hydrocarbon chain occurs by a sequential cleavage of two carbon atoms. Fatty acids are oxidized by most of the tissues in the body. However, brain, erythrocytes and adrenal medulla cannot utilize fatty acids for energy requirement. The β-oxidation of fatty acids involves three stages.

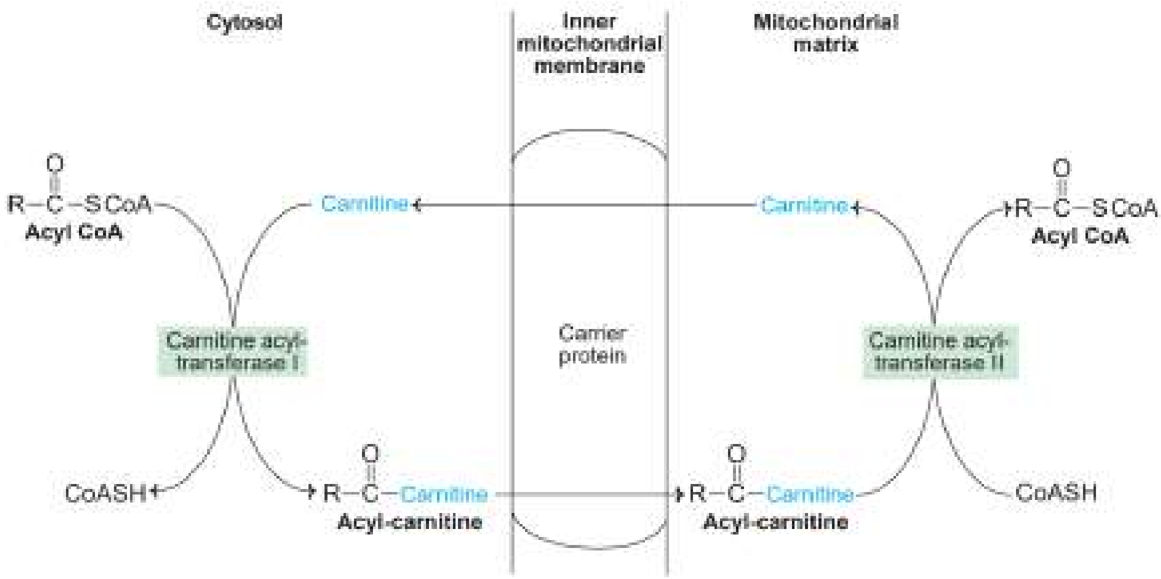
- Activation of fatty acids occurring in the cytosol.
- Transport of fatty acids into mitochondria.
- β-Oxidation proper in the mitochondrial matrix.

1. **Activation of fatty acids—** Fatty acids are activated to acyl CoA by thiokinase or acyl CoA synthetases. The reaction occurs in two steps and requires ATP, coenzyme A and Mg2+. Fatty acid reacts with ATP to form acyladenylate which then combines with coenzyme A to produce acyl CoA. In the activation, two high energy phosphates are utilized, since ATP is converted to pyrophosphate (PPi). The enzyme inorganic pyrophosphatase hydrolyses PPi to phosphate (Pi). The immediate elimination of PPi makes this reaction totally irreversible.



Activation of fatty acid to acyl CoA by the enzyme thiokinase.

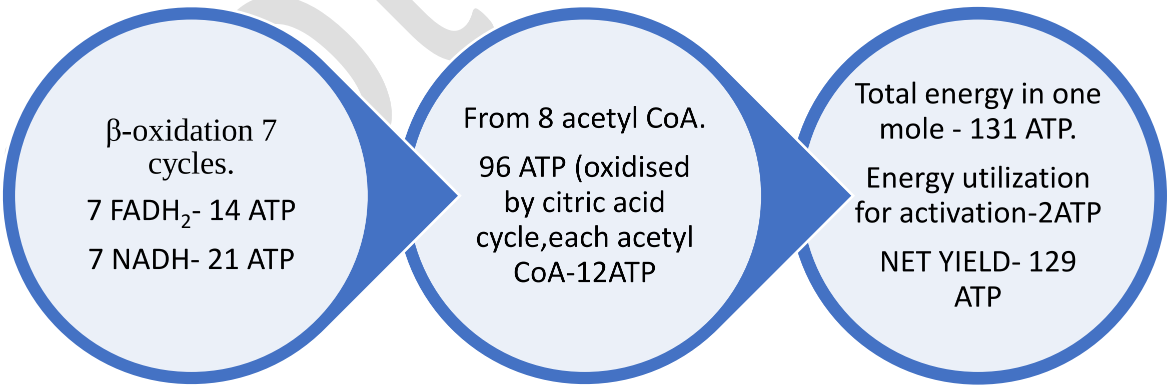
2. **Transport of fatty acids into mitochondria—** The inner mitochondrial membrane is impermeable to fatty acids. A specialized carnitine carrier system (carnitine shuttle) operates to transport activated fatty acids from cytosol to the mitochondria. This occurs in four steps.
   - Acyl group of acyl CoA is transferred to carnitine (β-hydroxy ϒ-trimethyl aminobutyrate), catalysed by carnitine acyltransferase I (present on the outer surface of inner mitochondrial membrane).
   - The acyl-carnitine is transported across the membrane to mitochondrial matrix by a specific carrier protein.
   - Carnitine acyl transferase II (found on the inner surface of inner mitochondrial membrane) converts acyl-carnitine to acyl CoA.
   - The carnitine released returns to cytosol for reuse



Carnitine shuttle for transport of activated fatty acid (acyl CoA) into mitochondria

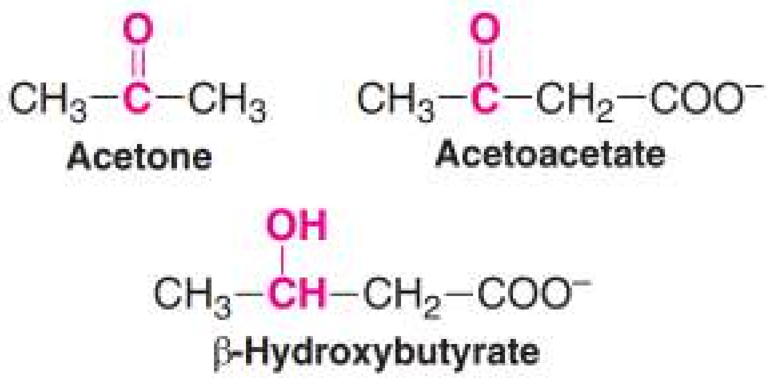
3. **β-Oxidation proper in the mitochondrial matrix—** Each cycle of β-oxidation, liberating a two-carbon unit-acetyl CoA, occurs in a sequence of four reactions.
    1. **Oxidation—**Acyl CoA undergoes dehydrogenation by an FAD-dependent flavoenzyme, acyl CoA dehydrogenase. A double bond is formed between α and β carbons (i.e., 2 and 3 carbons).
    2. **Hydration—** Enoyl CoA hydratase brings about the hydration of the double bond to form β-hydroxyacyl CoA.
    3. **Oxidation—** β-Hydroxyacyl CoA dehydrogenase catalyses the second oxidation and generates NADH. The product formed is β-ketoacyl CoA.
    4. **Cleavage—** The final reaction in β-oxidation is the liberation of a 2-carbon fragment, acetyl CoA from acyl CoA. This occurs by a thiolytic cleavage catalysed by β-ketoacyl CoA thiolase.

Energy of palmitic acid oxidation—



Ketogenesis

- The synthesis of ketone bodies occurs in the liver. The enzymes for ketone body synthesis are located in the mitochondrial matrix. Ketone bodies are water-soluble and energy yielding. Acetyl CoA, formed by oxidation of fatty acids, pyruvate or some amino acids, is the precursor for ketone bodies.
- The three main types of ketone bodies produced are acetone, acetoacetate, and beta-hydroxybutyrate. Ketone bodies can be used by the brain and other tissues as an alternative energy source when glucose is scarce, and they are also involved in regulating blood glucose levels and reducing inflammation.
- However, excessive production of ketone bodies can lead to a condition known as ketoacidosis, which is a potentially life-threatening metabolic state characterized by high levels of ketone



bodies in the blood.

Ketogenesis occurs through the following reactions.

1. Two moles of acetyl CoA condense to form acetoacetyl CoA. This reaction is catalysed by thiolase, an enzyme involved in the final step of E-oxidation. Hence, acetoacetate synthesis is appropriately regarded as the reversal of thiolase reaction of fatty acid oxidation.
2. Acetoacetyl CoA combines with another molecule of acetyl CoA to produce β-hydroxy β-methyl glutaryl CoA (HMG CoA). HMG CoA

synthase, catalysing this reaction, regulates the synthesis of ketone bodies.

3. HMG CoA lyase cleaves HMG CoA to produce acetoacetate and acetyl CoA.
4. Acetoacetate can undergo spontaneous decarboxylation to form acetone.
5. Acetoacetate can be reduced by a dehydrogenase to β-hydroxybutyrate.

Regulation of ketogenesis—

The ketone body formation (particularly overproduction) occurs primarily due to nonavailability of carbohydrates to the tissues. This is an outcome of excessive utilization of fatty acids to meet the energy requirements of the cells. The hormone glucagon stimulates ketogenesis whereas insulin inhibits. The increased ratio of glucagon/insulin in diabetes mellitus promotes ketone body formation.

Ketolysis

- Ketolysis is the metabolic process by which ketone bodies are broken down and converted into energy in the body's cells. This process occurs primarily in the mitochondria of cells, where the ketone bodies are broken down into acetyl-CoA, which can then enter the citric acid cycle to produce ATP, the energy currency of cells.
- This process is important for individuals who rely on ketone bodies as their primary source of energy, such as those on a ketogenic diet or during periods of prolonged fasting.
- The rate of Ketolysis is influenced by several factors, including the availability of ketone bodies and the metabolic state of the cells.
- In some metabolic disorders, such as diabetes, there can be a disruption in the balance between ketone production and utilization, leading to an accumulation of ketone bodies in the blood and potentially causing ketoacidosis.

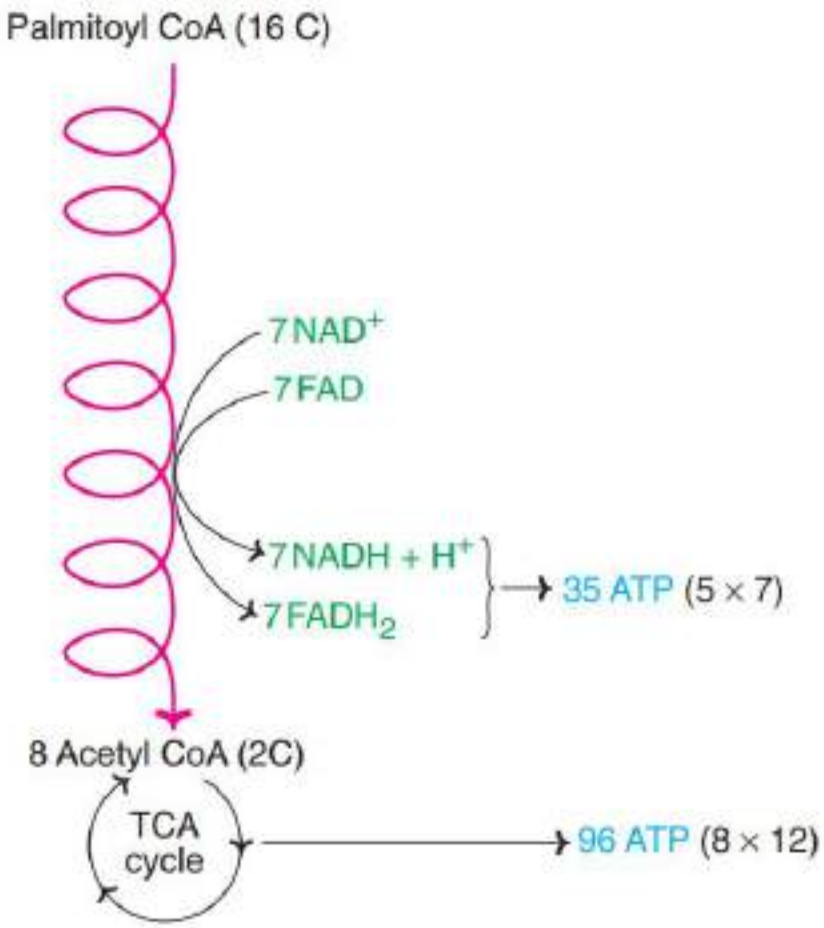
Diseases related to abnormal metabolism of lipids.

1. **Ketoacidosis—** Increase in concentration of both acetoacetate and β-hydroxybutyrate (strong acids) in blood would cause acidosis. The carboxyl group has a pKa around 4. Therefore, the ketone bodies in the blood dissociate and release H+ ions which lower the PH. Diabetic ketoacidosis is dangerous—may result in coma, and even death, if not treated. Ketosis due to starvation is not usually accompanied by ketoacidosis
2. **Hypercholesterolemia**—Increase in plasma cholesterol (> 200 mg/dl) concentration is known as hypercholesterolemia and is observed in many disorders
    - Diabetes mellitus— Due to increased cholesterol synthesis since the availability of acetyl CoA is increased.
    - Hypothyroidism (myxoedema)— This is believed to be due to decrease in the HDL receptors on hepatocytes.
    - Obstructive jaundice— Due to an obstruction in the excretion of cholesterol through bile.
    - Nephrotic syndrome— Increase in plasma globulin concentration is the characteristic feature of nephrotic syndrome. Cholesterol elevation is due to increase in plasma lipoprotein fractions in this disorder.
3. **Fatty liver—** The normal concentration of lipid in liver is around 5%. Liver is not a storage organ for fat, unlike adipose tissue. However, in certain conditions, lipids— especially the triacylglycerols—accumulate excessively in liver, resulting in fatty liver.

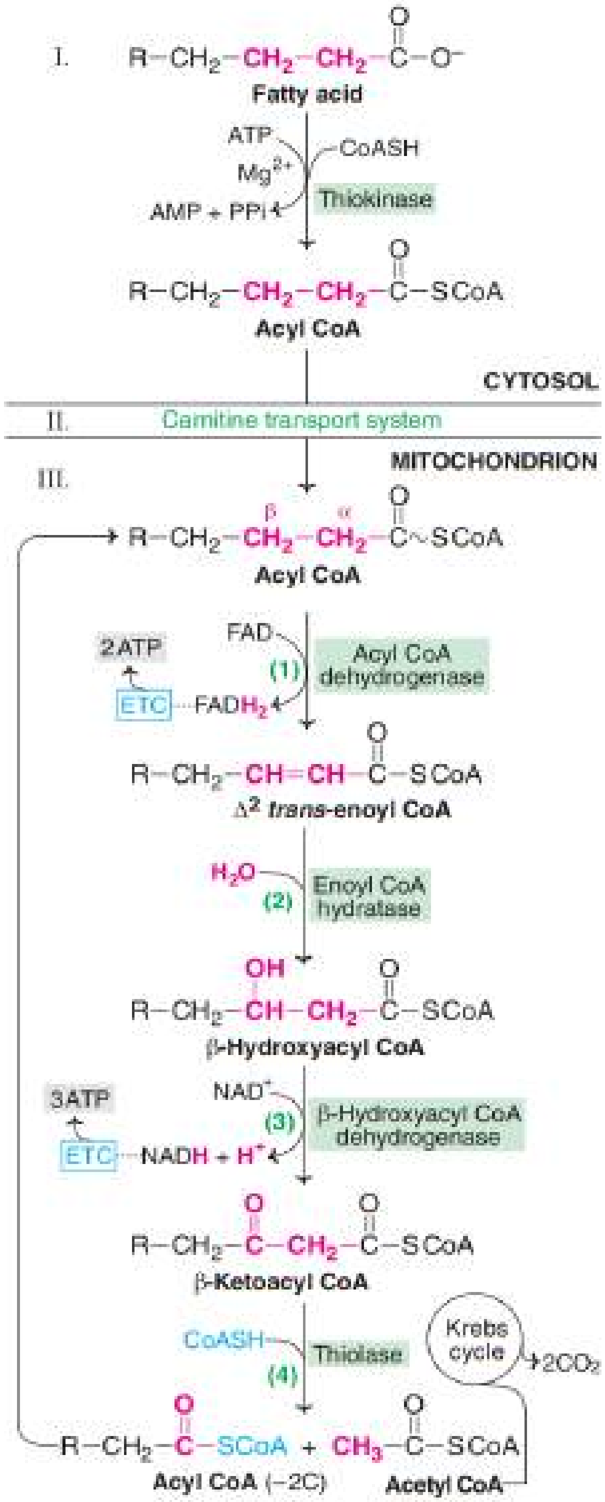
    In the normal liver, Kupffer cells contain lipids in the form of droplets. In fatty liver, droplets of triacylglycerols are found in the entire cytoplasm of hepatic cells. This causes impairment in metabolic functions of liver. Fatty liver is associated with fibrotic changes and cirrhosis, Fatty liver may occur due to two main causes

    - Increased synthesis of triacylglycerols
    - Impairment in lipoprotein synthesis.

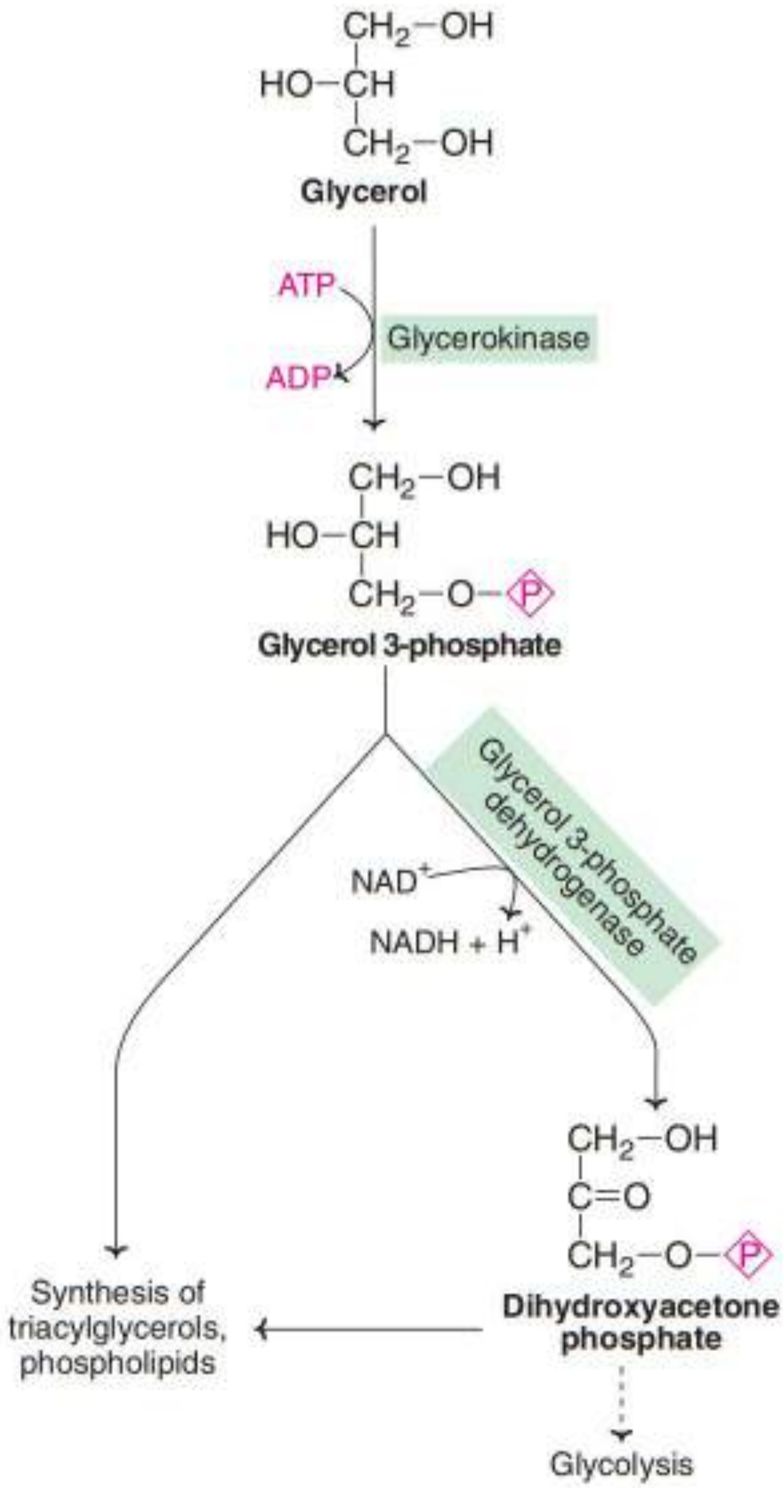
NOTE:



oxidation of palmitic acid.



β-Oxidation of fatty acids



Fate of glycerol

Chapter-8

Metabolism (Study of cycle/pathways without chemical structures)

(BIOCHEMISTRY & CLINICAL PATHOLOGY)

Metabolism (Study of cycle/pathways without chemical structures)

Unit-1

- **Metabolism of Carbohydrates:** Glycolysis, TCA cycle and glycogen metabolism, regulation of blood glucose level. Diseases related to abnormal metabolism of Carbohydrates.

Unit-2

- **Metabolism of lipids:** Lipolysis, β-oxidation of Fatty acid (Palmitic acid) ketogenesis and ketolysis. Diseases related to abnormal metabolism of lipids such as Ketoacidosis, Fatty liver, Hypercholesterolemia

Unit-3

- **Metabolism of Amino acids (Proteins): General reactions of amino acids and its significance– Transamination, deamination, Urea cycle and decarboxylation. Diseases related to abnormal metabolism of amino acids, Disorders of ammonia metabolism, phenylketonuria, alkaptonuria and Jaundice.**
- **Biological oxidation: Electron transport chain and Oxidative phosphorylation**

Unit-3

Metabolism of amino acids.

Proteins are the most abundant organic compounds and constitute a major part of the body dry weight (10-12 kg in adults). They perform a wide variety of static (structural) and dynamic (enzymes, hormones, clotting factors, receptors etc.) functions. About half of the body protein (predominantly collagen) is present in the supportive tissue (skeleton and connective) while the other half is intracellular.

Amino acid—Proteins are nitrogen-containing macromolecules consisting of L-D-amino acids as the repeating units. Of the 20 amino acids found in proteins, half can be synthesized by the body (non-essential) while the rest have to be provided in the diet (essential amino acids).

The proteins on degradation (proteolysis) release individual amino acids. Amino acids are not just the structural components of proteins. Each one of the 20 naturally occurring amino acids undergoes its own metabolism and performs specific functions. Some of the amino acids also serve as precursors for the synthesis of many biologically important compounds (e.g., melanin, serotonin, creatine etc.). Certain amino acids may directly act as neurotransmitters (e.g., glycine aspartate, glutamate).

General reactions of amino acids and its significance.

Amino acid pool—An adult has about 100 g of free amino acids which represent the amino acid pool of the body. Glutamate and glutamine together constitute about 50%, and essential amino acids about 10% of the body pool (100 g). The concentration of intracellular amino acids is always higher than the extracellular amino acids. The amino acid pool of the body is maintained by the sources that contribute (input) and the metabolic pathways that utilize (output) the amino acids.

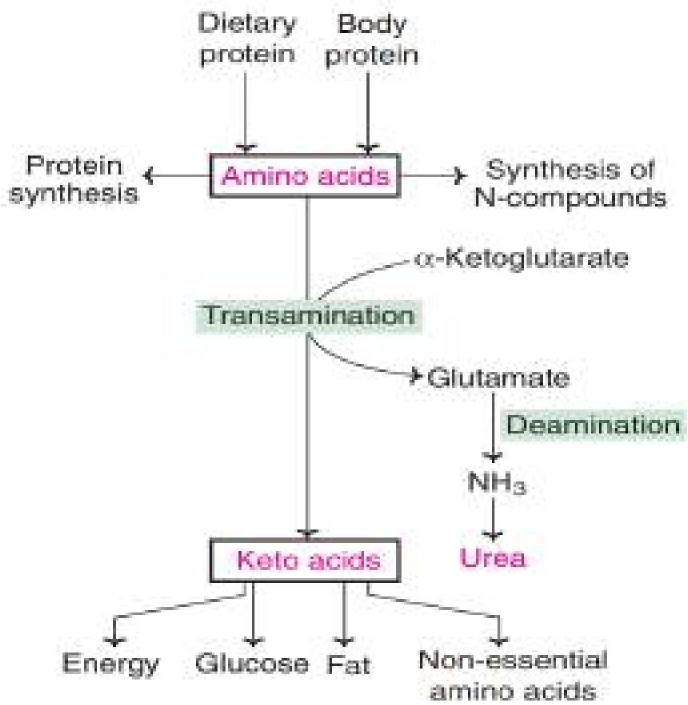
Sources of amino acid pool— Three major sources of amino acid pool.

- Protein turnover—The protein present in the body is in a dynamic state. It is estimated that about 300-400 g of protein per day is constantly degraded and synthesized which represents body protein turnover.

- Dietary protein— There is a regular loss of nitrogen from the body due to degradation of amino acids. In healthy adults, it is estimated that about 30-50 g of protein is lost everyday from the body. This amount of protein must, therefore, be supplied daily in the diet to maintain nitrogen balance. The purpose of dietary protein is to supply amino acids for the synthesis of proteins and other nitrogen compounds.
- Synthesis of non-essential amino acids—Ten out of the 20 naturally occurring amino acids can be synthesized by the body which contribute to the amino acid pool.

General metabolism and significance.

- Most of the body proteins (300-400 g/day) degraded are synthesized from the amino acid pool. These include enzymes, hormones, immunoproteins, contractile proteins etc.
- Many important nitrogenous compounds (porphyrins, purines, pyrimidines, etc.) are produced from the amino acids. About 30 g of protein is daily utilized for this purpose.
- Generally, about 10-15% of body energy requirements are met from the amino acids.
- The amino acids are converted to carbohydrates and fats. This becomes predominant when the protein consumption is in excess of the body



General reaction/metabolism.

requirements.

Transamination.

The transfer of an amino (NH2) group from an amino acid to a keto acid is known as transamination. This process involves the interconversion of a pair of amino acids and a pair of keto acids, catalysed by a group of enzymes called transaminases /aminotransferases.

Silent features of Transamination—

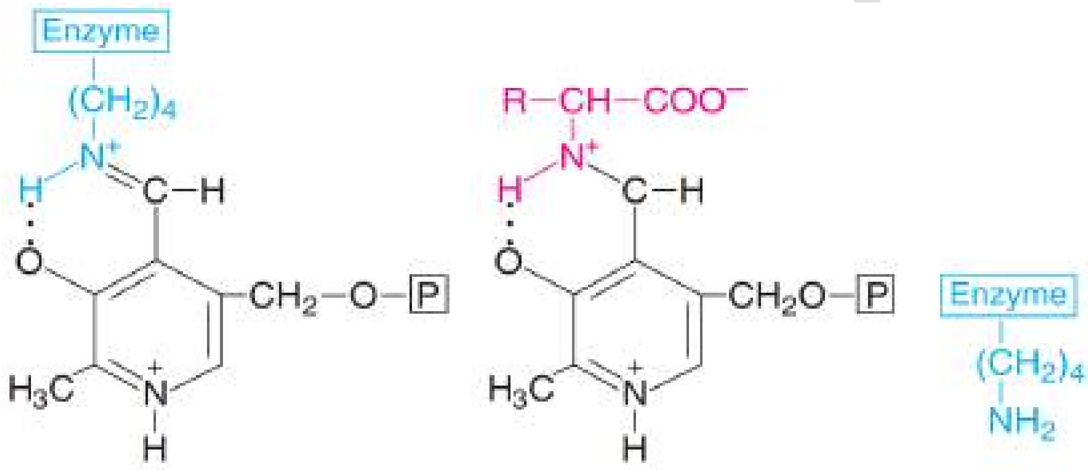
- All transaminases require pyridoxal phosphate (PLP), a coenzyme derived from vitamin B6. It is **reversible** and no free NH3 liberated, only the transfer of amino group occurs. Transamination diverts the excess amino acids towards energy generation.
- Specific transaminases exist for each pair of amino and keto acids. However, only two— namely, aspartate transaminase and alanine transaminase—make a significant contribution for transamination.
- Transamination is very important for the redistribution of amino groups and production of non-essential amino acids, as per the requirement of the cell. It involves both catabolism and anabolism of amino acids.
- The amino acids undergo transamination to finally concentrate nitrogen in glutamate. Glutamate is the only amino acid that undergoes oxidative deamination to a significant extent to liberate free NH3 for urea synthesis.
- All amino acids except lysine, threonine, proline and hydroxyproline participate in transamination. Serum transaminases are important for diagnostic and prognostic purposes.

Mechanism of transamination— it occurs in two stages.

1. Transfer of the amino group to the coenzyme pyridoxal phosphate (bound to the coenzyme) to form pyridoxamine phosphate.
2. The amino group of pyridoxamine phosphate is then transferred to a keto acid to produce a new amino acid and the enzyme with PLP is regenerated.

- All the transaminases require pyridoxal phosphate (PLP), a derivative of vitamin B6. The aldehyde group of PLP is linked with H-amino group of lysine residue, at the active site of the enzyme forming a Schiff base (imine linkage).

- When an amino acid (substrate) comes in contact with the enzyme, it displaces lysine and a new Schiff base linkage is formed. The amino acid-PLP-Schiff base tightly binds with the enzyme by noncovalent forces. **Snell and Braustein** proposed a Ping Pong Bi Bi mechanism involving a series of intermediates (aldimines and ketimines) in transamination reaction.



Enzyme-PLP Schiff base

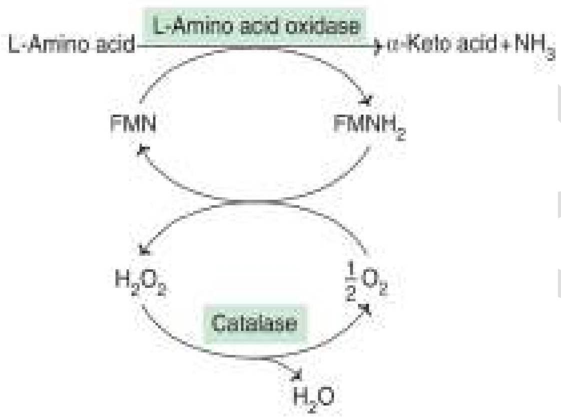
Amino acid PLP-Schiff base

Deamination.

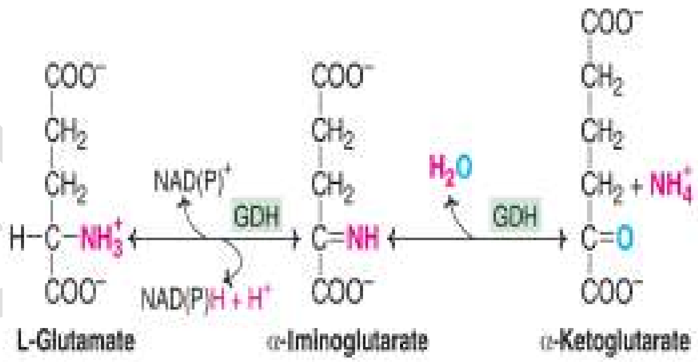
The removal of amino group from the amino acids as NH3 is deamination. Deamination results in the liberation of ammonia for urea synthesis (transamination involves only the shuffling of amino groups). It may be either oxidative or non-oxidative.

1. **Oxidative deamination—** Oxidative deamination is the liberation of free ammonia from the amino group of amino acids coupled with oxidation. This takes place mostly in liver and kidney. The purpose of oxidative deamination is to provide NH3 for urea synthesis and D-keto acids for a variety of reactions, including energy generation.

- **Oxidation of glutamate-by-glutamate dehydrogenase—**In the transamination process glutamate is form. Now, Glutamate rapidly undergoes oxidative deamination, catalysed by glutamate dehydrogenase (GDH) to liberate ammonia. This enzyme is unique in that it can utilize either NAD^+ or $NADP^+$ as a coenzyme. Conversion of glutamate to α-ketoglutarate occurs through the formation of an intermediate, α-iminoglutarate.
- **Oxidative deamination by amino acid oxidases—**L-Amino acid oxidase and D-amino acid oxidase are flavoproteins, possessing FMN and FAD, respectively. They act on the corresponding amino acids (L or D) to produce D-keto acids and NH3. In this reaction, oxygen is



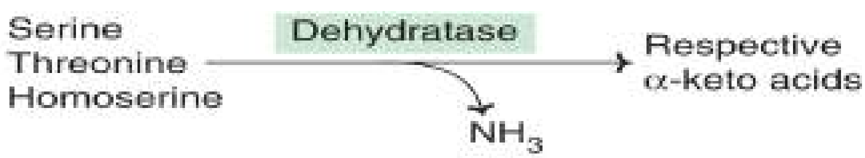
Oxidative deamination of amino acids.



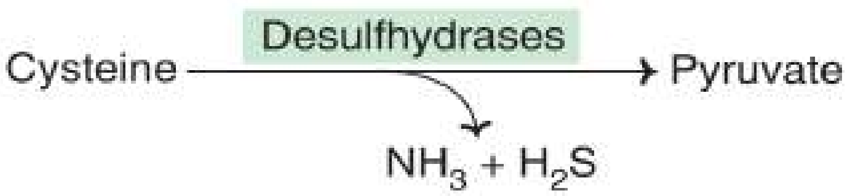
Oxidation of glutamate-by-glutamate dehydrogenase (GDH)

reduced to H_2O_2, which is later decomposed by catalase.

2. **Non-oxidative deamination—** Some of the amino acids can be deaminated to liberate NH3 without undergoing oxidation.
   a. **Amino acid dehydrases—** Serine, threonine and homoserine are the hydroxy amino acids. They undergo non-oxidative deamination catalysed by PLP-dependent dehydrases (dehydratases).

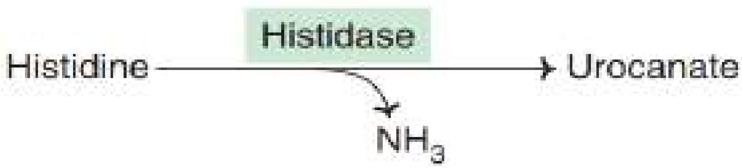


b. **Amino acid desulfhydrases—** The sulphur amino acids, namely cysteine and homocysteine, undergo deamination coupled with



desulfhydration to give keto acids.

c. **Deamination of histidine—**The enzyme histidase acts on histidine to liberate NH3 by a non-oxidative deamination process.



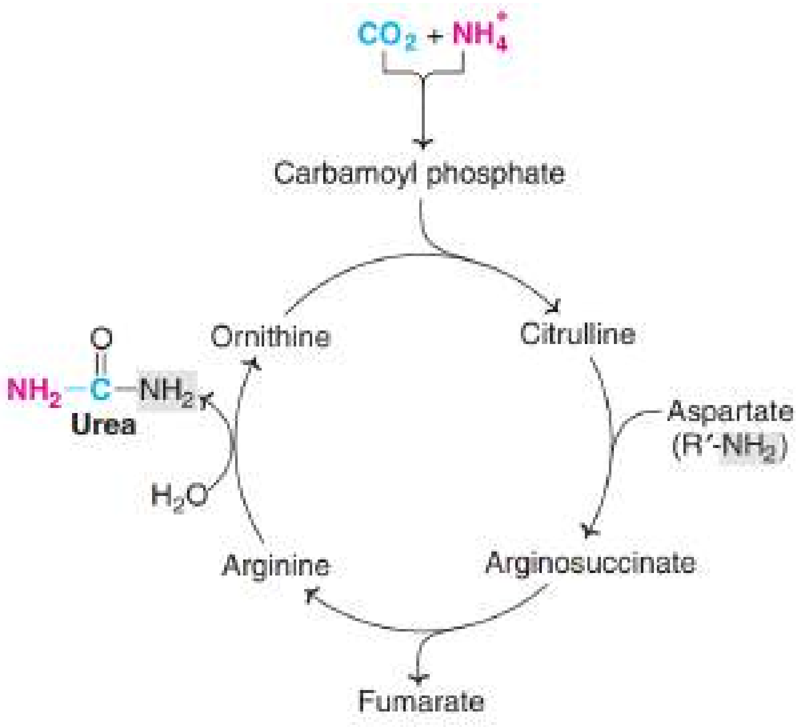
Urea cycle.

- Ammonia is constantly being liberated in the metabolism of amino acids (mostly) and other nitrogenous compounds. At the physiological pH, ammonia exists as ammonium (NH_4+) ion. Ammonium ions are very important to maintain acid-base balance of the body.
- The production of NH3 occurs from the amino acids (transamination and deamination), biogenic amines, amino group of purines and pyrimidines and by the action of intestinal bacteria (urease) on urea.

Introduction about urea cycle—

- Urea is the end product of protein metabolism (amino acid metabolism). The nitrogen of amino acids, converted to ammonia, is toxic to the body. It is converted to urea and detoxified. As such, urea accounts for 80-90% of the nitrogen containing substances excreted in urine.
- Urea is synthesized in liver and transported to kidneys for excretion in urine. Urea cycle is the first metabolic cycle that was elucidated by Hans Krebs and Kurt Henseleit (1932), hence it is known as Krebs-Henseleit

cycle. The individual reactions, however, were described in more detail later on by Ratner and Cohen. Urea has two amino (NH_2) groups, one derived from NH_3 and the other from aspartate. Carbon atom is supplied by CO_2. Urea synthesis is a five-step cyclic process, with five distinct enzymes. The first two enzymes are present in mitochondria while the rest are localized in cytosol.



Outline of urea cycle

Steps of urea cycle—

1. Synthesis of carbamoyl phosphate— Carbamoyl phosphate synthase I (CPS I) of mitochondria catalyses the condensation of $NH4^+$ ions with CO_2 to form carbamoyl phosphate. This step consumes two ATP and is irreversible, and rate-limiting.
2. Formation of citrulline— Citrulline is synthesized from carbamoyl phosphate and ornithine by ornithine transcarbamoylase. Ornithine is regenerated and used in urea cycle. Ornithine and citrulline are basic amino acids. Citrulline produced in this reaction is transported to cytosol by a transporter system.
3. Synthesis of arginosuccinate— Arginosuccinate synthase condenses citrulline with aspartate to produce arginosuccinate. The second amino

group of urea is incorporated in this reaction. This step requires ATP which is cleaved to AMP and pyrophosphate (PPi). The latter is immediately broken down to inorganic phosphate (Pi).

4. Cleavage of arginosuccinate— Arginosuccinase cleaves arginosuccinate to give arginine and fumarate. Arginine is the immediate precursor for urea. Fumarate liberated here provides a connecting link with TCA cycle, gluconeogenesis etc.
5. Formation of urea— Arginase is the fifth and final enzyme that cleaves arginine to yield urea and ornithine. Ornithine, so regenerated, enters mitochondria for its reuse in the urea cycle. Arginase is activated by Co2+ and Mn2+. Ornithine and lysine compete with arginine (competitive inhibition). Arginase is mostly found in the liver, while the rest of the enzymes (four) of urea cycle are also present in other tissues.

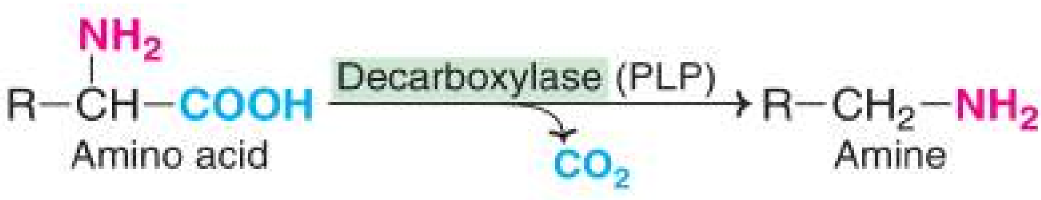
Regulation of urea cycle.

- Regulation of the urea cycle is depending on concentration of ammonium ions in the liver. When the concentration of ammonium ions is high, the urea cycle is activated to convert ammonia to urea and vice-versa.
- Another important regulator of the urea cycle is the availability of substrates. The urea cycle requires several substrates, including ammonia, bicarbonate, and ornithine.
- The activity of the urea cycle is also regulated by hormones, including insulin, glucagon, and cortisol. Insulin and glucagon, for example, can modulate the expression of urea cycle enzymes, while cortisol can increase the activity of the enzyme that converts arginine to ornithine.

Decarboxylation.

- Decarboxylation of an amino acid is a chemical reaction in which the carboxyl group (-COOH) of an amino acid is removed, leading to the formation of an amine group (-NH_2) and the release of carbon dioxide (CO2). This reaction is catalysed by enzymes called decarboxylases.
- In general, the decarboxylation of amino acids or their derivatives results in the formation of amines.
- The decarboxylation of amino acids plays an important role in many biological processes. For example, the amino acid histidine is

decarboxylated to form histamine, which is involved in regulating many physiological processes, including digestion and immune response. Similarly, the amino acid glutamic acid is decarboxylated to form the neurotransmitter gamma-aminobutyric acid (GABA), which is involved in the regulation of neuronal activity.



Disorders of ammonia metabolism.

- **Hyperammonemia—**Elevation in blood NH_3 level may be genetic or acquired. Impairment in urea synthesis due to a defect in any one of the five enzymes is described in urea synthesis. All these disorders lead to hyperammonemia and cause hepatic coma and mental retardation. The acquired hyperammonemia may be due to hepatitis, alcoholism etc. where the urea synthesis becomes defective, hence NH3 accumulates.

Diseases related to abnormal metabolism of amino acids.

Amino aciduria— The term amino aciduria is generally used to indicate the urinary excretion of amino acids. It is frequently associated with defects in amino acid metabolism. Most of the amino acidurias manifest in mental retardation

Phenylketonuria

- Phenylketonuria (PKU) is the most common metabolic disorder in amino acid metabolism. It is due to the deficiency of the hepatic enzyme, phenylalanine hydroxylase, caused by an autosomal recessive gene.
- Phenylketonuria primarily causes the accumulation of phenylalanine in tissues and blood, and results in its increased excretion in urine. Due to disturbances in the routine metabolism, phenylalanine is diverted to alternate pathways resulting in the excessive production of **phenylpyruvate, phenylacetate, phenyllactate and phenylglutamine.**

- All these metabolites are excreted in urine in high concentration in PKU. Phenylacetate gives the urine a mousey odour.
- Clinical condition considered as- mental retardation, hypopigmentation.

Alkaptonuria (Black urine disease).

- Alkaptonuria is a rare genetic disorder characterized by the inability of the body to break down certain amino acids, specifically phenylalanine and tyrosine, which leads to the accumulation of a pigment called homogentisic acid in the connective tissues, cartilage, and bones.
- The defective enzyme in alkaptonuria is homogentisate oxidase in tyrosine metabolism. Homogentisate accumulates in tissues and blood, and is excreted into urine. Homogentisate, on standing, gets oxidized to the corresponding quinones, which polymerize to give black or brown colour. For this reason, the urine of alkaptonuric patients resembles coke in colour.
- Homogentisate gets oxidized by polyphenol oxidase to benzoquinone acetate which undergoes polymerization to produce a pigment called alkapton. Alkapton deposition occurs in connective tissue, bones and various organs (nose, ear etc.) resulting in a condition known as **ochronosis.**
- **Symptoms** of alkaptonuria typically appear in early childhood and include dark urine that turns brown upon standing, as well as joint pain and stiffness, especially in the spine and large joints such as the hips and knees.

Jaundice.

- Amino acid metabolism disorders can lead to jaundice if there is a disruption in the pathway that converts bilirubin into a form that can be eliminated from the body. For example, a genetic disorder called **Crigler-Najjar syndrome** can lead to jaundice because it impairs the ability of the liver to convert bilirubin into a form that can be excreted in the bile. This can cause bilirubin to build up in the blood, leading to jaundice.
- Similarly, other genetic disorders such as **Gilbert's syndrome** can cause jaundice due to problems with bilirubin metabolism. In Gilbert's syndrome, there is a deficiency of an enzyme called UDP-glucuronosyltransferase, which is responsible for conjugating bilirubin so

that it can be eliminated from the body. Without this enzyme, bilirubin builds up in the blood and can lead to jaundice.

Biological oxidation: Electron transport chain and Oxidative phosphorylation

Biological oxidation

Prior to the understanding the biological oxidation we need to know that some important concept and terminologies.

Bioenergetics—Bioenergetics or biochemical thermodynamics deals with the study of energy changes (transfer and utilization) in biochemical reactions. The reactions are broadly classified as exergonic (energy releasing) and endergonic (energy consuming). Bioenergetics is concerned with the initial and final states of energy component of the reactants and not the mechanism of chemical reactions.

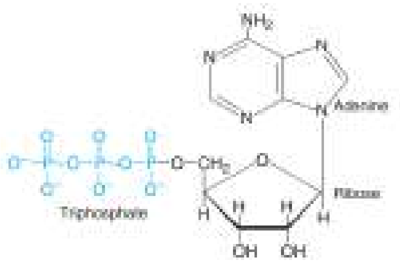
Free energy.

- The energy actually available to do work (utilizable) is known as free energy. Changes in the free energy (ΔG) are valuable in predicting the feasibility of chemical reactions. If free energy change (ΔG) is represented by a negative sign, there is a loss of free energy. The reaction is said to be exergonic, and proceeds spontaneously. On the other hand, a positive ΔG indicates that energy must be supplied to the reactants. The reaction cannot proceed spontaneously and is endergonic in character.
- Enthalpy (ΔH) is a measure of the change in heat content of the reactants, compared to products
- Entropy (ΔS) represents a change in the randomness or disorder of reactants and products. Entropy attains a maximum as the reaction approaches equilibrium.
- The relation between the changes of free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) are expressed as.

$$\Delta G = \Delta H - T\Delta S.$$

High energy compound—

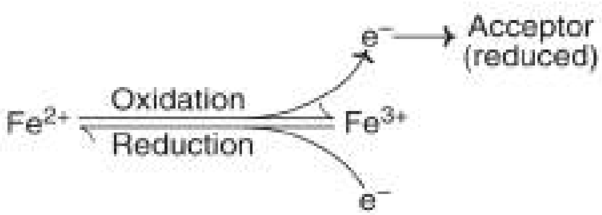
- Pyrophosphates e.g., ATP.
- Acyl phosphates e.g., 1,3-bisphosphoglycerate.
- Enol phosphates e.g., phosphoenolpyruvate.
- Thioesters e.g., acetyl CoA.
- Phosphagens e.g., phosphocreatine.



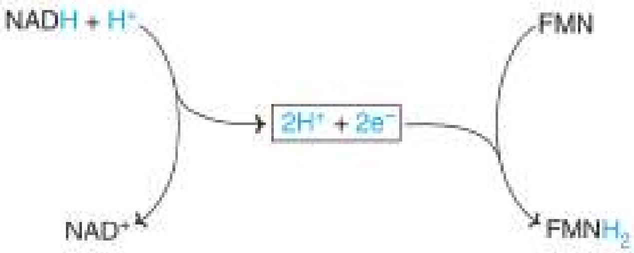
Structure of ATP

Biological oxidation

- Oxidation is defined as the loss of electrons and reduction as the gain of electrons. This may be illustrated by the interconversion of ferrous ion ($Fe2^{+}$) to ferric ion ($Fe3^{+}$).



- The electron lost in the oxidation is accepted by an acceptor which is said to be reduced. Thus, the oxidation-reduction is a tightly coupled process.
- The general principle of oxidation-reduction is applicable to biological systems also. The oxidation of NADH to NAD^{+} coupled with the reduction of FMN to FMNH2 is illustrated.

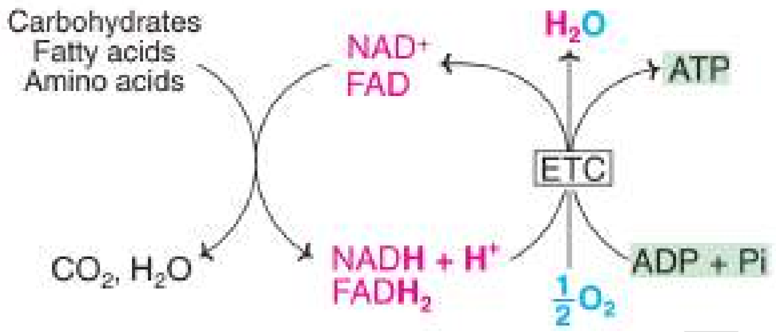


- In the above illustration, there are two redox pairs NADH/NAD+ and FMN/FMNH2. The redox pairs differ in their tendency to lose or gain electrons.

Redox potential.

- The oxidation-reduction potential or, simply, redox potential, is a quantitative measure of the tendency of a redox pair to lose or gain electrons. The redox pairs are assigned specific standard redox potential (E_0 volts) at pH 7.0 and 25°C.
- The more negative redox potential represents a greater tendency (of reductant) to lose electrons. On the other hand, a more positive redox potential indicates a greater tendency (of oxidant) to accept electrons. The electrons flow from a redox pair with more negative E0 to another redox pair with more positive E_0.

- The redox potential (E_0) is directly related to the change in the free energy ($\Delta G°$)



Overview on biological oxidation.

Electron transport chain.

The energy-rich carbohydrates (particularly glucose), fatty acids and amino acids undergo a series of metabolic reactions and, finally, get oxidized to CO_2 and H_2O. The reducing equivalents from various metabolic intermediates are transferred to coenzymes NAD^+ and FAD to produce, respectively, NADH and FADH2. The latter two reduced coenzymes pass through the electron transport chain (ETC) or respiratory chain and, finally, reduce oxygen to water. The passage of electrons through the ETC is associated with the loss of free energy. A part of this free energy is utilized to generate ATP from ADP and Pi

Mitochondrial organisation.

- The mitochondria are the centres for metabolic oxidative reactions to generate reduced coenzymes (NADH and FADH2) which, in turn, are utilized in ETC to liberate energy in the form of ATP. For this reason, mitochondrion is appropriately regarded as the power house of the cell.
- The mitochondrion consists of five distinct parts. These are the **outer membrane, the inner membrane, the intermembrane space, the cristae and the matrix.**

Organisation of respiratory chain.

- The inner mitochondrial membrane can be disrupted into five distinct respiratory or enzyme complexes, denoted as complex **I, II, III, IV and V**.
- The complexes I-IV are carriers of electrons while complex V is responsible for ATP synthesis.
- Besides these enzyme complexes, there are certain mobile electron carriers in the respiratory chain. These include NADH, coenzyme Q, cytochrome C and oxygen.
- The enzyme complexes (I-IV) and the mobile carriers are collectively involved in the transport of electrons which, ultimately, combine with oxygen to produce water. The largest proportion of the oxygen supplied to the body is utilized by the mitochondria for the operation of electron transport chain.

Components and reactions of the electron transport chain.

There are five distinct carriers that participate in the electron transport chain (ETC). These carriers are sequentially arranged and are responsible for the transfer of electrons from a given substrate to ultimately combine with proton and oxygen to form water.

A. **Nicotinamide nucleotides**— NAD^+ and $NADP^+$ derived from the vitamin niacin are the two coenzymes in this, NAD^+ is more actively involved in the ETC. NAD^+ is reduced to $NADH + H^+$ by dehydrogenases with the removal of two hydrogen atoms from the substrate (AH_2). The substrates include glyceraldehyde-3 phosphate, pyruvate, isocitrate, D-ketoglutarate and malate. $AH_2 + NAD^+ \rightleftharpoons A + NADH + H^+$.
B. **Flavoproteins**— The enzyme NADH dehydrogenase (NADH-coenzyme Q reductase) is a flavoprotein with FMN as the prosthetic group. The coenzyme FMN accepts two electrons and a proton to form FMNH2. NADH dehydrogenase is a complex enzyme closely associated with non-heme iron proteins (NHI) or iron-sulphur proteins (FeS).

$$NADH + H^+ + FMN \rightarrow NAD^+ + FMNH2$$

Succinate dehydrogenase (succinate-coenzyme Q reductase) is an enzyme found in the inner mitochondrial membrane. It is also a flavoprotein with FAD as the coenzyme. This can accept two hydrogen atoms ($2H^+ + 2e^-$) from succinate.

$$Succinate + FAD \rightarrow Fumarate + FADH_2.$$

C. **Iron-sulphur proteins**— The iron-sulphur (FeS) proteins exist in the oxidized ($Fe3^+$) or reduced ($Fe2^+$) state. About half a dozen FeS proteins connected with respiratory chain have been identified. However, the mechanism of action of iron-sulphur proteins in the ETC is not clearly understood. One FeS participates in the transfer of electrons from FMN to coenzyme Q. Other FeS proteins associated with cytochrome b and cytochrome c1 participate in the transport of electrons.

D. **Coenzyme Q**— Coenzyme Q is also known as ubiquinone since it is ubiquitous in living system. It is a quinone derivative with a variable isoprenoid side chain. The mammalian tissues possess a quinone with 10 isoprenoid units which is known as coenzyme Q_{10} (CoQ_{10}). Coenzyme Q is a lipophilic electron carrier. It can accept electrons from FMNH2 produced in the ETC by NADH dehydrogenase or FADH2 produced outside ETC (e.g. succinate dehydrogenase, acyl CoA dehydrogenase).

E. **Cytochromes**— The cytochromes are conjugated proteins containing heme group. The latter consists of a porphyrin ring with iron atom. The iron of heme in cytochromes is alternately oxidized ($Fe3^+$) and reduced ($Fe2^+$), which is essential for the transport of electrons in the ETC. Three cytochromes were initially discovered from the mammalian mitochondria. They were designated as cytochrome a, b and c depending on the type of heme present and the respective absorption spectrum. Additional cytochromes such as c1, b1, b2, a3 etc. were discovered later.

Oxidative phosphorylation.

The transport of electrons through the ETC is linked with the release of free energy. The process of synthesizing ATP from ADP and Pi coupled with the electron transport chain is known as oxidative phosphorylation. The complex V of the inner mitochondrial membrane is the site of oxidative phosphorylation.

P : O Ratio

- The P : O ratio refers to the number of inorganic phosphate molecules utilized for ATP generation for every atom of oxygen consumed. More appropriately, P : O ratio represents the number of molecules of ATP synthesized per pair of electrons carried through ETC.

- The mitochondrial oxidation of NADH with a classical P : O ratio of 3. Further, a P : O ratio of 2 has been assigned to the oxidation of FADH2.

Sites of oxidative phosphorylation in ETC.

There are three sites in the ETC that are exergonic to result in the synthesis of 3 ATP molecules.

1. **Oxidation of FMNH2 by coenzyme Q.**
2. **Oxidation of cytochrome b by cytochrome c1.**
3. **Cytochrome oxidase reaction.**

Mechanism of oxidative phosphorylation— Important hypothesis regarding the phosphorylation.

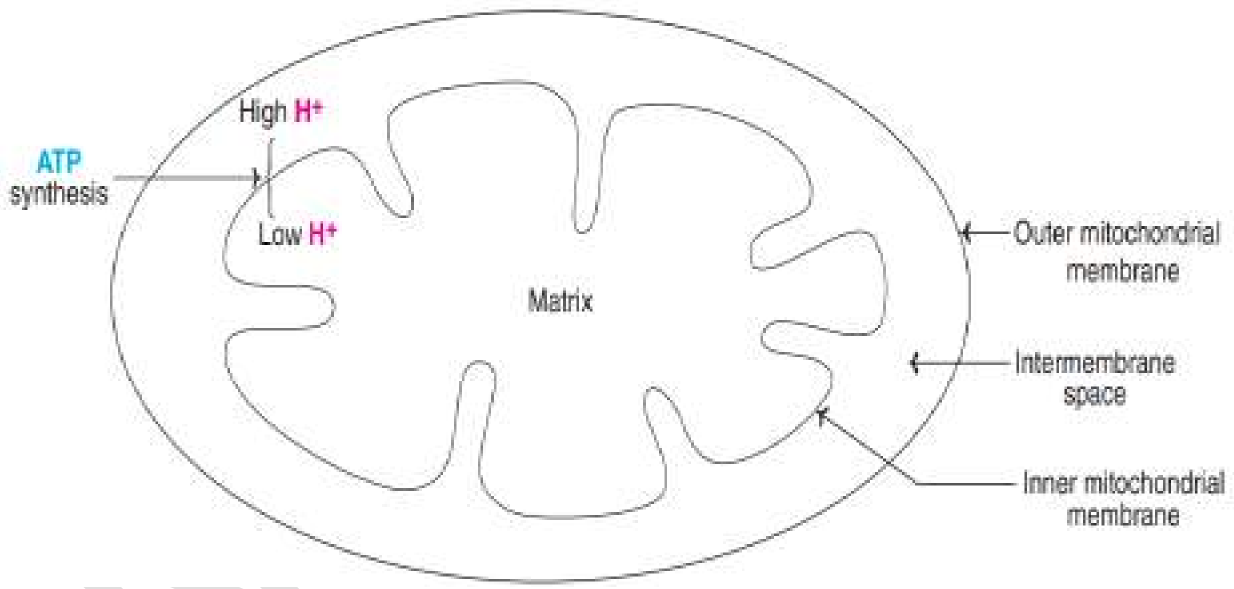
- **Chemical coupling hypothesis**— This hypothesis was put forth by Edward Slater (1953). According to chemical coupling hypothesis, during the course of electron transfer in respiratory chain, a series of phosphorylated high-energy intermediates are first produced which are utilized for the synthesis of ATP. These reactions are believed to be analogous to the substrate level phosphorylation that occurs in glycolysis or citric acid cycle. However, this hypothesis lacks experimental evidence, since all attempts, so far, to isolate any one of the high-energy intermediates have not been successful.

- **Chemiosmotic hypothesis**— This mechanism, originally proposed by Peter Mitchell (1961), is now widely accepted. It explains how the transport of electrons through the respiratory chain is effectively utilized to produce ATP from ADP + Pi. The concept of chemiosmotic hypothesis based on the positive and negative charges gradient **(Proton gradient) and enzymatic induction.**

Proton gradient— The inner mitochondrial membrane, as such, is impermeable to protons (H^+) and hydroxyl ions (OH^-). The transport of electrons through ETC is coupled with the translocation of protons (H+) across the inner mitochondrial membrane (coupling membrane) from the matrix to the intermembrane space.

The pumping of protons results in an electrochemical or proton gradient. This is due to the accumulation of more H^+ ions (low pH) on the outer side of the inner mitochondrial membrane than the inner side. The proton gradient developed due to the electron flow in the respiratory chain is sufficient to result in the synthesis of ATP from ADP and Pi.

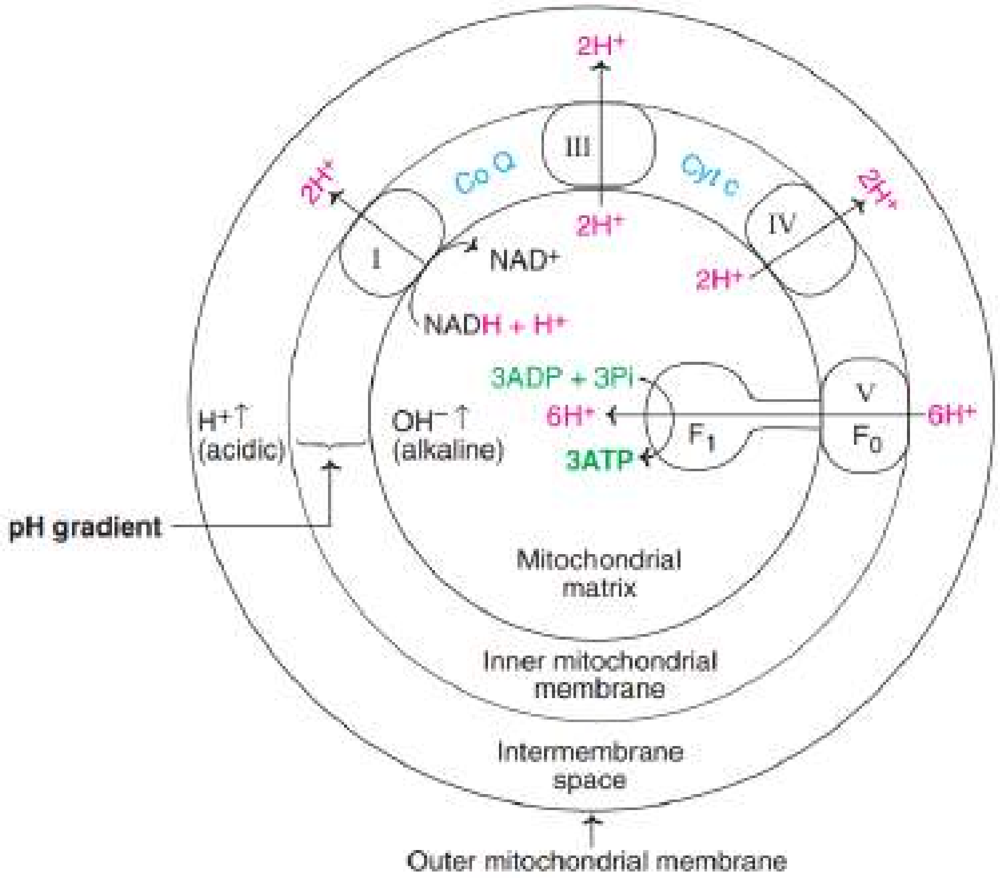
Enzyme system for ATP synthesis— ATP synthase, present in the **complex V**, utilizes the proton gradient for the synthesis of ATP. This enzyme is also known as ATPase since it can hydrolyse ATP to ADP and Pi. ATP synthase is a complex enzyme and consists of two functional subunits, namely F1 and F0. Its structure is comparable with 'lollipops'. The protons that accumulate on the intermembrane space re-enter the mitochondrial matrix leading to the synthesis of ATP.



Outline of chemiosmotic hypothesis for oxidative phosphorylation

Rotary motor model for ATP generation.

Paul Boyer in 1964 proposed (Nobel Prize, 1997) that a conformational change in the mitochondrial membrane proteins leads to the synthesis of ATP. The original Boyer hypothesis, now considered as rotary motor/engine driving model or binding change model, is widely accepted for the generation ATP.



Diagrammatic representation of chemiosmotic hypothesis for oxidative phosphorylation (I, III, IV and V–Respiratory chain complexes; F_0, F_1 –Protein subunits for phosphorylation).

Chapter-9
Minerals

Minerals: Functions, Deficiency diseases, recommended dietary requirements of calcium, phosphorus, iron, sodium and chloride

Minerals

Introduction.

- Minerals are essential for the normal growth and maintenance of the body. Many of the essential minerals are widely distributed in foods, and most people eating a mixed diet are likely to receive adequate intakes. daily requirement of mineral is more than 100 mg, they are called major elements or macro minerals. If the requirement of minerals is less than 100 mg/day, they are known as minor elements or microminerals or trace elements.
- Mineral availability depends on the region/soil or environmental condition because of mineral percentage vary region to region.

General functions of minerals.

- It is very essential components for day-to-day life in organism. These include calcification of bone, blood coagulation, neuromuscular transmission, homeostasis, acid-base equilibrium, fluid balance and osmotic regulation etc.
- Certain minerals are integral components of biologically important compounds such as haemoglobin (Fe), thyroxine (I), insulin (Zn) and vitamin B12 (Co). Sulphur is present in thiamine, biotin, lipoic acid and coenzyme A.
- Several minerals participate as cofactors for enzymes in metabolism (e.g., Mg, Mn, Cu, Zn, K). Some elements are essential constituents of certain enzymes (e.g., Co, Mo, Se).

Classification of minerals.

A. **Classification based on the requirement.**
   1. **Macrominerals.** The seven principal elements constitute 60-80% of the body's inorganic material. These are **calcium, phosphorus, magnesium, sodium, potassium, chloride and sulphur.**
   2. **Microminerals.**
      - Essential trace elements. Iron, copper, iodine, manganese, zinc, molybdenum, cobalt, fluorine, selenium and chromium.
      - Possibly essential trace elements. Nickel, vanadium, cadmium and barium
      - Non-essential trace elements. Aluminium, lead, mercury, boron, silver, bismuth etc

B. **Classification of minerals on the basis of the functions as.**
   1. Structural function— calcium, magnesium, phosphate.
   2. Membrane functions— sodium, potassium.
   3. Act as prosthetic groups in enzymes— cobalt, copper, iron, molybdenum, selenium, zinc.
   4. Regulatory action in hormone— calcium, chromium, iodine, magnesium, manganese, sodium, potassium.
   5. Essential, but function unknown— silicon, vanadium, bromine, nickel, tin, lithium.
   6. Present in normal diet but may cause the toxic in excess— aluminium, arsenic, antimony, cadmium, caesium, germanium, lead, mercury.

Calcium.

- Calcium is the most abundant among the minerals in the body. The total content of calcium in an adult man is about 1 to 1.5 kg. As much as 99% of it is present in the bones and teeth. A small fraction (1%) of the calcium, found outside the skeletal tissue.
- Absorption is taking place from the first and second part of duodenum. Calcium is absorbed against a concentration gradient and requires energy. Absorption requires a carrier protein, helped by calcium-dependent ATPase.

Sources.

- Milk and milk products are the good source of calcium (cow's milk, yogurt, cheese).
- Egg, fish and green vegetables, fruits (Papaya, orange, kiwifruit) are also medium sources for calcium.
- Other than these, cereals (wheat, rice), dry fruits (almond), beans and some pulses also contain the calcium.

Recommended dietary requirements.

Daily requirement of calcium by age

1-3 years of age	700 mg/day
4-8 years of age	1000 mg/day
9-18 years of age	1300 mg/day
19-50 years of age	1000 mg/day
50 years of age	1200 mg/day

Note- After the menopause condition calcium level in women decrease gradually so extra supplemental calcium require for women other than normal condition (1500 mg/day).

Functions of calcium.

- **Development of bones and teeth—** Calcium, along with phosphate, is required for the formation (of hydroxyapatite) and physical strength of skeletal tissue. Bone is regarded as a mineralized connective tissue. Bones which are in a dynamic state serve as reservoir of Ca. Osteoblasts are responsible for bone formation while osteoclasts result in demineralization.
- **Muscle contraction—** Ca^{2+} interacts with troponin C to trigger muscle contraction. Calcium also activates ATPase, increases the interaction between actin and myosin.
- **Nerve transmission—**It is necessary for the nerve transmission by activating the axonal vesicle in the neuron.

- **Membrane integrity and permeability—** Ca^{2+} influences the membrane structure and transport of water and several ions across it.
- **Activation of enzymes—** Ca^{2+} is needed for the direct activation of enzymes such as lipase (pancreatic), ATPase and succinate dehydrogenase.
- **Release of hormone and Secretory processes—** Ca^{2+} regulates microfilament and microtubule mediated processes such as endocytosis, exocytosis and cell motility and also helps in the release of certain hormone like insulin, PTH, calcitonin.

Deficiency diseases of calcium.

- **Hypercalcaemia—** Elevation in serum Ca^{2+} level (normal 9–11 mg/dl) is hypercalcemia. Hypercalcemia is associated with hyperparathyroidism caused by increased activity of parathyroid glands.
- **Hypocalcaemia—** Hypocalcaemia is a more serious and life-threatening condition. It is characterized by a fall in the serum Ca^{2+} to below 7 mg/dl, causing tetany. The symptoms of tetany include neuromuscular irritability, and convulsions.
- **Rickets—** Rickets is a disorder of defective calcification of bones. This may be due to a low levels of vitamin D in the body or due to a dietary deficiency of Ca^{2+}.
- **Osteoporosis—** Osteoporosis is characterized by demineralization of bone resulting in the progressive loss of bone mass.

Phosphorus

- An adult body contains about 1 kg phosphate and it is an intracellular component of cells. Most of it (about 80%) occurs in combination with Ca^{2+} in the bones and teeth. About 10% of body phosphorous is found in muscles and blood in association with proteins, carbohydrates and lipids.
- Calcitriol promotes phosphate uptake along with calcium and acidity favours while phytate decrease phosphate uptake by intestinal cells.

Sources.

- Best sources—milk and milk products, eggs, chicken meat, fish oil, seeds (sunflower, pumpkin), beans.
- Other sources— banana, vegetables oils, almond, nuts, cereals etc.

Recommended dietary requirements.

- It is based on the calcium intake. The ratio of calcium and phosphorus is required in 1:1 ratio means (generally 800 mg/day). But in infants it ratio about 2:1. Calcium and phosphate are distributed in the majority of natural foods in 1: 1 ratio. Therefore, adequate intake of Ca generally takes care of the P requirement also.

Functions of phosphorus.

- Phosphorus is essential for the development of bones and teeth.
- It plays a central role for the formation and utilization of high-energy phosphate compounds e.g., ATP, GTP, creatine phosphate etc.
- Phosphorus is required for the formation of phospholipids, phosphoproteins and nucleic acids (DNA and RNA).
- It is an essential component of several nucleotide coenzymes e.g., NAD+, NADP+, pyridoxal phosphate, ADP, AMP.
- Phosphate buffer system is important for the maintenance of pH in the blood (around 7.4) as well as in the cells.

Deficiency diseases of phosphorus.

- Serum phosphate level is increased in hypoparathyroidism and decreased in hyperparathyroidism.
- In severe renal diseases, serum phosphate content is elevated causing acidosis.
- Vitamin D deficient rickets is characterized by decreased serum phosphate.
- In diabetes mellitus, serum content of organic phosphate is lower while that of inorganic phosphate is higher.

Iron

- The total content of iron in an adult body is 3-5 g. About 70% of this occurs in the erythrocytes of blood as a constituent of haemoglobin. At least 5% of body iron is present in myoglobin of muscle. Haem is the most predominant iron-containing substance.
- Iron is mostly found in the foods in ferric form (Fe^{3+}), bound to proteins or organic acids. In the acid medium provided by gastric HCl, the Fe3+ is released from foods. Reducing substances such as ascorbic acid (vitamin C) and cysteine convert ferric iron (Fe^{3+}) to ferrous form (Fe^{2+}). Iron in

the ferrous form is soluble and readily absorbed. It is stored in body in the form of ferritin.

Sources.

- Rich sources— Leafy vegetables (spinach, silver beet, broccoli), pulses, apples, organ meats (liver, heart, kidney), fish, molasses, dry fruits, nuts, tofu.
- Other sources— Milk, wheat, polished rice, oats, egg etc.
- Cooking in iron utensils will improve the iron content of the diet.

Recommended dietary requirements.

- Adult man — 10 mg/day
- Menstruating woman — 18 mg/day
- Pregnant and lactating woman — 40 mg/day

Functions of iron.

- Iron mainly exerts its functions through the compounds in which it is present. Haemoglobin and myoglobin are required for the transport of O2 and CO2.
- Cytochromes and certain non-haem proteins are necessary for electron transport chain and oxidative phosphorylation.
- Peroxidase, the lysosomal enzyme, is required for phagocytosis and killing of bacteria by neutrophils
- Iron is associated with effective immune competence of the body

Deficiency diseases of iron.

- **Iron deficiency anaemia—**It is an important problem worldwide, because if iron losses from the body are relatively high (during heavy menstrual blood loss or intestinal parasites), it is difficult to achieve an adequate intake to replace losses. Iron deficiency anaemia mostly occurs in growing children, adolescent girls, pregnant and lactating women. It is characterized by microcytic hypochromic anaemia with reduced blood haemoglobin levels.
- **Nephrosis—** When kidney glomerular mechanism is damaged, proteinuria results. Then haptoglobin, hemopexin and transferrin are lost in urine, with consequent loss of iron.
- **Hemosiderosis—** This is a less common disorder and is due to excessive iron in the body. It is commonly observed in subjects receiving repeated blood transfusions over the years.

- **Hemochromatosis—** This is a rare disease in which iron is directly deposited in the tissues (liver, spleen, pancreas and skin).

Sodium

Sodium is the chief cation of the extracellular fluid. About 50% of body sodium is present in the bones, 40% in the extracellular fluid and the remaining (10%) in the soft tissues. Sodium is readily absorbed in the gastro intestinal tract.

Sources.

- common salt (NaCl) used in the cooking medium is the major source of sodium.
- Best sources— bread, whole grains, leafy vegetables, nuts, eggs and milk, sea fish, green vegetables.
- Other sources— fruits (Apple, mango, banana), tomato, cucumber.

Recommended dietary requirements.

- For normal individuals, the requirement of sodium is about 5-10 g/day.
- For patients of hypertension, around 1 g/day is recommended.

Functions of sodium.

- In association with chloride and bicarbonate, sodium regulates the body's acid base balance or homeostatic.
- It is necessary for the normal muscle irritability and cell permeability.
- Sodium is involved in the intestinal absorption of glucose, galactose and amino acids.
- It is necessary for initiating and maintaining heartbeat.

Deficiency diseases of sodium.

- **Hyponatremia—** This is a condition in which the serum sodium level falls below the normal. Hyponatremia may occur due to diarrhoea, vomiting, chronic renal diseases, adrenocortical insufficiency (Addison's disease).
- **Hypernatremia—** This condition is characterized by an elevation in the serum sodium level. The symptoms include increase in blood volume and blood pressure. It may occur due to hyperactivity of adrenal cortex (Cushing's syndrome), prolonged administration of cortisone, ACTH and/or sex hormones.

Chloride

Chlorine is a constituent of sodium chloride. Hence, the metabolism of chlorine and sodium are intimately related and in normal circumstances it is almost totally absorbed in the gastrointestinal tract.

Sources.

- Best sources— leafy vegetables, eggs and milk, olives, potato, yogurt.
- Other sources— tomatoes, banana, bread, orange etc.

Recommended dietary requirements.

- The daily requirement of chloride as NaCl is 5-10 g/day. Adequate intake of sodium will satisfy the chloride requirement of the body.

Functions of chloride.

- Chloride is involved in the regulation of acid-base equilibrium, fluid balance and osmotic pressure. These functions are carried out by the interaction of chloride with Na^+ and K^+.
- Chloride is necessary for the formation of HCl in the gastric juice.
- Chloride shift involves the active participation of chlorine.
- The enzyme salivary amylase is activated by chloride

Deficiency diseases of chloride.

- **Hypochloraemia**— A reduction in the serum chlorine level may occur due to vomiting, diarrhoea, respiratory alkalosis, Addison's disease and excessive sweating.
- **Hyperchloremia**— An increase in serum chlorine concentration may be due to dehydration, respiratory acidosis and Cushing's syndrome.

Chapter-10

Water and Electrolytes

Water and Electrolytes

- Distribution, functions of water in the body
- Water turnover and balance
- Electrolyte composition of the body fluids, Dietary intake of electrolyte and Electrolyte balance
- Dehydration, causes of dehydration and oral rehydration therapy

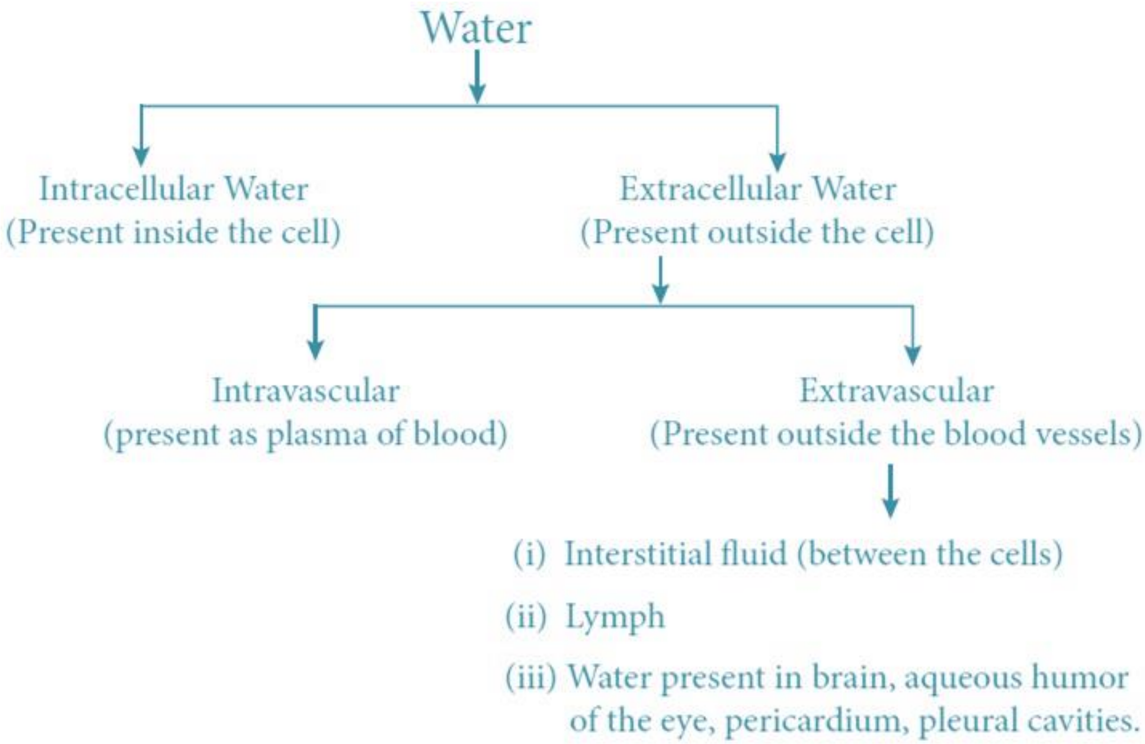
Water and Electrolytes:

Water and the principal electrolytes (sodium, potassium, and chloride) are often excluded from lists of nutrients, these substances are essential dietary components, in that they must be acquired from the diet either exclusively or—in the case of water—in amounts well in excess of that produced by metabolism in the body.

WATER

Water is the most abundant constituent of the human body, accounting for one-half to four-fifths of body weight, depending mainly on body fat content. Accordingly, body water, as a percentage of body mass, is higher in men than in women and tends to fall with age in both.

Distribution:



Functions of Water:

Major functions of water:

- **Carrier of Food Nutrients:** Every nutrient in soluble form in water is carried from intestines to tissues through blood.
- **Constituent of Liquid:** Water is the major constituent of all liquids of body as blood, urine, sweat, lymph.
- **Regulate body temperature:** Water helps to regulate and control body temperature. Heat is produced when food is burnt for energy. Water is evaporated through respiration and sweat and body temperature is maintained normal. Body's heat is lost through the skin, lungs, urine and faeces.
- **Safety/Security of Delicate Organs:** Water is around lungs, heart, brain which protects them from outer injury. Thus provide security to these organs and thereby to human being.
- **Water as lubricant:** Water acts as lubricant in joints. Water around joints help normal circulation process in cells. It is an essential constituent of all the cells of the body and the internal environment.

Water turnover and balance

- Water is an essential component of the human body, making up about 60% of body weight in adults. The balance of water in the body is regulated by several mechanisms to maintain proper hydration levels.
- Water turnover refers to the process by which water is continually exchanged between the body and the environment. This turnover can occur through various routes such as the skin, lungs, and gastrointestinal tract.
- Water balance refers to the balance between water intake and water excretion. The body must maintain water balance to prevent dehydration or overhydration.

Mechanisms involved in water turnover and balance:

1. Thirst mechanism: The body's thirst mechanism helps regulate water intake by stimulating the desire to drink water when there is a need to replace fluids lost through sweat, urine, and breathing.
2. Kidneys: The kidneys play a crucial role in water balance by regulating the amount of water excreted in urine. When the body needs to conserve water, the kidneys reduce urine output by reabsorbing more water back into the body.
3. Antidiuretic hormone (ADH): ADH is a hormone produced by the pituitary gland that regulates water balance by controlling the amount of water excreted in urine. When the body needs to conserve water, ADH levels increase, causing the kidneys to reabsorb more water and produce less urine.
4. Sweating: Sweating is a mechanism by which the body cools itself down and removes excess heat. However, excessive sweating can lead to dehydration, which can be dangerous.
5. Breathing: Water is lost through breathing as water vapor in the air we exhale. This loss is more significant in dry environments and during physical exertion.
6. Gastrointestinal tract: Water is lost through the gastrointestinal tract through feces. The body can reabsorb some of this water in the large intestine to maintain water balance.

Factors affecting water balance:

1. Temperature: Hot environments or physical exertion can increase water loss through sweating and breathing, leading to dehydration.
2. Dietary intake: Drinking fluids and eating foods with high water content, such as fruits and vegetables, can help maintain water balance.
3. Medications: Some medications can affect water balance by increasing or decreasing urine output.

4. Medical conditions: Certain medical conditions, such as diabetes insipidus, can affect the body's ability to regulate water balance.

Dietary intake of electrolyte:

- Electrolytes are minerals that carry an electric charge in the body and include sodium, potassium, calcium, magnesium, chloride, and phosphate.
- Electrolytes play a crucial role in various bodily functions such as maintaining fluid balance, regulating blood pressure, and facilitating muscle contractions and nerve impulses.
- Dietary sources of electrolytes include foods such as fruits, vegetables, dairy products, and meats.
- Sodium is commonly found in table salt, processed foods, and condiments, while potassium is abundant in fruits and vegetables.
- Calcium and magnesium can be obtained from dairy products, leafy greens, nuts, and seeds.
- Chloride and phosphate are found in foods such as processed meats, dairy products, and grains.
- Adequate electrolyte intake is important for overall health, but excessive intake of certain electrolytes such as sodium can lead to health issues such as high blood pressure and increased risk of cardiovascular disease.

Electrolyte balance:

- Electrolyte balance refers to the proper distribution of electrolytes in the body's fluid compartments, including the blood, cells, and extracellular spaces.
- Electrolyte balance is maintained by various mechanisms, including the kidneys, hormones such as aldosterone and antidiuretic hormone (ADH), and the thirst mechanism.
- Electrolyte imbalances can occur due to a variety of reasons, including excessive fluid loss through sweating, vomiting, or diarrhea, or due to certain medical conditions such as kidney disease.
- An electrolyte imbalance can have various symptoms depending on the specific electrolyte affected, including muscle weakness or spasms, cramping, irregular heartbeat, confusion, or seizures.
- Treatment of an electrolyte imbalance may involve dietary changes or supplements, medications, or in severe cases, hospitalization for intravenous electrolyte replacement.

Dehydration, causes of dehydration and oral rehydration therapy

Dehydration:

Dehydration refers to a condition where the body lacks adequate fluids to carry out normal physiological functions. It can occur when the body loses more fluids than it takes in, leading to an imbalance in the body's electrolytes and dehydration.

Causes of Dehydration:

There are several causes of dehydration, including:

1. Inadequate Fluid Intake: The most common cause of dehydration is not drinking enough fluids. This can occur if a person is not thirsty or if they are unable to access water.
2. Excessive Fluid Loss: Dehydration can also occur if the body loses too much fluid through sweating, urination, or diarrhea.
3. Medical Conditions: Certain medical conditions can also cause dehydration, including diabetes, kidney disease, and fever.
4. Medications: Some medications can cause dehydration as a side effect.

Oral Rehydration Therapy:

Oral rehydration therapy (ORT) is a simple and effective way to treat dehydration. It involves drinking a solution of water, salt, and sugar to replace fluids and electrolytes lost due to dehydration.

ORT is often used to treat dehydration caused by diarrhea, vomiting, and other illnesses that cause fluid loss.

ORT is recommended by the World Health Organization (WHO) as the first-line treatment for dehydration. It can be given at home or in a healthcare setting and is suitable for all age groups.

ORT solution can be made by dissolving the following in one liter of clean water:

- 6 level teaspoons of sugar
- 1/2 level teaspoon of salt

The solution should be sipped slowly over a period of four to six hours. If vomiting occurs, the solution should be paused for 10 minutes and then restarted. ORT should be continued until the individual is rehydrated, and fluid losses have been replaced.

Oral rehydration salt (ORS):

Oral rehydration therapy is a type of fluid replacement used to prevent and treat dehydration, especially due to diarrhea.

It involves drinking water with modest amounts of sugar and salts, specifically sodium and potassium. Oral rehydration therapy can also be given by a nasogastric tube.

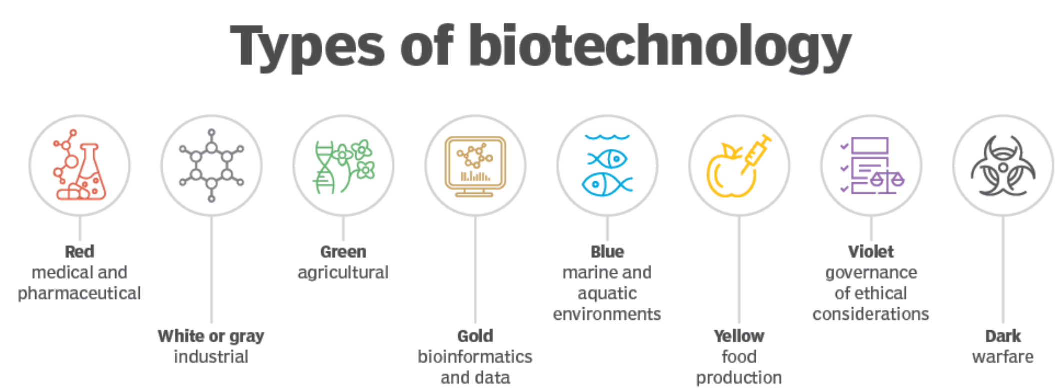
- It is expanded as Oral rehydration salt.
- It is composed of 4 constituents, sodium chloride, trisodium citrate, potassium chloride, and glucose.
- It helps in preventing dehydration caused by diarrhea.
- The combination improves fluid absorption in the intestines, allowing fluids to be restored more quickly.

Chapter-11

Introduction to Biotechnology

- Biotechnology is multidisciplinary field which has major impact on our lives.
- The technology is known since years which involves working with cells or cell-derived molecules for various applications.
- It has wide range of uses and is termed "technology of hope" which impact human health, well being of other life forms and our environment.
- Biotechnology is the use of living organisms or their products to create useful products, processes, or services. It involves the manipulation of biological materials, including cells, DNA, and proteins, to develop new medicines, foods, and other products. Biotechnology encompasses a wide range of fields, including genetic engineering, molecular biology, biochemistry, microbiology, and cell biology.
- The applications of biotechnology are vast and have the potential to benefit many industries and areas of life. In medicine, biotechnology has revolutionized the development of new drugs and therapies, including vaccines, gene therapies, and personalized medicine. In agriculture, biotechnology has improved crop yields, created drought-resistant crops, and enhanced livestock production.
- Biotechnology also plays a critical role in environmental conservation, with bioremediation technologies using microorganisms to clean up pollutants and biodegradable plastics and biofuels reducing dependence on fossil fuels.

Type of Biotechnology:



DNA technology:

- DNA technology is the use of various methods to manipulate, analyze, and modify DNA (deoxyribonucleic acid), which is the genetic material that carries the instructions for the development and function of all living organisms.
- DNA technology has revolutionized many fields of biology, including genetic engineering, molecular biology, and biotechnology.
- One of the most common uses of DNA technology is genetic engineering, which involves the manipulation of an organism's DNA to add, delete, or modify specific genes.
- This technology has allowed scientists to create genetically modified organisms (GMOs) with desired traits, such as resistance to pests, increased yield, or improved nutritional content.

Examples of DNA technologies:

- DNA cloning
- Polymerase chain reaction (PCR)
- Gel electrophoresis
- DNA sequencing

Chapter-12

Organ function tests

Organ function tests

- **Functions of kidney and routinely performed tests to assess the functions of kidney and their clinical significances**
- **Functions of liver and routinely performed tests to assess the functions of liver and their clinical significances**
- **Lipid profile tests and its clinical significances**

Organ function tests:

- Organ function tests are medical tests that are performed to assess the functioning of various organs in the body.
- These tests are usually recommended by doctors when a patient is experiencing symptoms that suggest a problem with a particular organ or when a patient has a condition that can affect the functioning of an organ.

Some common organ function tests include:

- **Liver function tests:** These tests are used to assess the functioning of the liver and to detect liver damage or disease. The tests measure levels of various enzymes, proteins, and other substances in the blood that are produced by the liver.

- **Kidney function tests:** These tests are used to assess the functioning of the kidneys and to detect kidney damage or disease. The tests measure levels of various substances in the blood and urine that are produced or excreted by the kidneys.

- **Lung function tests:** These tests are used to assess the functioning of the lungs and to detect lung diseases such as asthma, chronic obstructive pulmonary disease (COPD), and lung cancer. The tests measure how much air a person can breathe in and out, how quickly they can exhale, and how well their lungs are exchanging oxygen and carbon dioxide.

- **Cardiovascular function tests:** These tests are used to assess the functioning of the heart and blood vessels and to detect cardiovascular disease. The tests include electrocardiograms (ECGs), stress tests, and imaging tests such as echocardiograms and angiograms.
- **Endocrine function tests:** These tests are used to assess the functioning of the endocrine system, which includes glands such as the thyroid, adrenal glands, and pancreas. The tests measure levels of hormones in the blood or urine that are produced by these glands.
- **Gastrointestinal function tests:** These tests are used to assess the functioning of the digestive system and to detect gastrointestinal diseases such as ulcers, gastritis, and inflammatory bowel disease (IBD). The tests include imaging tests such as endoscopies and colonoscopies, as well as tests that measure the levels of enzymes and other substances in the blood.

Functions of kidney and routinely performed tests to assess the functions of kidney and their clinical significances:

Functions of kidney:

- The kidneys are two bean-shaped organs located in the abdomen that are responsible for filtering waste and excess fluid from the blood and excreting it as urine.
- They also help regulate blood pressure, maintain electrolyte balance, and produce hormones that help regulate red blood cell production and bone health.

The kidneys have several important functions in the body, including:

1. **Regulation of fluid balance:** The kidneys help regulate the balance of fluids in the body by filtering waste and excess fluids from the blood and excreting them as urine.
2. **Regulation of electrolyte balance:** The kidneys help regulate the balance of electrolytes in the body, such as sodium, potassium, and calcium, by filtering and selectively reabsorbing these ions as needed.
3. **Regulation of blood pressure:** The kidneys help regulate blood pressure by producing hormones that control the diameter of blood vessels and the volume of blood circulating in the body.
4. **Production of red blood cells:** The kidneys produce a hormone called erythropoietin, which stimulates the bone marrow to produce red blood cells.
5. **Regulation of acid-base balance:** The kidneys help regulate the pH balance of the body by excreting excess acids or bases in the urine.
6. **Excretion of waste products:** The kidneys filter waste products from the blood, such as urea, creatinine, and uric acid, and excrete them as urine.

Kidney Function Test:

The following are some commonly performed tests to assess kidney function and their clinical significances:

1. **Blood tests:** Blood tests that measure levels of creatinine and blood urea nitrogen (BUN) are commonly used to assess kidney function. Creatinine is a waste product that is produced by muscle metabolism and excreted by the kidneys, while BUN is a waste product of protein metabolism. Elevated levels of creatinine and BUN in the blood can indicate decreased kidney function or kidney damage.

2. **Urine tests:** Urine tests that measure levels of protein and albumin are commonly used to assess kidney function. Albumin is a protein that is normally present in the blood, but not in the urine, while proteinuria is the presence of excessive amounts of protein in the urine. Elevated levels of protein and albumin in the urine can indicate decreased kidney function or kidney damage.

3. **Glomerular filtration rate (GFR):** GFR is a measure of the rate at which blood is filtered by the kidneys. It is calculated based on a person's age, sex, and blood creatinine levels. A GFR of less than 60 mL/min/1.73m2 for more than 3 months indicates chronic kidney disease.

4. **Imaging tests:** Imaging tests such as ultrasounds, CT scans, and MRIs may be used to assess the size, shape, and structure of the kidneys and detect abnormalities such as cysts or tumors.

5. **Biopsy:** Kidney biopsy may be performed to collect a sample of kidney tissue for microscopic examination to diagnose certain kidney diseases.

- Abnormal results from these tests can indicate kidney disease or dysfunction. Early detection and management of kidney disease can help prevent or delay progression to end-stage kidney disease, which may require dialysis or kidney transplantation.
- It is important to consult with a healthcare provider to interpret test results and determine the appropriate course of treatment.

Clinical Significances:

You may also need a kidney function test if you have symptoms that indicate possible kidney problems. These symptoms might include:

- Problems with starting to pee: This is a measure of how much urine is produced over a certain period of time. Low urine output can indicate poor kidney function.
- Glomerular Filtration Rate (GFR): This is considered the most accurate measure of kidney function. It is a measure of how well the kidneys are filtering waste from the blood. GFR can be estimated through blood tests that measure levels of creatinine, a waste product produced by muscles.

- Blood Urea Nitrogen (BUN): BUN is a test that measures the amount of nitrogen in your blood that comes from the waste product urea. The kidneys are responsible for removing urea from the blood, so high levels of BUN can indicate poor kidney function.
- Serum Creatinine: This is a blood test that measures the level of creatinine in the blood. Creatinine is a waste product that is produced by the muscles and eliminated by the kidneys. High levels of serum creatinine can indicate poor kidney function.
- Urine Albumin: This test measures the level of albumin, a protein, in the urine. Albumin should not normally be present in urine, so its presence can indicate kidney damage.
- Urine Creatinine: This test measures the level of creatinine in the urine. This measurement can be used to estimate GFR.

Functions of liver and routinely performed tests to assess the functions of liver and their clinical significances:

Functions of liver:

The liver is a vital organ in the body that performs many essential functions. Some of the functions of the liver include:

1. **Bile production:** The liver produces bile, which helps in the digestion of fats.
2. **Protein synthesis:** The liver produces various proteins, such as albumin, that are essential for maintaining proper fluid balance in the body.
3. **Detoxification:** The liver detoxifies harmful substances, such as drugs, alcohol, and toxins, from the blood.
4. **Storage of vitamins and minerals:** The liver stores vitamins A, D, E, and K, as well as iron and copper.
5. **Metabolism:** The liver metabolizes carbohydrates, fats, and proteins to provide energy to the body.

Routine liver function tests are performed to assess the liver's health and function.

These tests include:

1. **Alanine transaminase (ALT) and Aspartate transaminase (AST):** These tests measure the levels of liver enzymes in the blood. High levels of ALT and AST are indicative of liver damage.
2. **Alkaline phosphatase (ALP):** This test measures the levels of ALP in the blood. Elevated levels of ALP may indicate liver or bone disease.
3. **Bilirubin:** This test measures the levels of bilirubin in the blood. High levels of bilirubin may indicate liver disease or hemolytic anemia.

4. **Albumin:** This test measures the levels of albumin in the blood. Low levels of albumin may indicate liver disease.

5. **Prothrombin time (PT):** This test measures the time it takes for blood to clot. Abnormal PT may indicate liver disease.

6. **Gamma-glutamyl transferase (GGT):** This test measures the levels of GGT in the blood. Elevated levels of GGT may indicate liver disease or alcohol abuse.

Clinical significance of liver function tests include:

1. **Detection of liver disease:** Liver function tests can help detect liver disease in its early stages, before symptoms appear.

2. **Monitoring of liver function:** Liver function tests are used to monitor the progress of liver disease and assess the effectiveness of treatment.

3. **Diagnosis of liver injury:** Liver function tests can help diagnose liver injury caused by drugs, toxins, or infections.

4. **Screening for liver disease:** Liver function tests may be used to screen for liver disease in people who are at risk, such as heavy drinkers or those with a family history of liver disease.

Lipid profile tests and its clinical significances:

A lipid profile test is a blood test that measures the amount of different types of lipids, or fats, in your blood. The lipids measured in a lipid profile test include:

1. **Total cholesterol:** This measures the total amount of cholesterol in your blood, including both high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol.

2. **HDL cholesterol:** This is often called the "good" cholesterol because it helps remove other types of cholesterol from your blood.

3. **LDL cholesterol:** This is often called the "bad" cholesterol because it can build up in your arteries and lead to heart disease.

4. **Triglycerides:** These are another type of fat that can build up in your blood and contribute to heart disease.

Clinical significances:

The clinical significance of a lipid profile test is that it can help your doctor assess your risk of developing heart disease.

- High levels of LDL cholesterol and triglycerides, and low levels of HDL cholesterol, are all risk factors for heart disease.

- By measuring these levels, your doctor can determine whether you need to make lifestyle changes, such as changing your diet and increasing your exercise, or whether you need medication to help lower your cholesterol levels.
- In addition to assessing your risk of heart disease, a lipid profile test may also be used to monitor the effectiveness of cholesterol-lowering medications, such as statins.
- If you are taking medication to lower your cholesterol, your doctor may order a lipid profile test periodically to ensure that the medication is working as it should.

Chapter-13

Poisoning Introduction to Pathology of Blood and Urine

- **Lymphocytes and Platelets, their role in health and disease.**
- **Erythrocytes - Abnormal cells and their significance.**
- **Normal and Abnormal constituents of Urine and their significance.**

Introduction to Pathology of Blood and Urine—

- The pathology of blood involves the study of the cellular and molecular components of blood and their interactions.
- Blood is composed of various cells and plasma, which contains nutrients, hormones, electrolytes, and other vital components that are essential for the body's normal functioning.
- Any abnormality in the composition or function of these components can lead to a wide range of medical conditions.

Common blood disorders include anemia, leukemia, and hemophilia.

- Anemia is a condition in which the body does not have enough red blood cells, causing fatigue and weakness.
- Leukemia is a type of cancer that affects the blood and bone marrow, leading to an overproduction of abnormal white blood cells.
- Hemophilia is a genetic disorder that causes the blood to clot abnormally, leading to excessive bleeding.

Lymphocyte's role in health and diseases.

- Lymphocytes are a type of white blood cell that plays a crucial role in the immune system. They are responsible for identifying and attacking foreign substances such as viruses, bacteria, and cancer cells. There are two main types of lymphocytes: B cells and T cells.
- B cells produce antibodies that help identify and neutralize pathogens. When a B cell encounters a foreign substance, it produces a specific antibody that binds to the pathogen and signals other immune cells to destroy it.
- T cells play a variety of roles in the immune system, including recognizing and destroying infected cells, regulating the immune response, and helping B cells produce antibodies.
- Abnormalities in lymphocytes can lead to various diseases, such as lymphoma, leukemia, and autoimmune disorders. Lymphoma and leukemia are cancers that affect lymphocytes, while autoimmune disorders occur when the immune system mistakenly attacks the body's own cells and tissues.

Platelet's role in health and diseases.

- Platelets, also known as thrombocytes, are small fragments of cells that help the blood clot to stop bleeding. They are formed in the bone marrow and circulate in the blood. When a blood vessel is damaged, platelets rush to the site and stick together, forming a plug to stop the bleeding.
- Abnormalities in platelet function can lead to bleeding disorders, such as hemophilia and von Willebrand disease, or thrombotic disorders, such as deep vein thrombosis and pulmonary embolism.
- Hemophilia and von Willebrand disease are inherited disorders that impair the blood's ability to clot, while deep vein thrombosis and pulmonary embolism occur when blood clots form in the veins and travel to other parts of the body, leading to serious complications.

Erythrocytes - Abnormal cells and their significance.

Red blood cells (RBCs) or erythrocytes are the most abundant type of blood cells in the human body, and their main function is to transport oxygen from the lungs to the body tissues and to carry carbon dioxide from the tissues back to the lungs. The shape of RBCs is an important characteristic that can provide information about a person's health status. A medical professional can evaluate the shape and size of RBCs as part of a complete blood count (CBC) test to diagnose and monitor blood disorders. Some abnormal shape of RBCs.

1. Normal RBCs— Normal RBCs have a biconcave shape with a flattened centre, which increases their surface area for efficient gas exchange. Normal RBCs are essential for oxygen transport and carbon dioxide removal.
2. Sickle cell-shaped RBCs— Sickle cell-shaped RBCs have a crescent or sickle shape and are characteristic of sickle cell anaemia, a genetic disorder that affects the production of haemoglobin. These abnormal RBCs can cause blockages in the blood vessels, leading to pain, organ damage, and other complications.
3. Schistocyte-shaped RBCs— Schistocyte-shaped RBCs have an irregular shape and are fragmented due to physical damage, such as from trauma or mechanical heart valves. These abnormal RBCs can cause haemolysis, anaemia, and other blood disorders.
4. Target-shaped RBCs— Target-shaped RBCs have a central dark spot surrounded by a lighter ring and an outer dark ring. Target-shaped RBCs can be caused by iron deficiency anaemia, liver disease, thalassemia, and other conditions.
5. Ovalocyte-shaped RBCs— Ovalocyte-shaped RBCs have an oval or elliptical shape and can be seen in various blood disorders, including hereditary elliptocytosis, thalassemia, and myelofibrosis.
6. Tear-drop-shaped RBCs— Tear-drop-shaped RBCs have a teardrop or pear-shaped appearance and can be seen in various blood disorders, including thalassemia, myelofibrosis, and other conditions.

Introduction to Pathology of Urine.

The pathology of urine involves the analysis of the chemical and physical properties of urine and their relationship to disease.

Urine is a waste product that is produced by the kidneys and excreted from the body through the urinary system. It contains a variety of components that can provide valuable diagnostic information when analyzed.

Examination of urine.

The investigation of urine, also known as urinalysis, is a medical test that evaluates the composition and properties of urine to diagnose various medical conditions. Here are some common investigations of urine:

1. Physical examination— This includes the colour, odour, and appearance of the urine. Normal urine is pale yellow in colour, clear, and has a mild odour. Abnormal urine may be cloudy, have a strong odour, or be a different colour, which can indicate various medical conditions.
2. Chemical analysis: A dipstick test is used to check for the presence of different chemicals in the urine, such as glucose, protein, ketones, blood, and bilirubin. Abnormal results can indicate conditions such as diabetes, kidney disease, or liver disease.
3. Microscopic examination: The urine sample is examined under a microscope to detect the presence of abnormal cells, bacteria, crystals, or other particles. This can help diagnose conditions such as urinary tract infections, kidney stones, or cancer.
4. Culture and sensitivity testing: If there is evidence of infection in the urine, a culture and sensitivity test may be performed to identify the type of bacteria causing the infection and determine the most effective antibiotic to treat it.
5. Urine cytology: This is a microscopic examination of the urine to detect abnormal cells that may indicate bladder cancer or other urinary tract cancers.
6. 24-hour urine collection: This test involves collecting all urine produced in a 24-hour period and measuring the volume, composition, and excretion of certain substances in the urine. It can be used to diagnose kidney disorders and other conditions.
7. Stone analysis: If a person has kidney stones, the stones can be analysed to determine their composition, which can help determine the cause and treatment of the stones.