21/10/24

Biochemistry → Greek word "bios" means life
Life

Introduction to Biochemistry → Biochemistry was launched by German chemist carl Alexander Neuberg (Father of Biochemistry) in 1903.

↓

The study of life's chemistry is a part of biochemistry.

Biochemistry is the branch of science that studies the chemical process-es and reactions occuring within living organisms. It fouses on understanding how biological molecules like enzymes, protein, carbohydrates, DNA, RNA, lipids work and contribute to the functions of living systems.

↓

[It is the connection b/w biology and chemistry at the molecular level of all the chemical processes associated with living cells.]

Objective of biochemistry → (1) To complete understanding
(2) To attempt to understand how life began.

Field of biochemistry → (1) metabolism → Sum of all reaction taking place in the cell is called metabolism.

Two parts → catabolism + anabolism = Metabolism (Carbohydrate → Glucose → Glycogen)

- Glycolysis (Glucose → energy (ATP) in mitochondria)

Scope of Biochemistry in pharmacy

⇒ Drug constitution → The drugs's composition, likelihood of degradation at different temperatures, how changes to medicinal chemistry can increase efficacy and reduce side effects, among other things are all revealed by biochemistry.

⇒ Half-life → This test is used to determine how long a medicine will remain stable at a specific temperature for biochemical drugs.

⇒ Drug storage → The storage condition required can be determined by biochemical test

⇒ eg many enzymes and hormones are stored for licensing and there get deteriorated over the time due to temp. or oxidation, contamination and also due to improper storage

⇒ Drug Metabolism → It also gives idea about how drug molecule are metabolised by many biochemical reactions in the presence of enzymes. This helps to avoid drugs which have poor metabolism or those with excessive side effect.

Cell and its biochemical organization

⇒ Protein, nucleic acids and polysaccharides are examples of complex chemical compounds that make up living things. For instance, the unicellular bacteria escherichia coli possesses almost 6000 different kinds of chemical compounds.

⇒ Small chemical substances called monosaccharides, nucleotides and amino acids serve as the monomeric units or building blocks for complex biomolecules like proteins, nucleic acids and polysaccharides.

Biomolecules		Building blocks	Major functions
(1) Protein	→	Amino acids	→ form basic framework of cells
(2) DNA	→	Deoxyribonucleotides	→ carrier of hereditary information
(3) RNA	→	Ribonucleotides	→ carry information related to protein biosynthesis.
(4) Polysaccharides	→	Monosaccharides	→ Body stores energy in this form and utilize it to meet short term needs.
(5) Lipid	→	Fatty acids	→ Second major source of energy.

The order of things

cells → tissue → organ → organ system → body

These are variety of molecules that are present in the living cell such as —

Micromolecules — they are very small in size for example amino acids, fatty acids.

They are small molecules that are present in the living cell. Each cell contains around 100 to 200 micromolecules.

Macromolecules → They are bigger in size and formed by polymerization monomer units.
e.g DNA, Proteins, RNA

Supramolecules → They are complex macromolecules.
e.g chromosomes, plasma membrane.

Biochemistry

Unit = 2

Carbohydrates :-

Definition → Carbohydrates are organic compounds with general formula $C_n(H_2O)_n$.

- They are composed of carbon, hydrogen and oxygen, but structurally they show substances with polyhydroxy aldehyde and ketones.
- Carbohydrates are primary source of energy as cell utilise carbohydrates for cellular respiration in the presence of oxygen.
- At the end of reaction, carbon-dioxide water & energy is obtained (in the form of ATP) as shown below:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Energy}$$

* Carbohydrates contain the following group :-

1. Alcoholic hydroxyl group ($-OH$)
   Aldehyde grp. ($-CHO$)
   Ketone grp. ($>C=O$)

Classification of Carbohydrates :—

1. On the basis of complexity
2. On the basis of Reactivity
3. On the basis of functional grp.

— On the basis of complexity

1. Monosaccharides
2. Oligosaccharides — Disaccharides, Trisaccharides.
3. Polysaccharides

— On the basis of Reactivity

1. Reducing Sugar.
2. Non-reducing sugar.

— On the basis of functional grp.

1. Aldoses.
2. Ketones.

* On the basis of Complexity :— (जटिलता)

1. Monosaccharides :—

Also known as simple sugar.

These are single unit carbohydrates (polyhydroxy aldehyde or ketones) that cannot be hydrolysed further.

Eg. — Glucose and fructose.

$C_6H_{12}O_6 + H_2O \xrightarrow{H^+}$ No rxn.

2.) Oligosaccharides :—
These are made up of 2-10 unit of monosaccharides or simple sugars.

a.) Disaccharides — These oligosaccharides consist of two monosaccharides units.
for eg :—
Sucrose ($C_{12}H_{22}O_{11}$) is a dissacharides and and on hydrolysis it gives one molecules of glucose or fructose.

$C_{12}H_{22}O_{11} + H_2O \xrightarrow{H^+} C_6H_{12}O_6 + C_6H_{12}O_6$
Glucose fructose.

b.) Trisaccharides :—
These oligosaccharides consist of three monosaccharides units.
for eg :— Raffinose is a trisaccharides and is hydrolysed into three simple sugar.

$C_{18}H_{32}O_{16} + 2H_2O \xrightarrow{H^+} C_6H_{12}O_6 + C_6H_{12}O_6 + C_6H_{12}O_6$
Glucose fructose Galactose

3.) Polysaccharides:—
A single molecule of a polysaccharides sugar is formed by polymerization of more than ten (10) monosaccharides unit.

These are molecule of starch or cellulose or hydrolysis yields a large number of glucose units.

The enzyme about inversion is named as invertase.

$$(C_6H_{10}O_5)_n + nH_2O \xrightarrow{H^+} (nC_6H_{12}O_6)$$

Starch or Cellulose — Water — Glucose

* On the basis of Reactivity:—

a) Reducing Sugar:— These sugars act as reducing agent and thus reduce Fehling's and Tollen's reagents.

b) Non-Reducing Sugar— These sugars do not reduce these reagents.

* On the basis of functional grp:-

a.) Aldose → These sugars have an aldehyde functional groups.
eg. — D-glucose.

b.) Ketose → These sugars have an ~~ato~~ Ketone functional grp.
Eg — D-fructose.

* Polysaccharides → Polysaccharides are considered to be the major class of biomolecules.
They are composed of long carbohydrates molecules chain ~~(~~ constituted of numerous simple Monosaccharides
These are structurally complex polysaccharides serve as an ~~oxygen~~ energy source in animal cells
Their general formula is $(C_6H_{10}O_5)$

Characteristics :-
They do not have a sweet flavour
Some are water soluble & and insoluble.

Chemical nature of Starch:—

Starch is the main storage polysaccharides of plant.
It is the most important dietry Soure for human beings.
Starch is found in cereals, roots and some vegetable
Starch which comes in two forms:—

a) Amylase b) Amylopectin.

→ Amylase :- Amylase is made up of 250–300 glucose residues that are joined together by α 1, 4 glycosidic linkage. Glycosidic linkage are present.

b) Amylopectin :-
It is a branched chain polymer of α-D glucose units in which chain is formed by α 1–4 glycosidic linkage (α 1, 4) whereas branching occurs by α 1–6 glycosidic linkage

Chemical nature of glycogen :-

living a storage polysaccharides glycogen occurs in a variety of animal tissue Including the liver and muscle.

It is polymer which is branched with around 8 to 12 glucose with present per branch.

* Structure :-

2 CARBOHYDRATES

2.1 INTRODUCTION

- Carbohydrates are organic compounds with **general formula $C_x(H_2O)_y$.**
- They are composed of **Carbon, Hydrogen and Oxygen** having the **ratio of Oxygen and Hydrogen atom 2:1**, e.g.: - Lactose, Glucose ($C_6H_{12}O_6$).
- Carbohydrates are the **primary source of energy** as cells utilize carbohydrate directly for cellular respiration in the presence of Oxygen.
- At the end of reaction, Carbon Dioxide, water and energy are obtained in the form of ATP, as shown below:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{Energy}$$

2.2 FUNCTIONS OF CARBOHYDRATE

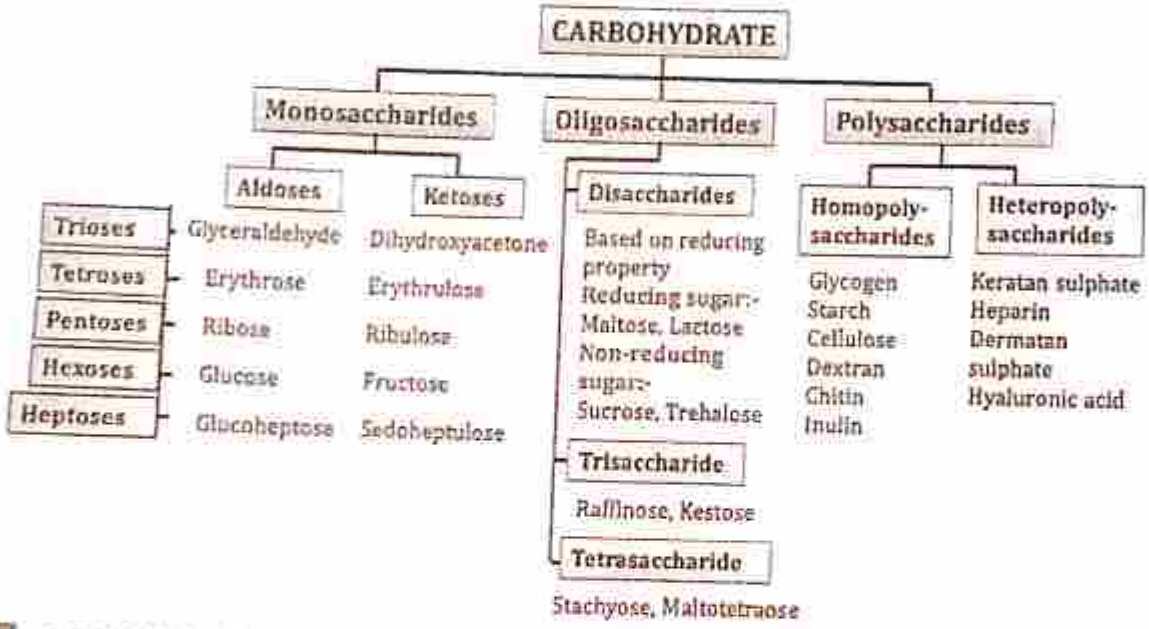
- They serve as primary source of energy for living beings, e.g., Glucose.
- They serve as structural component, e.g., Cellulose in plants and Chitin in insects.
- Non-digestible carbohydrates like cellulose, serve as dietary fibers.
- It is constituent of nucleic acids RNA and DNA, e.g., Ribose and Deoxyribose sugar.
- They play a vital role in lubrication, cellular intercommunication and immunity.
- Carbohydrates are also involved in detoxification, e.g., Glucuronic acid.
- They provide the carbon skeleton for the synthesis of some non-essential amino acids.
- Carbohydrates are precursors for many organic compound (Fat, Amino acid).
- Carbohydrate serves as storage form of energy, e.g., glycogen in animal tissue and starch in plants.

2.3 NOMENCLATURE OF CARBOHYDRATES

- Most carbohydrate nomenclature is based on historical trivial names. However, a few general rules are commonly used.
- The word saccharide [Greek-sakcharon (sugar)] is also used to refer to carbohydrates. It is primarily used to refer to the size of the molecule.
- "Monosaccharide" refers to the individual carbohydrates.
- "Oligosaccharide" and "Polysaccharide" refer to carbohydrate polymers of varying sizes.
  - ✓ Monosaccharide:- Molecules having only one actual or potential sugar group. **[$C_6H_{12}O_6$ Glucose]**

- ✓ Disaccharide:- When two monosaccharides are combined together. [$C_{12}H_{24}O_{11}$ Sucrose]
  Further addition of sugar group will produce –
  Trisaccharide [$C_{18}H_{32}O_{16}$ Raffinose].
  Tetrasaccharide [$C_{24}H_{42}O_{21}$ Stachyose], and so on. Commonly known as Oligosaccharides.
- ✓ Polysaccharide:- $C_n(H_2O)_n$ more than 10 sugar units are combined together.

2.4 CLASSIFICATION OF CARBOHYDRATES



2.5 MONOSACCHARIDES (GREEK: *MONO* - ONE)

- These are single unit carbohydrates with polyhydroxy aldehydes or ketones.
- General formula of carbohydrate is $C_n(H_2O)_n$.
- These sugars are simplest and non-hydrolysable. e.g., Glucose and Fructose.
- Glucose is the principal carbohydrate found in human circulation.

2.5.1 Classification and Nomenclature of Monosaccharides

- Sugar having aldehyde group are called aldoses and sugars with keto group are ketose.
- Depending on the number of carbon atoms, the monosaccharides are named as triose (C_3), tetrose (C_4), pentose (C_5), hexose (C_6), heptose (C_7) and so on.

Table 2.1 Based on type of carbonyl group

ALDOSE	KETOSE
Aldose are monosaccharides containing aldehyde group. Ex. Glyceraldehyde, Erythrose, Ribose, Glucose, Glucoheptose	Ketose are monosaccharide containing ketone group. Ex. Dihydroxyacetone, Erythrulose, Ribulose, Fructose, Sedoheptulose.

Physical properties

- Monosaccharides are crystalline solids at room temperature.
- Monosaccharides are water soluble and sweet in taste.
- They cannot be broken down into simpler sugars.
- They are reducing in nature. They reduce mild oxidizing agents, such as Tollens' or Benedict's reagents.

Chemical properties

Reducing properties

- Sugar are classified as reducing sugar and non-reducing sugar.
- Sugar containing free aldehyde or ketone group can reduce other reagents.
- They can reduce can reduce cupric ion of reagents into cuprous ions.
- Benedict, Fehling and Barfoed test are employed to identify the reducing action of sugar.
- Reduction is much more efficient in the alkaline medium than in acid medium.

$$C_6H_{12}O_6 \longrightarrow 2\,H_3C\text{-}CH_2OH + 2CO_2$$

- These tests are nonspecific, because these reagents reduce also by other hexoses or other reducing compounds as vitamin C.

2. Oxidation

- Under mild oxidation conditions (Hypobromous acid, Br_2/H_2O), the aldehyde group is oxidized to carboxyl group to produce Aldonic acid. Thus, glucose is oxidized to Gluconic acid, mannose to Mannonic acid, and galactose to Galactonic acid.
- Under strong oxidation conditions (Nitric acid + heat), the first and last carbon atoms are simultaneously oxidized to form dicarboxylic acids, known as saccharic acids.

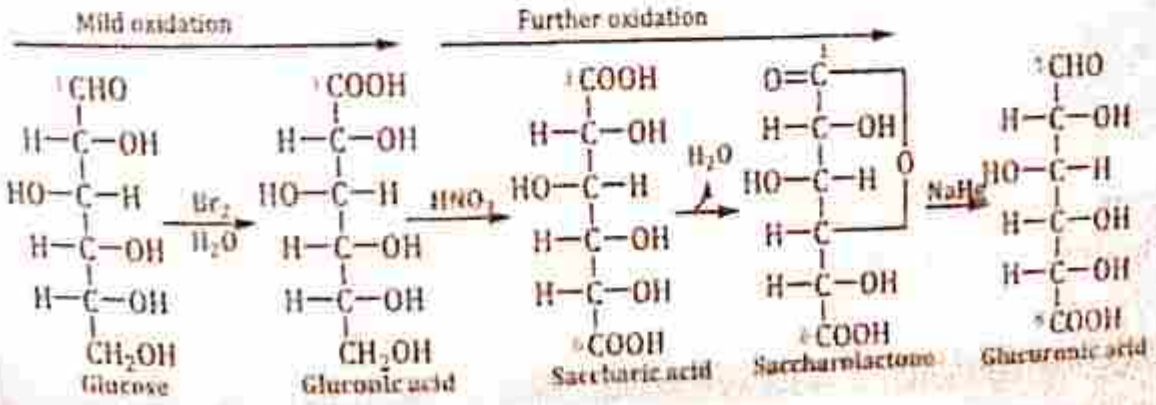


Fig.2.1 : Oxidation of glucose

3. Reduction

- When treated with reducing agents such as sodium amalgam, the aldehyde or keto group of monosaccharide is reduced to corresponding alcohol

- The important monosaccharides and their corresponding alcohols are given below:

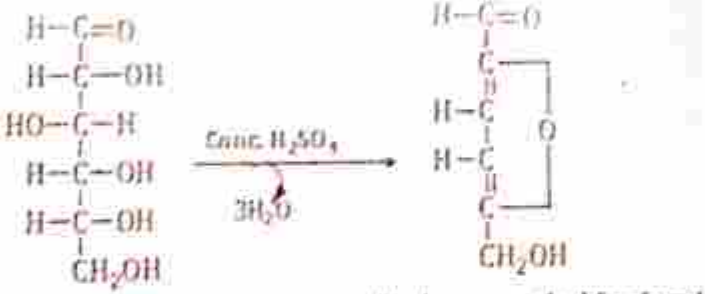
D-Glucose ⟶ D-Sorbitol
D-Fructose ⟶ D-Dulcitol
D-Mannose ⟶ D-Mannitol

D-FreeNose ⟶ D-Mannitol + D-Sorbitol
D-Ribose ⟶ D-Ribitol

- Sorbitol and dulcitol when accumulate in tissues in large amounts cause strong osmotic effects leading to swelling of cells.
- Mannitol is useful to reduce intracranial tension by forced diuresis.

4. Dehydration

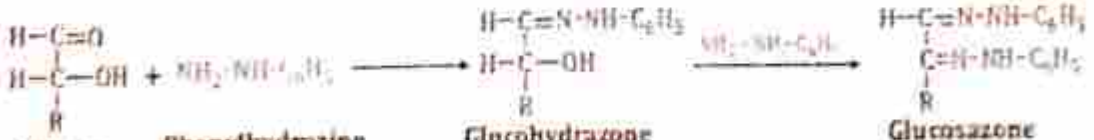
- When treated with concentrated sulfuric acid, monosaccharides undergo dehydration with an elimination of 3 water molecules, thus hexoses give hydroxymethyl furfural and pentose gives furfural on dehydration.



D-Glucose **Hydroxymethyl furfural**

5. Osazone formation

- Phenylhydrazine in acetic acid when boiled with reducing sugars forms osazones.
- All reducing sugars will form osazones.
- Each sugar will have characteristic crystal form of osazones.
- glucose, fructose and mannose give the needle shaped osazones.



Glucose **Phenylhydrazine** **Glucohydrazone** **Glucosazone**

2.5.3 Some Important Monosaccharide

Glucose

- Glucose is a sugar with the molecular formula $C_6H_{12}O_6$.
- Glucose is overall the most abundant monosaccharide.
- Glucose (sugar) is one of the main energy sources for living organisms.
- The primary source of energy for the brain.

- It is made up of glucose units combined with β-1 → 4 linkages. It has a straight-line structure, with no branching points.
- Molecular weight is in the order of 2 to 5 million. Cellulose has a variety of commercial applications- it is the starting material to produce fibers, celluloids, nitrocellulose, and plastics.

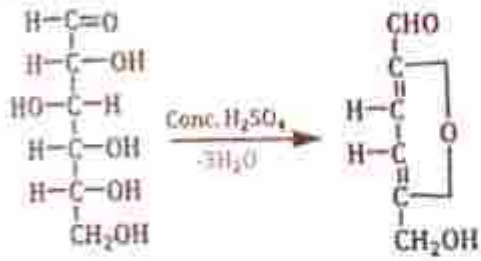
Heparin

- Heparin is an anticoagulant that occurs in blood, lung, liver, kidney, spleen etc.
- Heparin help in the releases of the enzyme lipoprotein lipase which helps in clearing the turbidity of lipemic plasma.
- Heparin is composed of alternating units of N-sulfo D-glucosamine 6-sulfate and glucuronate 2-sulfate.

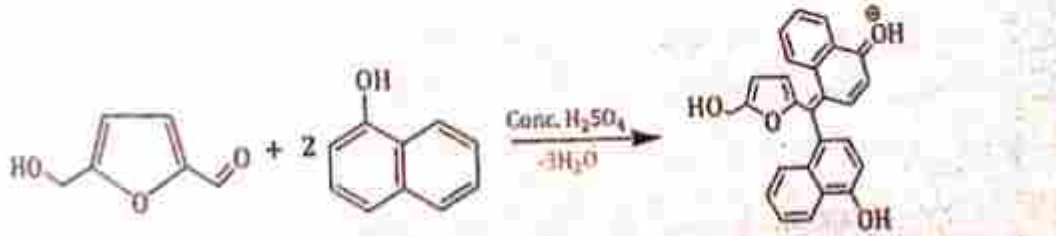
2.8 QUALITATIVE TESTS FOR CARBOHYDRATES

1. Molisch test

- It is a general test for the detection of carbohydrates.
- The strong H_2SO_4 hydrolyses carbohydrates (poly- and disaccharides) to liberate monosaccharides.
- The monosaccharides get dehydrated to form furfural (from pentoses) or hydroxy methyl furfural (from hexoses) which condense with α-naphthol to form a violet coloured complex.



D-Glucose — Hydroxymethylfurfural



Hydroxymethylfurfural — α-Naphthol — Purple/ Violet colored complex

2. Iodine test

- Polysaccharides combine with iodine to form a coloured complex. Thus, **starch gives blue colour while dextrin gives red colour with iodine.**

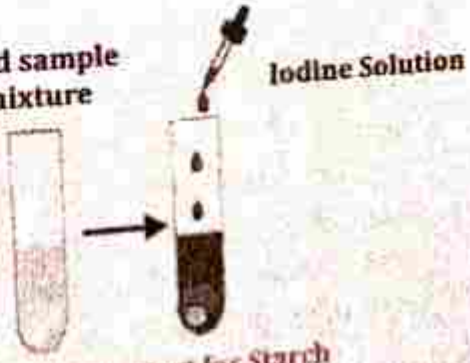
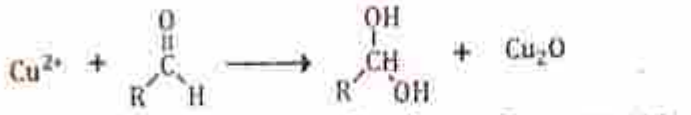


Fig.2.5: Iodine test for Starch

3. Benedict's test

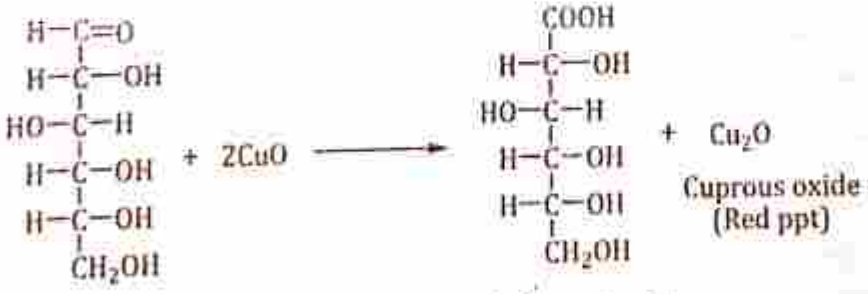
- This is a test for the identification of **reducing sugars.**
- The sugars reduce cupric ions (Cu^{2+}) of copper sulfate to cuprous ions (Cu^{+}) which form a yellow precipitate of cuprous hydroxide or a red precipitate of cuprous oxide.
- Sucrose contains two sugars (fructose and glucose) joined by their glycosidic bond in such a way as to prevent the glucose isomerizing to aldehyde, or the fructose to α-hydroxy-ketone form. Sucrose is thus a non-reducing sugar, which does not react with Benedict's reagent.
- **Benedict's reagent** ($CuSO_4.5H_2O$ (Copper sulfate pentahydrate) + Na_2CO_3 + $Na_3C_6H_5O_7$).



Cupric ion **Aldose** **Carboxylate ion** **Cuprous oxide** (Brick-red ppt)

4. Barfoed's test

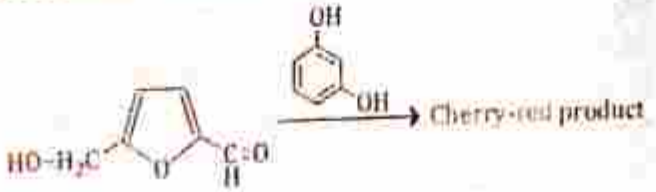
- This test is used for distinguishing only strong reducing sugars give this test positive.
- Monosaccharide usually react in about 1-2 min while the reducing disaccharides take much longer time between 7-12 min to get hydrolysed and the react with the reagent.
- Brick red colour is obtained in this test which is due to the formation of cuprous oxide.



D-Glucose D-Gluconic acid

5. Selliwanoff's test

- This is a specific test for ketohexoses.
- Concentrated hydrochloric acid dehydrates ketohexoses to form furfural derivatives which condense with resorcinol to give a cherry red complex.



Ketose

Proteins :- are naturally occuring polymers made-up of amino acids linked together by peptide bonds (-CONH-) having high molecular weight found in all living cells.

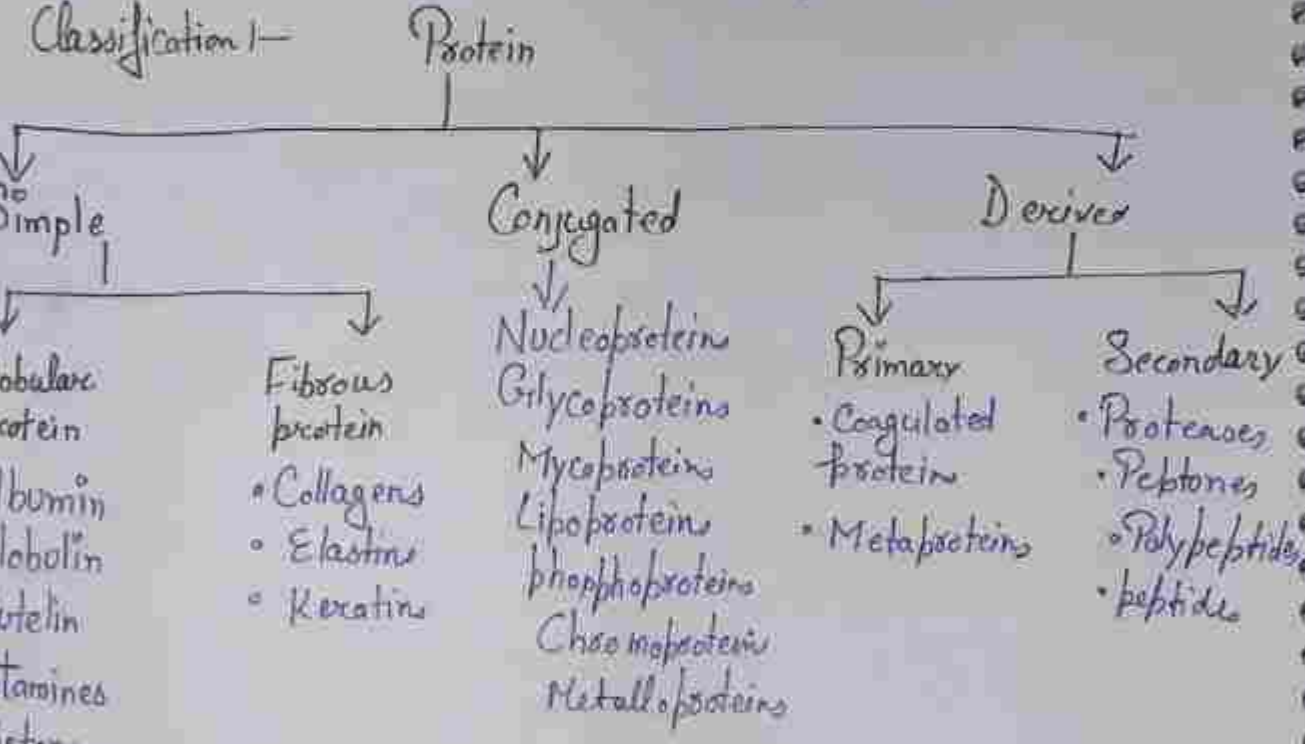
or

Proteins are high molecular organic compounds that consist of the long polypeptide chain of Amine acid monomers.
The amino acid monomers are joined together by the peptide bond b/w the carboxyl & amino group.

- Protein mainly consists of 20 different kinds of Amino acid molecule.
- Chemically a protein molecule consists of nitrogen, carbon, hydrogen, oxygen, Sulphur & phosphorus like -

Carbon - (50-55%) Nitrogen (15-17%) Sulphur (02-2%
Hydrogen (6-7.5%) oxygen (21-24%) phosphorus (0.1-1%.

Classification :-



1) Simple proteins - These proteins only consist of amino acid & are further classified into

a) Globular Proteins - These proteins are spherical or oval shaped.
- Soluble in water or other solvents and are digestible.

e.g

≡ Albumins - Soluble in water - eg - Serum albumin, Ovalbumin, Lactalbumin
≡ Globulin - Soluble in neutral and dilute Salt Sol.
eg - Serum globulin & Vitelline
≡ Glutelines - Soluble in dilute acid and alkalis, mostly found in plants.
eg - Glotelin, Oryzenin Insoluble in water & Alcohol
≡ Prolamines - Soluble in 70% Alcohol

b ≡ Fibrous Proteins - These Protein (fibre like in Shape) are water-insoluble and cannot be digested
eg - C

Conjugated protein - These are Simple protein Combine with some non-protein Substances Known as Prosthatic group
eg - Nucleoproteins - are Simple basic protein in Combination with nucleic acid at the prosthatic grup
They play a role in genetic information.
- Chromoprotein - These are protein containing colour prosthatic groups for eg - Hemoglobin, flavo protein, & Cytochrom
- Lipoprotein - These are protein conjugative with lipid such as neutral fat cholesterol / phospholipid
Cholesterol
- Phosphoprotein - These are protein containing phosphoric acid phosphoric acid linked to phosphoric group as linked to certain amino group like Serein in the protein eg - Kasein of milk

Derived protein - These are protein derived by partial to Complete Hydrolysis from the Simple or Conjugative protein by the action of acid alkalie or enzyme. They include two type of derivative. 1) Primary derived protein
2) Secondary derived protein.

Primary - These proteins are formed by Sali slightly. Changes in protein molecules & its property
- Little or no hydrolysis cleavage of peptide bonds acidor
eg - metaprotein :- These are formed of action of alkaline
They are insoluble in neutral solvent.

Coagulated protein are insoluble protein formed by heat of action or alcohol on natural protein.

eg- Cooked meat, Cooked albumin.

Proteans are insoluble product formed by the action of water dilute acid & enzyme. They are formed from globulins but insoluble in dilute Salt Sol. eg- Myosin from myosin.

Fibrin from ~~fibrinogen~~ ~~fibrinogen~~ fibrinogen.

Secondary - These protein are found in hydrolytic cleavage of peptide bonds of protein molecule.

- Proteoses are hydrolytic product of protein which are soluble in water & not coagulated by heat
- Peptones :- are hydrolytic product which have simplest than proteoses. Soluble in water & not coagulated by heat.

Biological Role of protein

- All Receptes are protein in nature.
- All Enzyme are protein in nature (Immunoglobulin)
- Some protein are protein in nature Inpulin & Grsowth hormone
- Some proteins are prestective eg — Caratein (Skin, hair, nails) make the skin resitant to chemical.
- Some proteins are Suppostive eg — Collagen.

⇒ Biological function of Protein -

- Catalytic function all chemical reaction in biological Systems Catalysed by Specific enzyme
- Transpost and Stosage
  e.g. Hemoglobin Transpost, Oxygen in blood
  Albomin transpost, free fatty acid in blood.
- Structure & Mechanical Suppost.
  eg — Collegen [A fibrous protein in Skin & bone].

LIPIDS

Lipids are broadly defined as fat soluble (lipophilic) naturally occuring molecules such as fats, oils, waxes, cholesterol, sterols, fat soluble vitamins (A, D, E, K), mono and diglycerides, phosphalipids etc.

The term lipids include any biological compound that is soluble in a lipid (i.e. non-polar organic solvent).

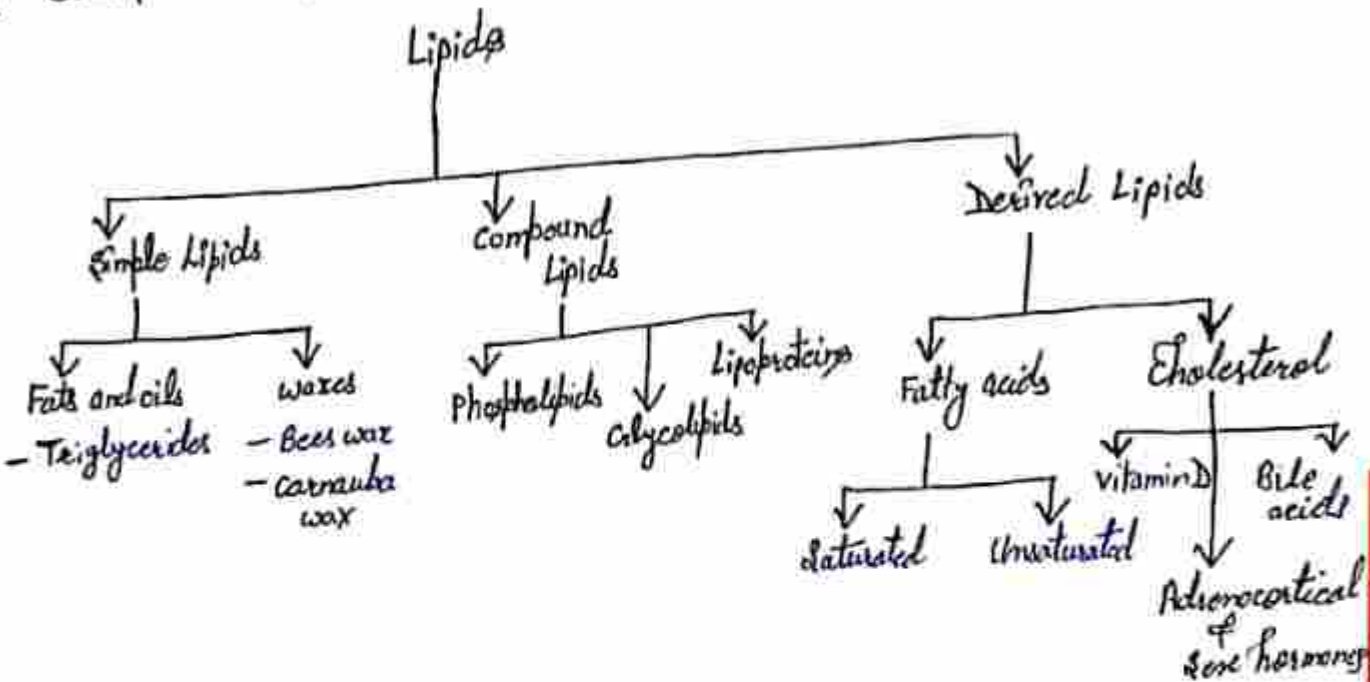
The lipids are hetergeneous group of compounds related to the fatty acids either actual or potential, insoluble in water, soluble in other solvents such as ether, chloroform and benzene and chemically are esters of fatty acid and some alcohols.

Sources: The lipids occur widely in plant and animal kingdom.

Examples: fats, oils, waxes and related compounds. oils are liquids at 20°c but fats are solid at 20°c

The word lipid is derived from Greek word lipos meaning fat.

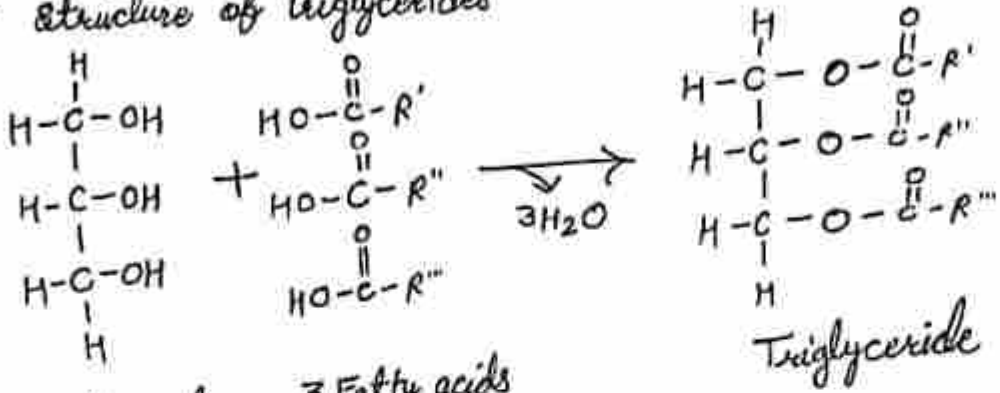
⇒ Classification of Lipids



Triglycerides

- They are esters of fatty acids with glycerol.
- Triglycerides are the main constituents of natural fats and oils.

⇒ Structure of Triglycerides

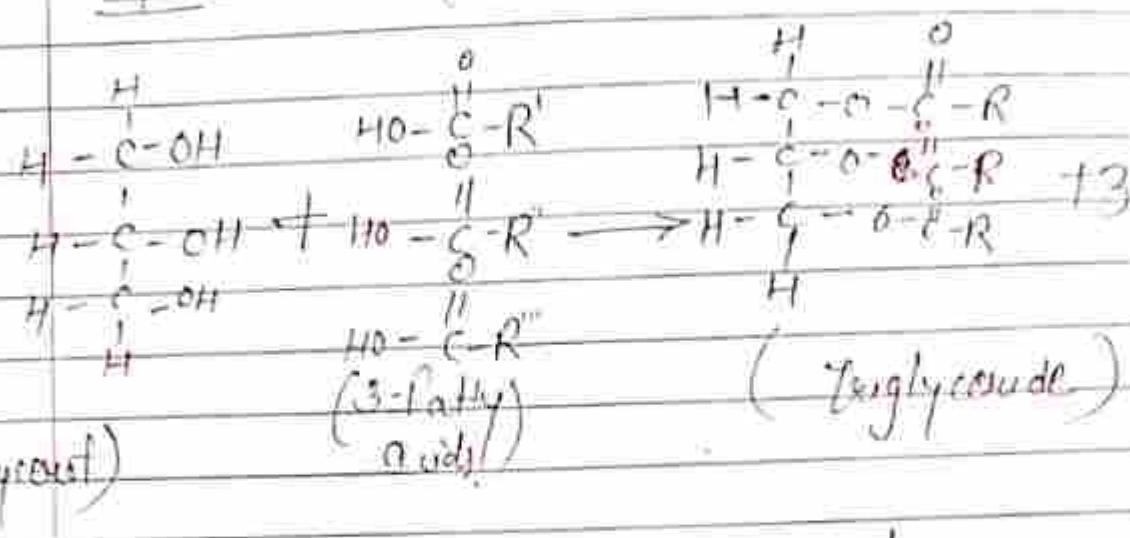


Glyceral 3 Fatty acids Triglyceride

⇒ Properties of Triglycerides :- The fats are insoluble in water, but readily soluble in ether, chloroform, benzene, carbon tetrachloride.

- They are readily soluble in hot alcohal but slightly soluble in cold.
- They are tasteless, odourless, colourless and neutral in reaction.
- Their melting points are low.

Lipids (Struct.)



→ Lipids are fatty, waxy or oily compounds that are organic solvents & insol. in water.
That are broad group of natural occuring molecules which includes fats, waxes, sterols, fat sol. vitamins A, D, E & K, monoglycerides, tri-glycerides, di-g. (fats & oils), phospholipids & others

or Phospholipids & other) Function :- Store energy, signaling and acting as a structural component of cell membrane.

→ Lipids have application in cosmetic & food industry as well as nano-technology.

→ The word Lipid is derived from 'Lipos means fat' present in all animal & plant cell.

Classification:

- Simple L. (homolipids)
  - Fat & oils (Triglycerides)
  - Waxes
- Compound L.
- Derived L.

→ Simple tri-glycerides
→ Mixed T.

→ Carnauva wax
Carnauva

Date
Page

Compound L. (Heterolipids)

Phospho L.
→ Phopho glycerides
→ ~~Cephalins~~
Cephalins, Lecithenes

Glyco Pl.
→ Kerasin
→ Phrenosin
→ Nervon

Derived L.

C = carbon

Steroids
→ C21, C28, C27 steroids
→ C24 s.
→ C21, C19, C18 steroids.

Terpenes
① Mono T. ② Poly T. ③ Di Terpenes

Carotenoids
→ Carotenes
→ Xanthophylls
④ Sesqui T. ⑤ Tri T.

⑥ Teta T.

Composition of Triglycerides

Glycerol

Fatty acids

Saturated F.A.
→ Palmitic acid
→ Stearic acid

Un. F. A.
Mono Un.
Poly un.

eg. of Mono un. F.A. → Oleic acid
" Poly " → Linolic acid

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Page:

Fatty acids :- These are organic acid consisting chain of alkyl group containing b/w four and 36 or more carbon atom with a terminal carboxyl group.

- Basic building units of body & principle constituting of body fat. There are more than 100 diff. fatty acids occuring naturally.
- 90% of fatty acids in our body occuring in form of ester of tri-glyceride & glycolipids, phospholipids.
- Fatty acids are also called carboxylic acid due to presence of carboxyl group.
- Degree of unsaturated fatty acids depends upon no. of double bonds present in hydro-carbon chain of fatty acids.
- Fatty acids may be saturated & unsaturated depending upon degree of unsaturation.
- Greater degree of unsaturation in fatty acids more would be lipid oxidation.

Introduction

~~Structure~~ & Properties of Triglycerides :-

→ Triglycerides are esters of glycerol & ~~three~~ 3 fatty acid group.
→ These are main constituents of natural fats & oils.
→ These are also named as triacylglycerol (acyl means fatty acid) esters of glycerol with 3-fatty acids.
→ Most abundant family of lipids in plants & animal cells.
→ Major compound of human diet. are present
→ Fatty acid in structure of triglyceride :- Mostly unbranched fatty acid. Their size ranges about 10-22 carbons.

- They contain even no of Carbon (10, 12, 14)
- Apart from carboxylic group, they have no functional group. except that some do have double bond (unsaturated fatty acid).

Properties :- Physical Properties :- P.P depends upon fatty acid component :-

1) Melting point ↑se as no. of Carbon in its hydrocarbon chain ↑ses & no. of double bond ↓ses.
2) Oils — Triglycerides rich in unsaturated fatty acids. U.F.A are generally liq. at room temp. eg → veg. oils :
3) Fats — T.G. are rich in SFA are generally semi solid or solids at room temp. eg → Animal fat.
4) Pure fats & oils are odourless, tasteless, colourless.
5) Melting P. differ due to difference in their 3-dimensional shape :- (a) Hydrocarbon chain of SFA can lie parallel with strong dispersion forces b/w their chains, they pack in well ordered, compact crystalline form & melt above room temp.
6) Because of cis configuration of double bond in UFA, their hydrocarbon chain have a less ordered structure & dispersion forces b/w them. These T.G. have melting P. below room temp.

Fatty acids :-

- Fatty acids are amphipathic in nature (hydrophilic & hydro
- It contains both non-polar hydro carbon chain & a polar carboxylic group. ∴ it act as hydrophilic & phobic.

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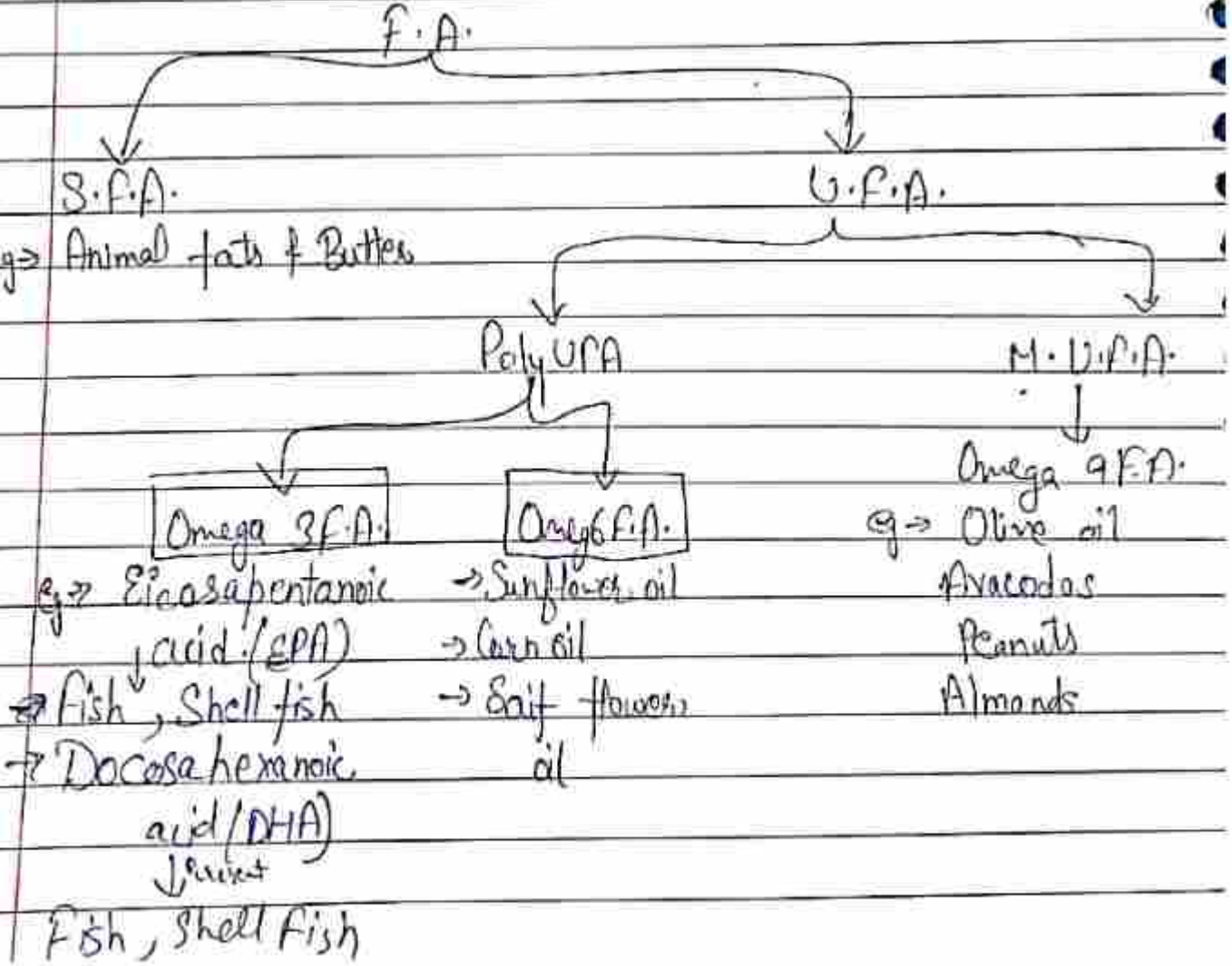
Fats Classification

Based on chemical structure :—

1. **Saturated Fatty A.** :— • These are F.A. that contain no double bond in their hydrocarbon chain.
   - They are solid at room temp.
   - They can rise risk of (CHD) coronary heart disease. eg → Butyric acid, Capric acid.

2. **UFA** :— • These are the F.A. that contain one or more double bond in their hydrocarbon chain.
   - These may either monounsaturated fatty acid (MUFA) or Poly U.F.A. (PUFA).
   - They are liq. at room temp.
   - Abundant in fish & veg. oils & reduce risk of CHD. eg → Stearic acid & oleic acid.



→ α-linolenic acid (ALA) found in walnut, Soyabean

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⇒ Based on nutritional req. :—

(Take through diet)

(1.) Essential amino acids :— These are F.A. that cannot be prepared by body & are obtained from diet.
- Our body is not capable to synthesise them.
eg → Linoleic acid, linolenic acid.

(2.) Non-E.F.A. :— These are F.A. that can be synthesised by our body & are not required from diet.
eg → Palmitic acid & Stearic acid.

Biological function of Lipids :— 1.) They are more palatable & storable to unlimited amount of compared to carbohydrates.
2.) They have high energy value (2x of body needs) & they provide more energy per gram than carbohydrates & proteins but carbohydrates are preferrable source of energy
3.) Supply essential F.A. that cannot be synthesise by body.
4.) Supply to body with fat sol Vit. (A, D, E & K).
5.) They are imp. constituents of N. system, thus tissue fat is essential constituent of cell membrane & Nervous system.
6.) Lipo-Proteins which are complex of Lipids & Proteins are imp cellular constituents that are present both in cellular & sub-cellular membrane.
7.) Cholesterol enters in membrane & used for synthesis of adrenal cortical hormones, Vit-D3 & Bile acids.
8.) Lipid provide bases for dealing with disease such as → Obesity, Atherosclerosis, Lipids storage disease, essential F.A. deficiency, respiratory distress syndrome.

Cholesterol :-

→ It is a waxy subs, fat like structural component of cell membranes & serves as building block for synthesing various steroid hormones of Vit-D, bile acids.
→ Besides, there structural role providing stability & fluidity cholesterol also plays a crucial role in regulating cell function.
→ It is a 27 Carbon compound with a unique structure with a hydrocarbon tail.
→ A central steroid nucleus made of 4-hydrocarbon ring & a hydroxyl group.
→ The central steroid nucleus or ring is a feature of all steroid hormones.
→ They hydrocarbon tail & central ring are non-polar & ∴ do not mix with water.
→ ∴ Cholesterol (lipid) is packaged together with apoproteins (protein) in order to be carried through blood circulation as a lipoproteins.
→ Humans can synthesise cholesterol 'de novo' & can also obtain it from diet.
→ D. Novo synthesis occurs in liver in intestine by all cells can synthesis Cholesterol to a small extent.
→ The liver is major site of cholesterol synthesis.

Functions of cholesterol :-

1.) Building & maintanence of cell membrane.
2.) Essential in determination of " " permeability.
3.) Necessary to production of sex hormone (estrogen & androgen).
4.) Imp. for production of adrenal gland released hormones like aldosterone, corticosterone etc.
5.) Helpful in production of bile.
6.) Conversion Vit. D from sunshine.
7.) Imp for metabolism of fat sol. Vitamins like (Vit - D, A, E, K).

Gluconeogenesis - Fatty [illegible]

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3.) Insulation of nerve fibres.

Lipo-Proteins :— These are particles made up of proteins + fats (lipids).

OR

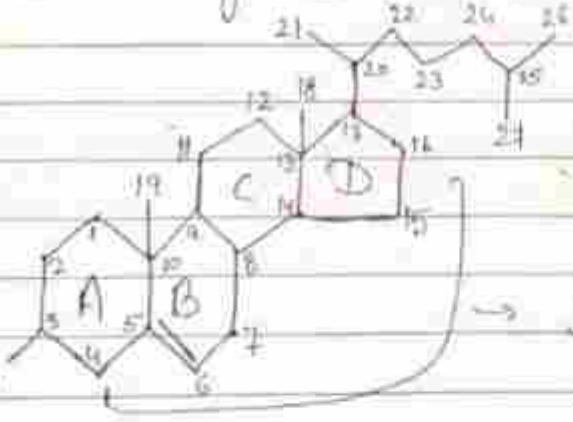
These are complex proteins + phospholipids that transport cholesterol + tri-glycerides in body. Outer surface of lipo-p contains apo-lipoproteins (Protein that bind lipids) of

⇒ Phospholipids + free cholesterol

Core of Tri-glycerides, Cholesterol esters.

⇒ Lipo-protein composition plasma lipoprotein particles contain variable proportion of 4 major elements of :- a) Cholesterol
b) T.G. c) Phospholipids d) Specific Proteins called apoprotein

⇒ Structure of Cholesterol :-



→ Aliphatic side chain

→ Steroid nucleus (6 members + 5 members)

⇒ Composition of Lipo-Protein :— 1.) Cholesterol.
~~Plasma LP~~ 2.) T.G. 3.) Phospholipids
4.) Specific proteins called apoproteins. (helps in metabolism + transportation of lip

Types of L.P. :— 1.) Chylomicrons → • they deliver dietary TG + cholesterol to liver + Peripheral tissues

2) Chylomicrons remnants :- They are produced when TGs are removed from chylomicrons thus they are rich in cholesterol ester.

3) Very low density lipo proteins :- They are made in liver & are rich in T.G. (VLDL)

4) Intermediate density lipo Proteins :- They are produced when TGs are removed from VLDL like chylomicrons remnants & they are rich in cholesterol because they are formed from VLDL. (IDL)

→ IDL sometimes are referred as VLDL remnants.

- types: composition and functions in the

Genetic Inf.
Store or transfer
Karta

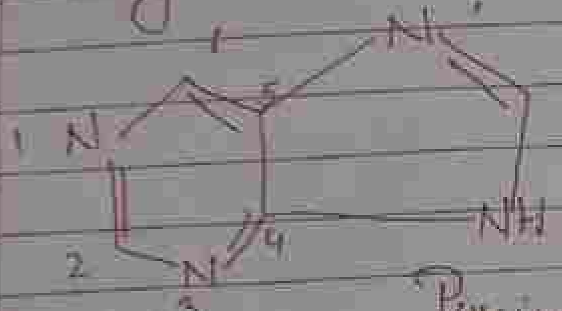
Nucleic acid

DNA → body pt. → genetic inform.
Protein synthesis in cell

Nucleic acid is the polymer in which the monomer unit are nucleotide.

Nucleotides are made up of 3-Components.

1) 5-Carbon Sugar.
2) Phosphate group. → help to combine the Sugar molecule
3) Nitrogenous waste. → Purine, Pyrimidine.



Pyrimidine ring

Purine ring

Nucleic acid follows into 2 Classes acc. to nature of Sugar they Contain.

Deoxyribonucleic acid (DNA)

Ribose Ribonucleic acid (RNA).

DNA is found primary in cell of certain viruses but may also occur in other portion of cell in mitochondria

Primary nucleic acid serves and and transmeter of genetic information

Definition

Nucleic acid are polymer of nucleotide 3'5' phosphoacids. Nucleic acid are build up by monomeric unit

Nucleic acid are mainly two type.

Deoxyribonucleic acid

Ribonucleic acid

DNA is present in nucleic Neucleï and small amount of mitochondric

90% of RNA is present in Cytoplasm & 10% in the Nucleolus.

Nitrogenous bases of DNA & RNA

Two classes of Nitrogenous bases mainly.

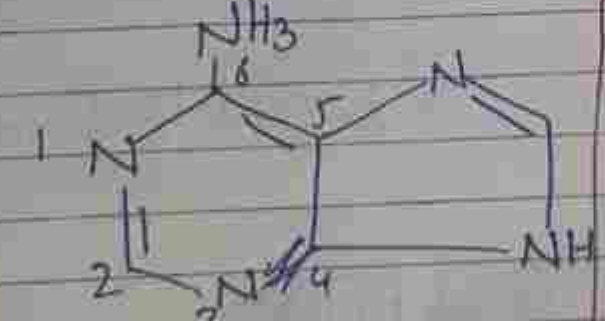
1. Purine
2. Pyrimidine

are present in DNA and RNA

Purine bases - Purine is a hetrocyclic compound and aromatic organic compound conipting of a pyrimidine ring fused to an imidazole ring

- Two principle ~~during~~ purine bases found in DNA as well as RNA

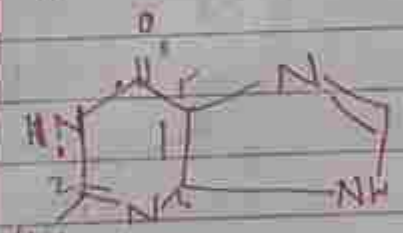
i) Adenine



Adenine

IUPAC (6-Amino purine)

ii) Guanine



Guanine

[2-amino 6oxy purine]

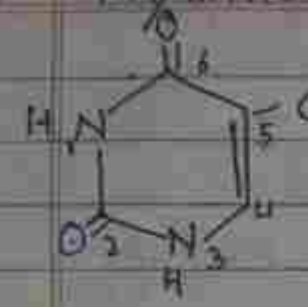
(2amino -1, 4 purine - 6one)

Pyrimidine bases. Pyrimidine is a hetrocyclic aromatic compound similar to benzene and pyridine ring containing 2- Nitrogen atom and position 1 and 3 of the 6 member ring.

Pyrimidine bases

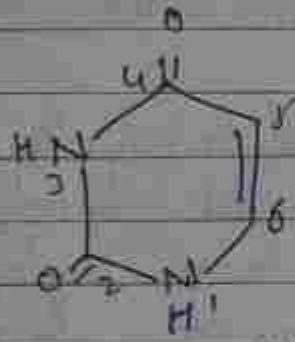
3 major pyrimidine bases are :-

(1) Thymine ii) Cytopine iii) Uracil



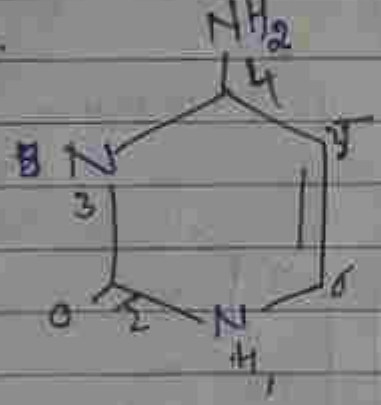
[2-dioxy, 5-methyl] pyrimidin

Uracil



[2-4-dioxy pyrimidin]

Cytopin.



[2-Oxy, 4-Amino pyrimi

Cytopine & Uracil are found in RNA
ii & Thymine are found in DNA
Both DNA & RNA

DNA containe thyamine whereas RNA contain Uracil.

Sugers of Nucleic acid / Pentose Sugar [Ribose / DeoxyRibose] & carbon monosaccharide (Pentoses) are found in the nucleic acid H.

RNA Contain Deribope, DNA contain Deoxyribope.

Ribope & Deoxyribope differ in 2^{nd} and C_2

Deoxyribope has 1 Oxygen less have Carbon 2 Compare to ribope.

Phopphate group -

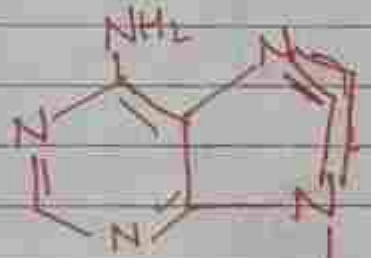
Mononucleotide are nucleotide in which single phopphate group attach to hydroxyl group of pentope sugar.

eg - AMP [Adinopin monophasphate]

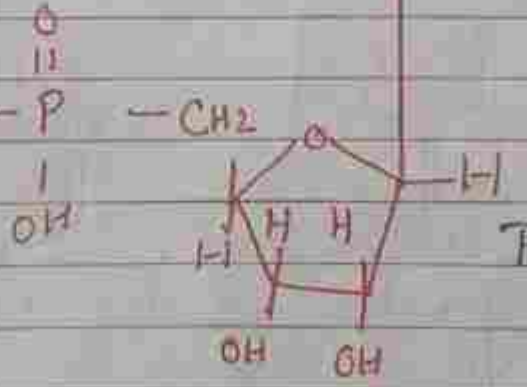
Adenine + Ribope + Phosphate.

if an additional phosphate group attach to pre existing mononucleotide

- A nucleopite diphopphate. eg - ADP
- A nucleopite triphopphate eg - ATP



Nitrogenous base



Phopphate grp

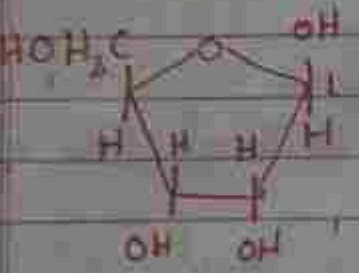
Pentope Sugar.

AMP

⇒ Sugars of Nucleic Acid.

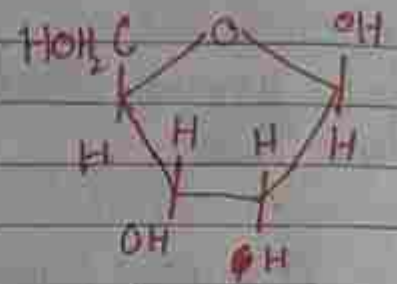
- A 5 Carbon monosaccharide (pentose) are found in nucleic acid ⇒

RNA Contain D-Ribose



D-Ribose

DNA Contains D-deoxyribose

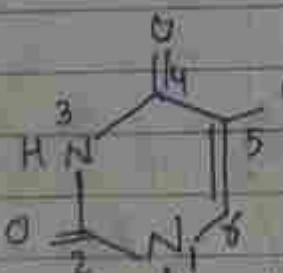


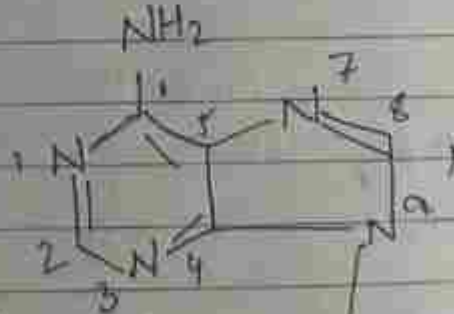
D-2-Deoxyribose

Nucleoside - A nucleoside consists of a nitrogenous base (purines and pyrimidine) and pentose sugar (ribose or deoxyribose) by the β-N-glycosidic bond b/w the first Carbon the pentose sugar and N_9 of a purine or N_1 of a pyrimidine

Nitrogenous base (Purine/Pyrimidine) + Pentose Sugar (Ribose/Deoxyribose) → Nucleoside

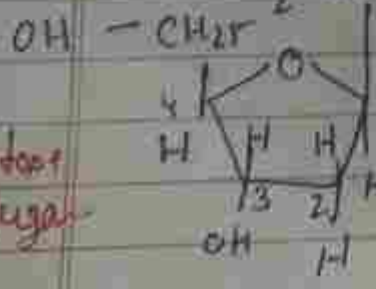
Nitrogenous base:

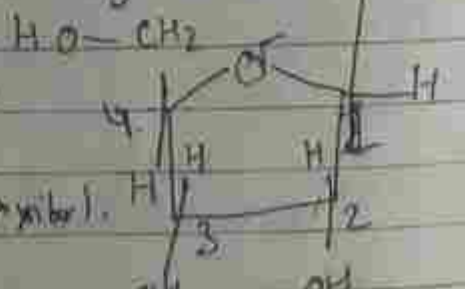




Nitrogenous base (Purine) II

Pentose Sugar





Pentose Sugar (Ribose/deoxyribose)

↓

Nucleoside

Deoxy

(Thymidine)

Adenosine

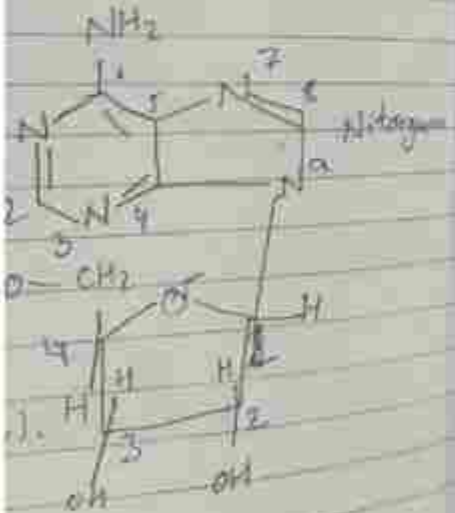
...nucleotides (Pentose) are

DNA contains D-deoxyribose

D-2-Deoxyribose

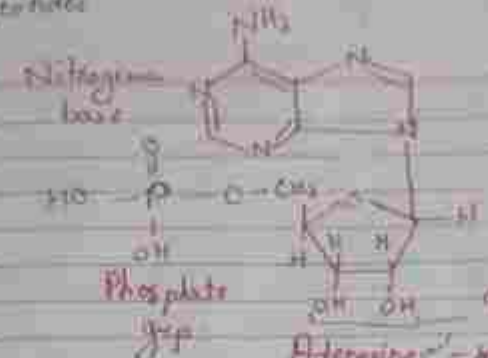
...consist of a nitrogenous base and pentose sugar (ribose ...-N-glycosidic bond b/w the ... and N9 of a purine or

...ar → Nucleoside
...ribose)

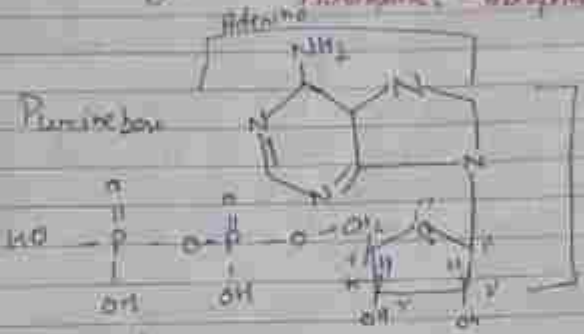


Adenosine

Nucleotides



Adenosine 5'-monophosphate [AMP]



A Nucleotides consist of nitrogenous base (Purine, Pyrimidine) and pentose sugar (Ribose, Deoxyribose Sugar)

The atom in the purine ring are numbered as 1 to 9 and Pyrimidine 1 to 6. The Carbon of Sugar are represented with an ' associated with purine for differentiation

The Pentose carbon are 1' to 5'. The pentose are bound with Nitrogenous base β-N-glycosidic bond

The ~~end line~~ of N9 of purine ring formed a covalent bond

In case of pyrimidine Nucleosides the glycosidic linkage b/w N_1 of pyrimidine C_1 of pentose.

- The Hydroxyl group of Adenosine are esterified with phosphate to form 5' or 3'-monophosphate.
- 5' hydroxyl is the most commonly esterified.

Thus AMP represent Adenosine 5'-Monophosphate
Adenosine 3'-Monophosphate is AMP is use

⇒ D/w Nucleosides & Nucleotides.

Defination - Nucleosides is a nucleobase linked to pentose sugar molecular.	Nucleotides is a monomer of Nucleic acid like DNA & RNA.
Component - A Nitrogenous base, Pentose Sugar.	A pentose Sugar, Nitrogenous base, phosphate grp.
Function - Nucleosides are good anticancer Compound as well as anti-Viral ~~doo~~ property	They polymerized and form nucleic acid as well as they act as energy storing molecular.
Eg - Cytidine, Adenosine, guanosine, uradin.	Deoxyribonucleotides, Ribonucleotides, ATP, CAM ADP etc

Function of Nucleotides:- They enter in the str. of nucleic acid DNA & RNA.
Purine enter in the structure of ATP which is a source of energy.
Cyclic adenosin monophosphate monophosphate AMP is a regulator of Carbo lipid metabolism.
Some Co-Enzymes are flavi & mononucleotide are the hydrogen carries

Nicotinamide adenine dinucleotide phosphate are hydrogen carrier. Co. A is a acid carrier.
Enzyme

- S Adenosin Methonine is a methyl donor.
- Pyrimidine derivative are puridine diphosphate glucose.
- Puridine diphosphate glactose
- Cytidine diphosphate acyl glycerol (CDP) - Acyl glycerol
- ~~Pre~~ nucleotide biological importance.
free

⇒ Natural Occuring Nucleotide -
Free Nucleotide which are not a part Nucleic acid also found in tissue many have imp. role - ATP & ADP

- These are the imp. compound as there participation in oxidative phosphorilation
- ATP is the high energy phosphate for ~~f~~ energy requiring rxn in the cell.
- ATP is the most abundant intracellular free nucleotide its conc. in living mamlian cell is near 1mM.

⇒ Cyclic AMP - 3,5 Adinosin monophosphate
This is present in most animal cell. It is formed by ATP by the

The activity which

It mediates series of rxn.

S Adinosin Etionin - It Serves a form of active litionin. It Serves as methyl doner. In many methylation rxn.

(4) Cyclic GMP (cGMP) — 3', 5' guanosin mono phosphate — It is formed from GDP by the enzyme Guaniade Cyclase.
It is catalysed by phosphodiesterase
It act antagonist to cAMP

(5) Enacinmonophosphate :—
which take place in the muscle

Enocintriphosphate, Enocin diphosphate play an Imp. role play an imp role in oxalo acetate, pyruvate Energy-trapping rxn., during oxidat. α-Ketoglutar.

(6) Uridine nucleotidederivative — These are the imp. Co enzyme in the metabolism of lactose in the mammary gland & polymerization of glucose to form glycogen in this rxn the substrate are UDP glucose, UDP galactose.
Another Co Enzyme Uridine diphosphate
Serve as active ~~glucotide~~ glucuronite active conjucative rxn glucuronite in the liver.

(7) Cytocin derivative — These derivative are CDP act as precursor for the polymerization of CMP into nucleic acid
CDP reg. for the biosynthesis of phosphoglyceride in the animal tissue.

Structure of DNA & RNA

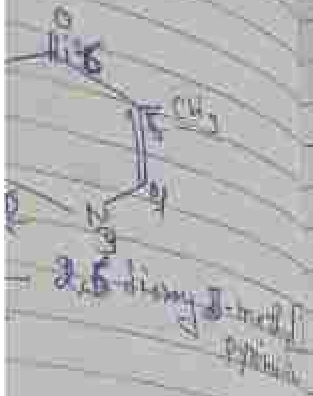
DNA & RNA are composed of 2 different classes of nitrogenous bases purine & pyrimidine. Purine are Most Common

Pyrimidine DNA are cytosin and thyamine
DNA & RNA are long polymers of Nucleotide.
DNA & RNA are long polymer of

The polynucleotide is connected by 5' to 3' phosphate linkage
Polynucleotide contain 5 phosphate and 3 hydroxy -ial terminal end
The Common representation of polynucleotide is 5' at the left 3' at the right

DNA — DNA is polymer of Deoxy -ribonucleotides. The monomeric deoxy nucleotide in DNA are held together 3' to 5' phospho diester bridges
DNA it is often represented in a Short hand form. The horizontal line repeated carbon chain of base attach to Carbon 1
Near the middle of Horizontal line is C3 phosphate linkage at the other of like C5 phosphate linkage

DNA Double Helix — The double helix of DNA was proposed by James Watson and Francis Crick in 1953
Noble price — 1962



hosphate backbone

- RNA Contain pyrimidine uracile in place of thyamine
- RNA is usually single standed polynucleotide however this stand may fold.
- If Compalmentary base pair
- Chargoff's rule — Not obeyed — due to single stand nature purine & pyrimidine thus guanine contain (As in the case DNA)
- Susceptibility to alkali hydrolysis — Alkali can hydrolysis to 2',3' Cyclic diester this is possible due to presence of hydroxy group at carbon 2' position
  D
- Orcinol colour rxn — RNA can be biological identify by orcinol colour rxn due to the presence of ribose.

Types of RNA: The three major type of RNA with their respective

1) mRNA — 5-10%
2) tRNA — 10-20%
3) rRNA — 50-80%

mRNA — Single standard complementary to the stan
mRNA Synthesis in nucleus (Eukaryote) as heterogenous nucleus RNA

Enzymes

Introduction → Enzymes are biocatalysts increase the velocity or rate of a chemical reaction without it self undergoing any change in the overall process.

→ The word enzyme was coined by Kühne.

Definition → Enzymes may be defined as biocatalysts synthesized by living cells. They are protein in nature (exception - RNA acting as ribozyme), colloidal and thermolabile in character, and specific in their action.

Properties of Enzymes

1. Enzymes are proteins. ie the amino, carboxyl and sulfhydryl groups of the side chains of amino acids are available for linkage b/w polypeptide chains.
2. The enzyme - substrate complex theory assumes combination of enzymes and substrate and the liberation of enzyme and the reaction product.

   Enzyme + Substrate = Enzyme - Substrate complex.

   Enzyme - Substrate Complex = Enzymes + Reaction product.

3. Alkaline phosphatase hydrolyses a number of phosphate esters to produce orthophosphate and an alcohol.

   Glucose - 6 - phosphate → Glucose + orthophosphate

- THE INTERNATIONAL UNION OF BIOCHEMISTRY AND MOLECULAR BIOLOGY (MB) CLASSIFICATION OF ENZYME
  - IUB appointed an enzyme commission in 1961.
  - This committee made a thorough study of existing enzymes and devised some basic principles for the classification and nomenclature of enzymes.
  - Since 1964, The IUB system of enzyme classification has been in force. Enzymes are divided in to six major classes.

1 Oxidoreductases → Enzymes involved in oxidation-reduction reactions.
eg Alcohol dehydrogenase (alcohol: NAD^+ oxidoreductase E.C. 1.1.1), cytochrome oxidase, L- and D- amino acid oxidases.

- Reaction Catalyst → oxidation → Reduction

$$AH_2 + B \rightarrow A + BH_2$$

2. Transferases → Enzymes that catalyse the transfer of functional groups.
eg Hexokinase (ATP : D-hexose 6-phosphotransferase, E.C. 2.7.1.1.), transaminases, transmethylases, phosphorylase.

- Reaction catalyst → $A-X + B \rightarrow A + B-X$

3. Hydrolases → Enzymes that bring about hydrolysis of various compounds by addition of water.
eg Lipase (triacylglycerol acyl hydrolase E.C. 3.1.1.3), Choline esterase, acid and alkaline phosphatases, pepsin, urease.

Reaction catalyst → $A-B + H_2O \rightarrow AH + BOH$

4. Lyases - Enzymes specialised in the cleavage of bonds (C-C, C-O, C-N), resulting in double bonds.
eg aldolase (Fructose-bisphosphate aldolase, E.C. 4.1.2.13), fumarase, histidase.
Reaction catalyst → Addition → Elimination
$A-B + X-Y \rightarrow AX-BY$

5. Isomerases → Enzymes involved in all the isomerization reactions.
eg retinal isomerase.
Triose phosphate isomerase (D-glyceraldehyde 3-phosphate ketoisomerase, EC 5.3.1.1), retinal isomerase, phosphohexose isomerase.
Reaction catalyst → Interconversion of isomers
$A \rightarrow A'$

6. Ligases - Enzymes catalysing the synthetic reactions (Greek: ligate - to bind) where two molecules are joined together and ATP is used.
eg → Succinate thiokinase
Glutamine synthetase (L-glutamate ammonia ligase, E.C. 6.3.1.2), acetyl CoA carboxylase, succinate thiokinase.
Reaction catalyst → condensation (usually dependent on ATP)

$$A + B \xrightarrow[ADP + P_i]{ATP} A-B$$

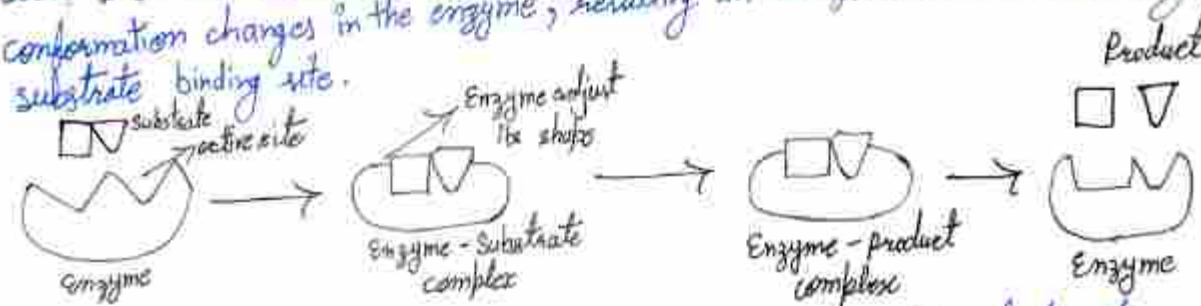
Mechanism of action of enzymes

Introduction

- Catalysis is the prime function of enzymes.
- Enzymes are powerful catalysts.
- The prime requisite for enzyme catalysis is that the substrate [S] combine with the enzyme [E] at the active site to form enzyme substrate complex [ES] which results in the product formation [P].

$$E + S \rightleftharpoons ES \rightarrow E + P$$

- Lock and Key Model → Proposed by a German biochemist, Emil Fischer.
  - → First model proposed to explain an enzyme catalysed reaction.
  - → Substrate fits to the binding site (active site) just as a key fit in to the Proper Lock
    - ↓
    - active site of an enzyme is a rigid and pre-shaped template where only a specific substrate can bind.
- Induced Fit theory → Koshland, in 1958, proposed a more acceptable and realistic model for enzyme substrate complex formation.
  - → The active site is not rigid and free-shaped.
  - → Essential features of the substrate binding site are present at the nascent active site. The interation of the substrate with the enzyme induces a fit or conformation changes in the enzyme, resulting in the formation of a strong substrate binding site.



- Substrate strain theory → In this model, the substrate is strained due to the induced conformation changes in the enzyme.
  - → Substrate binds to the performed active site, the enzyme induces a strain to the substrate.
  - → The strained substrate leads to the formation of product.

Factor affecting Enzyme activity

1. Concentration of Enzyme → The concentration of enzyme is increased (↑sc) the velocity of the reaction proportionately increases.

↓

This property is used for determining the activities of serum enzymes during the diagnosis of diseases.

2. Concentration of Substrate → In the presence of given amount of enzyme, the rate of enzymatic reaction ↑sc as the substrate conc^n increases until a limiting rate is reached, after which further increase in the substrate concentration produces no significant changes in the reaction rate.

3. Effect of temperature → The protein nature of the enzymes makes them extremely sensitive to thermal changes.

↓

Enzyme activity occurs with in a narrow range of temp. compared to ordinary chemical reactions.

↓

Each enzyme has a certain temp. at which it is more active.

↓

This point is called optimal temperature, ranges b/w 37 to 40°c.

4. Effect of pH → Enzymes are protein substances that contain acidic carboxyl groups (COOH) & basic amino groups (NH_2) So the enzymes are affected by changing the pH value.

- Each Enzyme has a pH value that it works at with maximum efficiency called the optimal pH, the enzyme activity decreases until it stops working.
  eg Pepsin works at low pH, ie it is highly acidic, trypsin works at high pH, ie it is basic. Most enzyme works at neutral pH 7.4

5. Effect of activators → Some of enzymes require certain inorganic metallic cations like Mg^{2+}, Zn^{2+}, Cu^{2+}, Na^{+}, K^{+}, for their optimum activity.
- Rarely, anions are also needed for enzyme activity, eg. a chloride ion (Cl^-) for amylase

Enzyme Inhibition

- Enzyme inhibitor is defined as a substance which binds with the enzyme and brings about a decrease in catalytic activity of that enzyme.
- The inhibitor may be organic or inorganic in nature.

There are three broad categories of enzyme inhibition.

1. Reversible inhibition
2. Irreversible inhibition
3. Allosteric inhibition

1. Reversible Inhibition → The inhibitor binds non-covalently with enzyme and the enzyme inhibition can be reversed if the inhibitor is removed

(i) competitive inhibition → The inhibitor competes with the substrate for the active group. This is known as competitive inhibition.

examples of competitive inhibition → Succinic acid ($HOOC-CH_2-CH_2-COOH$) is converted to fumaric acid when it combines with succinate dehydrogenase, the fumaric acid is released from the enzyme complex leaving the enzyme free to unite with more succinic acid.

Succinic acid —(Succinate dehydrogenase)→ Fumaric acid

Features of competitive inhibition →

- Competitive inhibition is Reversible.
- Km is increase
- V_{max} is same
- inhibitor cannot bind with ES complex
- complex is EI

(ii) Non-competitive inhibition — Some inhibitors are attached not to the active group but to some other groups of enzymes.
Under such conditions the activity of the unoccupied active group is affected; so that union with the substrate occurs less readily or not at all.

- This type of inhibition is called "Non-competitive inhibition"

⇒ Features of Non competitive inhibition →

(i) Non-competitive inhibition is reversible or irreversible.
(ii) V_{max} is lowered
(iii) K_m is unaltered
(iv) Inhibitor can bind with ES complex.
(v) Complex is E-I-S or E-I

(2) Irreversible Inhibition — The inhibitor bind covalently with the enzymes and inactivate them, which is irreversible.

- These inhibitors are usually toxic poisonous substances.

	INHIBITOR	ENZYME INHIBITION
(i)	Aspirin	COX-I and COX-II
(ii)	Diisopropyl Fluorophosphate	Serine proteases, Acetylcholine esterase
(iii)	Iodoacetate	Papain, Glyceraldehyde-3-phosphate dehydrogenase.
(iv)	Penicillin	Serine containing enzymes.

(3) Uncompetitive Inhibition — Inhibitor does not need resemblance with the substrate and it doesn't have any affinity for free enzyme.

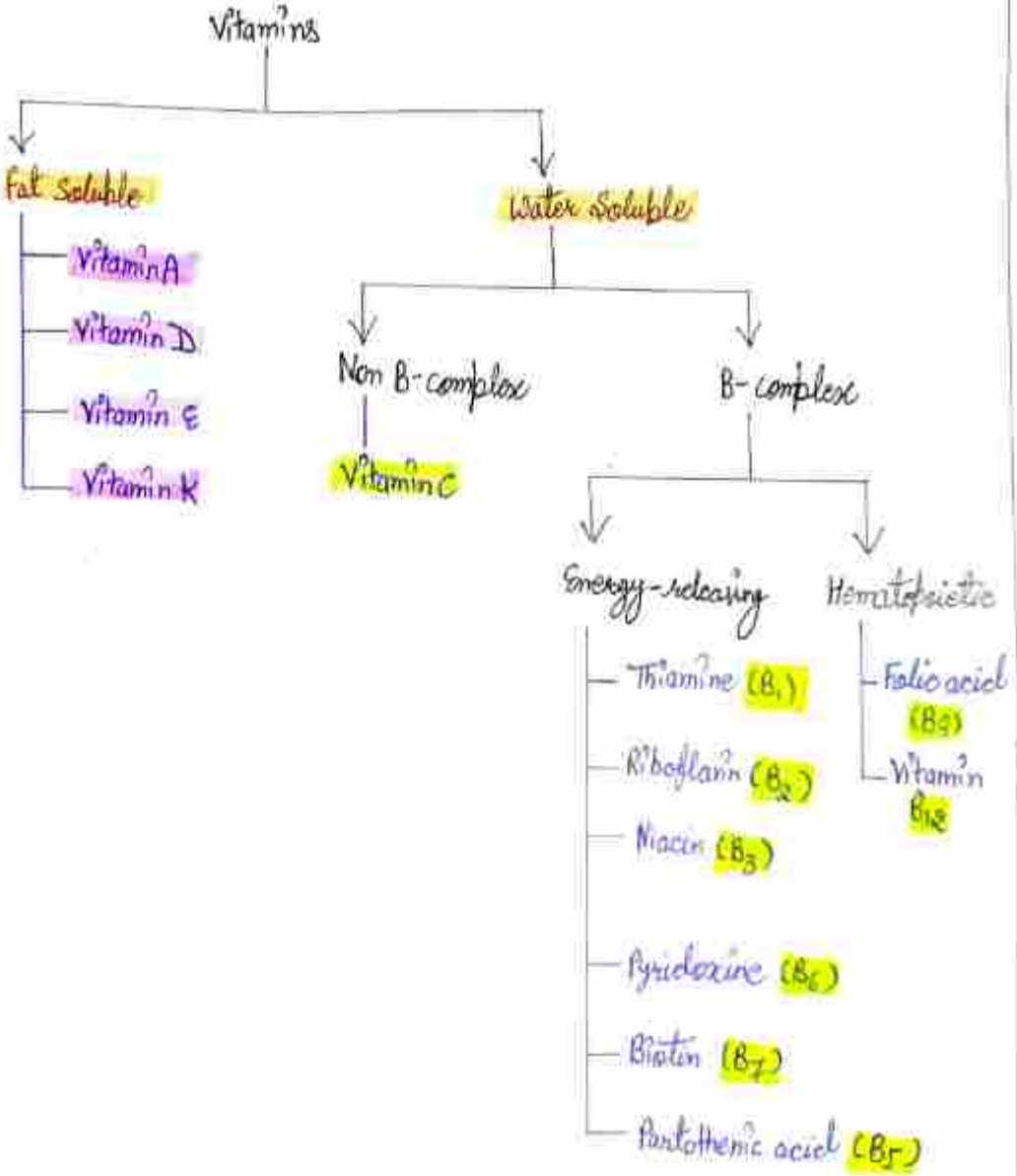
- Inhibitor binds to E-S complex.
- Allosteric inhibitors changes the substrate saturation curve to the right. The presence of activators shift the curve to the left.
- K_m increase but V_{max} is unchanged (Aspartate transcarbamylase and phosphofructokinase).
- K_m or substrate affinity is unchanged but V_{max} is decreased (Acetyl-CoA carboxylase).

Vitamins

Vitamins may be regarded as organic compound required in the diet in small amounts to perform specific biological functions for normal maintenance of optimum growth and health of the organism.

- Generally, vitamins are not synthesized by the body, and need to be supplied through the diet.

Classification of vitamins



Fat Soluble Vitamins

The four vitamins, namely vitamin A, D, E and K are known as fat or lipid soluble.

Their availability in the diet, absorption and transport are associated with fat.

↓

They are soluble in fats and oils and also the fat solvents (alcohol, acetone etc.)

↓

Fat soluble vitamins can be stored in liver and adipose tissue.

Water Soluble Vitamins

The water soluble vitamins are a heterogenous group of compounds, since they differ chemically from each other.

↓

The only common character shared by them is their solubility in water.

↓

Most of these vitamins are readily excreted in urine and they are not toxic to the body.

1. Fat soluble Vitamins

Vitamin A (Retinol, β-Carotene)

i) Source – Fish liver oil, carrots, butter and milk

ii) Function – growth and maintenance of skin, bone development, maintenance of retina & vision

iii) Dietary requirement – 750 µg

iv) Deficiency disease – Xerophthalmia (Hardening of cornea of eye)

v) Coenzyme form – retinal

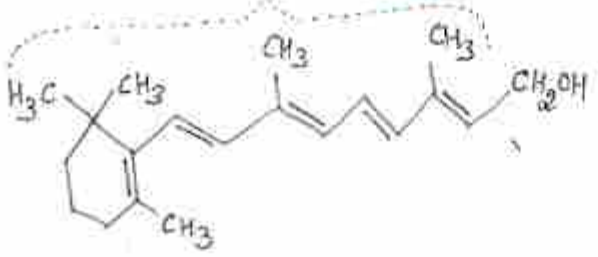
vi) Chemical nature →

- Natural form: A1 (retinol), A2 (3-dehydroretinol)
- Active form: retinol, retinal, retinoic acid
- Provitamin-A: β-carotene

- Vitamin A is a cyclic polyene alcohol which resembles the structure of diterpenoid

- The structure of Vitamin A constitute of a β-ionone ring.
- Four conjugated double bonds in the side chain of Vitamin A.
- They are in trans arrangement
- Synthetic retinol is a trans-isomer. It exists in 8 stereoisomeric forms.
- β-ionone ring and conjugated double bonds are essential for the biological activity of Vitamin A.

β-ionone ring



2. Vitamin D (Cholecalciferol)

i) Source – Exposure to sunlight, fish and egg yolk

ii) Function – Normal growth, Ca and P absorption, maintain serum calcium and Phosphorus level

iii) Dietary requirement – 2.5 µg

iv) Deficiency disease – Rickets, osteomalacia

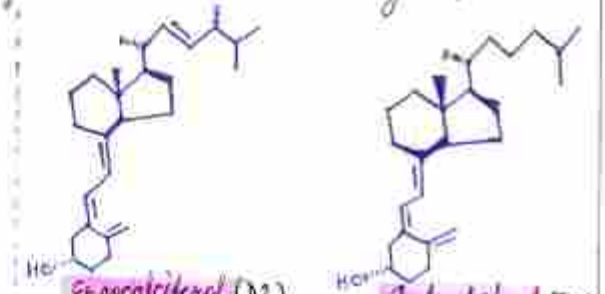
v) Coenzyme form – Nil

vi) Chemical nature – Two main forms of the vitamin D were discovered

a) Ergocalciferol Vitamin D_2 is formed from ergosterol, present in plants.

b) cholecalciferol (Vitamin D_3) is found in animals, and is formed by irradiating the animal sterol, 7-dehydrocholesterol.

- Both the sterols are similar in structure except that ergocalciferol has an additional methyl group and double bond.
- Both these compounds are source for vitamin D activity and are referred to as provitamins.
- The synthesis of vitamin D_3 in the skin is proportional to the exposure to sunlight. Dark skin pigment (melanin) adversely influences the synthesis of vitamin D_3.



Ergocalciferol (D_2) Cholecalciferol (D_3)

3. Vitamin E (Tocopherol)

i) Source – vegetable oils like wheat germ oil, sunflower oil etc.

ii) Function – Antioxidant, maintain muscular metabolism, aids absorption of unsaturated fatty acid

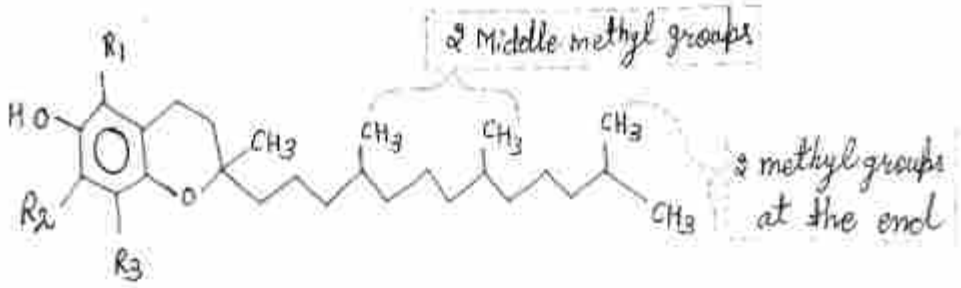
iii) Dietary requirement – 8–10 mg

iv) Deficiency Disease – Increased fragility of RBCs and muscular weakness.

v) Coenzyme form – nil

vi) chemical nature –

- Vitamin E refers to a family of eight molecules having a chromanol ring (chroman ring with an alcoholic hydroxyl group) and a 16-carbon aliphatic side chain containing two methyl groups in the middle and two more methyl groups at the end.
- For the four tocopherols the side chain is saturated, whereas for the four tocotrienols the side chain contains three double-bonds, all of which adjoin a methyl group.
- The four tocopherols and the four tocotrienols have an alpha, beta, gamma and delta form – named on the basis of the number and position of the methyl groups on the chromanol ring.
- The alpha form has three methyl groups, the beta & gamma forms have two methyl groups and the delta for has only one methyl group.



4. Vitamin K (Phylloquinones)

i) Source – green leafy vegetables
ii) Function – Blood clotting mechanism, prothrombin synthesis in liver, electron transport mechanism.
iii) Dietary requirement – 70–140mcg
iv) Deficiency disease – increased blood clotting time
v) coenzyme form – nil
vi) chemical nature – vitamin k family are naphthoquinone derivatives. phylloquinone and menaquinones, both have a long isoprenoid side chain.

The length of the side chain differs - Phylloquinone have a 20C side chain, where as menaquinone have a 30C side chain.

The isoprenoid chain makes these vitamin hydrophobic or lipophilic.

The synthetic vitamin K (menadione, menadiol diacetate) have only hydrogen in place of isoprenoid side chain that makes these vitamin water-soluble

⇒ Water Soluble Vitamins

1 Vitamin B1 (Thiamine)

(i) Source – Yeast, milk, green vegetables and cereals

(ii) Function – growth, appetite, digestion, nerve activity, energy production

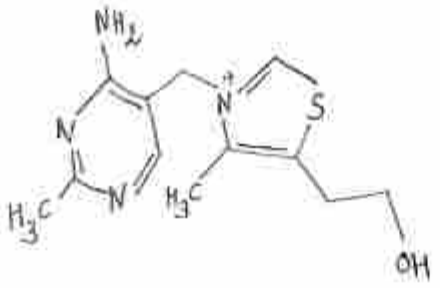
(iii) Dietary requirement – 1-1.5mg

(iv) Deficiency disease – Beri-Beri (loss of appetite, retarded growth)

(v) Coenzyme form – Thiamine Pyrophosphate (TPP)

(vi) Chemical nature – Thiamine consists of a thiazole and pyrimidine ring that are linked through a methylene group.

Thiamine is the only natural compound (apart from penicillin) that contains a thiazole ring.



2. Vitamin B2 (Riboflavin)

(i) Source → Milk, egg white, liver.

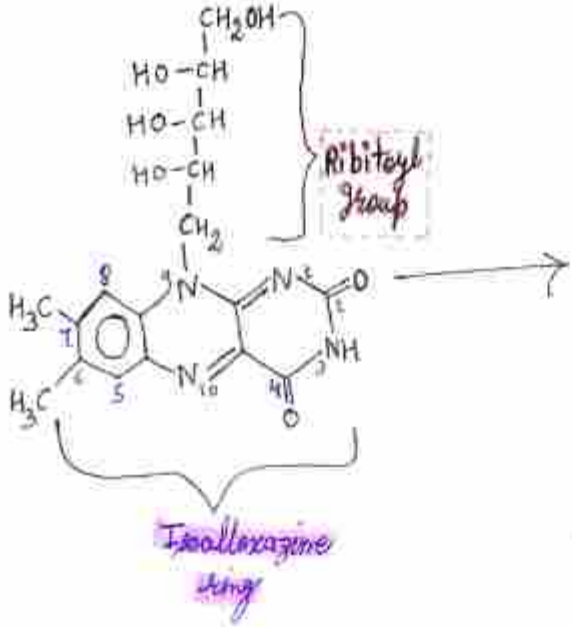
(ii) Function → growth and development of foetus, redox system, maintenance of mucosal, epithelial and eye tissues.

(iii) Dietary requirement → 1.5-2 mg

(iv) Deficiency disease → Cheilosis, digestive disorders and burning sensation of skin.

(v) Coenzyme form → flavin Mononucleotide (FMN), Flavin Adenine dinucleotide (FAD)

(vi) Chemical nature → Stable to heat, oxidation and acid.
→ Light and alkali destroy it.
→ It should be noted that bottled milk (which has large amount of B2) loses a significant amount of B2 if left in the sun light.



The structure of Riboflavin consists of 6,7-dimethyl-isoalloxazine (heterocyclic three ring system) to which a sugar alcohol group called ribitoyl group is attached at 9th position.

3. Vitamin B3 (Niacin)

(i) Source → Yeast, fish, pulses, cereals.

(ii) Function → converting carbohydrates in to glucose, metabolizing fats and proteins and keeping the nervous system working properly.

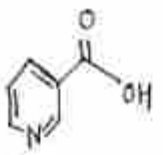
(iii) Dietary requirement → 10-20 mg

(iv) Deficiency disease → Pellagra includes the triad of dermatitis, dementia and diarrhea and can result in death.

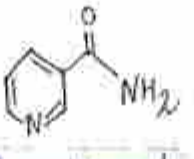
(v) Coenzyme form → Nicotinamide adenine dinucleotide (NAD$^+$), Nicotinamide adenine dinucleotide phosphate (NADP$^+$)

(vi) Chemical nature → Niacin is the generic term for nicotinamide and nicotinic acid

- Also known as Niacinamide / Nicotinamide / Vitamin PP (Pellagra preventive) / Vitamin G (after Goldberger).
- Is an organic compound with the formula $C_6H_5NO_2$
- Simple derivative of pyridine.
- Pyridine 3 carboxylic acid.
- White crystalline substance
- Water soluble.
- Resistant to heat, oxidation and alkalis.
- Nicotinamide is an important component of NAD and NADP.
- Niacin has a carboxyl group (COOH) at the 3-position, whereas in Nicotinamide the carboxyl group is replaced by a carboxamide group ($CONH_2$).



Niacin



Nicotinamide

4. Vitamin B5 (Pantothenic acid)

(i) Source – liver, yeast, egg yolk, mushroom, avocados.

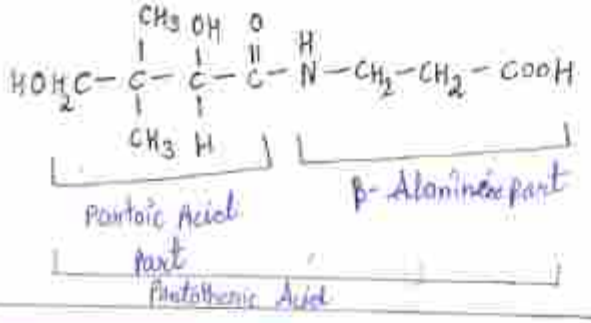
(ii) Function – breakdown of fats and carbohydrates for energy, manufacture of red blood cells, sex and stress related hormones produced in the adrenal glands.

(iii) Dietary requirement – 6 mg

(iv) Deficiency disease – paresthesia, dermatitis and adrenal insufficiency.

(v) Coenzyme form – Co-enzyme A

(vi) Chemical nature – Pantothenic acid is formed from β-alanine and Pantoic acid joined by a peptide bond.



5. Vitamin B6 (Pyridoxine)

(i) Source – yeast, milk, egg yolk, cereals and grams

(ii) Function – growth, protein, CHO and lipid metabolism, coenzyme in amino acid metabolism.

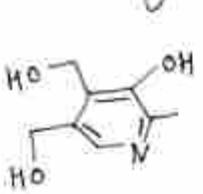
(iii) Dietary requirement – 1–2 mg

(iv) Deficiency disease – convulsions

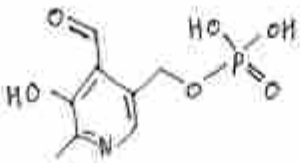
(v) Coenzyme form – Pyridoxal phosphate

(vi) chemical nature – Vitamin B_6 is used to collectively represent the three compounds namely pyridoxine, pyridoxal and pyridoxamine (the vitamers of B_6).

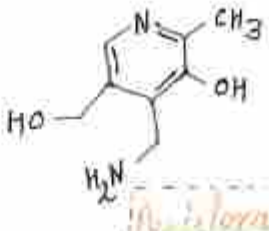
Chemistry – Vitamine B6 compounds are pyridine derivatives.



Pyridoxine



Pyridoxal PO_4



Pyridoxamine

They differ from each other in the structure of a functional group attached to 4th carbon in the pyridine ring. Pyridoxine is a primary alcohol, Pyridoxal is an aldehyde form while pyridoxamine is an amine.

Pyridoxamine is mostly present in plants while pyridoxal and pyridoxamine are found in animal foods.

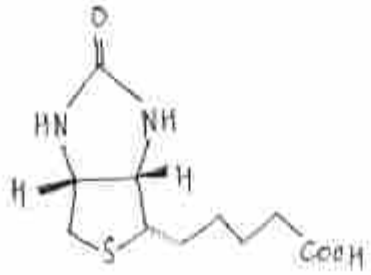
Pyridoxine can be converted to pyridoxal and pyridoxamine, but the latter two cannot form pyridoxine.

6. Vitamin B7 (Biotin or Vit-H)

(i) Source – egg, nuts, aracado, sweet potato
(ii) Function – metabolize carbohydrates, fats and amino acids.
(iii) Dietary requirement – 100–300 µg
(iv) Deficiency disease – Dermatitis and conjuctivitis
(v) coenzyme form – Biotin carboxyl carrier protein
(vi) Chemical nature – Biotin (formally known as anti-egg white injury factor) is a sulfur containing B-complex vitamin. It directly participates as a coenzyme in the carboxylation reactions.

Chemistry

Biotin is a heterocyclic sulfur containing monocarboxylic acid.



The structure is formed by fusion of imidazole and thiophene rings with a Valeric acid side chain. Biotin is co-valently bound to amino group of lysine to form biocytin in the enzymes. Biocytin may be regarded as the coenzyme of biotin.

7. Vitamin B9 (Folic acid)

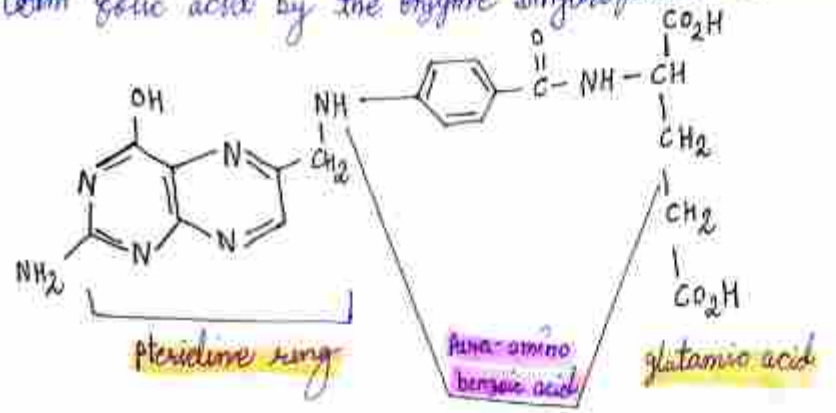
i) Source – Dark green vegetables, beans, pea nut, sunflower seed
ii) Function – formation of RBC
iii) Dietary requirement – 200 gm
iv) Deficiency disease – Megaloblastic anemia and diarrhea
v) coenzyme form – Tetrahydrofolic acid.
vi) Chemical nature – Folic acid is abundantly found in green leafy vegetables. It is important for one carbon metabolism and

is required for the synthesis of certain amino acids, purines, pyrimidine and thymine.

Chemistry

Folic acid consists of three components pteridine ring, p-amino benzoic acid (PABA) and glutamic acid (1 to 7 residues). Folic acid mostly has one glutamic acid residue and is known as pteroyl-glutamic acid (PGA).

The active form of folic acid is tetrahydrofolate (THF or FH4). It is synthesized from folic acid by the enzyme Dihydrofolate reductase.



8) Vitamin B12 (Cobalamin)

(i) Source – Meat, fish, egg and curd

(ii) Function – development, myelination and function of the central nervous system, healthy red blood cell formation and DNA synthesis.

(iii) Dietary requirement – 2-3 gm

(iv) Deficiency disease – Pernicious anemia

(v) Coenzyme form – Cobamide

(vi) Chemical nature – Vitamin B12 is also called cobalamin, cyanocobalamin and hydroxy cobalamin.

- It is built from:
  1. A nucleotide
  2. A complex tetrapyrrol ring structure (corrin ring)
  3. A cobalt ion in the center
  4. A R-group
- When R is cyanide (CN), vitamin B12 takes the form of cyanocobalamin
- In hydroxycobalamin, R equals the hydroxyl group (-OH)
- In the coenzyme form of vitamin B12,

- R equals an adenosyl group in adenosylcobalamin.
- R equals a methyl ($-CH_3$) group in methylcobalamin.

- Vitamin B_{12} is synthesized exclusively by micro-organisms (bacteria, fungi, and algae) and not by animals and is found in the liver of animals

↓

bound to protein as methylcobalamin or 5'-deoxyadenosylcobalamin.

9. Vitamin C (Ascorbic acid)

(i) Source – citrus fruit, amla and green leafy vegetables.

(ii) Function – Absorption of iron, antioxidants, growth, wound healing, formation of cartilage, dentine bone and teeth.

(iii) Dietary requirement – 30-45mg

(iv) Deficiency disease – Scurvy (bleeding gums)

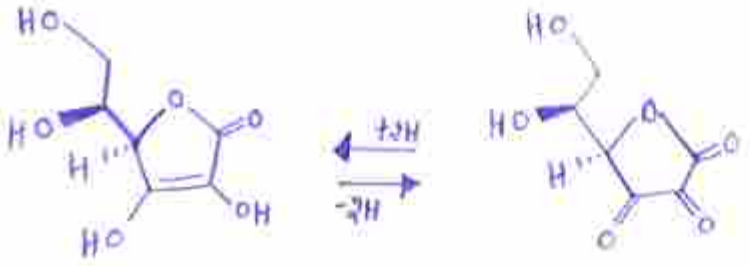
(v) Coenzyme form – Ascorbate

(vi) Chemical nature – Chemically it is known as ascorbic acid.

- Ascorbic acid is hexose derivatives and closely resembles monosaccharides in structure.
- It exists in two forms

  L-Ascorbic acid (Reduced form)

  L-dehydroascorbic acid (oxidized form)
- The acidic property of vitamin C is due to enolic hydroxyl group
- L-ascorbic acid undergoes oxidation to form dehydroascorbic acid and it is reversible reaction.



L-ascorbic acid

L-dehydroascorbic acid

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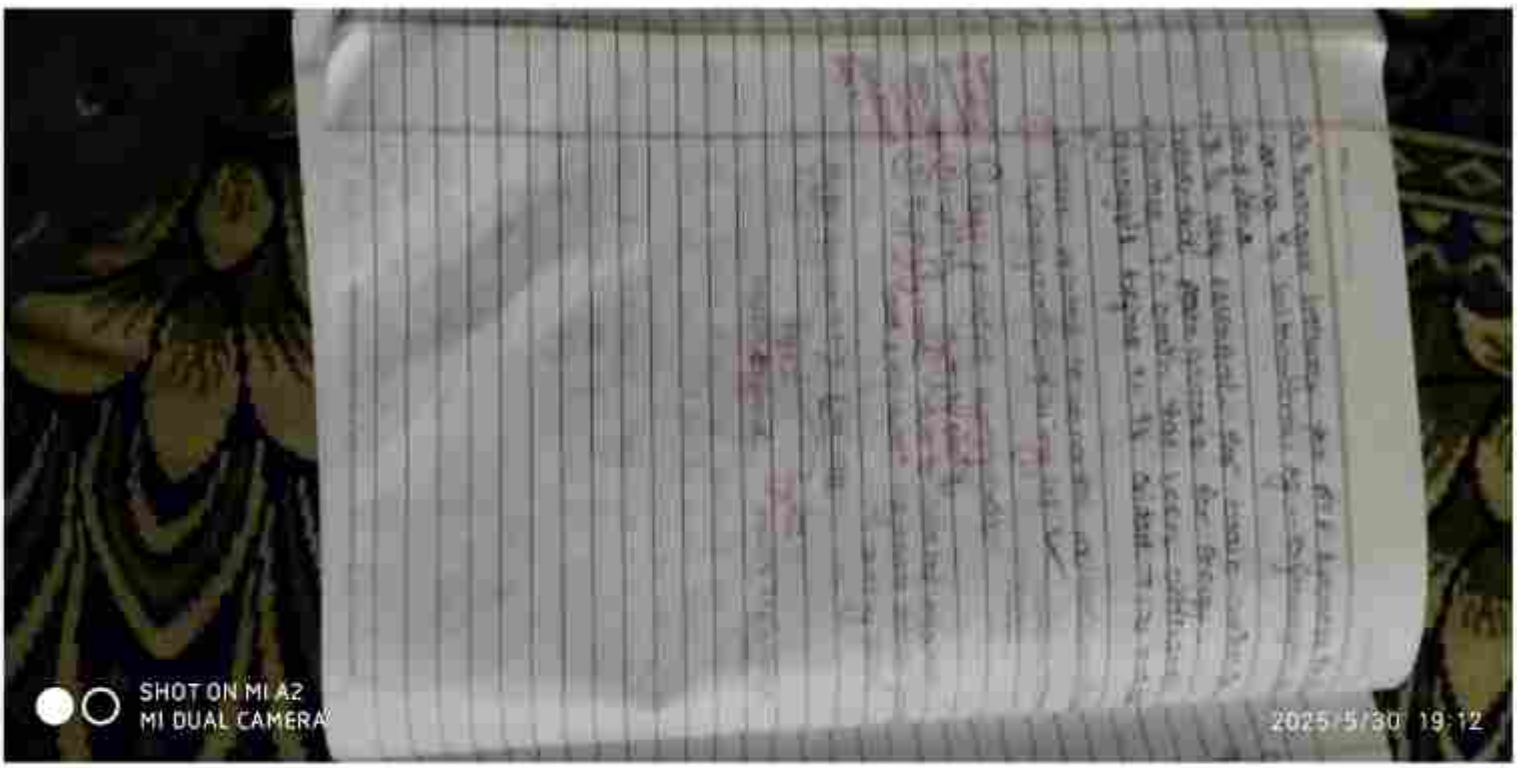
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Production of ATP in Glycolysis

Molecules of ATP utilised

Glucose ——→ Glucose-6-phosphate (1)

Fructose-6-phosphate ——→ Fructose 1-6-diphosphate (1)

Molecules of ATP synthesized

Glyceraldehyde-3-Phosphate → 1-3 bisphosphoglycerate — 6

1-3-diphosphoglycerate → 3-phosphoglycerate — 2

Phosphoenolpyruvate → Enol pyruvate — 2

Total ATP

No of ATP synthesized = 10

No of ATP utilised = 2

8

Krebs Cycle

- The citric acid cycle (Krebs cycle or Tricarboxylic ...) is the most imp. metabolic pathway energy supply to the body about 65 to 70% of ATP is synthesis in the Krebs cycle [illegible]

- Citric acid involve the acetyl CoA or [illegible]

→ **History**

The citric acid cycle (TCA) cycle was purpose by Hans Adolf Krebs in 1937 based on the study of conjuption of pigeon breast muscle.
The cycle is name is awared Nobel prize physiology in 1953

Location of TCA — The enzyme of TCA cycle located in mitochondria [illegible] involved Respiration in the electron transport chain
Synthesis of ATP was by phosphorylation [illegible] and [illegible]

- **Rxn of TCA cycle** — Oxidative D-carboxylation of pyruvate to AcCoA by pyruvate de-hydrogenase complex.

Step-1 — formation of citrate — Krebs cycle the condention of AcCoA or Oxaloacetate catalyze by enzyme synthese.

Step-2,3 — Citrate is isomerise to isocitrate by the enzyme aconitase this is achieved in 2 stage rxn of [illegible] followed by hydration to the formation isocitrate

Step 4,5 – Formation of α-ketoglutarate. The enzyme isocitrate dehydrogenase (ICD) catalysed the conversion (oxidative decarboxylation) of isocitrate to oxalosuccinate and then to α-ketoglutarate.

6 – The formation NADH and liberation of CO₂ α-ketoglutarate → Succinyl CoA occurs through decarboxylation.
This enzyme is dependent on five cofactors TPP (Thymine pyrophosphate), lipoic acid, FAD and CoA.
The mechanism of the rxn conversion of pyruvate → AcCoA.

Step-7 – Formation of succinate
Succinate CoA → Succinate by Succinate thiokinase. This rxn are coupled with phosphorylation the GDP to GTP or ADP to ATP
Guanosine Diphosphate
This is as substrate level phosphorylation. Two isoenzyme CoA of succinate thiokinase has been identified one dependent GDP is found in tissue like liver & kidney where gluconeogenic appears occurs. The 2nd isoenzyme ADP dependent in non-gluconeogenic tissue (brain and heart)

Conversion of succinate to fumarate – It is catalysed by succinate dehydrogenase to production of fumarate this rxn result in the production of $FADH_2$ and not NADH.
Formation of malate – The enzyme fumarase catalysed the conversion of fumarate to malate with the addition of water.

Urea Synthesis Urea

Urea cycle is 1st discovered by Hans Krebs and Kurt Henseleit (Cycle) ornithine cycle

It has amino group derived from ammonia and 2 from aspartate.
Carbon is supplied by CO_2

It is a 5 step cyclic process and 5 enzymes

- Two enzyme is present in mitochondria rest in Cytosol
- Urea is the end product of protein

- The nitrogen of amino acid converted to Ammonia is toxic to the body it is converted to urea and detoxified.
- Urea covers 80-90% of nitrogen containing pubs excreted in the form of Urine
- Urea is synthesis in liver and transported into the kidney and excreted in urine
- It is 1st metabolic cycle that was explained by Hans Krebs and Kurt Henseleit ornithine cycle.

- Disorders of aromatic metabolism
(1) phenylketonuria :- Deficiency of phenylalanine hydroxylase
(2) Albinism → tyrosinase
(3) Tyrosinemia →
(4) Jaundice

- phenylketonuria :- It is the most common disorder. It is due to the deficiency of hepatic enzyme. Phenylalanine hydroxylase caused by autosomal recessive gene. The enzyme deficiency impair the synthesis of tetrahydrobiopterin req. for the hydroxylation of phenylalanine. The net outcome PKU is phenylalanine is not converted to tyrosine.
- The name phenylketonuria is defined due to the fact that the metabolite phenylpyruvate is a keto acid. ($C_6H_5CH_2COCOO^-$)

Clinical and biochemical manifestation of PKU
- disturbs metabolism of phenylalanine resulting in the accumulation of phenylalanine and its metabolites in the body tissue. many clinical and biochemical manifestation

(1) Effect of CNS :- mental retardation failure to walk or talk, failure of growth seizures & tremors are findings in CNS

(2) effect on Pigmentation
Melanin. It is treatment synthesized from tyrosine by tyrosinase.

Diagnosis
mostly detect by the newborn baby for the presence level of phenylalanine (PKU) 20-65 mg/dl, abnormal is 2 mg/dl

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Disease Related to abnormal metabolism of carbonydrates.

① Hypoglycemia

Types

1 &

Post prandial hypoglycemia

Fasting hypoglycemia

② Galactosemia → hypoglycemia

Type I

Type-I

Type-II

(3) Lactose Intolerance :- It is also called lactase deficiency or hypolactasia.
It is the inability to digest lactose, the sugar found in the milk.
[illegible]. Intolerance does not make enough of digestive enzyme called lactase. It is in your food breakdown the lactose in food.

Symptoms :- Abdominal bloating or cramps, vomiting, diarrhoea and [illegible]

(4) Fructose Intolerance :- When body cannot digest or absorb fructose well.
Hereditary fructose intolerance is rare serious genetic disorder. This is when body is not able to help the body breakdown fructose.

(5) Galactosemia - It is condition in which body is unable to use a sugar.
It is a part of main sugar found in milk which is called lactose.
It is a problem for children because it is found in breast milk and baby formulas.
Classic galactosemia occurs when enzyme Galactose-1 phosphate uridyl transferase is missing or not functioning.

Glycogen storage disease is a genetic in which abnormal quantities of glycogen.
It is a genetic one abnormal deposits in heart, kidney and muscle.
This is due to enzyme deficiency.

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(8) Fatty liver :- The normal conc. of lipid in liver around 5%.
- Liver is not a storage organ for fat, unlike adipose tissue. However, in certain conditions lipid especially triglyceride accumulate excessively in liver resulting in fatty liver.
- In the normal liver, hepatic cell contain lipids in the form of droplets. In fatty liver droplet of triglyceride are found in entire cytoplasm of hepatic cells. This cause impairment of

*- Fatty acid is associated with fibrotic changes and cirrhosis, the many cause
(1) ↑ synthesis of triglyceride
(2) Impairment in glycoprotein synthesis

(1) - Mobilization of free fatty acids from adipose tissue and their influx into liver is much greater than oxidation. This leads to accumulation in liver. Diabetes mellitus, starvation, alcoholism & high fat diet are associated with ↑ mobilization of fatty acids.
- Alcohol also indicate fatty acid & promote fat synthesis and its deposition.

(2) Synthesis of VLDL actually take place in liver. VLDL formation req. phospholipid and
Fatty acid is caused by impaired in glycoprotein synthesis, may be due to a defect in phospholipid synthesis.

a [illegible] in [illegible] cytosol
(3) A failure of the [illegible] [illegible] of [illegible]

Hypercholesterolemia

↑ plasma conc. (> 200 mg/dl) can known as hypercholesterolemia.

Observed in (1) Diabetes mellitus — due to ↑ cholesterol synthesis since the availability of acetyl CoA is ↑

(2) Hypothyroidism — Dec. [illegible] receptors on hepatocytes.

(3) Obstructive Jaundice — Due to the obstruction in the excretion of cholesterol through bile

(4) Nephrotic Syndrome — ↑ in plasma globulin conc. is the characteristic feature of nephrotic syndrome. Cholesterol elevation is due ↑ in plasma lipoprotein fraction in this disorder.

Bad cholesterol & Good cholesterol:

Cholesterol is a natural molecule performing a wide range of function (membrane [illegible], precursor for steroid, bile acid)

These good & bad cholesterol are the [illegible] state are still in use.
The cholesterol in HDL [illegible] [illegible] considered that due to ↑ in LDL [illegible] complication in atherosclerosis and related complications, then the LDL [illegible] be regarded as [illegible] dangerous [illegible].

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Minerals

Minerals are chemical elements essential for life and are part of the four essential nutrient groups.

Major minerals — calcium, phosphorus, potassium, sodium and magnesium.

Trace elements → iron, chlorine, cobalt, copper, zinc, manganese, iodine and selenium.

minerals constitute about 8% body weight

it is essential for a number of metabolic processes like blood coagulation, muscle contraction and enzyme action.

Minerals are not energy source in the body but they are necessary for the maintenance of normal biochemical conditions in the body

Functions of minerals

They maintain an acid-base balance.
They regulate body fluids.
They help in muscle contraction.
They maintain osmotic pressure and cell permeability
They act as co-factor in enzymatic reactions

Types of Minerals.

Based on body requirements minerals are divided into two groups:

(i) Macrominerals
(ii) Microminerals.

(i) Macrominerals → it constitutes 60-80% of body's inorganic material
There requirement in body is greater than 100mg/day

They include seven elements:

1. calcium
2. Magnesium
3. Sodium
4. Potassium
5. phosphorus
6. Sulphur
7. Chlorine.

Microminerals

→ requirement in body → less than 100mg/day
→ include 10 elements

⇒ Further classified in to three types:

- Essential trace elements → iron, iodine, zinc, copper, cobalt
- Possibly essential trace elements → Silicon, nickel, tin.
- non-essential trace elements → Aluminium, cadmium, arsenic, lead and Mercury

MACROMINERALS

1 Calcium

Sources – Milk, Yogurt, cheese, fruits, nuts, Green leafy vegetables.

Normal plasma calcium level → 9-11 mg/dl.

Functions → bones and teeth formation, Muscle contraction, blood clotting, heart rhythm and nerve function.

Deficiency disease → Hypocalcemia, osteopenia, osteoporosis, Rickets.

Dietary Requirement → Adult males – 800mg/day
Woman during pregnancy, lactation – 1.5g/day
children – 0.8 – 1.2 g/day.

2. Phosphorus →

Sources → Red meat, milk, seafood and legumes, cereals, green leafy vegetables and eggs

Normal Serum phosphate level → whole blood – 40mg/dl.
Serum – 4mg/dl

Functions → Bone formation, energy production, DNA/RNA synthesis.

Deficiency disease → Hypophosphatemia, weakness, bone pain, loss of appetite.

Dietary requirement → 800mg/day

③ Potassium

⇒ Potassium an essential nutrient mineral, is major intracellular cation required by all body tissues.

⇒ It act as an electrolyte because it carries small electrical charge that activates various cell and nerve function.

⇒ Sources :- Leafy green vegetables, beans, nuts, dairy foods and rich sources Dry fruits, Beans and potato

Normal plasma level → 3.4 - 5.0 mg/dl

⇒ Functions

(i) It maintains acid-base balance.
(ii) It helps in maintaining the normal levels of fluids inside our cell.
(iii) It activates various cell and nerve function.
(iv) cardiac function.

Deficiency diseases

1. Hypokalemia ⇒ Deficiency of potassium leads to hypokalemia.
   causes ⇒ vomiting, diarrhoea,
2. Hyperkalemia ⇒ increase in serum potassium level is hyperkalemia
   It occurs in ⇒ Renal failure, Severe dehydration,
   Excess administration of intravenous potassium.

Symptoms of hyperkalemia :- ⇒ bradycardia, depression, mental confusion, and muscle weakness.

Dietary requirement ⇒ 3-4g / day

④ Magnesium

⇒ magnesium is naturally present in a variety of foods, available as a supplement, and ingredient in antacid and laxatives.

⇒ Adult body contain 25g magnesium, 70% found in bones in combination with calcium and phosphorus.

Sources — Cereals, Green leafy vegetables, Nuts and legumes

Normal serum level — 2-3 mg/dl

Functions → It act as a cofactor for a number of enzymes
eg Hexokinase, glucokinase,
- It is important constituents of bones and teeth.

Deficiency disease → mg deficiency leads to convulsions, neuromuscular irritation and weakness.
Rickets.

Dietary requirement → Adult man – 350mg/day
" female – 300mg/day.

(5) Sulphur
↓
Essential element, present in all the cells of the body.
→ Protein contains 1% of sulphur.

Sources
Egg, Fish and chicken
nuts, seeds grains and legumes.
walnut, oats

Functions → (1) It is constituent of sulphur containing amino acids.
Thiamine, biotin, coenzyme A contains sulfur.
formation of proteins like Keratins.
Sulfur is constituent of vitamins like biotin.

(6) Sodium → It is the principal cation of extracellular fluid.
→ Present in body as NaCl and $NaHCO_3$
→ Normal plasma level – 135-145 mEq/l
→ Functions → Acid-base balance, nerve transmission, muscle function, Maintains heartbeat, Maintain cell permeability.

→ Deficiency disease → Hyponatremia (Low sodium), confusion, Seizures.

→ Dietary Requirement →
Normal individual → 5-10g/day
Person with hypertension → 1g/day

(7) Chlorine

- Present in body as chloride ion.
- It has wide distribution in the body.
- Highest concentration is present in cerebro spinal fluid (CSF).

⇒ Sources → Table salt is main source of chlorine.

⇒ Normal Serum level → 95-105 meq/L.

⇒ Functions → (i) It regulates osmotic pressure of body.
(ii) Maintain normal pH level.
(iii) Stimulates nerve function
(iv) Maintains acid base balance
(v) Necessary for HCl secretion in gastric juice.

⇒ Deficiency disease → Hypochloremia

⇒ Dietary Requirement → 5-10g/day.

MICROMINERALS

(1) Zinc → Total zinc content of human body is 2g
Most of zinc is stored in skeletal muscle and bones.
Sources → Milk, eggs, cereals, pulses etc.
Normal Serum level — 100mg/dl.
Functions → Immune function, wound healing, DNA synthesis, taste & smell.
Deficiency disease → Anemia, loss of taste and sensation, growth retardation.
Dietary requirement → For normal adult — 10-15mg/day
In case of pregnancy its requirement increases

Iron

- Iron is most important trace elements that is required for maintenance of normal blood.
- It is most common nutritional deficiency in women.
- 70% of it is present in erythrocytes as hemoglobin.

Sources → organ meat (liver, spleen, heart).
→ leafy vegetables, legumes, Jaggery

Functions → major component of haemoglobin that carries oxygen throughout the body.

Deficiency disease → Iron deficiency anemia, fatigue, pale skin.

Dietary requirements → 10-15 mg /day

Copper

Adult human body contain 100mg of copper

Sources → whole grains
Beans and nuts
Organ meat
Shell fish

Normal serum level – 100 - 200 mg /dl

Functions → important constituents of certain enzymes [cytochrome oxidase, catalase, phenol oxidase etc.)
→ important for haemoglobin synthesis

Deficiency disease → Hypopigmentation of the skin.
Greying of the hair, anemia
Fragility of arteries, and myocardial fibrosis.

Menkes Disease → disorder result from a malfunction in the intestinal absorption of copper.

Dietary requirement
Adult – 2-3 mg / day
children – 0.5 – 2 mg / day.

Cobalt

Cobalt is crucial component of vitamin B_{12}.
Its total body content is 1.5 mg approx.

Sources

Fish and nuts
Green leafy vegetables, such as broccoli and spinach.
cereals, such as oats.

Functions → required for activation of certain enzymes like methyl tetrahydrofolate, methyl transferase etc.

⇒ important constituents of vitamin B_{12}.

Deficiency disease

(1) Pernicious anaemia → It is a type of autoimmune disorder

↓

intrinsic factor responsible for vit B_{12} absorption in intestine is destroyed by autoantibodies.

⇒ long-term cobalt administration causes polycythemia, or the red blood cells in the blood, which is toxic.

⇒ Dietary requirement

0.8 mg/day

Water and Electrolytes

⇒ Distribution and function of water in the body

- Introduction → water also known as solvent of life, is the fundamental elements of the human body, distributed throughout the body.
- The human body is composed of 60% water, which is essential for living and without which the body cannot function properly.

⇒ water Importance in human body :→

- Makes up 60% of total body weight.
- Vital for survival.
- Building block of cells, and body fluids.
- Serves as reactant, solvent and reaction medium.
- Transports nutrients and aids in urine waste removal.
- Essential for sweat evaporation to regulate body temperature.

⇒ Distribution of water

- Water the major component of body constitutes about 60% water.

  Men - 55 - 70%

  Women - 45 - 60%

- Normal human of average weight 70 kg contains 42 liters of water distributed throughout the body in both intracellular and extracellular compartments.

⇒ Functions of water

water provides aqueous medium to the organism which is essential for various biochemical reactions to occur.

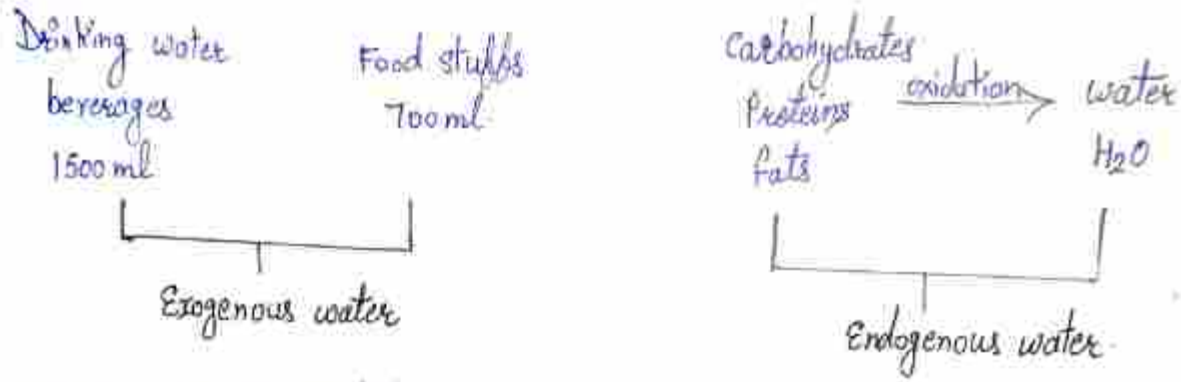
- It is a vital component of living cell.
- Regulate body temperature.
- Provides aqueous medium essential for various biochemical reactions.
- Act as a vehicle for physiological procedures (like absorption and transport).

Water turnover and balance

- Water homeostasis is essential for healthy living. Body water turnover, means replacement of body water that is lost in a given period of time.
- It depends upon the amount of water taken in and amount of water discharged from the body.

Water intake

Water intake - 2,500 ml



⇒ Most of water is ingested in our body through oral route. Source of water can be both exogenous and endogenous.

⇒ ingestion of water is controlled by thirst center located in hypothalamus.

(i) Exogenous water
→ 0.5 - 5 litres of water per day is ingested externally.
→ water ingested from outer source (drinking water, beverages, water content of solid foods etc.)

(ii) Endogenous water - 300-350 ml of water per day is achieved endogenously.
→ It constitutes metabolic water
→ Derived from oxidation of food stuffs water obtained from each gram oxidation of food product
a) carbohydrate - 0.6 ml water
b) Protein - 0.4 ml
c) Fats - 1.1 ml

Water output ⇒ water mainly gets eliminated from our body through four distinct routes.
(i) Urine
(ii) Skin
(iii) Lungs
(iv) Feces

ELECTROLYTES

- Electrolytes are minerals in the body that have an electric charge.
- They are present in blood, urine, tissues and other body fluids.
- Electrolytes are important because they help to balance the amount of water in our body.
- compounds which dissociate in solution and exist as ions, ie positively and negatively charged particles.

The concentration of electrolytes are expressed as milliequivalents per liter (mEq/l) rather than milligrams.

- Electrolytes are important because they help:

(i) Balance the amount of water in body.
(ii) Maintain body's acid-base balance.
(iii) Move nutrients in to cells.
(iv) Move waste out of cells.
(v) Support muscle and nerve function
(vi) Keep heart rate and rhythm steady.
(vii) Keep blood pressure stable.
(viii) Keep bones and teeth healthy.

- Composition of Electrolytes in body fluids

Main components of electrolytes are:

⇒ Potasium, main intracellular cation :→ helps in proper functioning of cells, heart, and muscles.
⇒ Sodium, main extracellular cation :→ controls the amount of fluid in the body and helps your nerves and muscles to work properly.
⇒ Calcium :→ make and keep bones & teeth strong.
⇒ Magnesium :→ helps muscles, nerves and heart work properly also control blood pressure and blood glucose.

⇒ Bicarbonate, maintain the body's acid and base balance (pH).

⇒ chloride, main extracellular anion → It controls the amount of fluid in the body and helps maintain healthy blood volume and blood pressure.

⇒ phosphate → in corporation with calcium builds strong bones and teeth.

- The concentration of molecules in relation to the osmotic pressure can be expressed in two ways :-

  Osmolarity : The number of moles/litre of solution is termed as osmolarity.

  Osmolality : The number of moles/kg of solvent is termed as osmolality.

- Osmolarity and osmolarity are similar in pure water solvents, but osmolality is more commonly used in biological fluids containing molecules like proteins.
- osmolality is about 6% greater than osmolarity.

⇒ Plasma Osmolality

- Presence of solute particles in fluid medium is measured by using osmolality.
- plasma osmolality ranges b/w 285 – 295 mOsm/kg.
- Sodium and its associated anions contribute approximately 90% to plasma osmolality.
- Osmolality is typically measured using an osmometer.

Dietary Intake of Electrolytes →

Main food source of electrolytes are fruits and vegetables. A common source of sodium and chloride is table salt.

Sodium → Pickled foods, cheese and table salt
Chloride → Table salt.
Potassium → Bananas, avocado and sweet potato
Magnesium → Dry fruits, beans.
Calcium → Dairy product, green leafy vegetables.
Bicarbonates → Breads and cookies.
Phosphates → Sea food, legumes and nuts.

⇒ Electrolyte Balance

The kidneys are primarily responsible for regulating the balance of water and electrolytes.

The majority of the regulation is fulfilled through the production of renin-angiotensin, aldosterone and ADH.

- Aldosterone
  Aldosterone, a mineralocorticoid produced by the adrenal cortex, increase Na^+ reabsorption by renal tubules, resulting in the retention of Na in the body
- Anti-Diuretic hormones (ADH)
  plasma osmolarity increase leads to stimulation of hypothalamus that leads to ADH release.
  ADH increases water reabsorption by renal tubules.
- Renin - angiotensin — The renin-angiotensin system regulates aldosterone secretion, with renin acting on angiotensinogen to produce angiotensin I, which is converted in to angiotensin II and at last in to aldosterone, maintaining normal fluid and electrolyte balance.
- Atrial - natriuretic factor (ANF)
  ANF, a 28 - amino acid peptide - is produced in the heart's atrium to regulate blood pressure, blood volume, and sodium excretion, offering renin and aldosterone action.

Enzyme	Disease	Function
① L-glutaminase	Acute lymphoblastic leukemia	Blocking glutamine uptake, cell death due to starvation.
② L-asparaginase	Acute lymphoblastic leukemia	Depletion of nutrient asparagine leads to tumor apoptosis
③ caspase	cancer	Regulates death of cancerous cell via apoptosis.
④ Lipase	cardiovascular diseases	Lower the level of triglycerides
⑤ Strepto Kinase	coagulation and thrombosis	Activates plasminogen.

Dehydration

Dehydration is a condition in which there is excessive loss of fluids or electrolytes in comparison to its intake which leads to negative water balance of our body.

Sym and symptoms Mild – Thirst, Dry mouth, less urine, weight loss,
Severe – coma, Rapid weak pulse
Moderate → Sunken eye, loss of skin elasticity

cause of dehydration → Excessive water loss due to vomiting, diarrhoea and sweating
→ excessive fluid loss due to burn
→ kidney disorders and diabetic insipidus.

oral Rehydration therapy

↓
is a type of fluid replacement therapy used to overcome dehydration
→ used in the treatment of diarrhoea and excessive fluid loss.

oral Rehydration Salts (ORS)

- ORS is most commonly used therapy for excessive water loss i.e dehydration.
- It is a combination of sodium chloride, potassium chloride, sodium citrate and glucose.
- Electral powder is the most common marketed ORS product.

→ old constituents of ORS:

- Sodium chloride – 2.5g
- Potassium chloride – 1.5g
- Trisodium citrate dehydrate – 2.9g
- Glucose anhydrous – 20g

⇒ New formula of ORS is tabulated below

Oral Rehydration Salts (WHO)

content	concentration
Nacl - 2.6g	Na^+ - 75 mM
Kcl - 1.5g	K^+ - 20 mM
Trisodium citrate - 2.9g	Cl^- - 65 mM
Glucose - 13.5g	citrate - 10 mM
water - 1 litre	Glucose - 75 mM

Total Osmolarity - 245 mOsm/litre

Introduction to Biotechnology.

Biotechnology is a branch of science in which using biology (living cell or bacteria or any part of them) and technology or scientific process, a new product is developed to improve human health and environment.

Biotechnology is also called "biotech"

There are many subfield of biotechnology, the main subfield are these:

1. Medical (red) biotechnology
2. Agriculture (green) biotechnology
3. Industrial (white) biotechnology
4. Marine (blue) biotechnology.

1) Medical biotechnology - It is used for medicinal purpose for example gene therapy is used to treat genetic or acquired disease like cancer this therapy utilize normal genes for replacing the defective gene.

2) Agriculture (green) biotechnology :- This is releated to agriculture product and processes for example one or two genes are combined together and developed a new variety of crop for increasing the yield.

3) Industrial biotechnology - This is releated to industrial process for example replacing gene of a microorganism a new organism id developed to ~~product~~ produce a useful chemical.

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(4) Marine biotechnology - this field involve in Marine resources to develop a novel pharmaceutical, drugs, chemicals product, enzyme or other industrial product.

Chapter - 12
Organ Function Tests

→ Organ function tests are the biochemical tests carried out to assess whether particular organ is functioning normally or not.

Renal Function Test (RFT)

→ Kidneys are the organ that filter waste products from the blood. The functional unit of kidney is nephron. Renal function may be assessed by measuring blood urea and serum creatinine.

Functions of kidney -

1) The most important function of the kidney is to filter the blood for urine formation.
2) It excretes metabolic wastes like urea and uric acid into the urine.
3) It secretes a number of hormones and enzymes such as
   a) Erythropoietin :- It is released in response to hypoxia.
   b) Renin - It controls the BP by regulation of angiotensin and aldosterone.
   c) Calcitriol - It helps in the absorption of Calcium in the intestines.
4) It maintains the acid-base balance of the body by reabsorbing bicarbonate from urine and excreting H^+ ions and acid ions into urine.
5) It also maintains the H_2O and salt levels of the body by working together with the pituitary gland thereby maintaining homeostasis.

Renal function Test (RFT)

RFT Tests are done
- to assess the functional capacity of kidney
- Early detection of possible renal impairment
- Severity and progression of the impairment
- Monitor response to treatment
- Monitor the safe and effective use of drugs which are excreted in the Urine

When should we assess Renal function -

- Older age
- Family history of chronic kidney Disease (CKD)
- Low birth weight
- DM
- HT
- Autoimmune disease
- Systemic infections
- UTI
- Drug toxicity.

Renal Functions tests are divided into the following :-

- Urine analysis
- Blood examination
- Glomerular Function test
- Tubular Function test

1) Urine analysis

→ Urine examination is an extremely valuable and most easily performed test for evaluation of renal functions. It includes physical or macroscopic examination, chemical and microscopic examination of the sediment.

1). Macroscopic examination -

a) Colour -

Normal - pale yellow in colour due to pigments urochrome, urobilin and uroerythrin.

→ Cloudiness may be caused by excessive cellular material e protein, crystallization e precipitation of salts, upon standing at room temp. e in the refrigerator.

→ If the sample contains many (RBC), it would be cloudy as well as red.

Colour of the Urine depending upon it's Constituents

Blue Green	Pink Orange Red	Red-brown black
- Methylene blue	- Hb	- Hb
- Pseudomonas	- Hyoglobin	- Hyoglobin
- Riboflavin	- Phenolphthalein	- RBC
	- Porphyrins	- L-DOPA
	- Rifampicin	- Melanin
		- Methyl Dopa

b) Volume :- Normal - ~~1-2~~ 1-2.5L/D

- Oliguria - Urine output < 400ml/day seen in acute glomerulonephritis
- Renal failure
- Polyuria :- Urine output > 2.5L/D seen in
  - used water ingestion
  - DM and insipidus
- Anuria :- Urine output < 100ml/day seen in renal shut down.

c) Specific gravity - Measured by Urinometer or refractometer

→ It is measurement of Urine density which reflects the ability of the kidney to Concentrate

Or, dilute the Urine relative to the plasma from which it is filtered.

- Normal :- 1.001 - 1.040

S.G.	Osmolality (mosm/kg)
1.001	100
1.010	300
1.020	800
1.030	1000
1.040	1400

→ Inc in specific gravity seen in
- Low water intake
- Diabetes Mellitus
- Albuminuria
- Acute nephritis

→ Dec in specific gravity is seen in
- absence of ADH
- Renal tubular damage

Isosthenuria - Persistant production of fixed low specific gravity Urine, iso-osmolar with plasma despite variation in water intake.

(pH) → Urine pH range from 4.5 to 8
- Normally it is slightly acidic
- After meal it becomes alkaline.
- On exposure to atmosphere, urea in Urine splits causing NH_4^+ release resulting in alkaline Ur.

Microscopic Examination - / Chemical Examination

a) Serum Creatinine - Creatinine is produced by muscle movements. Here the muscle mass & new Creatinine is produced.

→ A healthy kidney removes Creatinine present in the blood

→ kidney function blood test is conducted to check Creatinine level

If serum Creatinine is 1mg/dl then 100% renal fx
If serum Creatinine is 2mg/dl then 50% renal fx

Normal values -
- As the body muscle mass differs in each gender
For women, the creatinine level will be 0.6-1.1mg/dl
For men is 0.7 - 1.3 mg/dl

Clinical Symptoms -
- Serum Creatinine used in diagnosis of renal, muscle disease, prerenal azotemia and postrenal azotemia.
- Used in pregnancy.

b) Serum Urea - Urea is the end product of protein catabolism.
→ The Urea is produced from the amino group of the amino acids and is produced in the liver by means of the Urea cycle
→ Urea undergoes filteration at the glomerulus as well as secretion and re-absorption at the tubular level.
→ The rise in the level of serum urea is generally seen as a marker of renal dysfunction especially below 50%.
→ The normal serum urea level is b/w 20-45 mg/dl
→ But the level may also be affected by diet as well as certain non-kidney related disorders.
High protein diet → Increased Urea level
Low protein diet → Decreased Urea level
→ other causes of protein catabolism such as any hyper metabolic condn, starvation etc also increased blood Urea level.
→ Similarly the level of urea may also be used in case of hepatic injury.

→ So even though blood Urea is not an excellent marker of renal dysfx. it
→ for practical purpose serum urea level is still one of the most ordered test and forms an important part of the kidney fx test
→ Urea is measured in diagnostic Lab either by UV kinetic method using α ketoglutarate as an NH_4^+ acceptor in the presence of enzyme glutamate dehydrogenase
→ Also measured colorimetrically by Berthelot's end point method and is read in visible range using colorimeter.

C) Blood Urea Nitrogen (BUN) →
→ This test measures the BUN level in your Urine
→ Urea is the end product of protein breakdown reaction
→ this is passed out as waste in the Urine

Normal values -
Adults :- 6 - 20 mg/dl
Elderly patients - 8 - 23 mg/dl
Children - 5 - 18 mg/dl

Clinical symptoms -
↑ BUN level (Azotemia)
Symptoms are
- impaired renal fx, CHF salt and water depletion
- stress acute MI
- chronic renal disease
- Urinary tract obstruction
- Hemorrhage into GI tract
- DM

↓ BUN
- Liver failure
- acromegaly
- malnutrition

d) Calcium :-

→ The test measures the amount of calcium in your blood, not the calcium in your bones

→ The body needs it to build and fix bones and teeth, help nerves work, make muscle Contraction, help blood clot and help the heart to work.

→ The calcium test screens for problems with parathyroid glands or kidneys, certain types of Cancers and bone problems, inflammation of the pancreas and kidney stones

Normal — 8.5 to 10.2 mg/dl

e) Creatinine Test - Creatinine is a waste product of body metabolism which present in blood excreted out by kidneys

→ Normal range Creatinine in blood 1-2 mg/dl or 0.6 - 1.2 mg/dl

Significance - Increased level in blood and Low level in Urine indicates kidney dysfunction

Urine Creatinine normal range
For adult man - 0.74 - 1.35 mg/dl
For adult woman - 0.59 - 1.04 mg/dl

f) Creatinine clearance test - This test shows the ability of kidneys to clear (excrete out) Creatinine from blood through Urine.

→ In this test 24 hours collected urine sample is taken.

→ Along the test blood Creatinine Level also examined.

Normal range of Creatinine clearance/min in Urine

In adult men :- 90 - 139 ml/min
In adult women :- 80 - 125 ml/min
Significance - Lower than normal creatinine excretion indicate the kidney problem.

Urea clearance Test -
This test is also performed to check the kidney functions, In this test using blood, the amount of urea in blood checked, and along with two urine sample are collected with a gap of one hour to determine the amount of urea filtered by the kidney into urine.

Normal range of Urea clearance - 12-20 gm/24 hours
Significance - Low level of urea than normal range indicate kidney problems, protein deficiency in diet.
→ High level than normal range indicates excessive protein metabolism. or too much protein intake in diet.

Urine Osmolality Test - Urine osmolality is the number of dissolved particles in urine (creatinine, urea, potassium, sodium etc)
Normal range :- 500 - 850 m Osm/kg
Significance - If level indicates - kidney problem and can cause dehydration.

6) Urine Cmc Test - kidney maintain the osmolarity of body fluid (290 - 300 m Osmol/L) and excrete urine with avg 500 - 850 m Osm/l normally.
Significance - High Cmc Urine indicate kidney problems, dehydration, heart failure.

Function of Liver -

1) Metabolism of Carbohydrate - Liver plays an important role in the metabolism of carbohydrates and release glucose into blood, in low blood glucose level is high liver convert glucose into glycogen and store it

2) Metabolism of protein and lipids - In case glycogen is not enough to fulfill the body requirement of glucose, liver makes glucose from protein and fat which is called gluconiogenesis.

3) Detoxification - Liver detoxifies the toxic substance like alcohol drug and steroid hormone and prevents other tissue from damage.

4) Storage - Liver stores glycogen, certain vitamins (fat soluble) and minerals (iron and copper)

5) Phagocytosis - The aged RBCs, WBCs and some bacteria undergo phagocytosis by kuffer cells of liver and destroyed.

6) Formation of Urea - The ammonia is obtained during metabolism of protein, which is highly toxic is converted into urea which is less toxic.

7) Formation of RBC in foetal life.

8) Destruction of aged RBC and formation of bile pigment.

9) Formation of plasma proteins - like albumin, globulin, prothrombin, fibrinogen

10) Formation of heparin - It is a natural anti-coagulant present in the blood.

Liver Function Test (LFT)

→ LFT is performed to estimate the dysfunction of liver several tests are performed under liver function test, like Serum, bilirubin, serum (plasma) proteins, alkaline phosphatase (ALP), Serum Glutamic Oxaloacetic transaminase (SGOT) or Aspartate transaminase (AST), Serum Glutamate Pyruvate Transaminase (ALT) etc.

Uses – 1) Screening of liver dysfunction
2) To recognize pattern of liver disease
3) To assess prognosis of patient
4) Follow up of disease
5) To evaluate the response to therapy

Classification of Liver Function tests –

1) Test based on excretory Fx^n
   a) Serum bilirubin
   b) Urine bilirubin
   c) Urine and fecal urobilinogen
   d) Urine bile salts
   e) Dye excretion tests
2) Test based on detoxification Fx^n –
   a) Hippuric acid test
   b) Determination of blood ammonia
3) Test based on synthetic function –
   a) Prothrombin time
   b) Protein time
4) Test based on metabolic function –
   a) Test related to Carbohydrate metabolism → (Glucose tolerance test)
   b) Test related to Lipid metabolism → Serum cholesterol

c.) Tests related to protein metabolism → Serum protein, Aminoaciduria

5) Enzymes in Diagnosis of Liver diseases –

- SGOT - ALP
- ALP
- AST

1) Serum billirubin –

- Billirubin - It is the end product of heme degradation derived from breakdown (aging) erythrocytes by monocular phagocyte system specially in the spleen, liver and bone marrow
→ the major pigment present in bile is the yellow compound billirubin
→ It is highly soluble in all cell membranes (hydrophobic) and is also very toxic
→ therefore, its excretion in the bile is one of the very important function of the liver.
→ Classification of bilirubin into direct and indirect bilirubin is based on original Van den bergh method of measuring bilirubin

Extravascular Pathway for RBC destruction –

RBC, (Liver, Bone marrow, and spleen)

↓ Phagocytosis / Lysis

Hemoglobin

(Recycled and converted into aa)

Globin → aa → aa pool

Heme → Fe++ → Recycled

Heme → Bilirubin → Excreted

Bilirubin Metabolism –

→ Bilirubin is the excretory product formed by the catabolism of heme part of Hb.

→ RBC, Breakdown
↓
Hb produces and breakdown
↓
Heme ——— Heme
oxygenase ↓
Biliverdin
↓ ← ——— Biliverdin reductase
Bilirubin

Difference b/w the Conjugated and unConjugated bilirubin –

Conjugated	Unconjugated
– water soluble	– Insoluble
– Present normally in bile	– Present normally in plasma
– Loosely bound to albumin	– Tightly combine to albumin
– Filtered through renal glomeruli and excreted in urine	– Not filtered through renal glomeruli, is not excreted in urine
– Non-toxic	– toxic substance

Diagnostic Importance of Bilirubin –

– Disruption of bilirubin metabolism and excretion can cause hyperbilirubinaemia and subsequent jaundice.

Hyperbilirubinaemia

Conjugated (Direct) | Unconjugated (Indirect)

A) Unconjugated Hyperbilirubinemia

→ It is due to overproduction of bilirubin

by reticuloendothelial system over the capacity of the liver to remove and clear from blood
→ It is characterized by high level of indirect or unconjugated bilirubin
→ this type of bilirubin can cross the BBB into the CNS and cause kernicterus.

B) Conjugated Hyperbilirubinemia - It is due to reflux of direct or conjugated bilirubin into blood due to biliary obstruction, conjugated bilirubin is water soluble, so it excreted in Urine and darken its colour.

Jaundice classification

Pre-hepatic ← Over production
Impaired uptake by the liver

Hepatic → Impaired conjugation

Post hepatic → Impaired excretion (intra or extrahepatic obstruction)

Normal Range -

Bilirubin TYPE	Bilirubin Level
Total bilirubin	0.0 - 1.4 mg/dL
Direct bilirubin	0.0 - 0.3 mg/dL
Indirect bilirubin	0.2 - 1.2 mg/dL

Significance - high level indicates haemolysis, jaundice (Liver problem)

2) Serum (plasma) proteins - Albumins and globulins proteins are the major protein of plasma and produced by Liver.

Normal Range -
Albumins :- 3.5-5.1gm/dl
Globulins - 1.8-3.1gm/dl
Significance - Red Level indicates dehydration liver problem.
Red Level indicates Oedema, haemorrhage, Prot protein breakdown

3) ALP (Alkaline phosphate) - This enzyme produced in liver, bones, small intestine and kidneys, It catalyses splitting of phosphate group from monophosphate ester.
Normal range :- 29-92 IU/L
→ High level indicate :- ~~hypothyroidism~~ Rickets, Osteomalacia, Abnormal absorption of Vit D.
→ Low level indicates - hypophosphatasia.

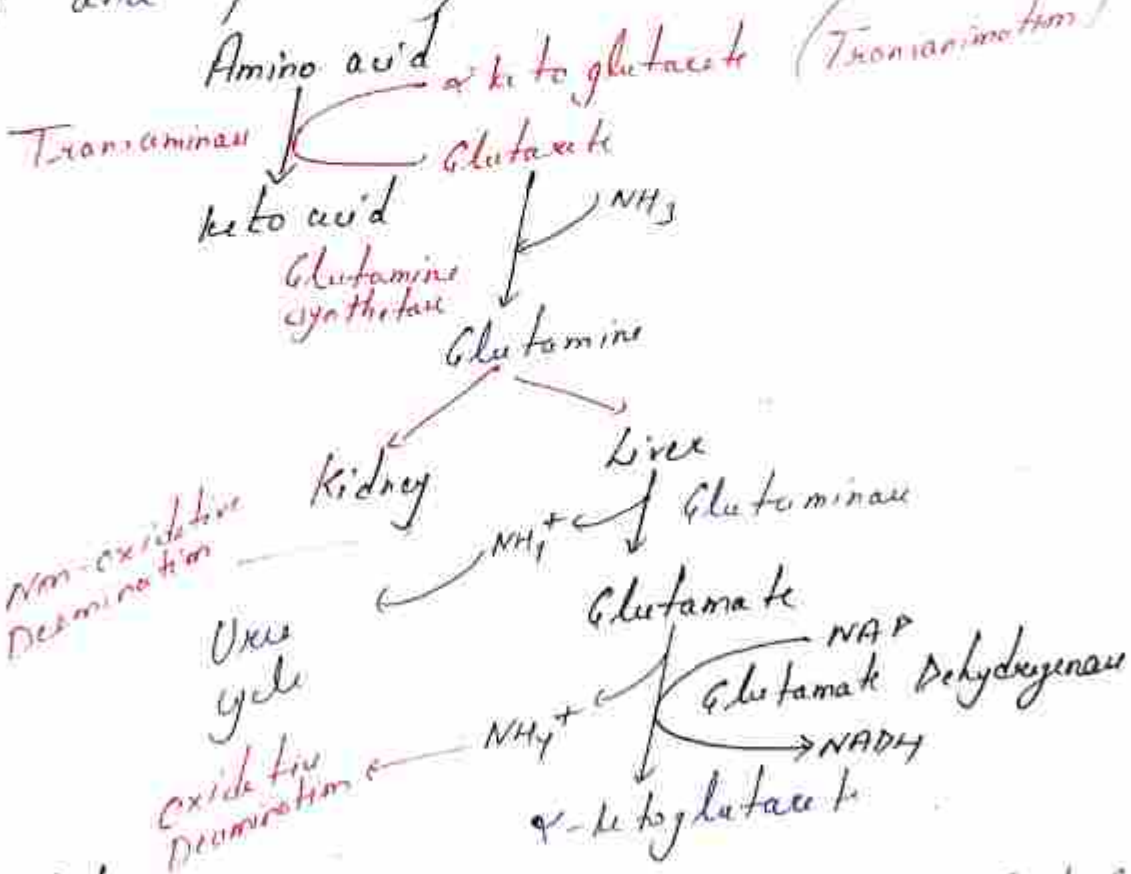
4) Serum Glutamic Oxaloacetic Transaminase (SGOT) or Aspartate Transaminase (AST)
→ This enzyme is produced by liver and it helps in energy production.
Normal Range - 0.40 U/L
Significance - Red Level of SGOT indicates liver disease (Hepatitis, Cirrhosis)

5) Serum Glutamate Pyruvate Transaminase (ALT) - this enzyme produced by liver. and helps in formation of alanine
Normal range :- 5-36 U/L

L-Glutamate + Pyruvate $\xrightarrow[\text{Alanine aminotransferase (ALT)}]{\text{Glutamate Pyruvate transaminase (GPT) OR}}$ α-ketoglutarate + L-Alanine

L-Glutamate + Oxaloacetate $\underset{\text{ALT}}{\overset{\text{GPT}}{\rightleftharpoons}}$ α-ketoglutarate + L-Aspartate

Significance – ALT and AST are clinically significant aminotransferases. Both are markers of liver disease however, ALT is more liver specific than AST, AST levels are also significantly raised in skeletal muscular disorders and pulmonary embolism.



Decarboxilation – Glutamate $\xrightarrow{-CO_2}$ Gamma Aminobutyric Acid (GABA)

(eg) It is inhibitory Neurotransmitter

Salient features of transamination –

1) All transaminases require PLP. In this Rx, no free NH_3 is liberated, only the transfer of amino group take place.
2) Transamination is reversible
3) There are multiple transaminase enzymes, which vary in substrate specificity. Aspartate aminotransferase (AST) make a significant contribution for transamination.
4) Transamination is important for redistribution of amino group and production of non-essential aa
5) Amino Acid undergo transamination to finally concentrate nitrogen in glutamate. Glutamate undergoes oxidative deamination to liberate free NH_3 for urea synthesis.
6) All aa except, lysine, threonine, proline and hydroxyproline participate in transamination. It involves both anabolism and catabolism, since - reversible.

Mechanism of transamination –

→ It occurs in 2 stages

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

Pathology

- Pathology one of important branch of medical science that mainly deals with the study and diagnosis of a particular disease or disorder.

⇒ It generally involves the analysis of removed organ cell or tissue body fluids like urine blood and other fluids and sometimes the whole body is also analyzed.

⇒ Through pathology testing we can diagnose any abnormalities or disorder of our body

Blood

- Blood is a specialized fluid connective tissue that consist of plasma, corpuscles (RBC and WBC) and platelets.
- It circulates throughout the body and perform various function such as:

⇒ Transportation of vital elements (such as nutrients, oxygen and hormones etc.) to cells, tissues and organs.

⇒ Elimination of waste from cells, tissues or organs.

⇒ Regulation of body temperature (Thermo-regulation).

Blood Composition

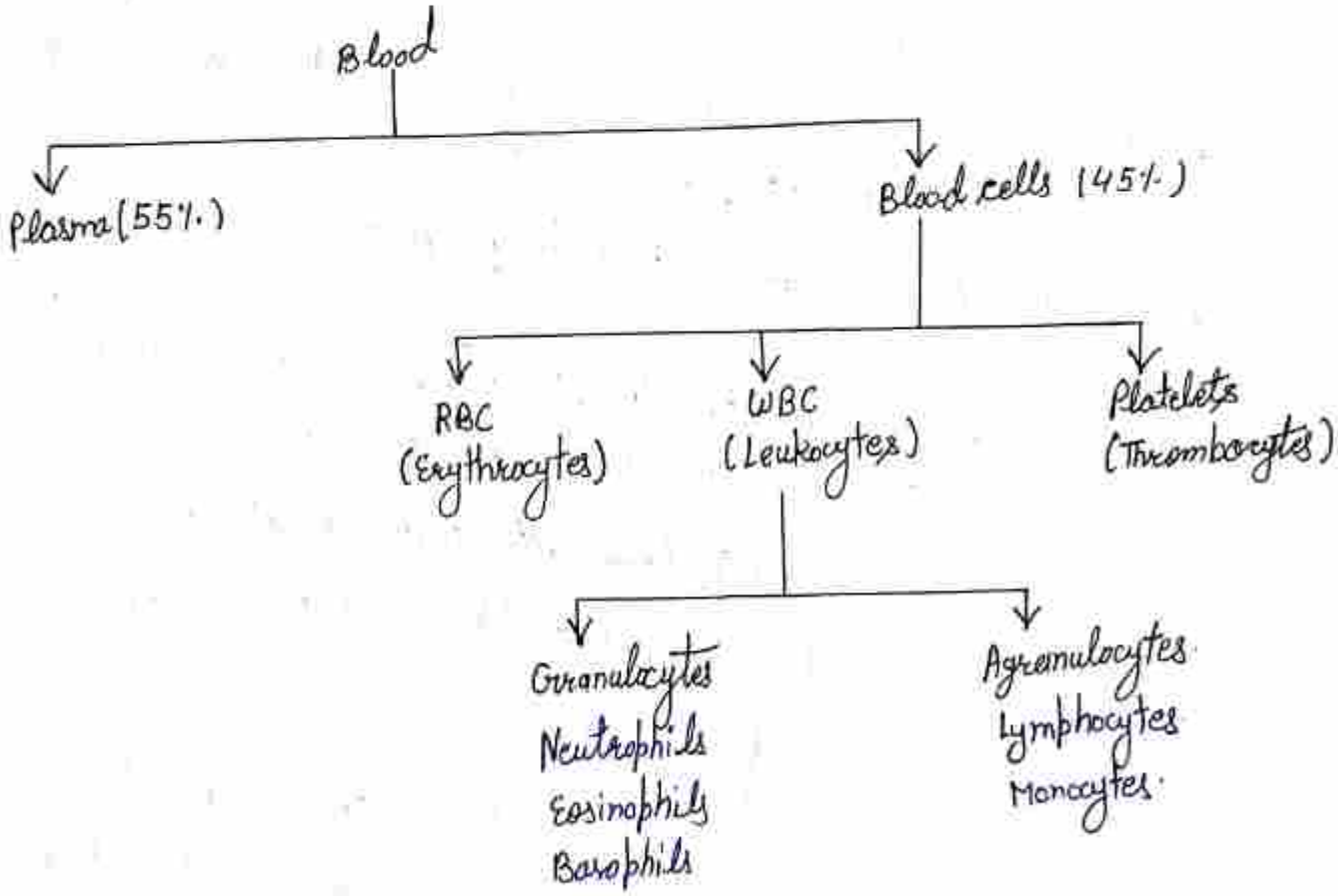
Blood is made up of several different types of blood cells suspended in a clear, straw-coloured fluid called plasma.

Plasma: 55%
of total plasma content 91% is water and rest 8% is solid comprising coagulant, plasma protein etc.

- cellular substances (Blood cells) 45%.
  out of total cellular substances i.e. blood cells 44%. RBC and the rest 1%.
  comprises both WBC and platelets.
- WBC are divided in to two types and constitute the number as mentioned against each of them.

⇒ Granulocytes
  Neutrophils → 40-70%.
  Eosinophils → 01-04%.
  Basophils → 0-01%.

⇒ Agranulocytes.
  Lymphocytes - 20-45%. and Monocytes - 02-10%.



Introduction to Pathology of Blood

Pathology of blood :-

→ The study of blood related diseases, causes and progression is called Pathology of blood.

→ The tests are performed for pathology of blood are called Haematological Tests.

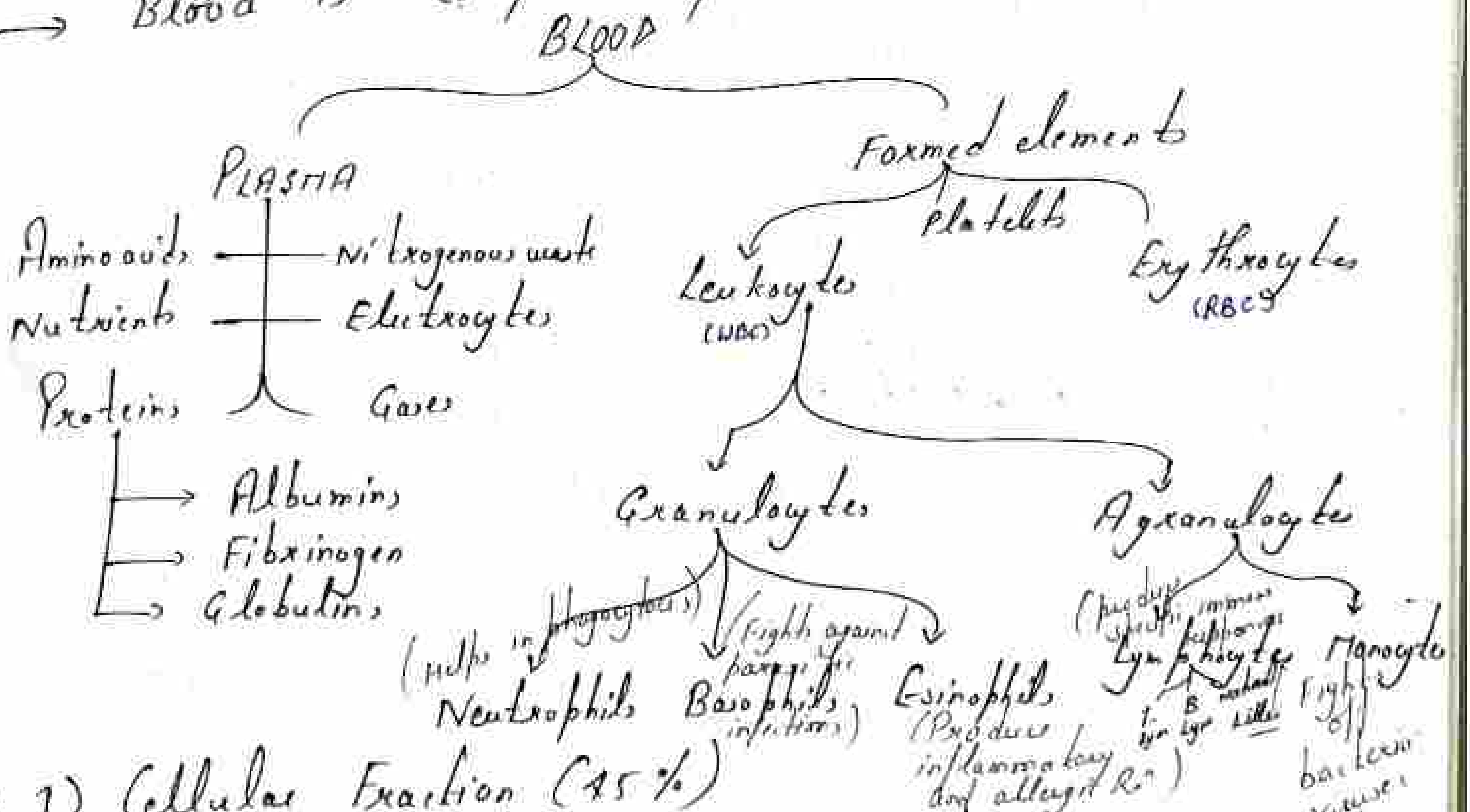
→ Blood is a fluid connective tissue. It circulates in closed system of vessels. Total volume app. 6 litre, pH 7.4 (alkaline slightly). The major fxⁿ of blood include :-

Functions of blood

1) transporting oxygen and nutrients to the lungs and tissues
2) Carrying cells and antibodies that fight infection. (Contains WBC - protect body from disease)
3) bringing waste products to the kidneys and liver, which filter and clean the blood.
4) Regulating body temperature.
5) Redistributes water
6) Carries hormones from glands
6) clotting of blood protects against haemorrhage.

Composition of Blood :-

→ Blood is composed of Plasma and blood cells



1) Cellular Fraction (45%)

a) RBCs (Erythrocytes) ⟶ 5,000,000 per. cu.mm
b) WBCs (Leukocytes) ⟶ 6,000 per. cu. mm
c) Platelets (Thrombocytes) ⟶ 250,000 per. cu. mm

2) Plasma fraction (55%)

a) Non-diffusible Constituents :- albumin, globulin, fibrinogen, enzymes

b) Diffusible Constituents :- Albumin, globulin, fibrino Hormones, vitamins, glucose aa, fatty acid.

c) Electrolytes - Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, HCO_3, HPO_4

d) Catabolic products - Urea, Uric acid, Creatinine etc.

Function of RBCs (Erythrocytes)

→ Is to carry oxygen from the lungs to the body tissue and CO_2 as a waste product, away from the tissues and back to the lungs.

→ Hemoglobin (Hb) is an important protein in the red blood cells that carries oxygen from the lungs to all parts of our body.

Function of WBCs (Leukocytes)

→ The main function of WBC's are to fight infection.

→ There are several types of WBCs and each has its own role in fighting bacterial, viral, fungal and parasitic infections.

Function of Platelets - (Thrombocytes)

→ The main function of platelets in blood clotting.

→ Platelets are much smaller in size than the other blood cells.

→ They group together to form clumps, or a plug, in the hole of a vessel to stop bleeding.

Lymphocytes – Role in Health and Disease

→ Lymphocytes are WBCs and one of the body's main types of immune cells. They are made in the bone marrow, and found in the blood and lymph tissue.

→ These cells work together to defend the body against foreign substances, such as bacteria, viruses and cancer cells.

→ Lymphocytes includes natural killer cells (which function in cell-mediated, cyto-toxic innate immunity), T cells (for cell-mediated, Cytotoxic adaptive immunity) and B cells (for humoral antibody-driven adaptive immunity).

Disorders –

1) Lymphocytosis – It is a high lymphocyte Count, is an increase in WBC called Lympho-cytes.

→ Indicates your blood is during with an infection or other inflammatory Condition.

2) Lymphocytopenia – It is an abnormally low number of lymphocytes in the blood.

→ Various diseases and conditions including infections with viruses such as HIV virus (caused by AIDS, the influenza virus, SARS-CoV-2 can be the number of lymphocytes in the body.

Haematological tests

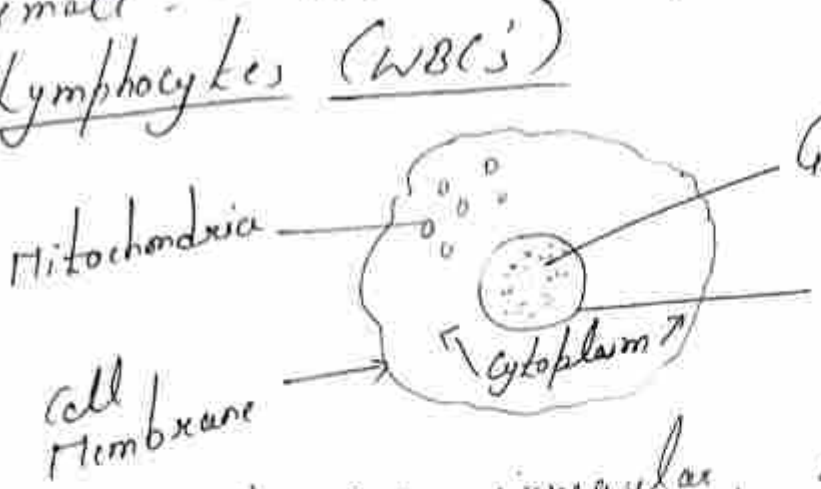
1) WBC or total Leucocyte Count (TLC –

This test is performed to check conditions like

infection, allergic Rx, inflammation, Blood Cancer (Leukemia)

Normal range -
male :- 4500 to 11000/ml
Female :- 4500 to 11000/ml

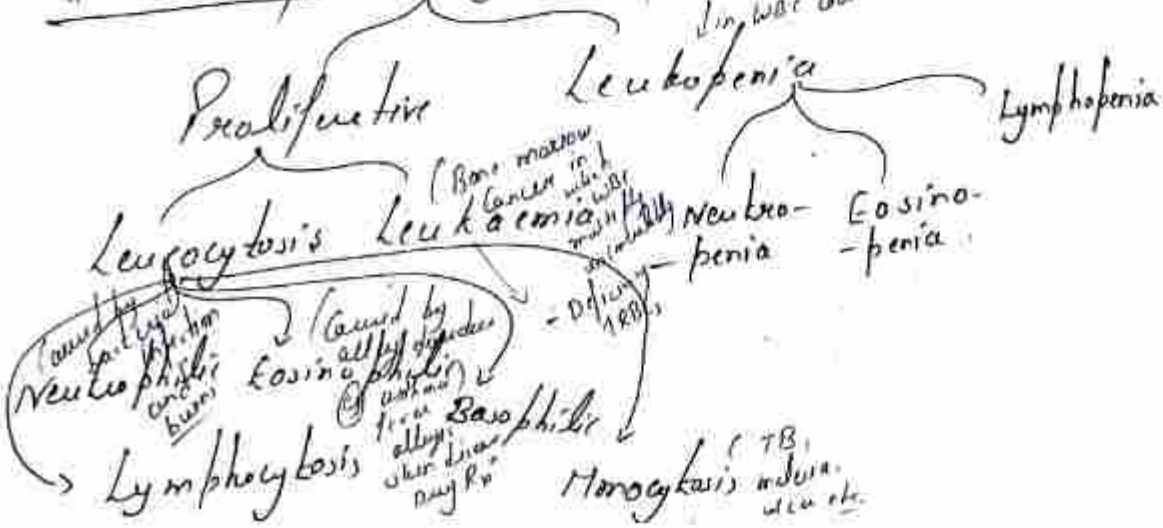
Lymphocytes (WBC's)



→ WBC's are irregular, nucleated, colourless
→ These are larger in shape than RBC
→ Diameter are 10-14 μ
→ It produced in bone-marrow, lymph nodules, tonsils and spleen.
→ Leucocytes eat-up diseases causing micro-organism (Phagocytosis)
→ It also take parts of fragments of dead cells and helps in cleaning the body.

TYPES OF Lymphocytes

Disorders of Leucocytes

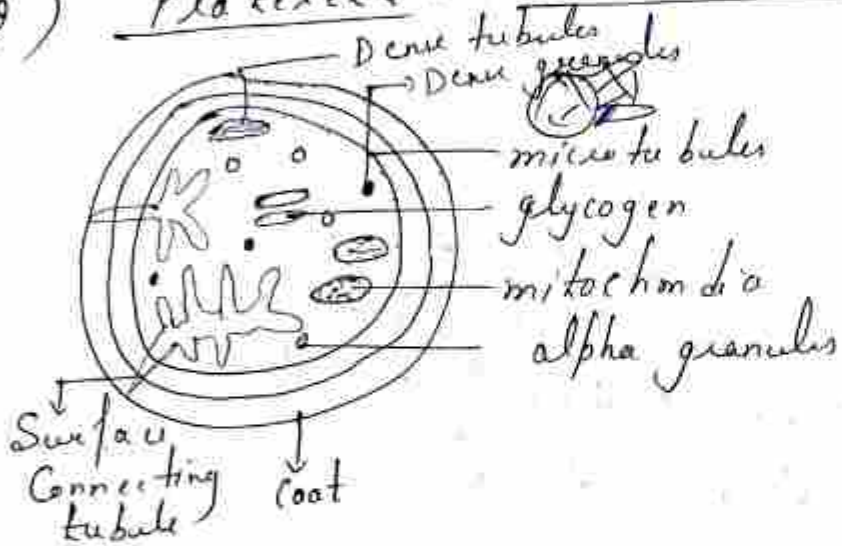


Disorders of WBCs

1) **Lymphocytosis** - It is a high lymphocyte count, is an Inc in WBCs called lymphocytes. Indicates your body is dealing with an infection or other inflammatory Condition.

2) **Lymphocytopenia** - It is an abnormally low number of lymphocytes in the blood. Various diseases and Cond including infection with viruses such as (HIV) - the virus that Causes AIDS, the influenza virus, SARS-CoV-2 Can Lu the number of lymphocytes in the blood.

2) Platelets - Role in Health and Disease



→ Platelets are non-nucleated round and oval, biconvex discs with varying sizes and covered by single membrane.
→ They are about 2-4 μm in diameter
→ Formed in red bone marrow.
→ Their cytoplasm Contain numerous granules
→ The average life of platelets about 5-10 days
→ It destroys in splen.
→ Platelets Contain thromboKinase and thromboplast.

→ Important role in clotting of blood.

Functions -

1) They initiate blood clotting
2) They repair capillary endothelium
3) They involve in the haemostatic mechanism
4) They hasten clot retraction
5) when they disintegrate, 5-HT and histamine are liberated.

Disorders related to Platelets

1) Thrombocytopenia - when number of platelets in the blood are below 1,00,000/μl that Cond^n called as thrombocytopenia.

→ It is caused due to the production of platelets.

→ the survival of platelets

→ Certain Cancers, Cancer treatments, medications and autoimmune diseases can cause the Cond^n.

(eg) Heparin (a blood thinner) is the most common cause of drug-induced immune thrombocytopenia.

Thrombocytosis - It is a Cond^n in which there are an excessive number of platelets in the blood.

→ Platelets are blood cells in plasma that stop bleeding by sticking together to form a clot

→ Too many platelets can lead to certain conditions such as stroke, heart attack or a clot in the blood vessels.

Platelets

⇒ Platelets also known as thrombocytes are small disc shaped non-nucleated bodies formed by cytoplasm of megakaryocytes in red bone marrow.

⇒ They contains variety of substances that are important for blood clotting.

⇒ General features of platelets.

2-4 μm in diameter

2,00,000 – 3,50,000 mm^3

Life span 8-11 days

⇒ Physiological function:

- Platelets helps in the process of blood coagulation thus helps in maintaining homeostasis.
- Hemostasis ⇒ it's body's natural response to an injury which stops bleeding and fixes the damage.
  usually the ability helps in preserving blood and avoiding infections.
- Normal range:
  1,50,000 to 4,50,000 platelets per ml of blood.

Disorders related to platelets

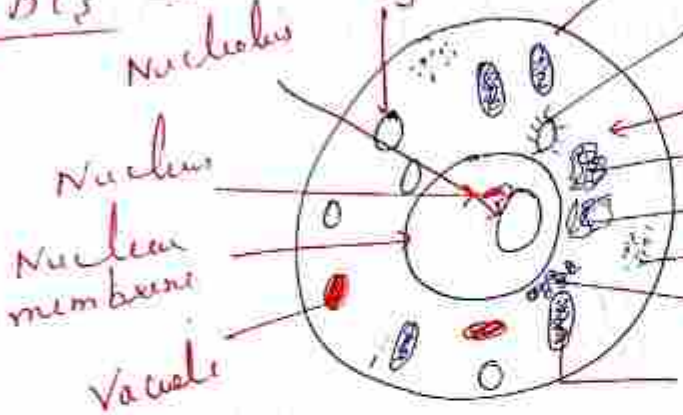
- Purpura ⇒ purpura also known as blood spot or skin hemorrhages, are purple colour spots on the skin, organ or mucous membranes.
- They occur when small blood vessels burst, causing blood to pool under the skin. These spots can range in size from small dots to large patches and may indicate a serious medical condition.

3). Purpura :- If platelet count is below the normal, the disease is called as purpura.

Symptoms - Bleeding from mucous membrane, appearance of lesions.

→ Color of lesions in first red, gradually dark, then purple to brownish yellow.
→ Clotting time remain normal. (2-9 min)
→ Bleeding time is prolonged. (2-7 min)
→ Blood clot does not retract.

RBCs :-



Normal value (Hb)
Adult male - 14-17 g%
" female - 12-16 g%

Haemoglobin (Hb)
- It is the respiratory pigment found in RBC's of the blood.
- It is colouring matter i.e. it is a red pigment of blood.
- It is chromoprotein consisting of two parts:
  i) globulin (96%), a histone, and
  ii) a non-protein prosthetic group - an iron containing pigment called 'Haem'.

→ Erythrocytes diameter about 8 µm
→ It's biconcave and without nucleus containing hemoglobin in cytoplasm, hence red in color.
→ RBC's produced in bone marrow, spleen and kidney.
→ Life span about 120 days.
→ It destroy in liver and spleen.
→ It transport oxygen and CO_2.
→ Normal RBC present in Men - 4.7 to 6.1 million cells per microlitre (mL)
Women - 4.2 to 5.4 million cells per mL
Children = 4.0 - 5.5 mL

Abnormal Erythrocytes and Their Significance :-

1) Anaemia → Anemia is Condition in which the oxygen carrying Capacity of blood reduced
→ Low number of RBC's.
→ Low Conc^n of Hb
Symptoms - Fatigue, intolerance to cold, breathlessness, loss of appetite

Several types of Anaemia

1) Pernicious Anaemia - Stomach produce intrinsic factor which required for absorption of Vit B_{12} in small intestine
→ Inability of stomach is to produce intrinsic factor.
→ Leads to insufficient hemopoiesis that Cond^n called pernicious anemia
Sign and Symptoms - Weakness, Bleeding of gums, Jaundice, Loss of appetite

2) Sickle Cell Anaemia - It is also called as Haemoglobinopathic haemolytic anaemia
→ Abnormal formation of RBC
→ RBC are sickle shaped (S or C)
→ S shaped are sensitive to Lower O_2 supply
→ C shaped are Lowest O_2 Conc^n
→ This cells (RBC's) does not pass through small blood capillaries it block the blood supply

3) Megaloblastic Anaemia
- It is caused due to inadequate intake of Vit B_{12} or folic acid.
- The red bone marrow produce large abnormal RBCs called megaloblasts.
- It may be caused due to used in treatment of cancer.

4) Iron deficiency anaemia
- It is caused due to inadequate absorption of iron
- Excessive loss of iron.
- insufficient intake of iron
- women are higher risk for this type
- Because of menstrual blood loss
- Increased iron demand during pregnancy.

5) Aplastic Anemia - It is a anaemic Condⁿ in which the bone marrow fails to form the RBCs.
Caused by radiation, toxins

6) Hemorrhagic Anemia (HA)
- Bleeding due to large wounds, stomach ulcer, heavy menstruation leads to excessive loss of RBCs. → This Condⁿ called (HA)

7) Hemolytic Anemia - RBCs plasma membranes ruptures prematurely

→ Due to rupture of plasma membrane, the Hb in RBC, released in plasma.
→ It may damage glomeruli in kidney.

Polycythemia → The Conc^n of blood ↑se abnormally, also ↑se in Hb level, this Cond^n call polycythemia.

(↑se Conc^n of red cells)
1. Relative
due to ↓se in the volume of plasma
- Plasma volume ↓se by vomiting, dehydration, diarrhea

2. absolute
~~due ↑se in the~~ [no of RBC greatly ↑sed in, i.e. polycythemia
→ It is due to ↑sed secretion of erythroproteins

Introduction of URINE

→ Urine is an excretory product of the body.
→ It's ~~formed~~ eliminated through the kidney.
→ Normal urine Contains like waste products like urea, uric acid, creatinine
→ Certain salts such as chlorides, sulphates
→ It does not Contain substances which are required by the body or tissues
→ Such Urine is k/s physiological or normal Urine
→ Urine examination helps in the diagnosis of various renal as well as systemic disease

→ The abnormal or pathological Urine is the Urine Containing essential of body like glucose, ketone, bile, blood, protein etc, apart from normal organic and inorganic substances
→ They are defined as high threshold substances and are not filtered easily during glomerular filteration

Urine Constants -

1) Volume - 600 - 2500ml/24 hours
2) Colour - Pale Yellow or Amber
3) Reaction - Acidic
4) pH - 4.5 - 8.2
5) odour - Aromatic

→ In disease Cond^n the substances essential as tissues may also be excreted in Urine
→ Substances like sugar, bile salts, albumin etc.
→ If such abnormal substances are excreted in Urine, then such urine is described as abnormal or pathological Urine.
→ Urine pH slightly acidic.

Urine Colour chart -

1. Clear - Very good
2. Light Yellow - Good
3. Yellow - Fair
4. Dark Yellow - Light dehydrated
5. Amber - Dehydrated
6. Brown - Very dehydrated
7. Red - Severe Dehydrated

Abnormal Constituents of Urine and their Significance.

1) **Protein** - The appearance of proteins in the Urine is called (Proteinuria). if albumin appears it is referred as albuminuria.

Causes - Severe exercise, high protein diet, pregnancy, kidney disease, Lower urinary tract damage, fasting etc.

2) **Glucose** - The appearance of glucose (sugar) in Urine is called as glycosuria and the disease Condⁿ is called DM.

3) **Ketone bodies** - The presence of ketone bodies such as acetone, β-hydroxybutyric acid and aceto acetic acid in the Urine is called ketonuria and the pathological Condⁿ is called ketosis.

Causes - Excessive fat metabolism, usually seen in diabetes, during starvation, pregnancy etc.

4) **Bile salts and bile pigments** - It may appear in the Urine in Condⁿ like obstructive hemolytic jaundice as hepatic jaundice.

5) **Blood** - The appearance of blood in the Urine is called haematuria. The blood may appear in Urine, due to TB, Cancer, renal stones or acute inflammation of kidney.

6) **Pus** - Pyuria refers to having WBC or pus cells in Urine. A UTI is the most Common Cause of Pyuria.

Urine - Normal and Abnormal Constituents

Introduction

Urine is excretory product of our body is eliminated by the kidney. Normally urine contain urea, uric acid, creatinine and few salts (like chloride and sulphate).

Urine Analysis

Normal	Abnormal	Significance
1,500 ml per day or 800 to 2,000 ml of urine (during 24 hrs period)	Polyuria - urine volume 2.5 L/day	It occurs in diabetes mellitus. Diabetes Insipidus
	oliguria - less than 400 ml/day	occur in case of fever diarrhea, acute nephritis etc.
	Anuria - urine output approx nil	occur in case of acute tubular necrosis, renal stone or surgical shock.

Table 1 : Urine output

Table 2 : Urine Colour

Normal Colour	Abnormal Colour	Clinical significance
Transparent Pale yellow or Amber colour	Pale yellow	Dilute urine
	Deep orange	Concentrated urine
	Red or red Brown	Hematuria
	Yellow - brown	Jaundice
	Deep orange	Fever

Table 3: Urine pH

Normal pH	Abnormal pH	clinical significance
4.5 - 7.8	Less than 6 acidosis	⇒ High Protein diet ⇒ Metabolic and respiratory acidosis ⇒ Urinary tract infection
	More than 6 Alkalosis	⇒ Vitamin C rich food ⇒ Potassium depletion ⇒ Dehydration

Specific gravity ⇒ The concentrating ability of Kidney is known as specific gravity.

Table 4: Specific gravity

Normal Specific gravity	Abnormal specific gravity	clinical significance
4.5 - 7.8	Low specific gravity (Isosthenuria)	Chronic renal disease
	very low specific gravity (Hyposthenuria)	Diabetes Insipidus Glomerulonephritis Diuretics
	very high Specific gravity (Hypersthenuria)	Vomiting Sweating Diarrhoea

odour ⇒ Normally urine has aromatic odour, bacterial infection leads to foul smell.

Normal Constituents of Urine

1. Nitrogenous non-Protein constituents

(A) Urea

Normal range
- 20 – 40g/day

Description
- End product of protein metabolism.
- Formed in liver excreted by Kidney.
- It is altered by protain content diet.

(B) Uric acid

Normal range
- 7.2 mg/dl)

Description
- uric acid is produced when purines, compounds found in certain foods and beverages, are broken down by the body.

(C) Creatinine

Normal range
Male : 0.8 – 1.8g/day
Female : 0.6 – 1.6g/day

Description
Creatinine phosphate present in muscle is metabolized to yield creatinine.

2. Organic Constituents of urine
Ketone bodies, oxalic acid, phenols, Hormones, Vitamins and Enzymes.

3. Inorganic constituents of urine
Sodium, Potassium, chloride, Bicarbonate, phosphates and sulphates.

Abnormal Constituents of urine and its clinical Significance

1. Protein

⇒ clinical Significance

- Presence of Protein in urine is termed as proteinuria.
- occur in case of renal disorders, heavy exercises, urinary tract infection, fever, dehydration, myeloma etc.
- Heat test is employed for protein analysis of urine.

⇒ Heat test

- Urine is taken 2/3 full in a test tube.
- Holding the bottom of the test tube, top portion of urine is heated.
- Then 1-2 drops of acetic acid is added.
- Turbidity or precipitate in the heated portion indicates the presence of protein.

2. Sugar

clinical Significance

- Glucose in urine is termed as glycosuria
- Diabetes mellitus is the condition in which glucose comes out with urine.
- Detected by glucose-oxidase reagent strip.

3. Ketones

Clinical Significance

- High level ketone in urine is termed as ketonuria
- Ketoacidosis, a common complication of diabetes, is characterized by High ketones levels in urine, often leading to rapid development and potential fatalities.
- Rothera's test can be employed to test for presence of ketone in urine.

Rothera's test

An alkaline solution of sodium nitroprusside reacts with acetoacetic acid and acetone to form a complex that is purple in colour.

Acetone and acetic acid conc^n above 1-5 mg/dl and 10-20 mg/dl.

4. Bilirubin

clinical significance

mainly occur in case of jaundice and other liver disorders.

It can be tested by Fouchet's test, smith's test or Gmelins test.

Fouchet's test

1ml urine + Barium chloride (Few ml)

↓

Filter the urine

↓

Add one drop of ferric chloride on the filtrate obtained on filter paper

↓

Green blue spot appears

↓

Confirms bilirubin in urine