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Introduction to biochemistry

Biochemistry is a branch of biology. Biochemistry is defined as the chemistry of living things. It deals with the structure of chemical compounds that make up part of living matter, the transformation of these chemical compounds and the physico-chemical processes that constitute the basis of vital activity. In biochemistry, biological phenomena are analyzed in terms of chemistry.

Anatomy is the study of structure and physiology is the study of function. Biochemistry integrates both these aspects to describe the structure and function of living things in molecular terms.

Biochemistry, as the name implies, is the chemistry of life. It thus bridges chemistry and biology. The term biochemistry was introduced by Carl Neuberg in 1903.

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Structure of water molecule

1) Water is an inorganic compound.

2) The molecular formula of water is H_2O.

3) It contains 2 hydrogen atoms and one oxygen atom.

4) The hydrogen and oxygen atoms are held together by covalent bond (bonds formed by sharing of two electrons).

5) The three atoms in the water molecule (2 hydrogen atom and one oxygen atom) are not in a line. But they are arranged in the form of the letter V, with oxygen atoms at the tip and the hydrogen atoms at the ends of the two limbs.

6) The bond angle between hydrogen and oxygen atom is 105°.

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7) The central property of water molecule is its electrical polarity.

8) The oxygen atom is negatively charged and the hydrogen atoms are positively charged.

9) As this molecule has two different poles like that of a magnet, the water molecule is a dipole. In other words, water is a polar compound.

10) The polar molecule have the property of attracting each other. owing to this attractive force, water molecule aggregate together.

11) As a result of this force, a water molecule can link with 4 adjacent water molecules.

12) The linking between two water molecules is effected by the formation of hydrogen bond [O···H] b/w the oxygen atom of one water molecule and hydrogen atom of another water molecule.

13) The oxygen atoms forms a tetrahedron with the four hydrogen atoms of the neighbouring 4 water molecules

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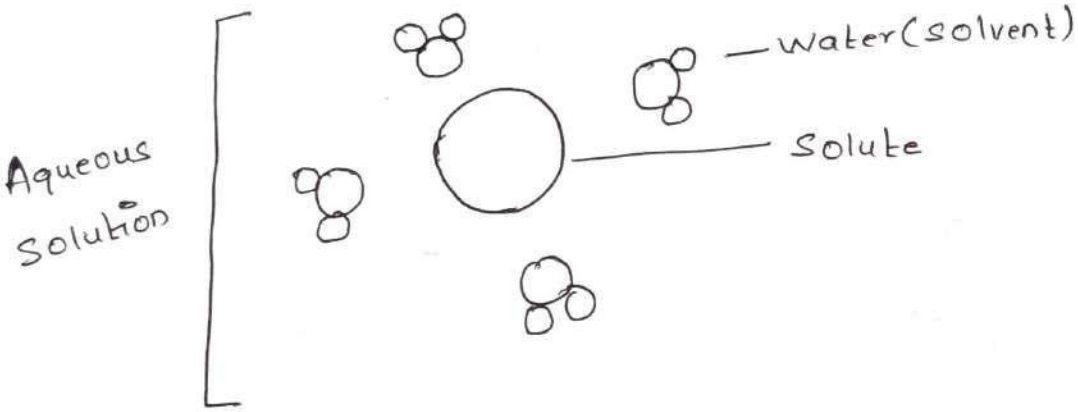
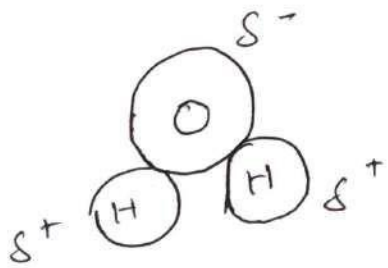
Water as a biological solvent

-> The liquid inside cells and in fluids such as blood and sap is not just a pure water. It is an aqueous solution.

-> Aqueous solution are formed when solutes are dissolved in the solvent water.



-> Water is a powerful solvent because it is a polar molecule which allows it to easily dissolve ionic and polar molecules.



-> When ionic substances are added to water, the water molecules are attracted to the ions that are in contact with the water.

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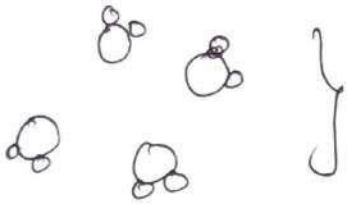
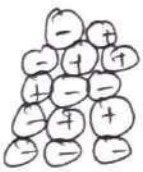
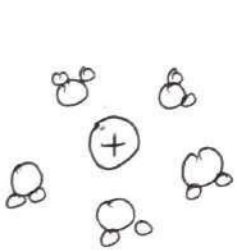
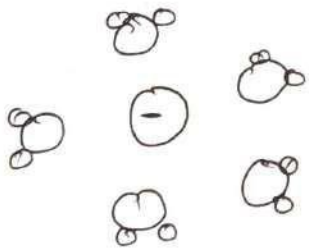


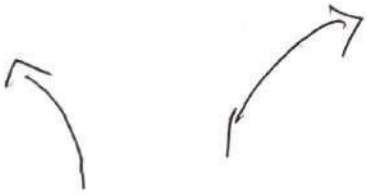


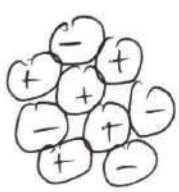
ionic substances -> made up of ions [+/-]

-> The water molecules cluster around each ion, seperating them from the ionic lattice.









-> The slightly positive hydrogen in each water molecule are attracted to negative ions.

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-> The slightly negative oxygen in each water molecules is attracted to the positive ions.

-> When all the ions have been surrounded by water molecules the solute is fully dissolved.

-> Because water is a solvent, it can be used as transport medium for solutes.

-> Water in the blood carries a range of important solutes, including:

Gases eg: CO_2 and oxygen

Biomolecules eg: Aminoacids and glucose

Inorganic ions eg: sodium, chloride, potassium.

-> The cytoplasm in cells is an aqueous solution where many chemical reactions happen. When solutes dissolved in water, they are able to freely move around
-> This allows molecules like enzymes to collide and interact with substrates to catalyse reactions.
-> The ability of water to act as a solvent therefore makes it an excellent reaction medium in cells.

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Weak acids and bases

-> Molecules the release protons (hydrogen ions) in solution are termed acids, while substances that accept a hydrogen ion (proton) are called bases.

-> An acid is a substance that when added to water, increases the number of H^+ ions in the water.

eg:- addition of HCl

-> A base is a substance that when added to water, increases the number of OH^- ions in the water.

eg:- addition of NaOH

Some acids and their conjugate bases present in body.

$H_2CO_3 \longleftrightarrow H^+ + HCO_3^-$

(Carbonic acid) (Bicarbonate)

$NH_4^+ \longleftrightarrow H^+ + NH_3$

(Ammonium ion) (Ammonia)

-> All substances shown on the left are acids because they donate hydrogen ions. All the substances on the right are bases since they combine with hydrogen ions (note the two-way arrows). By combining with hydrogen ions bases lower the hydrogen-ion concentration of a solution.

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$HCl \longrightarrow H^+ + Cl^-$

[Hydrochloric acid] — Hydrogen ion — chloride ion

→ When hydrochloric acid is dissolved in water, it completely diassociates into hydrogen and chloride ions which do not unite again in solution (note the one-way arrow in the equation above).

→ An acid or base which can completely ionises into ions is called a strong acid or strong base.

Strong acid : HCl, H_2SO_4, HNO_3

Strong base : $NaOH$, KOH

→ An acid or base which undergo incomplete or partial ionisation, when it is dissolved in aqueous medium is known as weak acid or weak base.

Weak acid : Acetic acid

Weak base : NH_4OH

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pH

Acids are substances which furnish hydrogen ions (H^+) in the solution, whereas bases are substances that furnish hydroxide ions (OH^-) in the solution. Substances that dissociate in water into a cation (positively charged ion) and an anion (negatively charged ion) are classified as electrolytes. Whereas sugar (or alcohols which dissolve in water but donot carry a charge on dissociate into species with a positive and negative charge are classified as non electrolytes.

Strong electrolytes are completely ionized in aqueous solution ions whereas weak electrolytes are partially ionized in aqueous solutions.

pH of a solution is defined as the negative logarithm of its hydrogen ion concentration.

$$pH = -\log_{10}[H^+]$$

$$= \frac{1}{\log_{10}[H^+]}$$

Pure water has equal concentration of H^+ and OH^- ions, the concentrations of each is very

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Small and each being equal to 10^{-7} mole/liter at room temperature.

Water dissociates into

$$H_2O \rightleftharpoons H^+ + OH^-$$

From the law of mass action, the disassociate of water can be represented as

$$K_w = \frac{[H^+]\,[OH^-]}{[H_2O]}$$

The bracket indicates the concentration of each component in moles per liter

The concentration of undissociated water is so large as compared to the concentration of H^+ and OH^- ions, so that for all the practical purposes is fairly constant. This simplifies the above equation to

$$[H^+]\,[OH^-] = K\,[H_2O]$$

$$[H^+]\,[OH^-] = K_w$$

Where K_w is ionic product of water or the dissociation constant of water.

Ionic product of water is usually taken as 10^{-14} at the room temperature (25°c)

Then $[H^+]\,[OH^-] = 10^{-14}$

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Taking logarith on both sides

$$\log [H^+] + \log [OH^-] = -14$$

By rearrangement

$$-\log [H^+] - \log [OH^-] = 14$$

According to the definition of pH, the above equation simplifies to

$$pH + pOH = 14$$

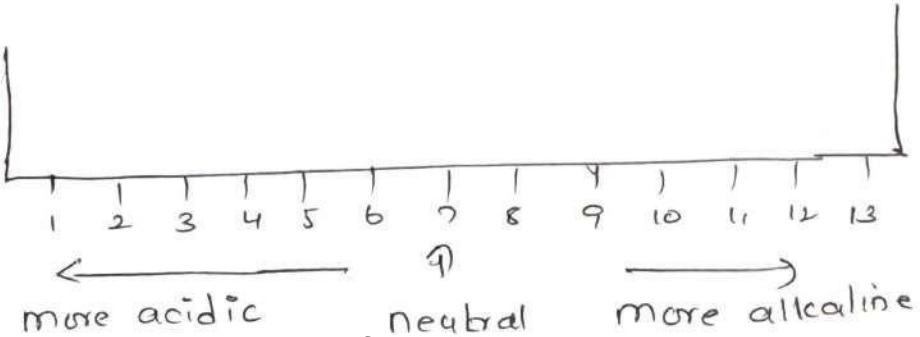
At neutrality, both hydrogen and hydroxide ions have equal concentration i.e

$$pH = 7$$

$$pOH = 7$$

There exists an inverse relationship between $[H^+]$ and $[OH^-]$ ions in solution. As hydrogen ion concentration increases, the hydroxide ion concentration decreases and viceversa.

The acidity or alkalinity of a solution is determined by the amount of $[H^+]$ and $[OH^-]$ ions present



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Average pH values of some body fluids are

Gastric juice - 1.4

Urine - 6.0

Blood - 7.4

Buffer:-

A solution that resists change in pH or addition of a small amount of an acid or a base is called as buffer solution. The capacity of a solution to resist alteration in its pH value is known as buffer capacity. The capacity to resist changes in pH depends upon

(i) The actual concentration of salt and acid present in the buffer and

(ii) the salt acid concentration ratio

Eg:- Ammonium acetate

When a drop of HCl is added to a litre of pure water, the pH of the water changes immediately from 7 to 2.2.

Similarly, if a drop of NaOH solution is added to a liter of pure water, the pH of the water increases from 7 to about 13.

The same type of changes occurs for an aqeous solution of Nacl. However, such change will

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not occur in some solutions like ammonium acetate. Even when 1ml of acid (or) alkali is added to this solution, the pH of the solution will remain nearly 7.

The buffer solutions possess reserve acidity as well as reserve alkalinity.

Thus ammonium acetate (CH_3COONH_4) has reserve acidity due to the presence of NH_4^+ ions and reserve alkalinity due to the presence of CH_3COO^- ions.

Any solution containing a weak acid together with one of its salts (or) a weak base with one of its salts, function as a buffer.

Two types of buffer

1) Acid buffer 2) basic buffer

Acid buffer:- It consists of a weak acid and its salt

Eg:- $CH_3COOH + CH_3COONa$ (Acetic acid + sodium acetate)

Basic buffer:- It is a mixture of a weak base and its salt

Eg:- $NH_4OH + NH_4Cl$ [Ammonium hydroxide + Ammonium chloride]

A buffer can be represented by placing the acid (or) base as the numerator and its salt as the denominator

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Acid buffer = $\frac{\text{Acid}}{\text{Salt}}$

Basic buffer = $\frac{\text{Base}}{\text{Salt}}$

Examples

1) Acetate buffer - $\frac{\text{Acetic acid}}{\text{Sodium acetate}} = \frac{CH_3COOH}{CH_3COONa}$

2) Bicarbonate buffer - $\frac{\text{Carbonic acid}}{\text{Sodium bicarbonate}} = \frac{H_2CO_3}{NaHCO_3}$

3) Ammonium hydroxide - $\frac{\text{Ammonium hydroxide}}{\text{Ammonium chloride}} = \frac{NH_4OH}{NH_4Cl}$

Biological buffer

1) Bicarbonate buffer system

Bicarbonate buffer system consists of carbonic acid and sodium bicarbonate.

$$\frac{\text{Carbonic acid}}{\text{Sodium bicarbonate}} = \frac{H_2CO_3}{NaHCO_3}$$

-> It is present in blood.
-> It operates with the help of lungs.
-> Carbonic acid is a weak acid and sodium bicarbonate is a weak base. Hence they dissociate into ions slightly.
-> Lactic acid is an acid. Hence it releases large amount of H^+ ions. It changes pH of blood.
-> When lactic acid enters the blood, it is handled by the sodium bicarbonate.

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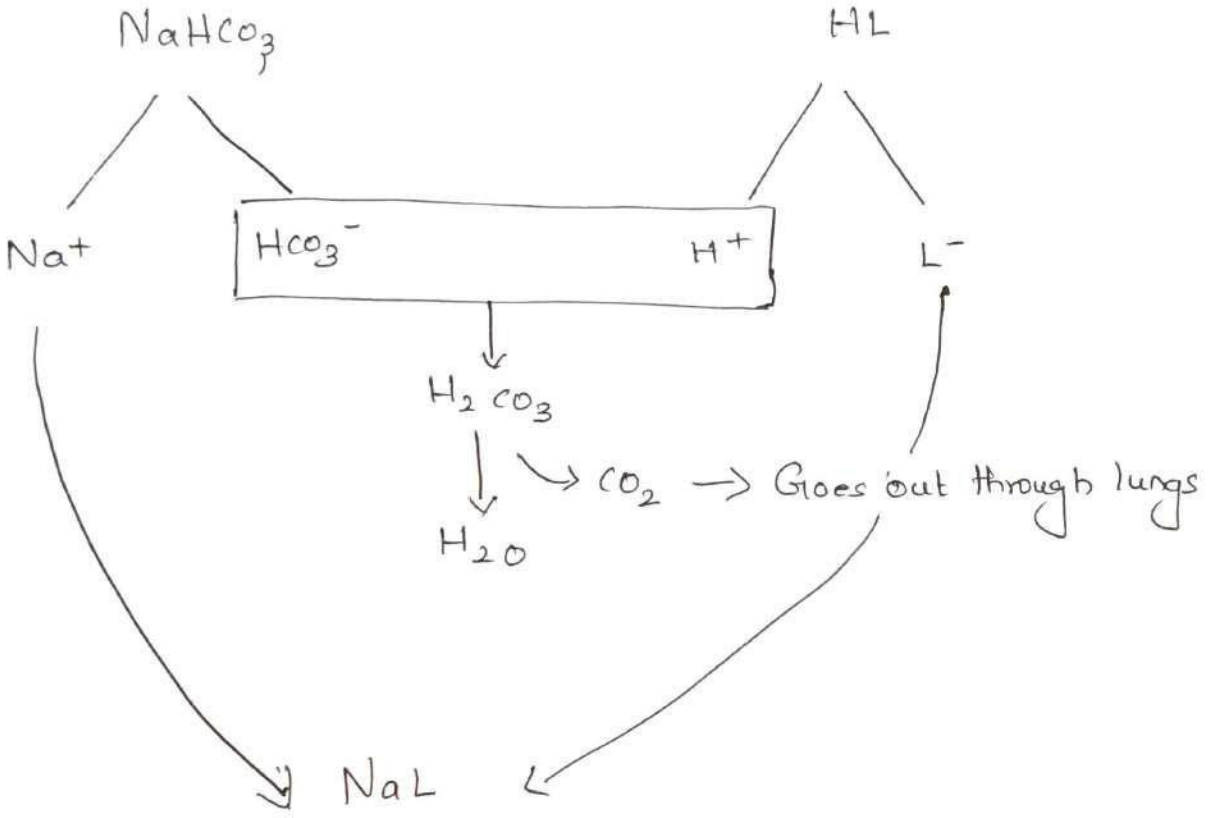
-> The sodium bicarbonate ionizes into Na^+ ions and HCO_3^- ions.



-> The lactic acid ionizes into H^+ ions and L^- (Lactate) ions.

-> The HCO_3^- ions combine with H^+ ions to form carbonic acid.

-> The Na^+ ions combine with L^- ions to form sodium lactate.

-> The carbonic acid is volatile and is converted into CO_2 and H_2O by the enzyme anhydrase.

-> CO_2 diffuses out through the lungs.

$$H_2CO_3 \xrightarrow[\text{anhydrase}]{\text{Carbonic}} H_2O + CO_2$$

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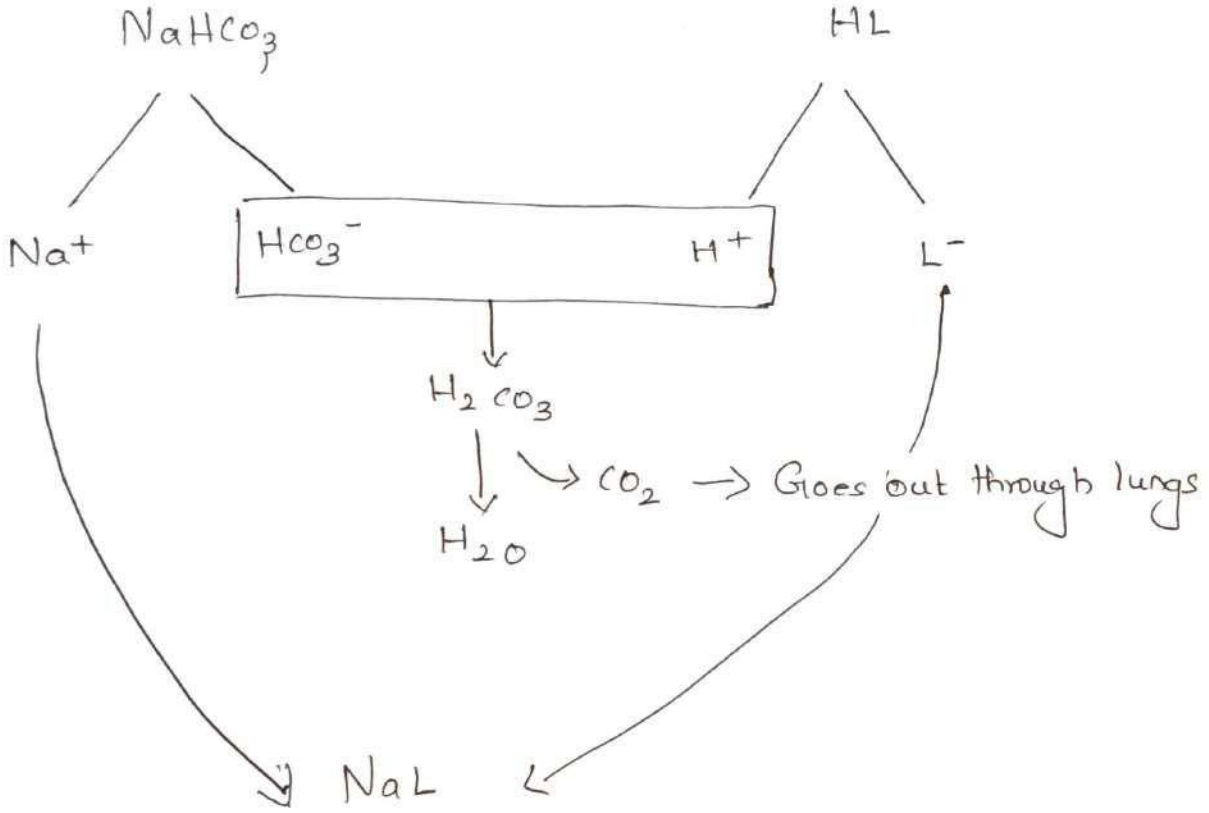
-> The sodium bicarbonate ionizes into Na^+ ions and HCO_3^- ions.



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-> CO_2 diffuses out through the lungs.

$$H_2CO_3 \xrightarrow[\text{anhydrase}]{\text{Carbonic}} H_2O + CO_2$$

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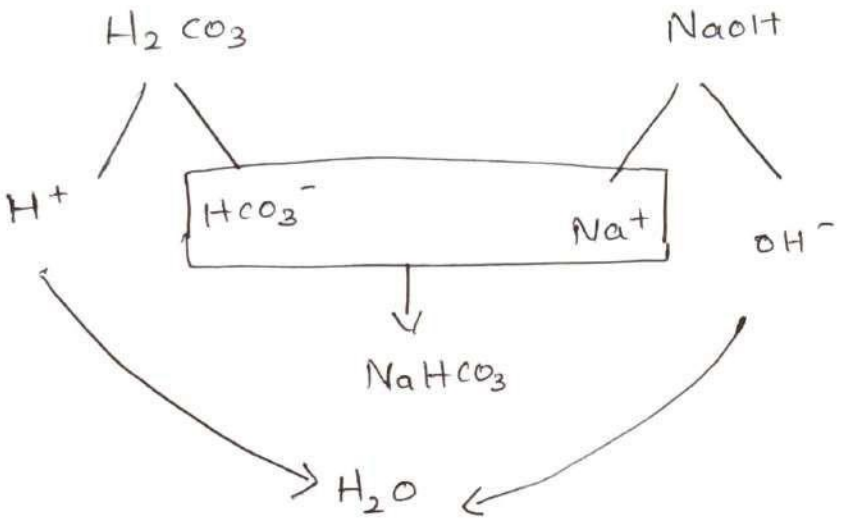
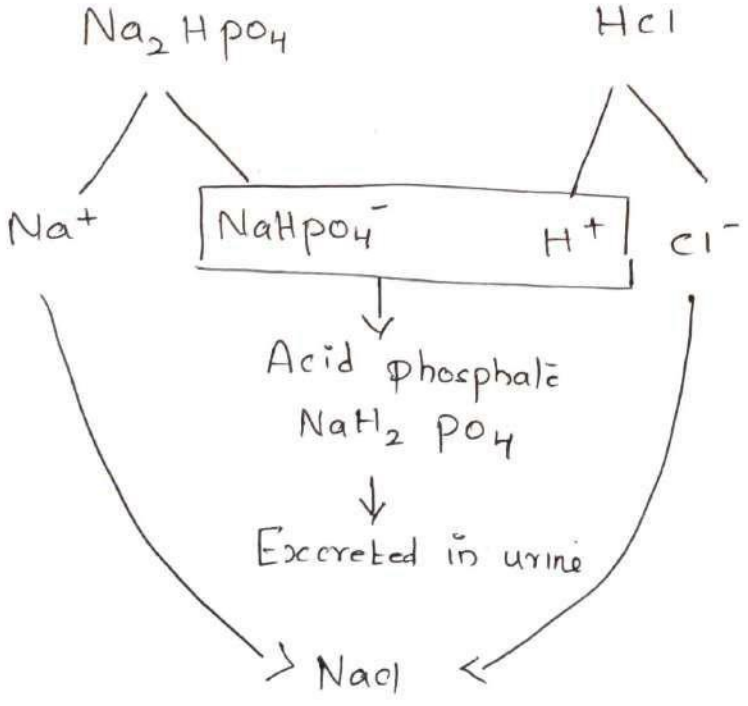
Similarly, when an alkali, NaOH enters the body fluid, it is handled and removed by carbonic acid.



2. Phosphate Buffer System

Phosphate buffer system consists of acid phosphate and alkaline phosphate.



$$\frac{\text{Acid phosphate}}{\text{Alkaline phosphate}} = \frac{NaH_2PO_4 \text{ [Sodium monophosphate]}}{Na_2HPO_4 \text{ [Disodium phosphate]}}$$

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-> It is present in the blood.

-> It operates with the help of kidney.

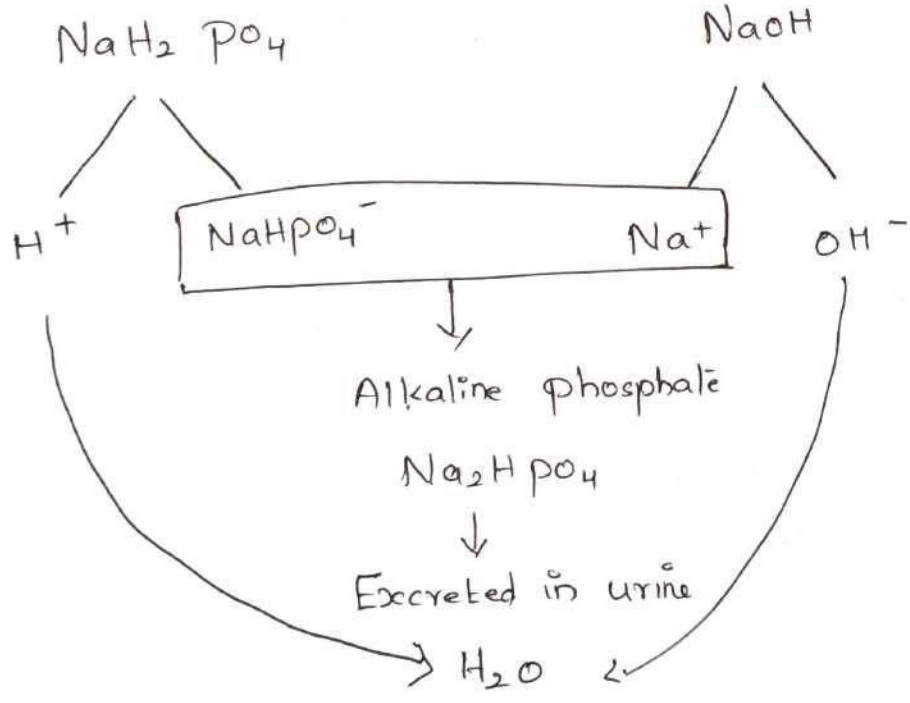
-> Acid phosphate is a weak acid and alkaline phosphate is a weak base. Hence they dissociate into ions slightly.

-> When an acid, Hcl enters the blood, it is handled by the alkaline phosphate.

-> The acid phosphate thus produced is excreted by the kidney.

-> When an alkali, NaOH enters the blood, it is handled and moved by the acid phosphate.

-> The acid phosphate formed is removed by the kidney.



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Henderson - Hasselbalch equation:

Consider a weak acid HA ionises as follows

$$HA \rightleftharpoons H^+ + A^- \rightarrow (1)$$

The equilibrium constant for this dissociation is

$$K_a = \frac{[H^+][A^-]}{[HA]} - (2)$$

$$[H^+][A^-] = K_a [HA]$$

÷ both sides by $[A^-]$ we get

$$[H^+] = K_a \frac{[HA]}{[A^-]} - (3)$$

Take log on both sides

$$\log [H^+] = \log K_a + \log \frac{[HA]}{[A^-]} - (4)$$

x equ (4) by -1 we get

$$-\log [H^+] = -\log K_a - \log \frac{[HA]}{[A]} - (5)$$

we know that

$$pH = -\log [H^+], \quad pK_a = -\log [K_a]$$

$$pH = pK_a - \log \frac{[HA]}{[A^-]} - (6)$$

$$pH = pK_a + \log \frac{[A^-]}{[HA]} - (7)$$

equ (7) Represents Henderson - Hasselbalch equation.

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Energy in living organisms

Every organism, be it a primitive form of life or the most complex form like a human being, needs energy for its survival. The status of a living organism is basically characterised by the maintenance of a relatively different (higher) concentration of molecules and ions from those of its surroundings. The maintenance of this concentration gradient is important for life. In other words, an organism is never at equilibrium with its surroundings. Living organisms come to equilibrium with their surrounding only after death. To maintain this concentration gradient vital for life, the organism needs to spend energy.

Energy is also needed to perform mechanical work, to synthesis biomolecules for maintaining its structural integrity and to store and transfer genetic information during replication for the continuity of the species. Living organisms must work to stay alive and to reproduce. They require energy to work. Living organisms derive energy from sunlight or through consumption of nutrients from their surroundings. They exchange energy and matter with the environment. The basic mechanism of energy generation in living cells is through the flow of electrons in oxidation-reduction reactions.

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Photosynthetic cells (plants) absorb solar energy in the form of light and utilise it to transfer electrons from water to carbondioxide, forming energy-rich products and releasing oxygen into the atmosphere. Non-photosynthetic cells (animals) garner energy by oxidising the energy-rich products of photosynthesis and transferring electrons to oxygen to form water, carbondioxde and other end products. These are recyled in the environment. Thus, sunlight is the ultimate source of energy for all forms of life.

Though the chemical composition of an organism is always constant, the molecules present in the cell or the organism are not static. Instead, they are continously synthesized and broken down using energy. The rate of synthesis always balances the rate of degradation, thereby maintaining a constant internal environment, known as homeostasis.

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Introduction

Carbohydrates are defined as optically active Polyhydroxy aldehydes (or ketones; or substances that yield one of these compounds on hydrolysis. Carbohydrates are the most abundant class of biomolecules in nature. They are also known as saccharides (sugars). They are widely distributed in plants and animals.

Carbohydrates are hydrates of carbon. They contain carbon, hydrogen and oxygen in the ratio of 1:2:1. Carbohydrates are represented by the general formula $C_x(H_2O)_y$. For example glucose has the molecular formula $C_6H_{12}O_6$.

Physical properties of carbohydrates

1) Carbohydrates act as energy reserves, also store fuels, and metabolic intermediates.
2) Ribose and deoxyribose sugars form the structural frame of the genetic material RNA and DNA.
3) Polysaccharides like cellulose are the structural elements in the cell wall of bacteria and plants.
4) Carbohydrates are linked to proteins and lipids that play important roles in cell interactions.
5) Monosaccharides - These are crystalline compounds, soluble in water, sweet to taste, and need digestion in order to be absorbed into the blood stream.
6) Disaccrides - These are crystalline, water soluble, sweet to taste and must be digested to monosaccharides before they can be absorbed and used for energy.

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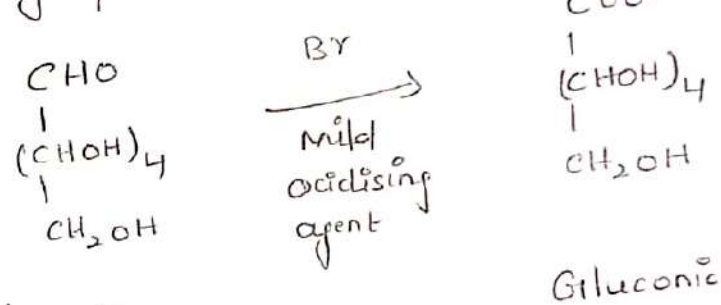
iii) polysaccharides - These are not water soluble and are not crystalline. They form colloidal suspensions instead of solutions. They are not sweet and must be digested before being absorbed.

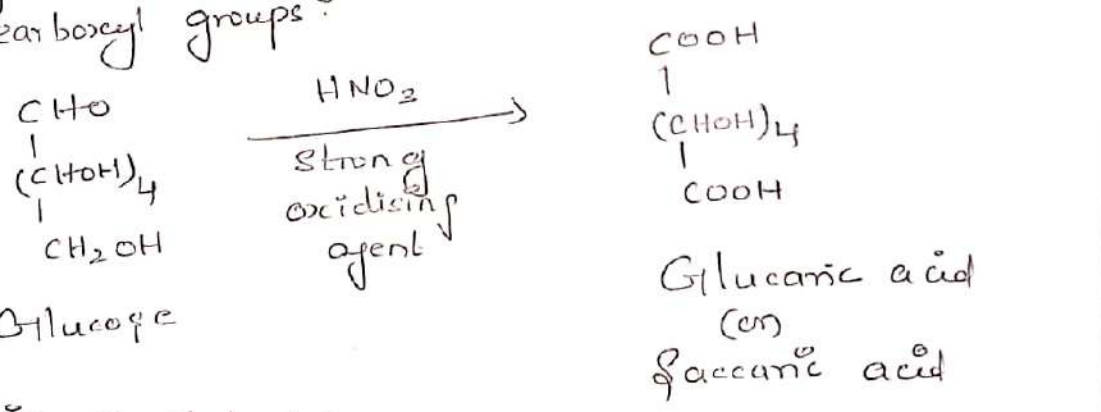
Chemical properties of carbohydrates

1) Oxidation of sugars

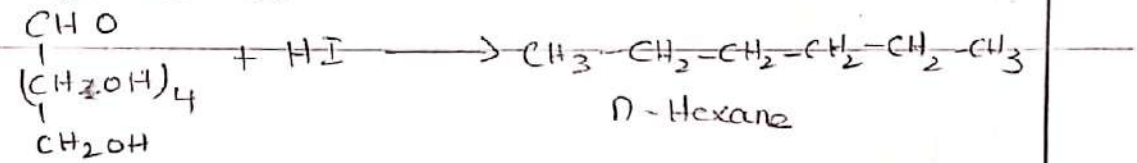
a) When glucose is treated with bromine water, it forms gluconic acid. The aldehyde group is oxidised to carboxyl group



b) When glucose is treated with nitric acid, both aldehyde and primary alcohol groups are oxidised to carboxyl groups.



2) Action of Hot HI



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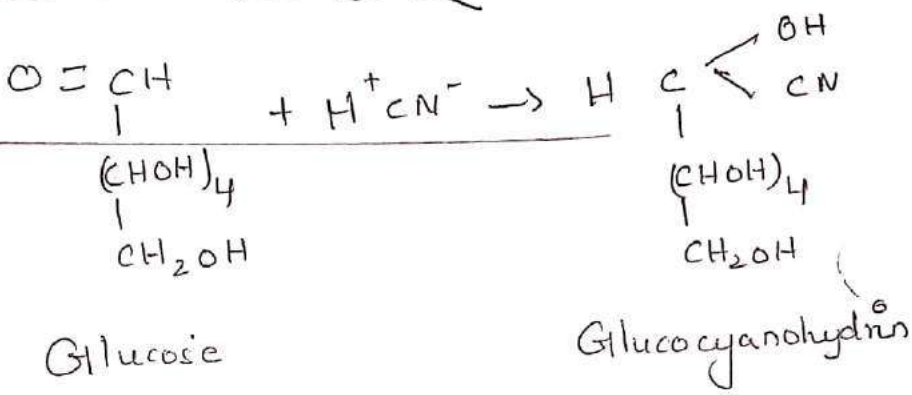
Oxygen is removed and hydrogen is added. Open chain structure is converted into a straight chain structure.

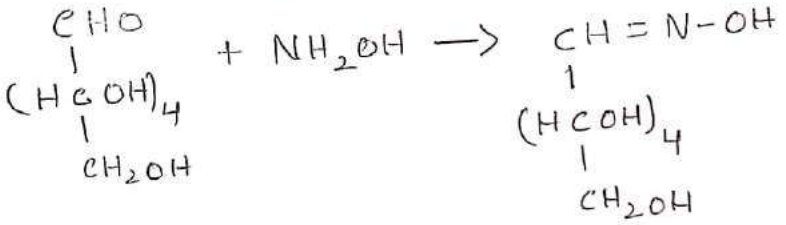
3) Action of hydrogen cyanide



Glucose

Glucocyanohydrin

4) Action of hydroxylamine



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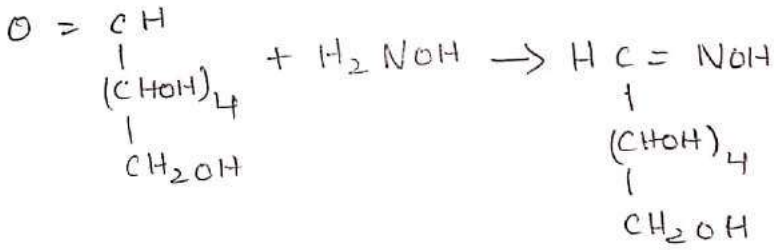
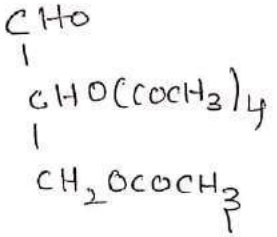


5) Action of acetic anhydride



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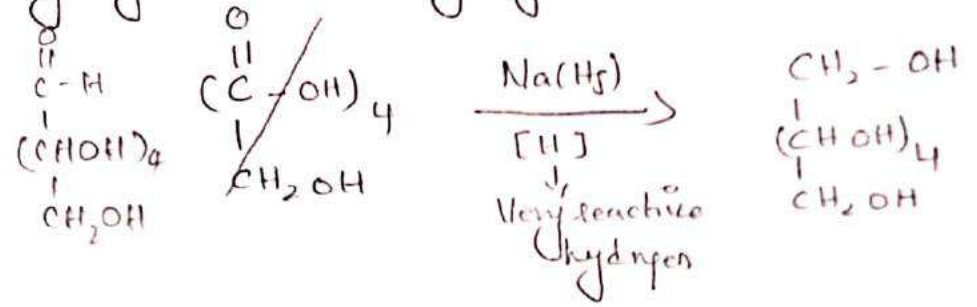
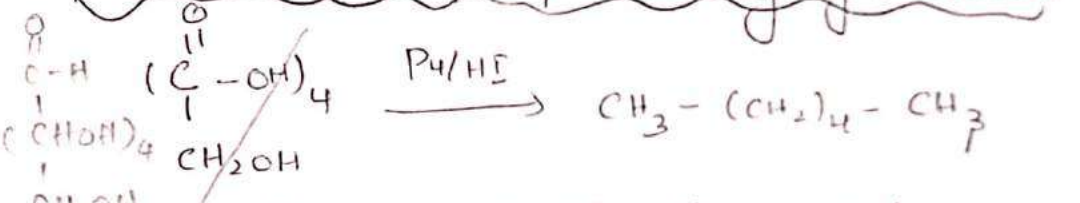
6) Reduction of Sugar

a) Reduction with Na/Hg (Sodium amalgam) converts the monosaccharides to corresponding alcohols. Glucose is reduced to Sorbitol when sodium displaces hydrogen, Nascent hydrogen is released.



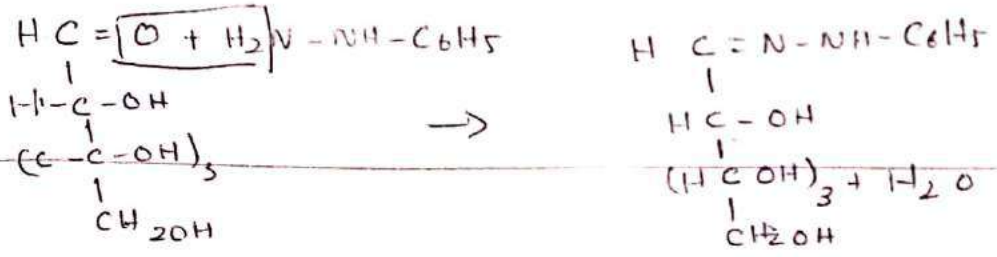
b) Reduction with Phosphorous hydrogen Iodine



Being more Reactive, it can reduce any functionality (-OH (or) -CHO) into alkene.

7) Formation of Osazone

Osazone is yellowish, crystalline compound, produced as a result of heating Sugar solution with Phenylhydrazine. Osazones are formed by those Sugars which Contain a free aldehyde (or) ketone group. For eg: one molecule of glucose reacts with three molecules of Phenyl hydrazine to form glucosazone



Glucosazone

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Structure of carbohydrates.

The carbohydrates can be structurally represented in any of the three forms.

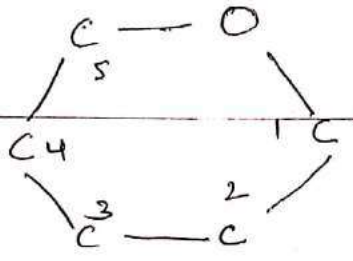
1) Open chain structure
2) Hemi-acetal structure
3) Haworth structure

1) Straight chain structure (or) open chain structure

In straight chain structure, the 6 carbon atoms of glucose are arranged in a straight line. It is also called open chain structure because the two ends remain seperate and they are not linked.

2) Hemi-acetal structure- Here the 1st carbon of the glucose condenses with the OH group of the 5th carbon to form a ring structure.

3) Haworth structure:- It is the presence of pyranose ring structure. It is a 6-membered ring. It is a hexagonal ring. This ring resembles the ring of a compound called Pyran and hence the ring is called pyranose ring.



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Classification of carbohydrates

Carbohydrates are optically active polyhydroxy aldehydes or ketones. They are classified into two types, namely sugars and non sugars. Sugars are sweet in taste and soluble in water. They are of two types, namely

* Monosaccharides
* Oligosaccharides

Monosaccharides are simple sugars. They cannot be hydrolysed into simple sugars. They are sweet in taste and soluble in water. Eg: glucose, fructose, galactose etc.

Oligosaccharides are sugars which yield 2 to 10 monosaccharides on hydrolysis. They are sweet in taste and soluble in water. Eg:- Maltose, lactose, sucrose etc.

Depending on the number of sugars, oligosaccharides are classified into disaccharides, trisaccharides and so on.

Non sugars do not have sweet taste and they are insoluble in water. Eg: Starch, glycogen, cellulose, Chitin etc.

Non-sugars are formed by the linking of many monosaccharides. Hence non-sugars are called polysaccharides.

Polysaccharides are of two types, namely

Homopolysaccharides

Heteropolysaccharides

A Homopolysaccharide is formed by the linking of a single type of monosaccharide.

A heteropolysaccharide contains two types of monosaccharide.

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i) Monosaccarides -> [Simple Sugars]

-> Single Sugar unit
-> which cannot be hydrolysed further

Disaccharides -> [two Sugar unit] -> connected to each other by glycocytic link

Oligosaccharides -> 3 - 10 Sugar unit

Polysaccharides -> > 10 Sugar unit

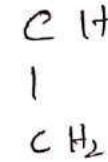
Monosaccharides

They are further subdivided according to the number of carbon atoms contained in their structure

1) Diose

Diose has two carbon atoms and its molecular formula is $C_2H_4O_2$.

Example :- Glycolaldehyde



2) Triose

Triose contains three carbon atoms ($C_3H_6O_3$) Since triose contains Polyhydroxylic groups, it is considered as a true carbohydrate.

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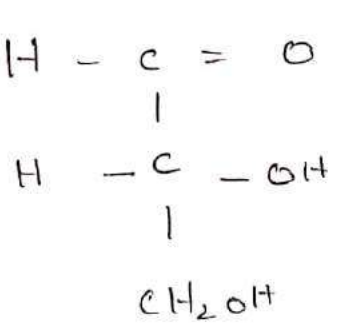
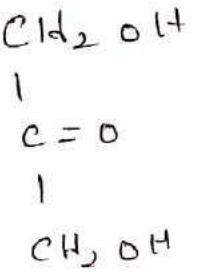
eg:-



Glyceraldehyde

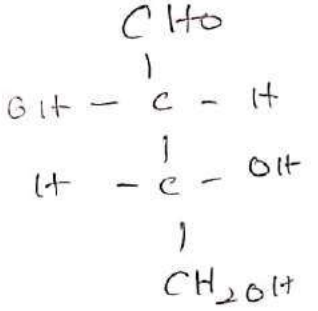


Dihydroxyacetone

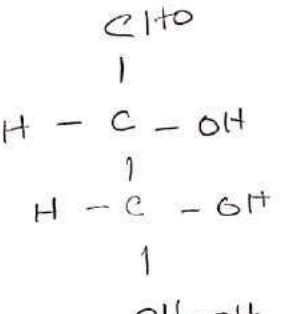
3. Tetrose

It contains four carbon atoms ($C_4H_8O_4$)

eg:-



D - threose



D - Erythose

4 Pentose

Pentose contains five carbon atoms ($C_5H_{10}O_5$). Pentoses are physiologically important because ribose and deoxyribose are constituents of nucleic acid. Ribose is also a constituent of the Vitamin riboflavin

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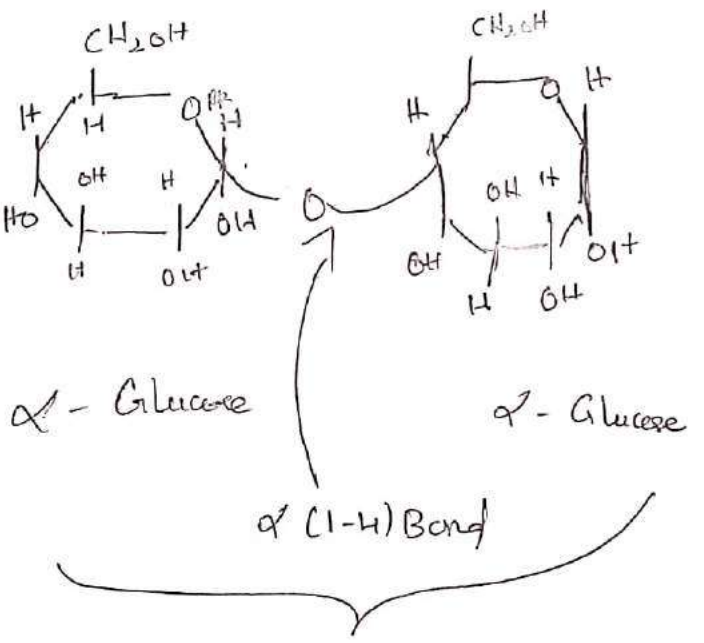
Disaccharides

Disaccharides are sugars containing two molecules of monosaccharides. When condensation occurs between monosaccharides, union takes place between C-1 of the second Monosaccharide & C-4 of the first Monosaccharide

Eg :- Maltose

Maltose



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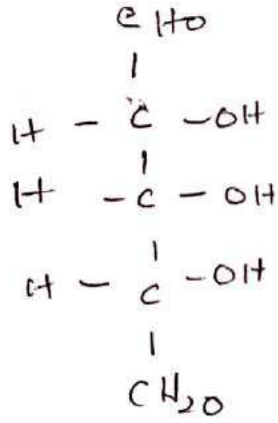
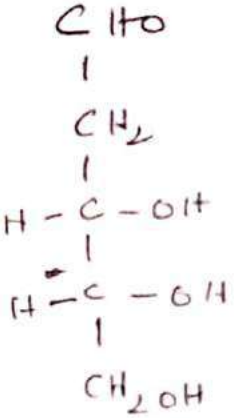
Eg :-



D - Ribose

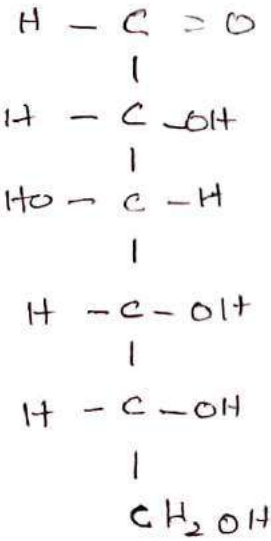


deoxyribose

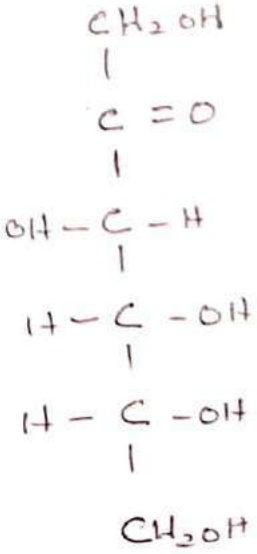
5) Hexoses

These are physiologically important compounds. They contain six carbon atoms Molecular formula $C_6H_{12}O_6$.

Eg.



D - Glucose



D - fructose

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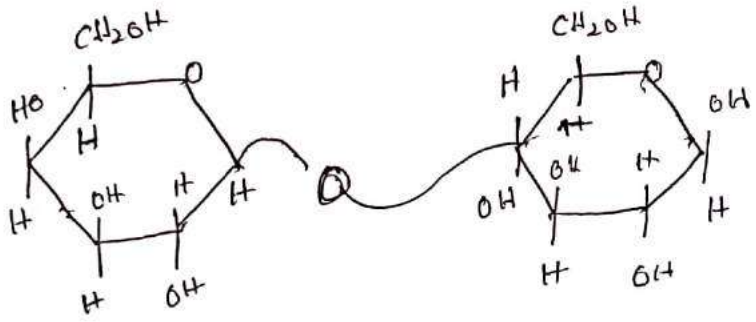
Lactose (found in milk)

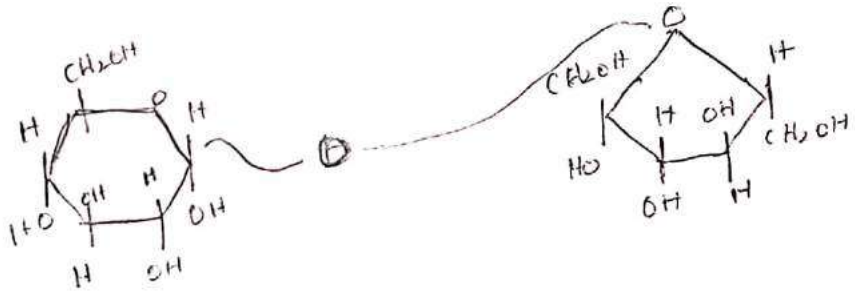


β - Galactose β Glucose

β (1-4) Bond

Lactose

Sucrose (Table sugar) formed by plants



α - Glucose β - fructure

Oligosaccaride :- Short chain of monosaccharides

less than 20 monosaccharics.

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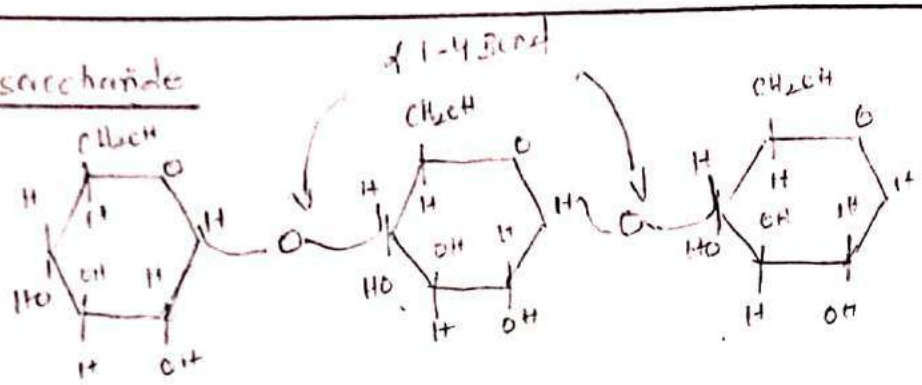
Oligosaccharide



Maltotriose

Polysaccharides :- Also known as Glycans.
most carbohydrates found in nature occur as Polysaccharides

Homopolysaccharide :

contains only a single type of monosaccharide

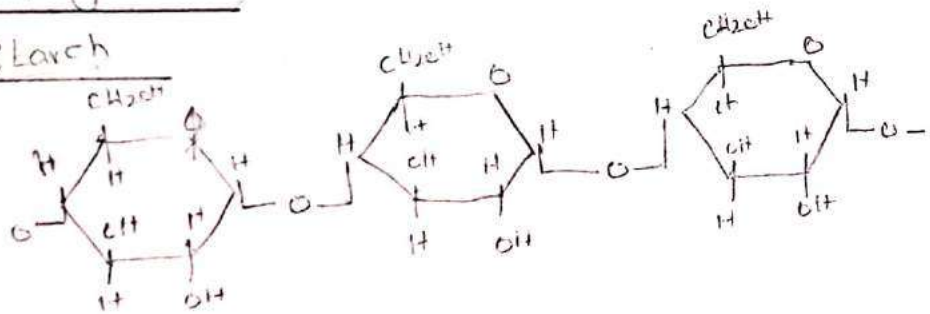
eg:- only contains glucose molecules linked together eg: Starch, glycogen.

Heteropolysaccharides contains two or more type of monosaccharides. eg:- dextrins, cellulose

eg :- long chain of glucose & fructose molecules

Homopolysaccharides

Starch



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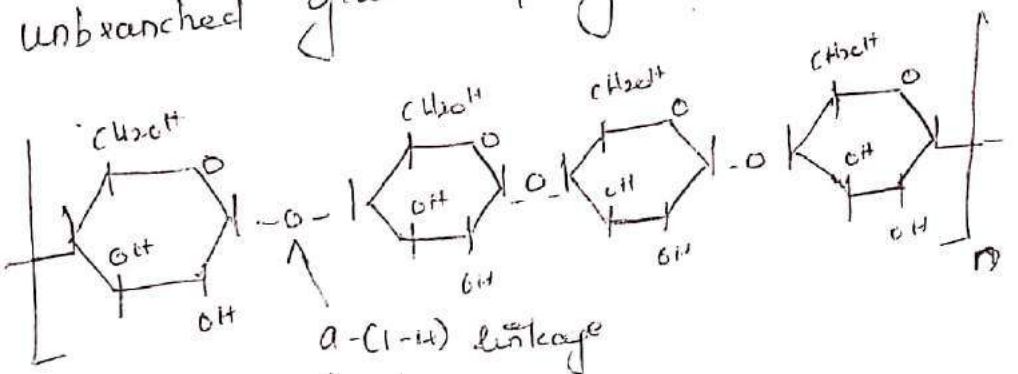
Two types

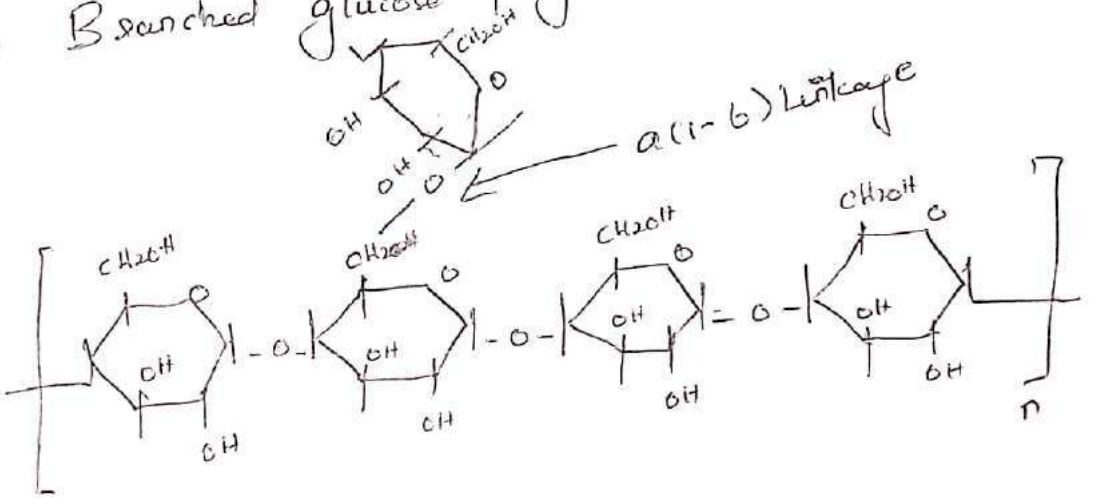
1) Unbranched glucose polymer



α-(1-4) linkage

Amylose

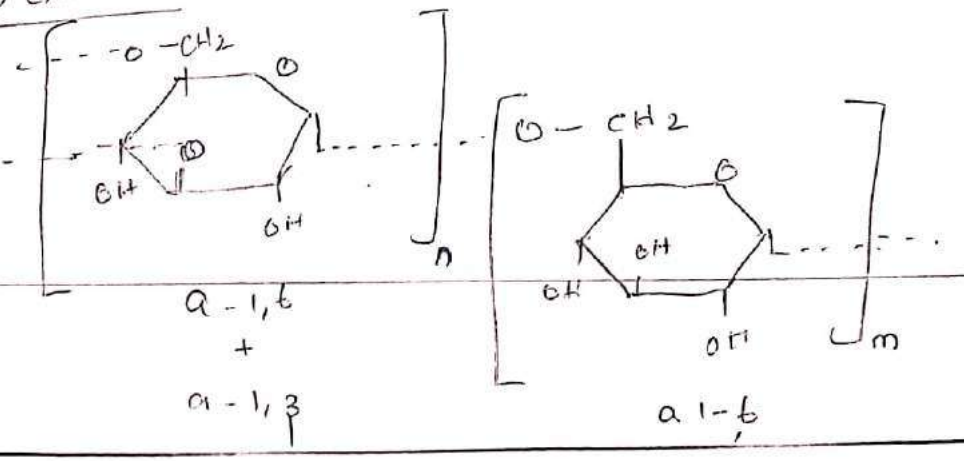
2) Branched glucose polymer



Amylopectin

Heteropolysaccharides

Dextran



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Glycolysis

1) Glycolysis is the process of breaking down glucose
2) Glycolysis can take place with (or) without oxygen.
3) Glycolysis produces two molecules of Pyruvate, two molecules of ATP, two molecule of NADH, & two molecules of water.
4) Glycolysis takes place in the cytoplasm.
5) There are 10 enzymes involved in breaking down Sugar.
6) The 10 steps of glycolysis are organized by the order in which specific enzyme act upon the system.
7) It is the first stage of cellular respiration

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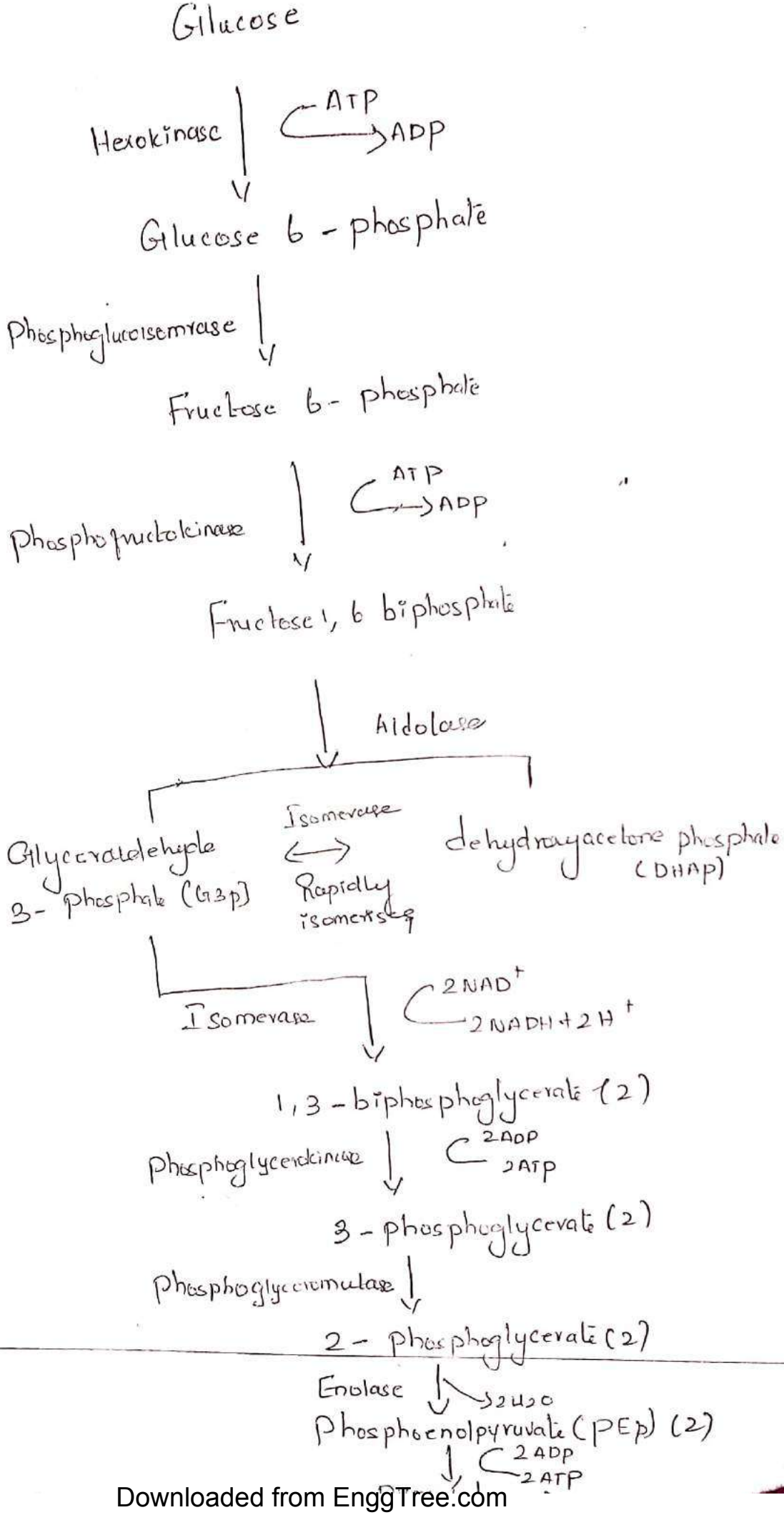

NAD
↓
nicotinamide adenine dinucleotide

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Steps of glycolysis

1) The first step in glycolysis is the conversion of D-glucose into glucose-6-phosphate. The enzyme that catalyzes this reaction is hexokinase.

2) The second reaction of glycosis is the rearrangement of glucose 6-phosphate (G6P) into fructose 6-phosphate (F6P) by glucose phosphate isomerase.

3) Phosphofructokinase, with magnesium as a cofactor, changes fructose 6-phosphate into fructose 1,6-biphosphate

4) The enzyme Aldolase splits fructose 1,6 biphosphate into two sugars that are isomers of each other. These two sugars are dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP).

5) The enzyme triophosphate isomerase rapidly inter-converts the molecules dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP). Glyceraldehyde phosphate is removed/ used in next steps of glycolysis.

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6. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) dehydrogenates and adds an inorganic phosphate to glyceraldehyde 3-phosphate, producing 1,3-biphosphoglycerate.

7. Phosphoglycerate kinase transfers a phosphate group from 1,3-biphosphoglycerate to ADP to form ATP and 3-phosphoglycerate.

8. The enzyme phosphoglyceromutase relocates the P from 3-phosphoglycerate from the 3rd carbon to the 2nd carbon to form 2-phosphoglycerate.

9. The enzyme enolase removes a molecule of water from 2-phosphoglycerate to form phosphoenolpyruvic acid (PEP)

10. The enzyme pyruvate kinase transfers a P from phosphoenolpyruvate (PEP) to ADP to form pyruvic acid and ATP Result in Step 10

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Glycogenesis

The food we eat are turned into glucose and released as energy to be able to use by the body. The molecule of glucose that is stored in the important organs of the body is called glycogen.

It is stored in various parts of the body such as the kidney, liver, and muscles. It is only released if the glucose in the blood is used up for all physical activities. Once the body runs out of glucose supply, additional energy is immediately released in the form of glycogen.

Glycogen is a polysaccharide deposited in the tissues and stored as a carbohydrate. During hydrolysis, glycogen is converted into glucose.

Glycogenesis

It is process by which glycogen is formed from glucose. Glycogen is synthesized accordingly as per the demand of energy. If there is sufficient amount insulin in the body, excess glucose will not be used and will only be stored in the form of glycogen.

If the body runs out of insulin, the stored glucose will be released to supplement the body's need of energy. in the form of glycogen and the through the process of glycogenesis

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Steps

1) Activation of glucose
2) Initiation of glucose synthesis
3) Elongation
4) Branching
5) Elongation & Branching [continues]
6) Glycogen

1) Activation of glucose/synthesis of UDP-G

Glucose

↓ Glucokinase

Glucose 6-phospate

↓ Mutase

Glucose 1-phosphate

UTP ↓

UDP-G

UTP - Uridinetriphosphate
UDP - Uridinediphosphate

2) Initiation of glucose Synthesis

For the synthesis of glycogen, Glycogen prime is required. Glycogen primer is short glucose molecule (or) pre existing glycogen molecule. to which there is a addition of glucose (or) in the absence of glucose primer, there is one substance

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called glycogenin, is a protein with oligosaccride side chain, 7 glucose residues are attached to glucogenin. In the absence glycogen primer, glycogenin can initiate the synthesis of glycogen

G - G - G - G - G — Glycogenin

3) Elongation :- With the help of glycogen synthase, the UDP-G gives glucose molecules to elongate the glucogen primer. In each step there is addition of one glucose molecules which is carried by the UDP-G, so there is lengthening of molecules

G - G - G - G - G - G - G — Glycogenin

↓ glycogen synthase

G - G - G - G - G - G - G - G - G - G - G — Glycogenin

4) Branching :- When the glucose chain is long [11-12] G residues, 6-8 glucose molecules from this chain get detached from linear glucose molecule and it get attached to another side by glucosidic linkages between the first and fourth carbon atoms (1-4) of adjacent glucose molecules

G - G - G - G - G - G - G - G - G - G - G - G

↓ ← Branching enzyme

G - G - G - G - G - G - Glycogen

G - G - G - G

5) Elongation & Branching continues ↓

6) Formation of ⟶ Glycogen

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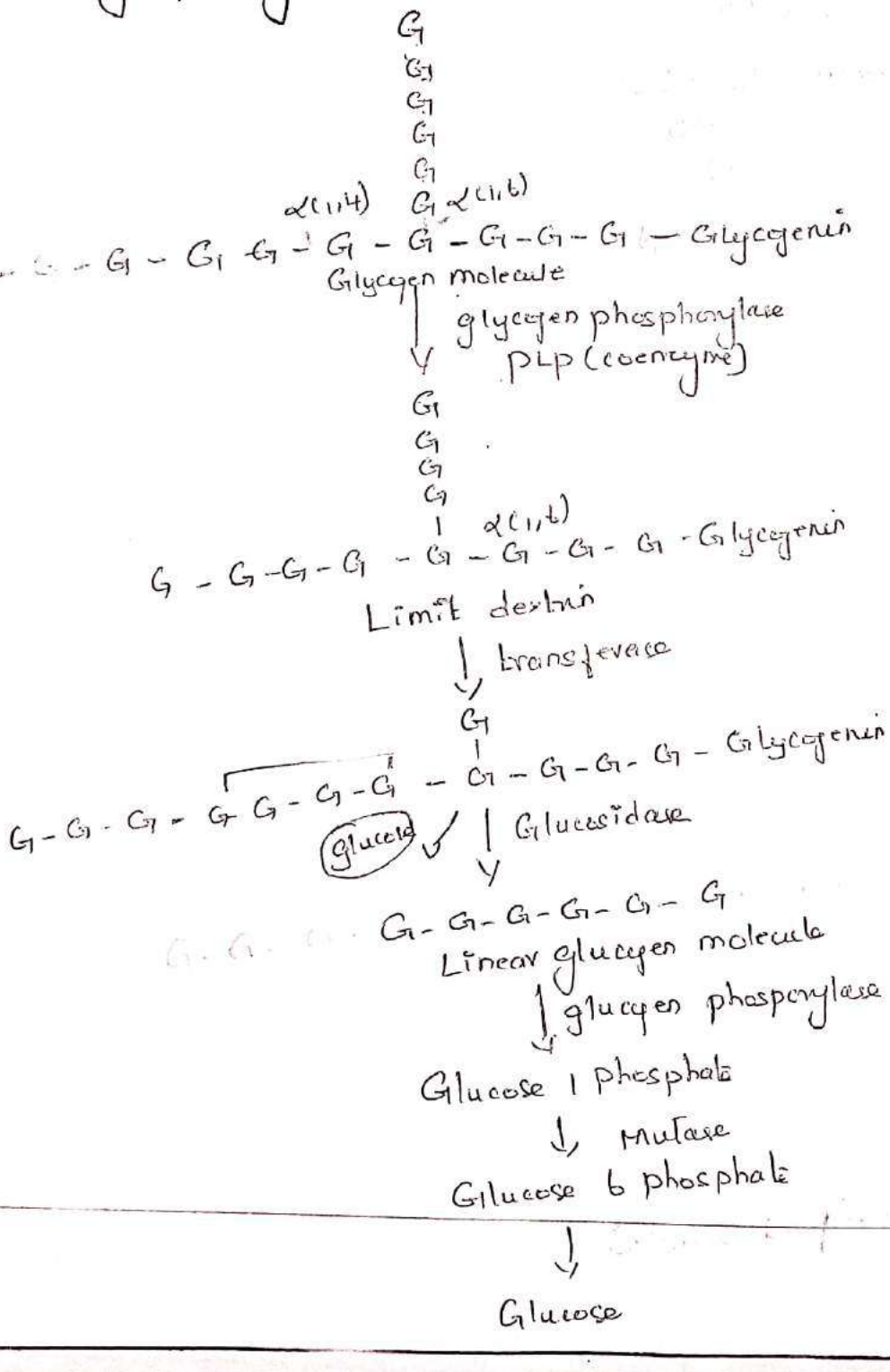
Glycogenolysis

Glycogenolysis, process by which glycogen, the primary carbohydrate stored in the liver and muscle cells of animals, is broken down into glucose to provide immediate energy and to maintain blood glucose levels during fasting.



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Steps

1) Phosphorolysis / Shortening of chains

-> Glycogen is a branched polymer of glucose units in chains are linked by α-1,4-glycosidic bonds with a branch point created by α-1,6-glycosidic bonds at approximately every 10 residues of glucose.

-> The key enzyme for glycogenolysis, glycogen Phosphorylase, will leave the α-1,4-glycosidic bonds of the terminal glucose residues at the non-reducing end of glycogen, until only four glucosyl units remain on each chain before a branch point.

2) Debranching / Removal of branches

-> Glycogen phosphorylase can only carry out the glucogen breakdown process by itself until a limited extent before encountering a obstacle.

-> When phosphorylase reaches a terminal residue four residues away from a branch point, it will stop cleaving and the α-1,6 linkages are not susceptible to cleavage by phosphorylase.

-> The branches of the glycogen molecules are removed by the debranching enzyme, a single bifunctional protein with two enzymic activities.

-> The debranching enzyme can act as a transferase as well as an α-1,6-glucosidase to aid the continued degradation by phosphorylase.

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-> A block of three glycosyl residues from one outer branch was shifted by the transferase.

-> The remaining single glucose molecule has α-1,6- glucosidase will cleave the linkage and results in the release of a free glucose molecule.

-> The glycolytic enzyme, hexokinase will phosphorylate this free glucose molecule. Thus, the net result is a linear structure which can be continue degraded by glycogen phosphorylase.

3) Recovery

-> As in the glycolysis pathway, phosphoglucomutase is used to convert glucose 1- phosphate formed in the clevage of glycogen into glucose 6- phosphate to enter the metabolic mainstream.

4) Release

-> This process will occur in lives.

-> In contrast with glucose, the phosphorylated glucose produce in the glycogen breakedown is not readily to be transported out of the cell.

-> The liver containg a glucose 6- phosphatase, ~~locate~~ which conver the glucose 6- phosplate into glucose by cleaving the phosphoryl group.

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Digestion and absorption of carbohydrates

1) The mechanical and chemical digestion of carbohydrates begins in the mouth. chewing crumbles the carbohydrate food into smaller and smaller pieces.

2) The salivary glands in the oral cavity secrete saliva that coats the food particles.

3) Saliva contains the enzyme salivary amylase This enzyme breaks the bonds between the monomeric sugar units, disaccharide: oligosaccharides and starches.

4) The salivary amylase breaks down amylose and amylopectin into smaller chains of glucose, called dextrins and maltose, only about 5% of starches are broken down in the mouth.

5) From the stomach carbohydrate gradually expelled into the upper part of the small intestine the pancreas releases pancreatic juice through a duct.

4) This pancreatic juice contains the enzyme pancreatic amylase, which starts again the breakdown of dextrins into shorter and shorter carbohydrate chains.

5) Additionally, different enzymes secreted by intestine cells namely, sucrase, maltase and lactase breakdown the sugar chains into simple sugar units. They are then [illegible] inside of the intestinal cells.

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Absorption of carbohydrates

1) The cells in the small intestine have membrane that contain many transport proteins in order to get the monosaccharides and other nutrients into the blood

2) From blood it can be distributed to the rest of the body

3) The first organ to receive glucose, fructose and galactose is the liver.

4) The liver takes them up and converts galactose to glucose, breaks fructose into even smaller Carbon containing units and either stores glucose and glycogen or exports it back to the blood.

Biochemical aspects of diabetes mellitus

Diabetes mellitus (DM) is one of the world's most important public health problems. It is a metabolic disorder resulting either from deficiency of insulin or resistance to its action, causing increased blood glucose level.

Diabetes mellitus is broadly classified into two categories. They are type-I and type-II

a) Type-I Diabetes mellitus

It is also known as insulin dependent diabetes mellitus. About 5% to 10% of all cases of diabetes mellitus belongs to this Category 1. This disease is due to loss of pancreatic

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β-cell function resulting in deficiency of insulin. Hence, these patients are dependents on insulin injections.

Type I DM is considered as autoimmune disease in which autoreactive T-cells of the immune system destroy β-cells of islets of the pancreas.

b) Type-II Diabetes Mellitus

About 90% diabetes patients belong to this category. It usually affects the individual 40 years of age. In this case patients are not dependent on insulin, hence it is also called non-insulin dependent diabetes mellitus. In this type of disease insulin is not deficient, but its action is impaired (i.e, insulin resistance). Insulin resistance is defined as a decreased biological response to normal levels of circulating insulin. Type-II DM is most commonly associated with obesity.

Metabolic Abnormalities in Diabetes

Metabolic alterations in carbohydrate metabolism:

1) Insulin resistance or insulin deficiency in decreased glucose uptake and underutilization of glucose by cells.

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2) All enzymes dependent on insulin [illegible] less active.

3) As a result, glycolysis and glycogenesis, are decreased.

4) Glycogenolysis and gluconeogenesis are increased. The net result is elevated. When glucose levels cross the renal threshold it spills into urine (glycosuria)

Metabolic alteration in lipid metabolism

1) underutilisation of glucose ~~levels~~ leads to increased utilization of fatty acids forming more acetyl CoA that can be handled by the krebs cycle.

2) Further, increased glycogenesis depletes the levels of oxaloacetate, as a result, the availability of oxaloacetate to start TCA cycle is limited.

3) The excess of acetyl CoA is directed for the synthesis of excess ketone bodies resulting in Ketoacidosis

Metabolic alterations in protein Metabolism

Protein breakdown is increased causing muscle wasting. Amino acids formed from protein degradation are used as substrates for glucogenesis

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Glycogen storage disease

→ The enzyme defects causing altered glycogen synthesis and degradation result in inborn error of glucogen metabolism

→ These disorders are characterised by the accumulation or altered function of glucogen in the liver, muscle and other organs associated with glycogen metabolism.

About 12 glycogen storage diseases have been identified and explored till date

Type 0 (Lewis disease) - Liver

Type I (Liver, kidney, intestines) - Von Gierke's disease

Type II (pompe's disease) - Muscles, heart, liver, nervous system, blood vessels

Type IV (Anderson's disease) - Liver, heart, Skeletal muscles, blood cells.

Type III (Forbes-cori disease) - Liver, heart, Skeletal muscles

Type V (McArdle's disease) - Skeletal muscles

Type VI (Hers disease) - Liver, blood cells

Type VII (Tarui's disease) - Skeletal muscles, blood cells

Type IX - Liver

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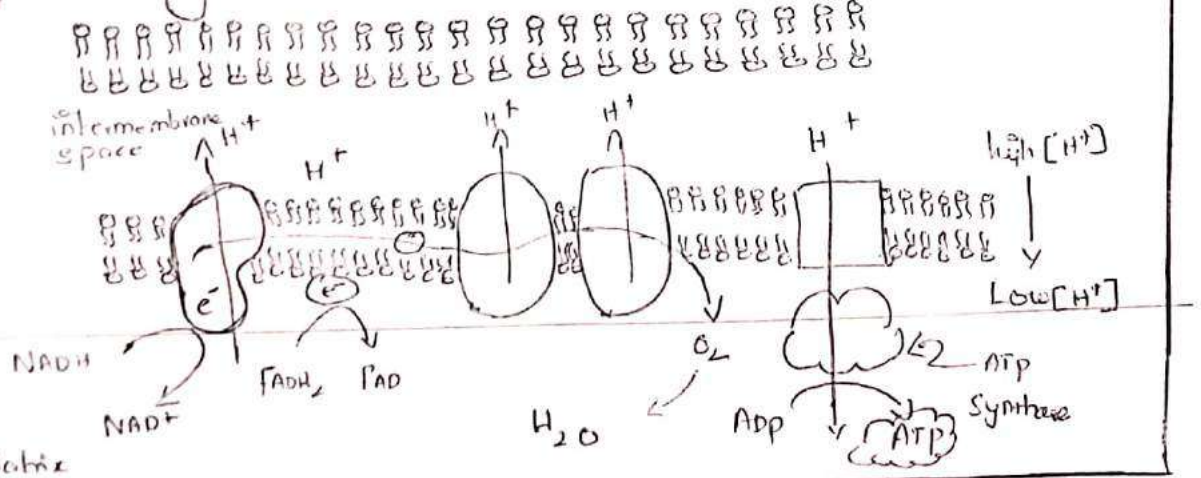
Oxidative Phosphorylation

Oxidative phosphorylation is the process in which ATP is formed as a result of the transfer of electrons from NADH or $FADH_2$ to O_2 by a series of electron carrier. The NADH and $FADH_2$ formed in glycolysis, fatty acid oxidation, and the citric acid cycle are energy rich molecules because each contains a pair of electrons having a high transfer potential. When these electrons are used to reduce molecular oxygen to water, a large amount of free energy is liberated, which can be used to generate ATP. The energy released in these reactions is captured as a proton gradient, which is then used to make ATP in a process called Chemiosmosis. Together, the electron transport chain and chemiosis make up oxidative phosphorylation



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The key steps of the process are

1) Delivery of electrons by NADH and $FADH_2$

Reduced electron carriers (NADH and $FADH_2$) from other steps of cellular respiration transfer their electrons to molecules near the beginning of the transport chain. In the process, they turn back into NAD^+ and FAD, which can be reused in other steps of cellular respiration.

2) Electron transfer and proton pumping.

As electrons are passed down the chain, they move from a higher to a lower energy level, releasing energy. Some of the energy is used to pump H^+ ions, moving them out of the matrix and into the intermembrane space. This pumping establishes an electrochemical gradient.

3) Splitting of oxygen to form water. At the end of the electron transport chain, electrons are transferred to molecular oxygen, which in half and takes up H^+ to form water.

4) Gradient-driven Synthesis of ATP. As H^+ ions flow down their gradient and back into the matrix, they pass through an enzyme called ATP Synthase, which harnesses the flow of protons to Synthesize ATP

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Electron transport chain

The electron transport chain is the final component of aerobic respiration and is the only part of glucose metabolism that uses atmospheric oxygen. Electron transport is a series of redox reactions that resemble a relay race. Electrons are passed rapidly from one component to the next component of the chain, where the electrons reduce molecular oxygen producing water.

A complex is a structure consisting of a central atom, molecule (or protein weakly connected to surrounding atoms, molecules (or proteins. The electron transport chain is an aggregation of four of these complexes (labeled I through IV), together with associated mobile electron carriers. The electron transport chain is present in multiple copies in the inner mitochondrial membrane of eukaryotes and the plasma membrane of prokaryotes

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Complex I

All of the electrons that enter the transport chain from $NADH_2$ and $FADH_2$ molecules produced during early stages of cellular respiration: glycosis, pyruvate oxidation, and the citric acid cycle.

→ NADH, is very good at donating electrons in redox reactions (that is, its electrons are at a high energy level), so it can trasfer its electrons directly to Complex I, turning back to NAD^+ As electrons move through complex I in a series of redox reactions, energy is released, and the complex uses this energy to pump protons from the matrix to the intermembrane space

→ $FADH_2$ - is not as good at donating electrons as NADH (that is, its electrons are at a lower energy level), so it cannot transfer its electrons to Complex I. Instead, it feeds them into the transport chain through Complex II, which does not pump protons across the membrane

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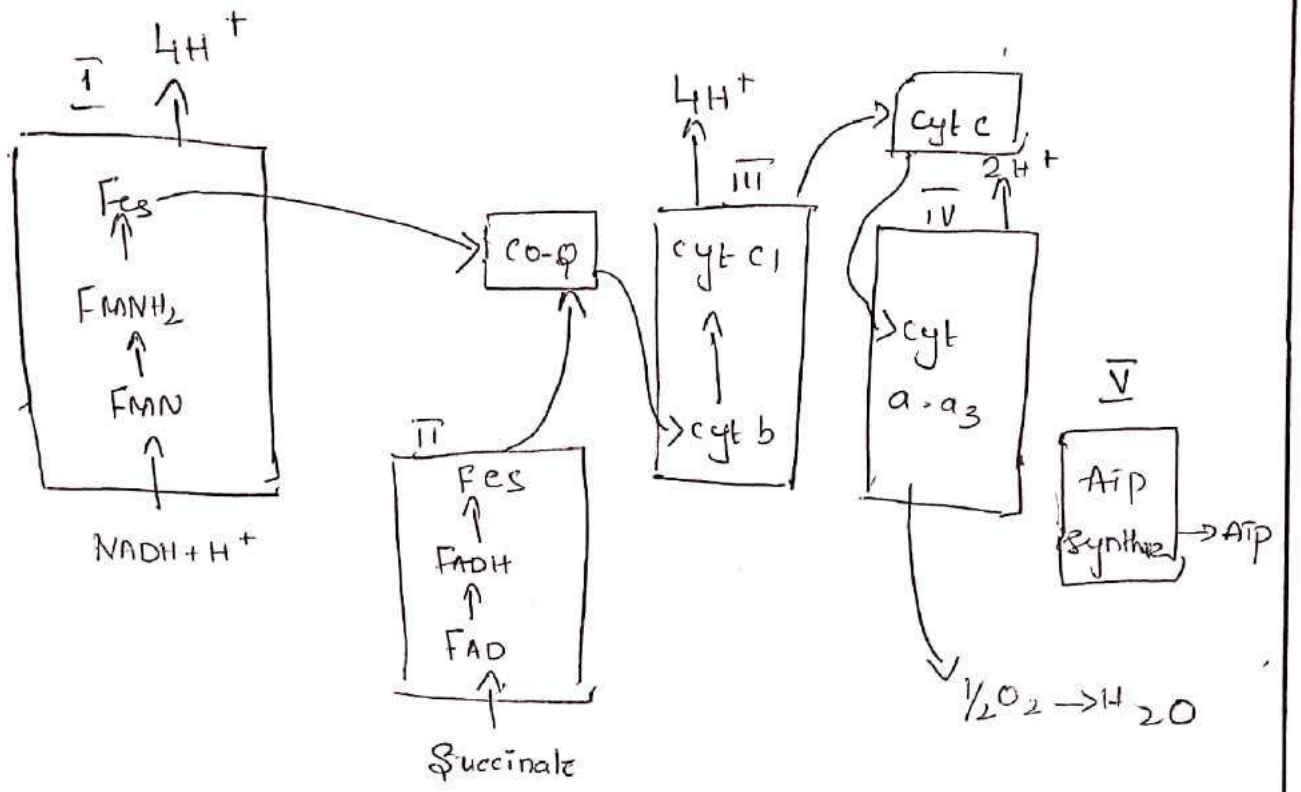

Complex I [NADH -co-Q- oxidoreductase]

1) Two electrons are carried to the Complex I from NADH

2) This complex is composed of flavin mononucleotide [FMN) and an iron-Sulfur [Fe-s)-containing protein.

3) The enzyme in complex I is NADH dehydrogenase and is a very large protein, containing 45 amino acid chains.

4) Complex I can pump four hydrogen ions across the membrane from the matrix into the intermembrane space.

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Q and Complex II [Succinate dehydrogenase]

1) Complex II directly receives $FADH_2$, which does not pass through complex I.
2) The compound connecting the first and second complexes to the third is ubiquinone (Q).
3) The Q molecule is lipid soluble and freely moves through the hydrophobic core of the membrane.
4) Once it is reduced, (QH_2), ubiquinone delivers its electrons to the next complex in the electron transport chain
5) Q receives the electrons from NADH (complex I) and the electrons from $FADH_2$ (complex II) including Succinate dehydrogenase.

Complex III [co Q cyt c oxidoreductase]

1) The third complex is composed of cytochrome b, another Fe-S protein, and cytochrome c proteins; this complex is also called cytochrome oxidoreductase
2) Complex III pumps protons through the membrane and passes its electron to cytochrome c for transport to the fourth complex.

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Complex 4 [cytochrome oxidase]

1) The fourth complex is composed of cytochrome proteins c, a and a_3.

2) This complex contains two heme groups and three copper ions.

3) The cytochromes hold an oxygen molecule very tightly b/w iron & copper ions until oxygen is completely reduced.

4) The reduced O_2 then picks up two hydrogen ions from the surrounding medium to form water (H_2O).

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Isomerism

The compounds having identical molecular formulae but different structures are referred to as isomers. The phenomenon of existence of isomers is called isomerism. Isomers differ from each other in physical and chemical properties.

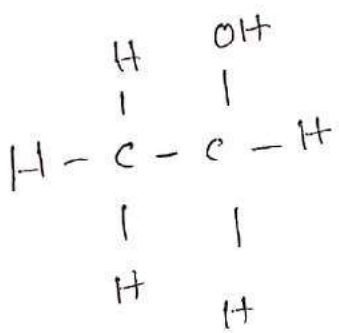
Consider the molecular formula

C_2H_6O.

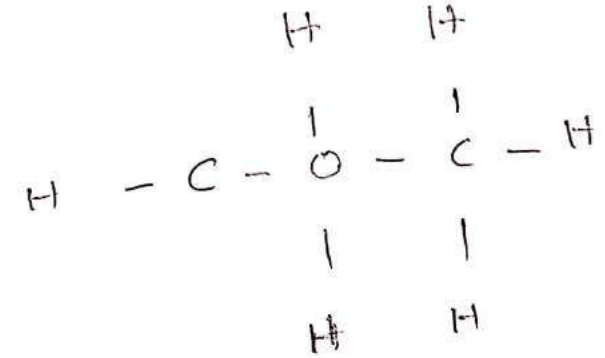
There are two important isomers of this

-> Ethyl alcohol

-> diethyl alcohol.



Ethyl alcohol



Dimethyl alcohol.

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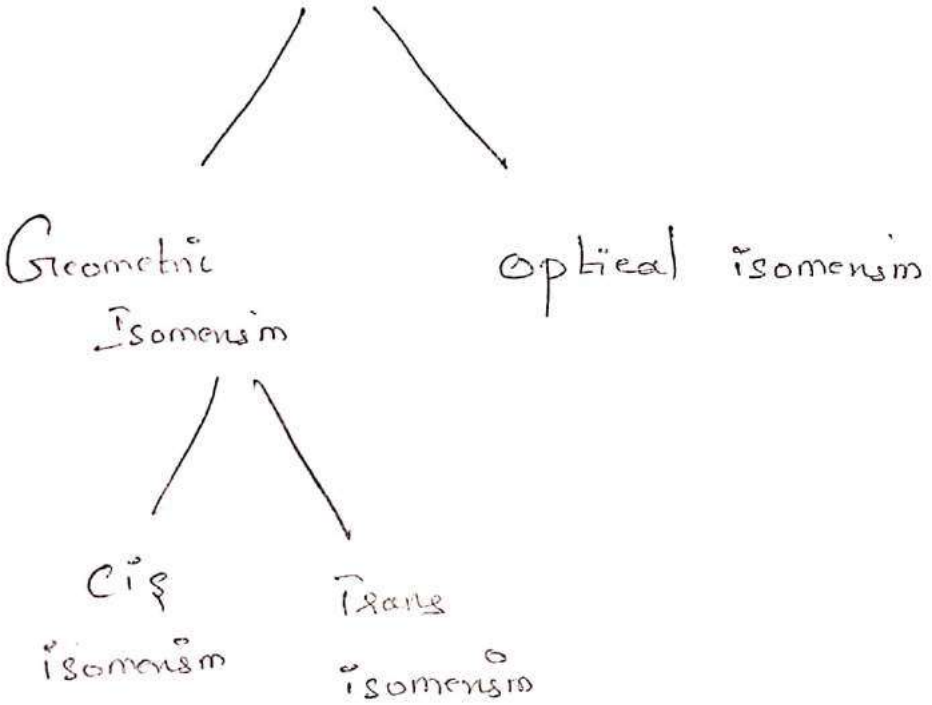
Isomerism is broadly classified into

1) Structural Isomerism

2) Stereoisomerism



1) Structural Isomerism [connection b/w atoms]

The difference in the arrangement of the atoms in the molecule is responsible for Structural isomerism. This may be due to Variation in carbon chain (or) difference in the position of functional groups (or) difference in both molecular chain and functional groups

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eg. $CH_3-CH(OH)-CH_3$ $CH_3-CH_2-CH_2OH$

2 - propanol 1 - propanol

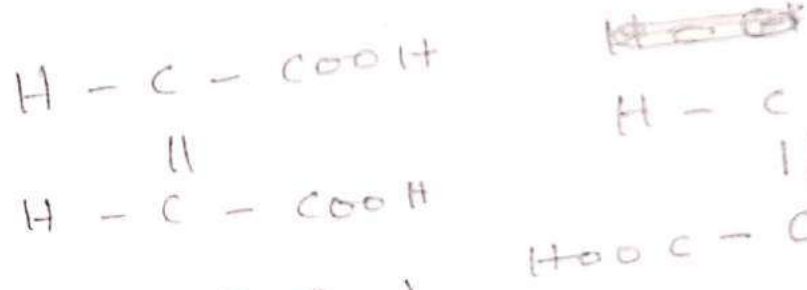
Stereoisomerism [Arrangement of atoms in space]

The differential space arrangement of atoms (or) groups in molecules gives rise to Stereoisomerism. Thus, Stereo isomers have the same structural formula but differ in their Spatial arrangement.

Geometric isomerism

This is also called cis - trans isomerism and is exhibited by certain molecules possessing double bonds. Geometric isomerism is due to restriction of freedom of rotation of groups around a Carbon Carbon double bond.

Eg:



Malaic acid (cis) Fumaric acid (trans)

When similar group lie on the same side, it is called cis- isomerism when similar group lie on the opposite

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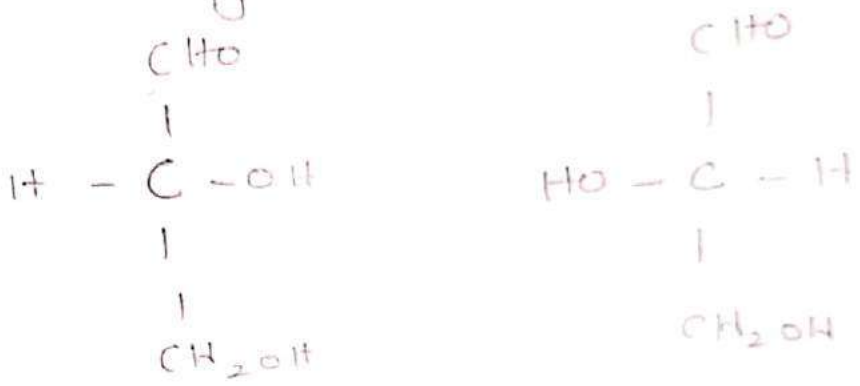
Optical isomerism

Optical isomerism or enantiomerism occurs due to the presence of an asymmetric carbon (achiral carbon). Optical isomers differ from each other in their optical activity to rotate the plane of Polarized light



D- glyceraldehyde L- glyceraldehyde

i) The change in optical rotation is called muta-rotation.
ii) Monosaccharides exhibit muta-rotation
iii) When a M.S is dissolved in H_2O, the optical rotating power of the solution gradually changes until it reaches a constant value.
iv) A freshly prepared aq. soln of α-D-glucose has a specific rotation of $+112.2^\circ$.
v) When this soln is allowed to stand, the rotation falls to $+52.7^\circ$ and remains constant at this value.
vi) The final stage can be attained more quickly either by heating the soln or by adding some catalyst ($+18.7^\circ$)

$+112.2^\circ \rightarrow +52.7^\circ \rightarrow +18.7^\circ$
(less stable) (more stable).

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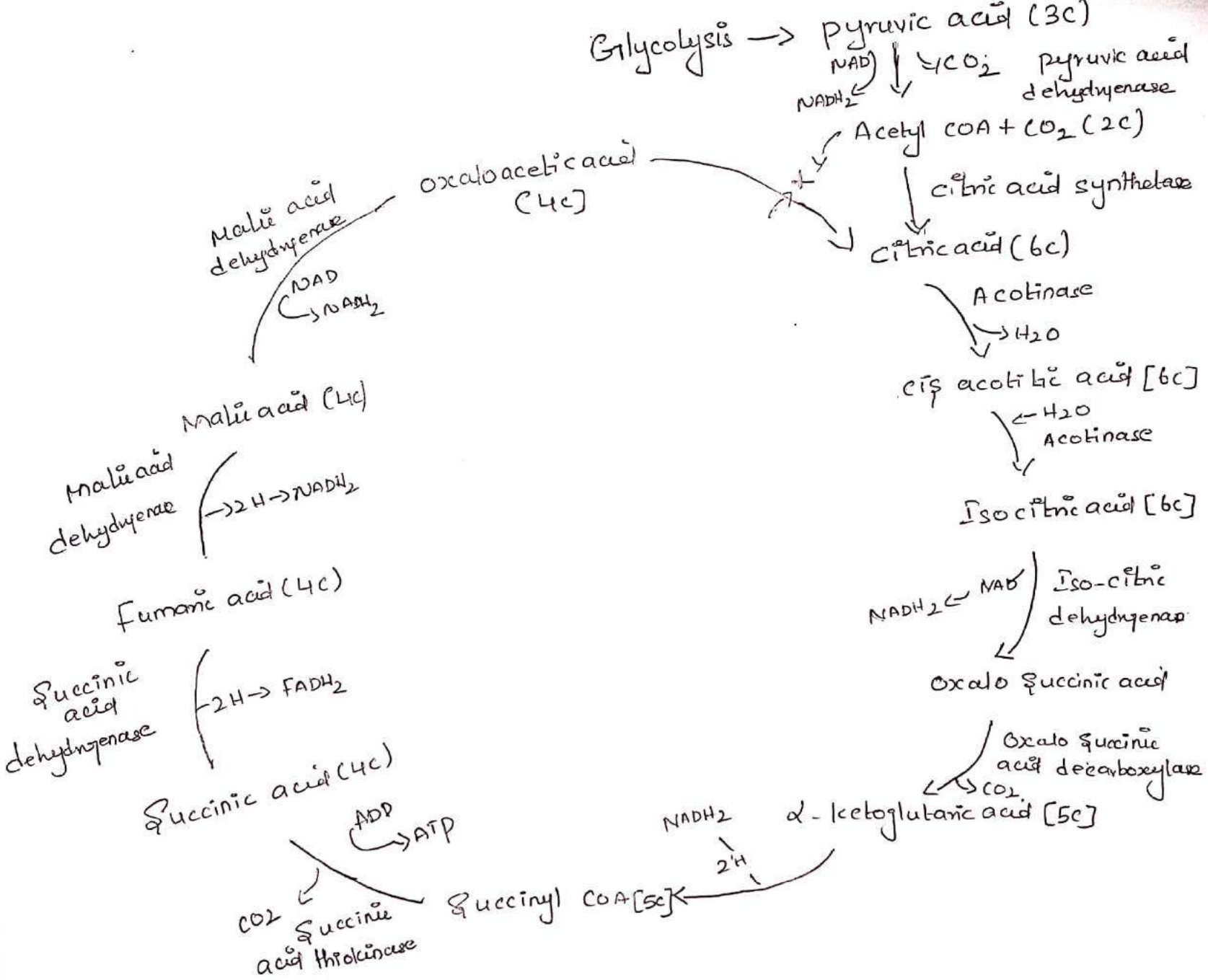

Krebs cycle

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Steps

In order for pyruvate from glycolysis to enter the krebs cycle it must be converted into acetyl CoA by the pyruvate dehydrogenase complex which is an oxidative process wherin NADH and CO_2 are formed. Another source of acetyl-CoA is beta oxidation of fatty acids.

1) Acetyl-CoA enters kreb cycle when it is joined to oxaloacetate by citrate synthase to produce citrate. This process requires the input of water. Oxaloacetate is the final metabolite of the krebs cycle and it joins again to start the cycle over again, hence the name kreb's cycle.

2) citrate is then converted into isocitrate by the enzyme aconitase. This is accomplished by the removal and addition of water to yield an isomer.

3) Isocitrate is converted into alpha-ketoglutarate by isocitrate dehydrogenase. The byproducts of which are NADH and CO_2.

4) Alpha-ketoglutarate is then converted into Succynl-CoA by alpha-ketoglutarate dehydrogenase NADH and CO_2 are once again produced

5) Succynl-CoA is then converted into Succinate by Succynl-CoA Synthetase which yields one ATP per Succynl-CoA

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6) Succinate converts into fumerate by way of the enzyme succinate dehydrogenase and [FAD] is reduced to [$FADH_2$] which is a prosthetic group of succinate dehydrogenase.

7) Fumerate is the converted to malate by hydration with the use of fumerase.

8) Malate is converted into oxaloacetate by malate dehydrogenase the byproducts of which are NADH.

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Lipids and their classification

Lipids are greasy materials occuring widely in nature. They are generally insoluble in water but soluble in fat solvents. Lipids are widely distributed in plants and animal tissues, from which they can be extracted by fat solvents like alcohol (or) ether.

Lipids are organic compounds that contain hydrogen, carbon and oxygen atoms, which forms the framework for the structure and function of living cells.

Classification

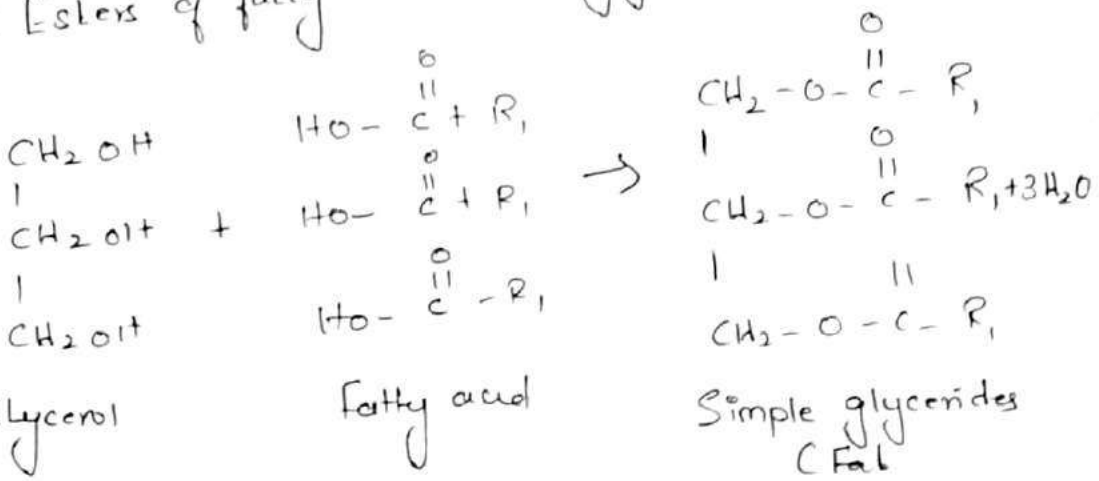
1) Simple lipids
2) Compound lipids
3) Derived lipids
4) Substance associated with lipids

1. Simple Lipids

Esters of fatty acids with various alcohols.

a) Fats: Esters of fatty acid with glycerol.

Eg:



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b) Waxes: Esters of fatty acid with higher molecular weight alcohol other than glycerol

eg:. Bees wax [palmitic acid and myricyl alcohol]

(ii) Compound lipids: Esters of fatty acids with alcohol, but they also contain other groups.

A) Phospholipids: They contain fatty acid, glycerol, phosphoric acid and a nitrogenous compound.

eg:- Lecithin, cephalin

b) Glycolipids: It containg carbohydrates and nitrogen, but phosphoric acid and glycerol are absent.

c) Sulpholipids:- It contains sulphate group

d) lipoproteins: These are lipids attached to proteins. They are present in plasma and tissues.

3) Derived lipids: These are substances which are derived either from phospholipids, glycolipids, sulpholipids (or) lipoproteins by hydrolysis. They are

a) Fatty acids b) Alcohol other than glycerol c) glycerides

d) Bases

4) Substances associated with lipids:

They are (i) carotinoids (ii) Tocopherols (iii) Vitamin A, D, E and K (iv) Steroids

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Physical properties of lipid (They exhibit isomerism due to presence of double bond

1) It may be either liquid or non crystalline solids at room temperature.

2) Colorless, odorless and tasteless in their pure state.

3) Color of fat is due to other substances
   - Yellow color of butter is due to keratin.

4) Lighter then Water

5) Insoluble in Water

6) Readily soluble in organic solvents

7) Fats have specific gravity less than 1, and therefore, they float on water.

8) Melting points of fats are usually low, but higher than the solidification point.

9) The hardness or consistency of the fat depends upon the relative amount of saturated and unsaturated fatty acids present in the fat. Fats containing saturated fatty acids are solids at room temperature. Fats containing unsaturated fatty acids are liquids at room temperature and those are oils.

10) Spreading of fat : When a liquid fat is placed on water, it spreads uniformly over the surface of water and if the quantity is sufficiently small, it will form

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a layer of one molecule thick. The spreading is due to the presence of carboxyl group (COOH) and hydrocarbon chain (CH_3) in the fatty acid

ii) Emulsification:

Though fats are insoluble in water, they can be broken down into minute droplets and dispersed in water. This is emulsification. A satisfactory emulsion is one which is stable and which contains very minute droplets with a diameter less than 0.5m. Naturally occuring emulsions are milk and yolk of egg.

Chemical properties of liquids

Alkali hydrolysis (saponification)

$$R^1-\overset{O}{\overset{\|}{C}}-OR^2 + NaOH \rightarrow R^1-\overset{O}{\overset{\|}{C}}-O^-Na^+ + R^2OH$$

Ester Base Salt Alcohol

-> The process of alkali hydrolysis is called saponification

-> Saponification is the process of breaking down (or) degrading a neutral fat into glycerol and fatty acids by treatment with alkali

-> The alkali salt of fatty acid resulting from saponification is soap.

$$\begin{array}{l} CH_2-O-\overset{O}{\overset{\|}{C}}-R_1 \\ CH-O-\overset{O}{\overset{\|}{C}}-R_1 \\ CH_2-O-\overset{O}{\overset{\|}{C}}-R_3 \end{array} + 3KOH \rightarrow \begin{array}{l} CH_2OH \\ CH_2OH \\ CH_2OH \end{array} + \begin{array}{l} R_1-\overset{O}{\overset{\|}{C}}-OK \\ R_2-\overset{O}{\overset{\|}{C}}-OK \\ R_3-\overset{O}{\overset{\|}{C}}-OK \end{array}$$

Glycerol Soap

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Saponification number is defined as the mg of KOH required to saponify 1g of fat.

Acid hydrolysis

$$R^1-\overset{O}{\overset{\|}{C}}-OR^2 + HO-H \xrightarrow[heat]{H^+} R^1-\overset{O}{\overset{\|}{C}}-OH + R^2OH$$

Ester Water Fatty acid Alcohol

-> It is the reaction of water with a substance such as fats.

-> This results in the splitting of some of the fatty acids from the oil or fat, yielding some free fatty acids, monoglycerides and diglycerides.

Oxidation

-> It is the reaction of an oil or fat with O_2 in the air, and with the food

-> It occurs at the double bonds.

-> The rate of oxidative increases with increase in temperature, exposure to O_2 in air, the presence of light.

-> oxidation induced by air at room temperature is referred to as Autooxidation

Hydrogenation

-> unsaturated fats can be combined with hydrogen under the influence of a suitable catalyst, such as finely divided nickel, Platinum or copper at high

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temperature and fats become more saturated.

$$CH_3-(CH_2)_7CH=CH-COOH \xrightarrow{2[H]} CH_3-(CH_2)_{16}COOH$$

Oleic acid — Stearic acid

Esterification

$$R'-\overset{O}{\overset{\|}{C}}-OH + HOR^2 \xrightarrow[\text{heat}]{H^+} R'-\overset{O}{\overset{\|}{C}}-OR^2 + H_2O$$

Fatty acid — Alcohol — Ester

-> Reverse of hydrolysis

-> It is combining or recombining of free fatty acids with glycerol to form triglycerides

Rancidity

It is the oxidation of fats that is caused by hydration (water), oxidation (oxygen), metallic atoms or microbes

eg :- potato chips when kept in air for a long time gives unpleasant smell and bad taste.

Kries test :- It is used to detect oxidative rancidity. In this test, the fat is treated with ether, Phloroglucinol and hydrochloric acid. positive test is indicated by the development of a red color. This is due to the presence of epihydrine aldehyde which is one of the oxidation product

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Addition Reaction

Fats containing unsaturated fatty acids, readily add on elements such as halogen and hydrogen at their double bonds.

$$-CH=C=CH- \xrightarrow{+3I} -CHI-CHI-CHI$$

Iodine Value

The number of grams of iodine taken up by 100g of a given fat is called its iodine value as iodine number.

Classification of fatty acid

Fatty acid is an important constituent of fat. It is a monocarboxylic acid with a hydrocarbon chain. It is obtained by the hydrolysis of fats by acids, alkalies or enzymes.

Fatty acids may be divided into three classes

(i) Saturated fatty acids

(ii) unsaturated fatty acids

(iii) cyclic fatty acids

Saturated fatty acid

-> These are fatty acids which donot contain double bond.

-> They donot exhibit addition reactions and their iodine values are nil. Generally, presence of saturated fatty acid makes the fat solid with some exceptions

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For example: butter which is a solid fat has a higher iodine value (35 to 50) than coconut oil which is a liquid fat (Iodine value 6-16)

General formula for saturated fatty acid

$C_nH_{2n+1}COOH$

$CH_3(CH_2)_{10}CO_2H$ lauric acid

$CH_3(CH_2)_{12}CO_2H$ Myristic acid

2. unsaturated fatty acid

These are fatty acids which contain double bond. Iodine value vary according to the degree of unsaturation. They are generally liquid at room temperature. But there are certain exceptions. For example lard contains unsaturated fatty acids, but it is solid.

unsaturated fatty acids are further divided according to the degree of unsaturation.

They have a general formula of $C_nH_{2n-1}COOH$, with a double bond.

They are subdivided into

(i) one double bond - Oleic acid - $CH_3(CH_2)_7CH=CH(CH_2)_7CO_2H$

(ii) Two double bond - Linoleic acid - $CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7CO_2H$

(iii) Three double bond - Linolenic acid - $CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7CO_2H$

(iv) Four double bond - arachidonic acid - $CH_3(CH_2)_4(CH=CHCH_2)_4(CH_2)_2CO_2H$

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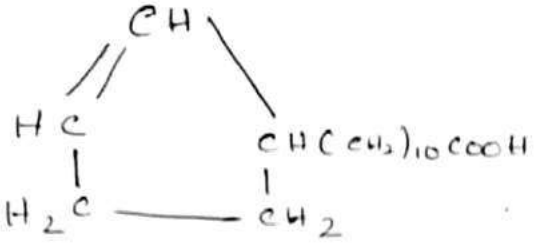
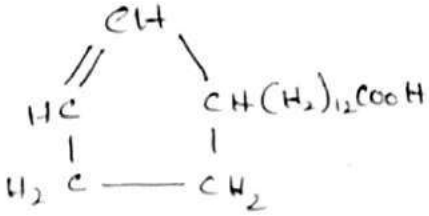
3) Cyclic acids

These are fatty acids having a cyclic ring structure containing five carbon atoms. They are unsaturated.



Hydnocarpic acid



Chaulmoorgic acid

4) Essential fatty acids

-) It cannot be synthesized in the body of human beings.

-) They must be included in the diet for maintaining normal health.

-) The three essential fatty acids are linoleic acid, linolenic acid and arachidonic acid

-) Most of the animal system can interconvert these three essential fatty acid. Therefore, the diet should contain atleast any one of these essential fatty acids.

-) The essential fatty acids are unsaturated fatty acids with one (or more double bonds.

Linoleic acid (2 double bonds) $C_{17}H_{31}COOH$

$CH_3(CH_2)_4-CH=CH-CH_2-CH=CH-(CH_2)_7-COOH$

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Linolenic acid (3 double bond) $C_{17}H_{29}COOH$

$CH_3-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH(CH_2)_7COOH$

Arachidonic acid (4 double bond) $C_{19}H_{31}COOH$

$CH_3(CH_2)_4CH=CH-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH-(CH_2)_3-COOH$

-> The essential fatty acids are present in Vegetable oil. The best known source is the Safflower oil.

Non essential fatty acid

-> Certain fatty acids can be synthesized in the tissues from other fatty acids. These fatty acids need not to be included in the diet.

-> palmitooleic acid and oleic acid are non essential fatty acid.

-> Non-essential fatty acids are synthesized from their corresponding (Stearic acid) saturated fatty acids by the introduction of a single double bond. They are synthesized in the liver.

Palmitoleic acid

$CH_3-(CH_2)_5CH=CH-(CH_2)_7-COOH = C_{15}H_{29}COOH$

Oleic acid

$CH_3-(CH_2)_7CH=CH-(CH_2)_7-COOH = C_{17}H_{33}COOH$

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Physical Properties of Fatty acids

1) Fats are solids or semisolids

2) Fats are sparingly soluble in water, i.e. fats are hydrophobic. They are highly soluble in organic solvents like alcohol, ether etc. Solubility decreases with increase in molecular weight. Fats containing hydroxyl groups are more soluble than fats without hydroxyl groups.

3) They are oily to touch and leave an oily impression on paper.

4) They ~~are sparingly~~ float on water

5) They have less specific gravity

6) They exhibit isomerism due to the presence of double bonds.

Chemical Properties

1) Emulsification

In water fats are broken into minute droplets and dispersed. This is called emulsification. Emulsion is a mixture of lipids and water. Milk is a naturally occuring emulsion. Emulsion greatly increases the surface area of fats. It is an essential requisite for digestion of fats.

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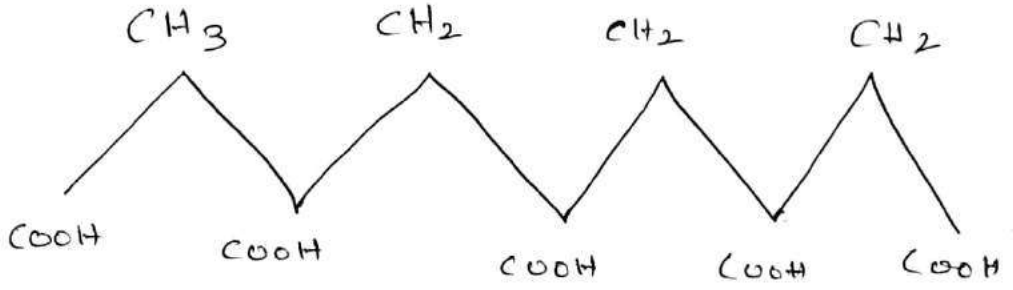
2) Spreading of fat

When a liquid fat is placed on water, it spreads uniformly over the surface of water to form a thin layer. This phenomenon is called spreading.

The spreading of fat is due to the presence of carboxyl group (COOH) and hydrocarbon chain (CH_3) in the fatty acids.

The carboxyl group is hydrophilic (water loving) and the hydrocarbon is hydrophobic (water hating)

When placed in water, the hydrophilic carboxyl group lies below the surface of water and the hydrophobic hydrocarbon lies above the surface of water.



3) Hydrolysis of water

Fats on hydrolysis with the enzyme lipase, give fatty acids and glycerol. The hydrolysis is a stepwise process. Fats (triglycerides are first converted into diglycerides). The diglycerides are hydrolyzed to monoglycerides and finally monoglycerides are hydrolyzed to glycerol and fatty acids.

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4) Saponification

The conversion of fat into glycerol and 3 molecule of sodium salt of higher fatty acids (soaps) by boiling with sodium hydroxide is called Saponification.

$$CH_2-OOC-C_{17}H_{33} \quad CH-OOC-C_{17}H_{33} \quad CH-OOC-C_{17}H_{33}$$

Triolein

$$\xrightarrow{3NaOH} \underset{\text{Glycerol}}{CH_2OH-CHOH-CH_2OH} + 3C_{17}H_{33}COONa$$

Sodium Oleate

5) Rancidity

Rancidity is the ill-smelling of fat. It is caused by rancidification. Rancidification is due to auto-oxidation of fats. The fat which has become rancid has a disagreeable odour and taste and is unfit for consumption. Rancidification occurs more frequently in summer. The chemical changes which occur during rancidification are called rancidity.

6) Addition of H_2

The unsaturated fatty acids are saturated by the reaction with H_2 in the presence of nickel or platinum or palladium as catalyst. If there is one double bond, one molecule of H_2 will be taken. If two bonds are present, 2 hydrogen molecules will be taken.

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7. Addition with Halogen

unsaturated fatty acids and esters are giving addition reactions with halogens in the presence of acetic acid (or Methanol at room temperature.

Linoleic acid + $2I_2$ ——————> 9,10,12,13 tetraiodo Stearic acid

8. Acrolein test

When fats are heated with $NaHSO_4$ (sodium bisulphate) (or potassium hydrogen sulphate, acrolein (unsaturated aldehyde) having a pungent odour is formed. This is a test for fat containing glycerol.

Functions of fats

1) They provide energy for living organisms.
2) They insulate body organs from heat and cold.
3) They supply heat to the body.
4) They transport fat soluble vitamins through blood.
5) They form steroid harmones.

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Synthesis of fatty acids

Fatty acids are synthesized by De-Novo Synthesis. The carbohydrates and aminoacids, obtained is during diet, after fulfilling their calorie requirements are converted to fatty acids and stored as triglycerides.

Chief precursor – Acetyl CoA

Site – cytosol [liver, intestine, kidney, lung lactating Mammary gland, Adipose tissue]

Acetyl CoA is generated from pyruvate by pyruvate dehydrogenase complex in mitochondria.

Steps

1) Transport of Acetyl CoA
2) Requirements
3) Activation of acetyl CoA
4) pathway

1) Transport of acetyl CoA

Acetyl CoA + oxaloacetate

↓

citrate

↓ ATp citrate lyase

Acetyl CoA + oxaloacetate

2) Requirements :- NADH, ATp, Acetyl CoA

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3) Activation of acetyl CoA

Acetyl CoA $\xrightarrow{\text{Acetyl CoA carboxylase}}$ Malonyl CoA

(requires CO_2, Biotin, ATP)

4) Path way

Acetyl CoA + Enz cys-SH $\xrightarrow{\text{Acetyl transacylase}}$ Acetyl-Enz + CO ASH

Malonyl CoA + Acp-SH $\longrightarrow$ Malonyl Acp + CO ASH

Acetyl Enz + Malonyl Acp

CO_2, CoA ← ketoacyl synthase

β-ketoacyl Acp

$NADPH+H^+$, $NADP^+$ ← ketoacyl reductase

β-hydroxy acyl-Acp

H_2O ← Dehydratase

Enoyl Acp

$NADPH+H^+$, $NADP^+$ ← Enoyl reductase

Butyl Acp

6 cycles

Palmitate

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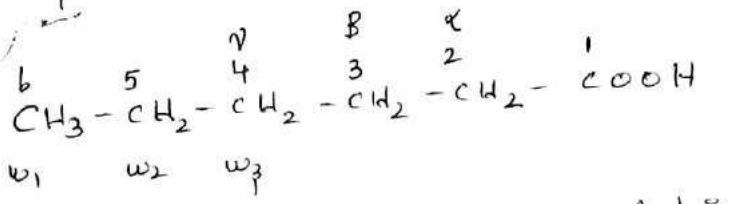
Degradation of fatty acids (β-oxidation) -> Mitochondria

Fatty acids are broken down by a system of degradation is called β-oxidation



The mechanism of the oxidation of fatty acids was primarily explained by knoop in 1904 and is known as knoop's theory of β-oxidation. According to him, the oxidation of fatty acids takes place at the carbon atom in the β-position to the carbonyl group with the splitting of the two terminal carbons leaving a fatty acid chain shorter by two carbons than original fatty acid

Site :- mitochondria [liver, adipose tissues, muscle]

Three steps: Activation, transport & steps

Activation: FA [R-CH2-CH2-CH2-COOH]

ATP, Thiokinase, ipp

Acyladenylate [R-CH2-CH2-CH2-CO~S-CoA]

CoASH, AMP, CoASP, Amp

Acetyl CoA

R-CH2-CH2=CH-CO~S CoA

ipp - Inorganic pyrophosphate
AMP - Adenosine monophosphate

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2) Transport

Acetyl CoA
Carnitine
+ Carnitine

CAT-I

CAT - carnitine acyl transferase

Acyl carnitine

CAT-II

Carnitine + Acyl CoA

3) Steps [oxidation, Hydration, oxidation, Clevage]

Acyl CoA
FAD → FADH2
Acyl CoA DH [oxidation]

Trans enoyl CoA
H2O
Enoyl CoA hydratase [hydration]

β hydroxyacyl CoA
NAD+ → NADH
β-hydroxyacyl CoA dehydrogenase [oxidation]

Ketoacyl CoA

Thiolase - clevage [clevage]

Acyl CoA + Acetyl CoA → TCA cycle

2 carbon compound

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Ketogenesis

The process of formation of these ketone bodies is known as ketogenesis. Acetoacetate, 3-hydroxybutyric acid and acetone are collectively referred to as ketone bodies. Ketogenesis occurs in the mitochondria of liver and the ketone bodies which are water soluble, lipid fuels are released continuously.

Ketogenesis occurs when fatty acid undergo excessive oxidation in the liver, producing large amount of acetyl CoA. The entry of acetyl CoA into Kreb's cycle depends on the availability of oxaloacetate.

When the amount of oxaloacetate is less, the acetyl CoA is diverted to form ketone bodies. In normal conditions, when carbohydrates are plenty and glucose is readily available to the tissues, the amount of ketone bodies in the blood is very low (1mg/100ml of blood) and the average excretion in the urine for 24 hours is less than 125 mg. But, if breakdown of fat predominates, acetyl CoA is diverted to form ketone bodies.

Certain chemical substances such as ammonia and phlorhizin are found to increase the formation of ketone bodies and such substances are known as ketogenic substances.

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Ketosis

The overall condition of increased concentration of ketone bodies in tissues and fluids is termed as ketosis. Excretion of abnormally high amount of ketone bodies in urine is known as ketonuria and appearance of high levels of ketone bodies in the blood is known as ketonemia.

ketosis may occur due to many different physiological as well as pathological factors such as prolonged starvation, availability of less amount of carbohydrate (or high amount of fat in the diet, severe excretion in the post absorptive state, increased metabolic demand (as in pregnancy and lactation) glycogen storage diseases, continued fever, mild pancreatic dysfunction, deficiency of insulin, diabetes mellitus and toxemia of pregnancy. In all the above cases, there is a diminished utilization of carbohydrate and increased mobilization of fats.

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Ketogenesis

Acetoacetate, β-hydroxybutyr and acetone are collectively called ketone bodies (or) acetone bodies, and the process of their formation is known as ketogenesis. The main site of ketogenesis is the liver. These substances pass into the blood stream in very small amount under normal circumstances. Normally, the ketone bodies are carried in the blood stream mainly to the kidney and muscles, where acetoacetate is oxidised after conversion to aceto acetyl CoA. When two moles of acetyl CoA are formed, it is cleaved by thiolase. This process of oxidation of ketone bodies is called ketogenesis.

Acetyl CoA + Acetyl CoA

↓ Thiolase

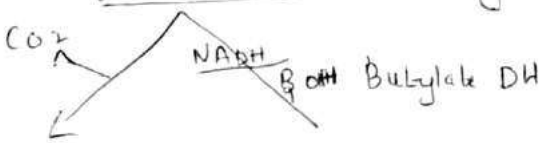
AcetoAcetyl CoA

Acetyl CoA ↓ HMG CoA synthase

HMG CoA

↓ Lyase

Acetoacetate + Acetyl CoA



Acetone β-OH - Butyrate

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Formation of Ketone Bodies

There are 3 steps in the formation of ketone bodies.

1) In the first step two molecules of acetyl coA condense to form acetoacetyl COA with the loss of one molecule of CoA and this reaction is catalyzed by 3-keto thiolase.

2) Next, acetoacetyl CoA reacts with one more molecule of acetyl CoA and H_2O to form 3-hydroxy-3-methylglutaryl COA (HMG-CoA) in the presence of hydroxy methyl glutaryl Synthase. In this process one molecule of CoA is also split off.

3) 3-hydroxy-3-methylglutaryl CoA is then cleaved to acetyl CoA and Acetoacetate in the presence of hydroxy methyl glutaryl CoA lyase. Acetoacetate that is formed, spontaneously decarboxylates to form acetone. The odour of acetone may be detected in the breath of a person who has a high level of acetoacetate in the blood. In the liver acetoacetate may also be reduced to 3-hydroxybutyric acid in the presence of NADH and this reaction is catalysed by the enzyme 3-hydroxybutyric acid.

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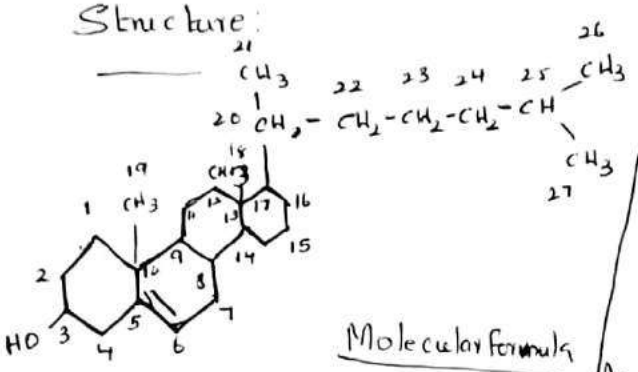
Biosynthesis of cholesterol.

Structure:



Molecular formula

$C_{27}H_{45}OH$

Cholestrol is composed of the cyclopentanoperhydrophenanthrene ring system, which is combination of cyclopentane and perhydroanthracene ring. The ring system has a total 17 carbon atoms. In addition to this, cholesterol has 8 asymmetric carbons in position 3, 5, 8, 9, 10, 13, 14 and 17.

Site :

Steps

2 Acetyl CoA

↓ Thiolase (CoASH)

Acetoacetyl CoA

↓ HMG CoA synthase (CoASH)

β - HMG CoA

↓ HMG CoA reductase ($NADH + H^+$ / NADP)

Mevalonate

↓ kinase (ATP)

5' - Phosphomevalonate (5'-PMV)

↓ kinase (ATP)

5' pyrophosphomevalonate (5'-PPMV)

↓ kinase (ATP)

3 Phospho 5' Pyrophsphomevalonate

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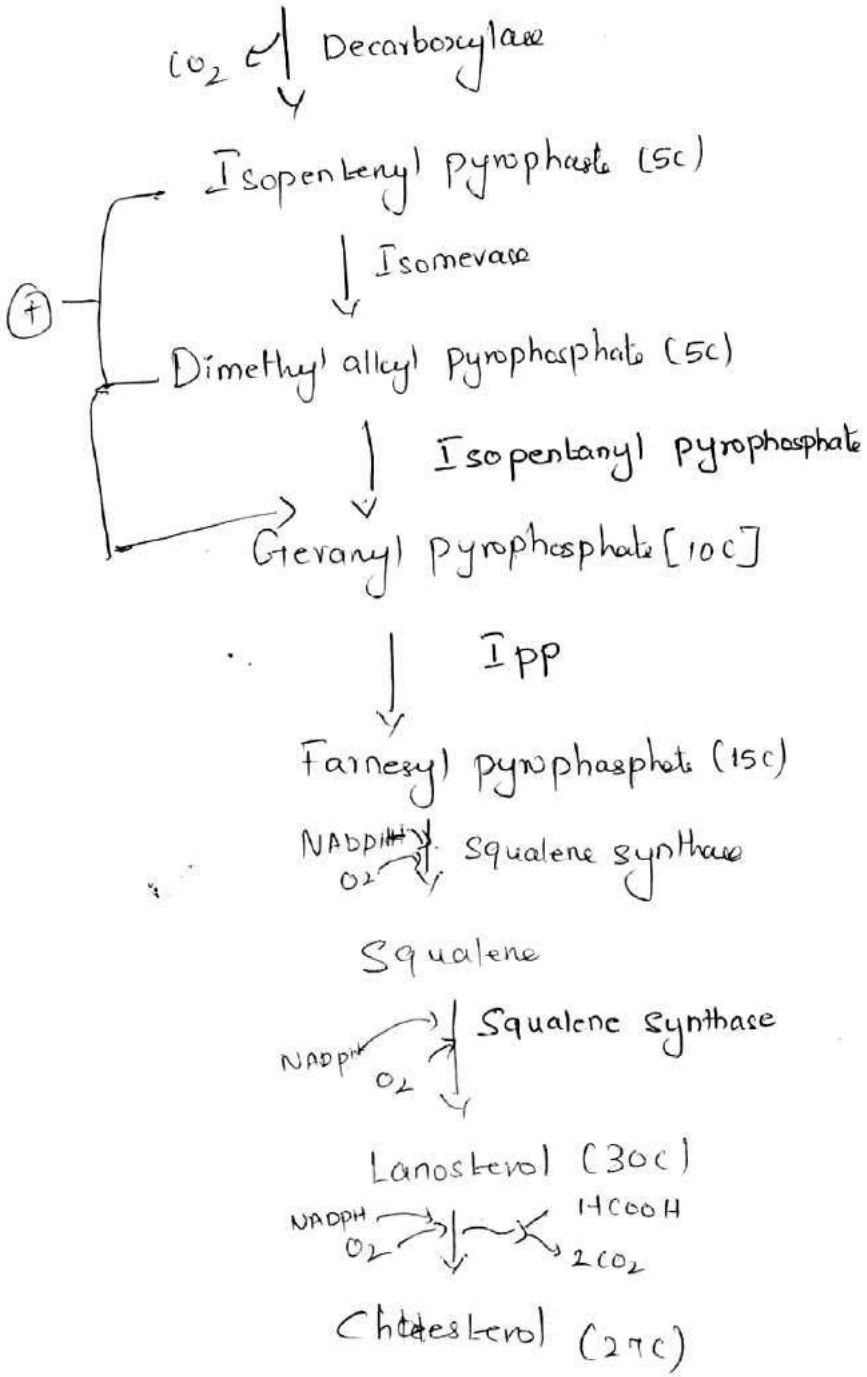

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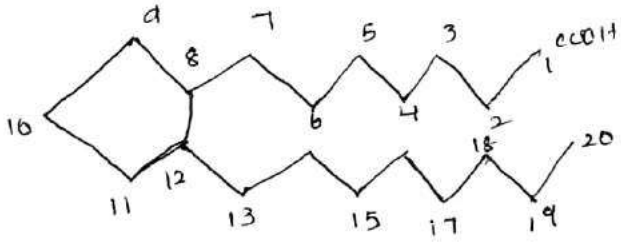
Disorders of lipid metabolism

Inherited defects in lipo protein metabolism seen in some individuals cause primary hyper and hypo-lipoproteinemias. They usually occur due to genetic defects that impair the lipoprotein metabolism at any stage. In addition, there are secondary lipoproteinemias which observed in some diseases such as diabetes mellitus, hypothyroidism and nephrotic syndrome. The clinical manifestation of primary and secondary lipoproteinemias are almost similar.

Prostagladins

Prostagladins are biologically active lipids widely distributed in mammalian tissues and body fluids.

All prostagladins are derived from a parent compound known as prostenoic acid. This is a 20-carbon carboxylic acid consisting of a cyclopentane ring and two aliphatic side chains.



Prostanoic acid

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Hormonal Regulation of fatty acids

It has long been held that hormone sensitive lipases (HSL) is the enzyme that hydrolyses triglyceride to free fattyacids from fats (lipolysis). However, More recently it has been shown that at most HSL converts diacyl glycerides to monoglycerides and free fatty acids.

Monoglycerides are hydrolysed by monoglyceride lipases. Adipose triglyceride lipase may have a special role in converting triglycerides to diacyl glycerides. While diacyl glycerides are the best substrate for HSL. HSL is regulated by the harmones insulin, glycogen, nonepinephrine and epinephrine.

Glycogen is associated with low blood glucose and epinephrine is associated with increased metabolic demands. In both cases energy is needed and the oxidation of fatty acid is increased to meet that need. Glycogen, nonepinephrine, epinephrine bind Gi protein coupled receptors that activate adenylate cyclase to produce cyclic AMP. As a consequence cAMP activates protein kinase A which phosphorylate (and activates) harmone sensitive lipase.

When blood glucose is high, lipolysis is inhibited by insulin. Insulin activates protein phosphate 2A, which phosphorylates HSL, thereby inhibiting its activity. Insulin also activates the enzyme phosphodiesterase which breaks down cAMP and stops the phosphorylation effects of protein kinase A.

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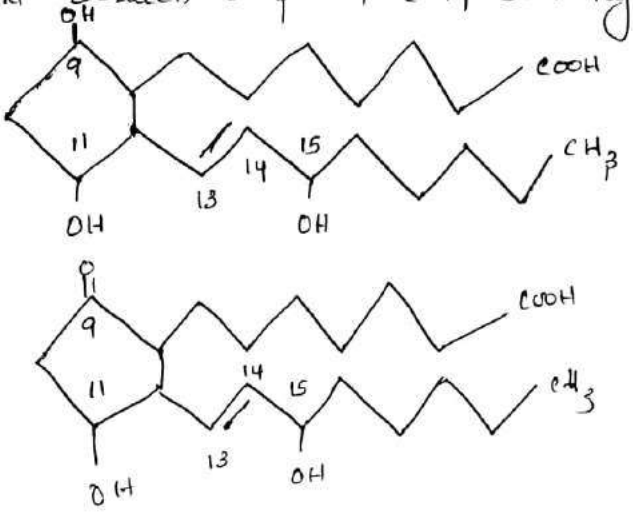
There are several series of prostaglandins present in various tissues, each exerting different effects and actions. Of these two groups classified as PGE and PGF are considered to be the primary compounds. PGE series has a ketone group at C-9 and hydroxyl at C-11 of the ring. PGF series has two hydroxyl groups on the ring, one at C-9 and the other at C-11. Also both PGE and PGF series have a double bond between C-13 and C-14 and hydroxyl at C-15



Metabolism:

The major site for the metabolism of prostaglandins are the lungs on entering the circulation, they are rapidly converted into inactive metabolites and excreted through urine.

Function:

They control the production of cyclic Amp. They act as the stimulators of endocrine glands.

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Biological effects

1) Lack of prostaglandin causes muscle contraction
It also causes
2) Lowering of blood pressure
3) Control of inflammation
4) Relief of asthma and nausal congestion
5) Prevention (or alleviation of peptic ulcer
6) Inhibit platlet aggreviation

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UNIT - IV

Purine and pyrimidine

-> purine and pyrimidines are the two families of nitrogenous bases that make up nucleic acid

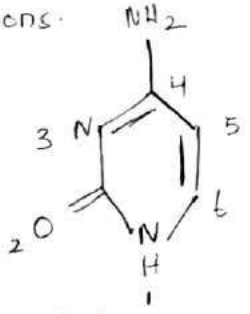
-> In other words, they are the building blocks of DNA and RNA.

-> Each DNA strand has a 'backbone' that is made up of sugar-phosphate chain.

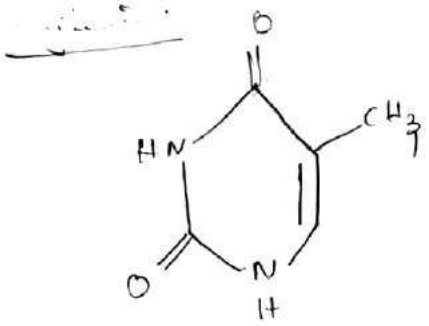
-> Attached to each one of these sugars is a nitrogenous base that is composed of carbon and nitrogen rings.

Pyrimidine

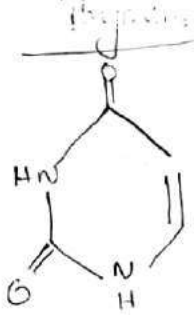
The pyrimidine bases have a 6-membered ring with two nitrogens and four carbons.



Cytosine



Thymine



Uracil

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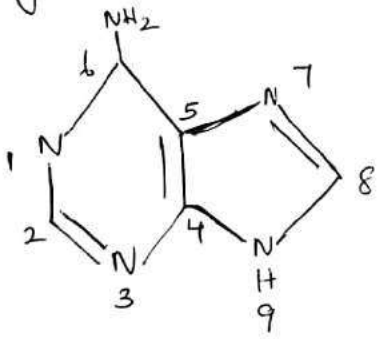
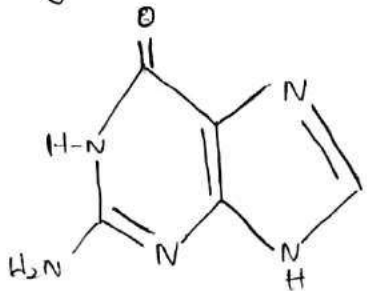
Purine

The purine bases have a 9- membered double-ring system with four nitrogens and five carbons



Adenine



Guanine

Nucleotide

-> A nucleotide is an organic molecule that is the building Block of DNA and RNA.

-> A nucleotide is made up of three part

1) phosphate ~~Sugar~~ group
2) 5- carbon Sugar
3) Nitrogenous base

-> The four nitrogenous bases in DNA are adenine, cytosine, guanine and thymine RNA contains uracil, instead of thymine.

-> The Sugar and Phosphate group makeup the backbone of the DNA double helix, while the bases are located in the middle.

-> A chemical bond between the phosphate group of one nucleotide and the Sugar of a neighboring nucleotide holds the backbone together.

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→ Chemical bonds (hydrogen bonds) between the bases that are across from one another hold the two strands of the double helix together.

Nomenclature of nucleotides

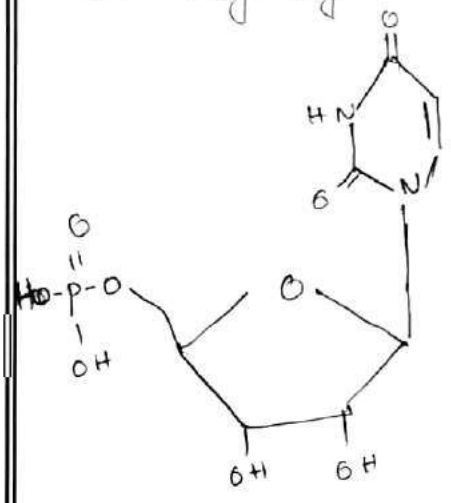
The nucleotides are named according to the purines and pyramidines contained in them. The purines are adenine and guanine. The pyramidines are uracil, cytosine and thymine.

Nucleotides containing purines are

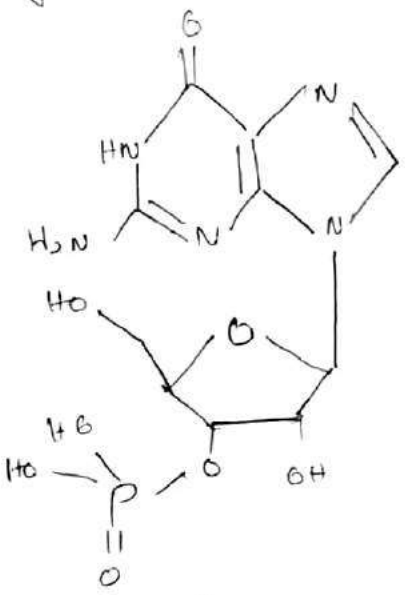
(1) Adnylic acid (Adenine nucleotide)
(2) Guanylic acid (Guanine nucleotide)

Nucleotide containing pyramidines are

(1) uridylic acid (Uracil nucleotide)
(2) Cytidylic acid (cytosine nucleotide)
(3) Thymidylic acid (Thymine nucleotide)



Uridylic acid



Guanylic acid

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Besides the nucleotides which forms integral component of the nucleic acid the following nucleotides exist in free state in the tissues.

1. Adenylic acid (AA) or AMP, also known as adenosine monophosphate. It helps in the activation of phosphorylase.
2. Adenosine diphosphate (ADP) and triphosphate (ATP).

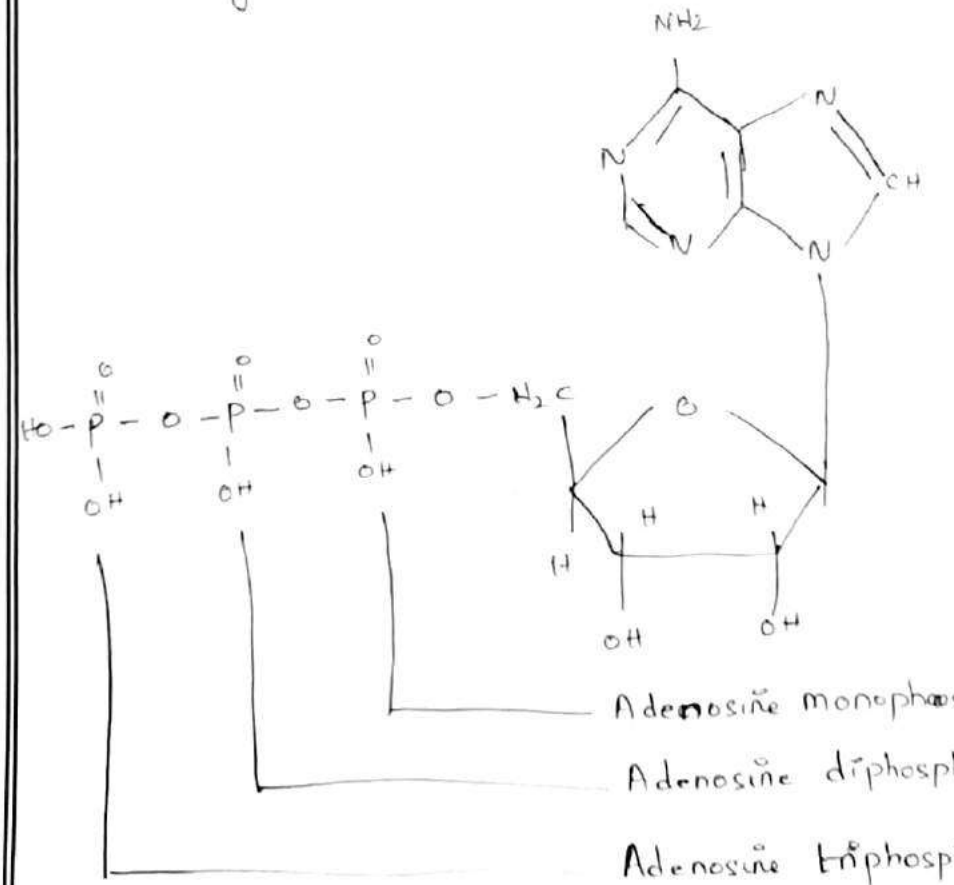
Both of them act as the transfer agents for phosphate group and involved in oxidative phosphorylation. They serve as source of high energy phosphate.

3. Adenosine - 3', 5' - cyclophosphate (CAMP)

Helps in the activation of phosphorylase

4. Guanosine diphosphate (GDP)

Helps in the oxidation of α-ketoglutaric acid to Succinyl CoA.



Adenosine monophosphate (AMP)

Adenosine diphosphate (ADP)

Adenosine triphosphate (ATP)

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5. Guanosine triphosphate (GTP)

Act as a source of high energy phosphate

6. cytosine derivative

Act as a high energy phosphate compound.

Nucleosides:

-> Nucleoside is composed of a purine (or pyrimidine base linked to pentose sugar, either ribose or deoxyribose through a glucoside linkage.

-> originally it was thought that nucleoside is hydrolysed by enzyme Nucleosidase to its components like nitrogenous base and pentose sugar.

-> But recently, it has been proved that the reaction does not take place by hydrolysis but by a reversible phosphorolysis by enzyme phosphorylase.

The reaction will be as follows

Adenine ribose + Inorganic phosphate $\rightleftharpoons$ Adenine + Ribose

Guanine ribose + Inorganic phosphate $\rightleftharpoons$ Guanine + Ribose

Examples of nucleosides

Nucleosides are named according to the purine and pyrimidines contained in them. Nucleosides containing purines are

1. Adenosine (Adenine + Ribose) – AR
2. Guanosine (Guanine + Ribose) – GR

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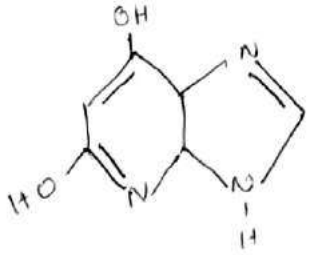
Nucleosides containing pyramidines are

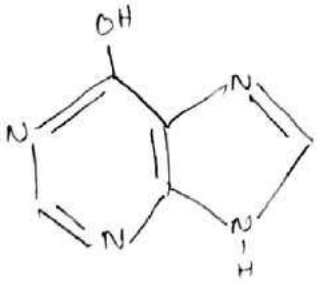
1. Uridine (Uracil + ribose) – UR
2. Cytidine (cytosine + ribose) – CR
3. Thymidine (Thymine + ribose) – TR

S- Adenosyl methionine, also known as active methionine, is a biologically important nucleoside containing adenine.

In addition to the purines and pyramidines, there are some nitrogenous base which are formed as product of metabolism. They are hypoxanthine, xanthine, uric acid and orotic acid



Xanthine



Hypoxanthine

DNA act as a genetic material

Genetic material

Genetic material of a cell (or) an organism refers to

-> those material found in the nucleus, mitochondria and cytoplasm.

-> which play a fundamental role in determining the structure and nature of cell substances and

-> capable of self- propagating and variation

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The basic requirements for genetic material (i.e the material that determines the inherited characteristics of a functional organism

-> It must be stable
-> It must be capable of being expressed when needed
-> It must be capable of accurate replication
-> It must be transmitted from parent to progency without change.

Mendel helped to establish the heredity was controlled by "factors" and chromosomes.

Miescher identified DNA in 1869 and in 1914 Feulgen discovered a specific DNA strain, known as Feulgen strain However the connection between DNA and heredity was established many years later only.

Griffith finds a transforming principle
-> He experiment with the bacteria that cause pneumonia.
->He used two forms
Griffith injected mice with bacteria
S form (deadly) and the R form (not deadly)
A transforming material passed from deads bacteria to live R bacteria, making R bacteria deadly.

Avery identified DNA as the transforming principle
->He performed three test on the transforming principle
-> Qualitative tests showed DNA was present
-> Chemical tests showed the chemical makeup matched that of DNA

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Hershey and chase confirm that DNA is the genetic material.

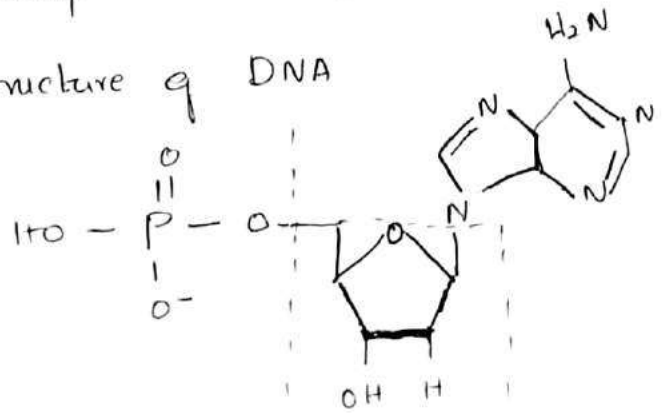
-> They studied Viruses that inject bacteria, called bacteriophages.

-> They tagged Viral DNA with radioactive phosphorus.

-> They tagged Viral protein with radioactive sulfur.

-> Tagged DNA was found inside the bacteria: tagged protein were not present.

Structure of DNA



1. Each nucleotide contains three parts a) A phosphate group b) Sugar deoxyribose c) Four nitrogen bases.
2. The Four bases of DNA are adenine (dATP), guanine (dGTP), thymine (dTTP) and cytosine (dCTP)
3. In 1950, chargaff developed the principle of basepairing

A = T [Adenine pairs with thymine]

C ≡ G [Guanine pairs with cytosine]

Adenine pairs with thymine through two hydrogen bond

Guanine pairs with cytosine through three hydrogen bond

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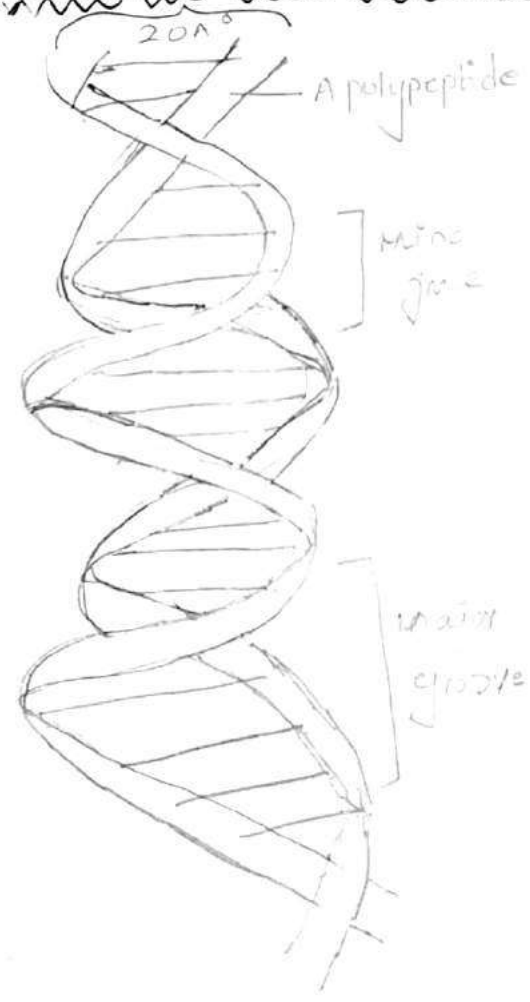
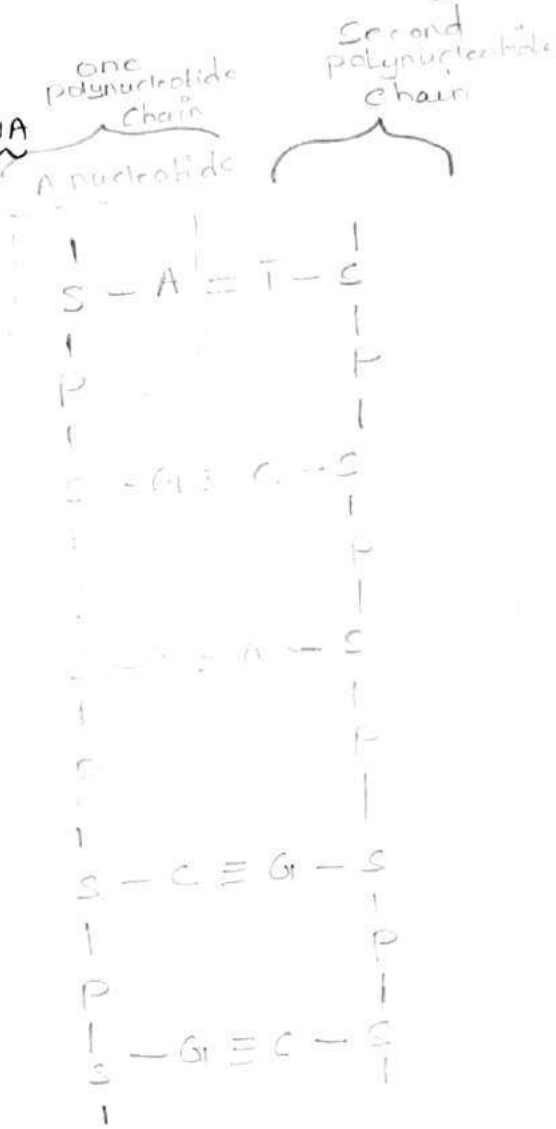
-> They studied Viruses that infect bacteria, called bacteriophages.
-> They tagged Viral DNA with radioactive phosphorus.
-> They tagged Viral proteins with radioactive sulfur.
-> Tagged DNA was found inside the bacteria; tagged proteins were not.

Watson and Crick Model of DNA



Structure of DNA



A Fragment of DNA Molecule

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Watson and Crick Model of DNA

1. The DNA Molecule consists of two polynucleotide chains (or Strands that spirally twisted around each other and coiled around a common axis to form a right-handed double-helix.

2. The two strands are antiparallel ie. they ran in opposite direction so that the 3' end of one chain facing the 5' end of the other.

3. The sugar-phosphate backbone remain on the outside, while the core of the helix contains the purine and pyrimidine bases.

4. The two strands are held together by hydrogen bonds between purine and pyrimidine bases of the opposite strands.

5. Adenine (A) always pairs with thymine (T) by two hydrogen bonds and guanine (G) always pairs with Cytosine (C) by three hydrogen bonds. This complimentarily is known as the base pairing rule. Thus, the two strands are complementary to one another.

6. The base sequence along a polynucleotide chain is Variable and a specific sequence of bases carries the genetic information.

7. The diameter of DNA is 20 nm (or 20 A. Adjacent bases are seperated 0.34 nm (or 3.4 A along the axis. The length of a Complete turn of helix is 3.4 nm (or 34 A

8. The DNA helix has a shallow groove called minor groove (~1,2 nm) and a deep groove called major groove (~2.2 nm) across.

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Structure of RNA

Introduction

-> RNA is a ribonucleic acid that helps in the synthesis of proteins in our body.

-> This nucleic acid is responsible for the production of new cells in the human body.

-> It is usually obtained from the DNA molecule.

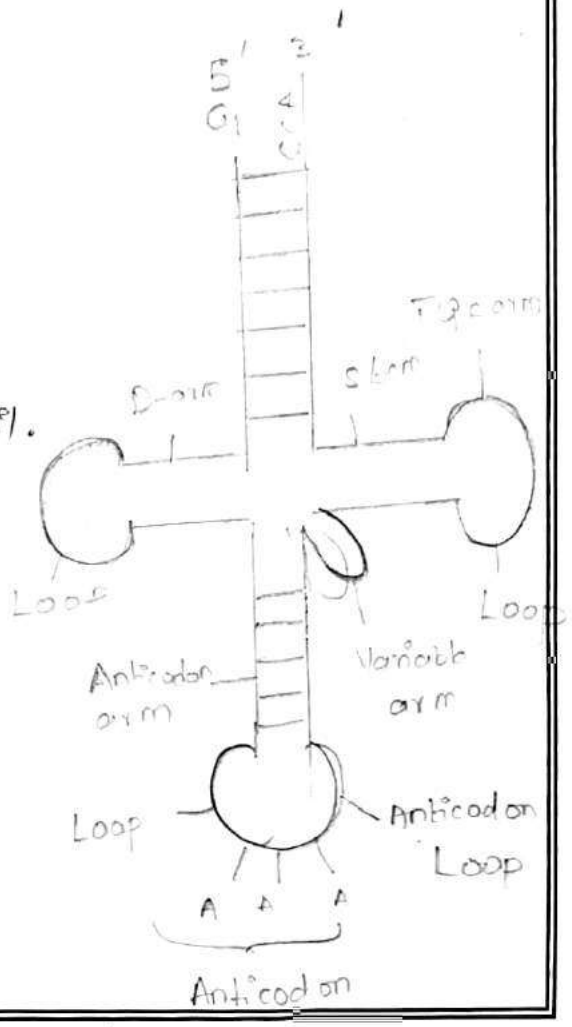
-> RNA resemble the same as that of DNA, the only difference being that it has a single strand unlike the DNA which has two strands and and it consists of an only single ribose sugar molecule in it. Hence is the name Ribonucleic acid.

-> RNA is also referred to as enzyme as it helps in the process of chemical reactions in the body.

Structure

i) The ribonucleic acid has all the components same to that of the DNA with only 2 main differences within it. RNA has the same nitrogen bases called the adenine, guanine, cytosine as that of the DNA except for the thymine which is replaced by the uracil. Adenine and Uracil are considered as the major building blocks of RNA and both of them form base-pair with the help of 2 hydrogen bonds

2) RNA resembles a hairpin structure and like the nucleotides in DNA, nucleotides are formed in the ribonucleic material (RNA)



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Function of RNA

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

Types of RNA

The three primary types of RNA molecules are messenger RNA, transfer RNA and ribosomal RNA.

T-RNA

-> The transfer RNA (t-RNA) molecules are the smallest of the RNAs. 15% of the RNAs are t RNA. They are generated in the nucleus and are passed onto the cytoplasm.

-> t RNA molecules transport specific activated amino acids from the activated amino acyl complex to the ribosomes where proteins are synthesized. They serve as adaptors.

-> There are about 20 species of t RNA present in every cell, each corresponding to each of the 20 amino acids required for protein synthesis.

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-> A t RNA molecule consists of a single RNA strand that has about 75-80 nucleotides folded upon itself to form a three dimensional structure.

-> All t RNA molecules have four arms and three loops.

The four arms are

1) Acceptor arm 2) Anticodon arm 3) D-arm 4) TψC arm

-> Acceptor arm is the long arm. It has a base paired stem consisting of 7-base pairs. At one of the free end of this stem, at 3'- terminal, the nucleotide base sequence CCA which represents cytidine and adenine residues. The activated amino acid is attached to this end. The other free end of the stem, at the 5' terminal is phosphorylated (5'p)

-) The anticodon arm is at other end of the stem.

-> The D arm has the base dihydrouridine. It has three or four base pairs

-> The TψC arm (thymidine pseudouridine cytidine) named so, for the presence of thymidine, pseudouridine and cytidine, ψ stands for base pseudouridine.

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mRNA

-> mRNAs or messenger RNAs are single stranded RNA molecule having high molecular weight. They are processed from nuclear RNA precursors known as hnRNA.

-> mRNAs are the direct carriers of genetic information from the nucleus to the cytoplasmic ribosome and present about 2% of the cellular RNA.

-> The information within mRNA lies in a linear sequence.

-> The 5' end of mRNA is attached to a 7-methylguanosine by a 5-5' pyrophosphate linkage. [cap]

-> The translation of mRNA on the ribosomes begin at the 5'end and proceed towards the 3'-end. To the 3'end of the mRNA is attached to Adenylic acid (AAA).

rRNA

-> rRNA are called as ribosomal RNAs which are found in Cytoplasm and are the most abundant type of RNAs present in cells.

-> They are classified into four types that differ in their size.

-> They are 5S, 5.8S, 18S and 28S rRNAs

-> The rRNAs in the ribosomes are necessary for

(i) Ribosomal assembly

(ii) their important role in the binding of mRNA to Ribosomes and its translation.

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Protein [Macromolecule composed of one (or) more polypeptide chain possessing a characteristic of amino acid sequence). It is a polymer of amino acid

Introduction

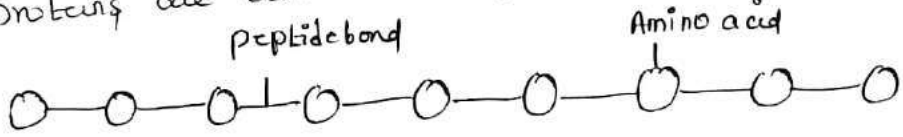
-> Proteins are biological polymers composed of amino acids.
-> Aminoacids, linked together by peptide bonds, form a polypeptide chain.
-> one (or) more polypeptide chains twisted into a 3D shape. form a protein.
-> Proteins have complex shapes that include various folds, loops and curves
-> There are two general classes of protein molecules

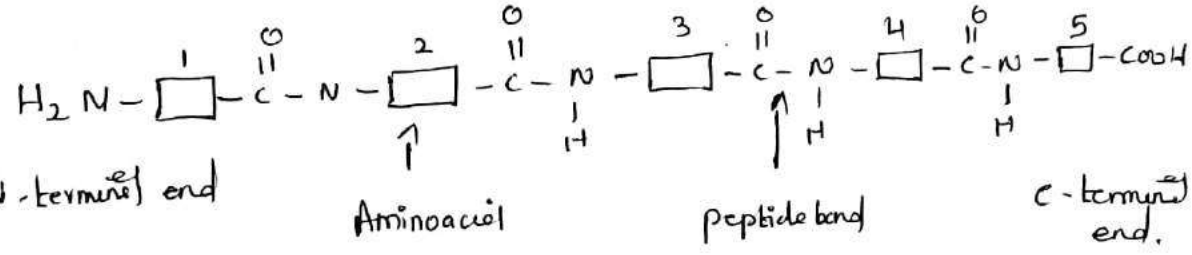
1) Globular protein - compact, soluble & spherical, branched, tightly folded
   Eg:- Enzymes, Protein hormones, Antibodies
2) Fibrous protein - Elongated and insoluble, unbranched, linear molecules
   Eg: Collagen, Elastin, Actin, myosin

Four Protein Structure types

1) Primary structure

-> The primary structure of a protein molecule refers to the sequence of aminoacids in the polypeptide chain.
-> The aminoacids are linked by covalent peptide bonds.
-> Proteins are constructed from a set of 20 amino acids.





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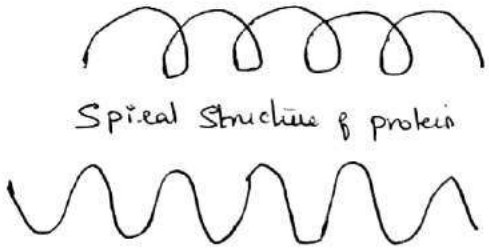
2) Secondary Structure

-> It refers to the coiling (or) folding of a poplypeptide chain that gives the protein its 3-D shape.

-> There are two types of secondary structures observed in proteins

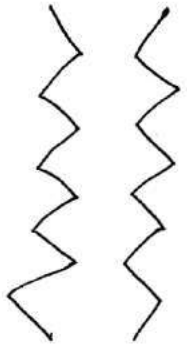
-> one type is the alpha (α) helix structure. This structure resembles a coiled spring and is secured by hydrogen bonding in the polypeptide chain.

-> The second type is the (β) pleated sheat. This structure appears to be folded (or) pleated and is held together by hydrogen bonding between polypeptide units of the folded chain that lie adjacent to one another.



Spiral Structure of protein

Helically coiled polypeptide chain



-> Pleated Sheat

Tertiary structure

-> The polypeptide chain with secondary structure may be further folded and twisted producing structure coils of different size and shapes to form a compact three dimensional globular molecule called the tertiary structure. There are several types of bonds and forces that hold a protein in its tertiary structure

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Hydrophobic interactions - greatly contribute to the folding and shaping of a protein.

Hydrogen bonding - in the polypeptide chain and between amino acid "R" groups helps to stabilize protein structure by holding the protein in the shape.

Ionic bonding - Due to protein folding, ionic bonding can occur between the positively and negatively charged "R" groups that come in close contact with one another.

Disulfide bridge - Folding can also result in covalent bonding between "R" groups of cysteine amino acids. This type of bonding forms is disulfide bridge.

4. Quaternary structure - refers to the structure of a protein macromolecule formed by interactions between multiple polypeptide chains. Proteins with quaternary structure may consists of more than one of the same type of protein subunit. They may also be composed of different subunits.

Hemoglobin is an example of a protein with quaternary structure.

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Classification of proteins

Proteins are conveniently classified on the basis of their physical properties like solubility and decomposition. Accordingly these are classified into three main groups.

(i) Simple proteins (ii) Conjugated proteins

(iii) Derived proteins

Simple proteins

These proteins on hydrolysis yield only α-amino acids. Simple proteins are again classified into different classes. They are

(a) Albumin and Globulin

These proteins contain most of the amino acids. They coagulate on heating. They differ from one another in their solubility. Both of them are soluble in dilute neutral solutions of salt and alkali.

Albumin is soluble in water but globulin is insoluble. Albumins are precipitated from solution by full saturation with ammonium sulphate, whereas globulins are precipitated by half saturation with ammonium sulphate.

(b) Glutelin and Gliadin

Both glutelin and gliadin are present in cereals, especially in wheat. They form the protein of wheat. Both glutelin and gliadin contain large amount

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of glutamic acid. Gliadin contains a high concentration of amino acids and therefore it is also known as Prolamine. Both glutelin and gliadin are insoluble in water and alcohol, but soluble in dilute acids and alkali.

c. Scleroproteins (or) Albuminoids

These proteins are called albuminoids because of their essentially similar structure of albumins and globulins. They form most of the supporting structures of animal proteins. Insoluble in water, soluble in long boiling conc. acids

Eg: hoofs, nails, hair, dentine in teeth, skin & bone

d. Histones : Histones are more complex than protamines. They are basic proteins and contain fairly large amount of aminoacid histidine. Soluble in water & dilute acid. Insoluble in ammonia. They are not coagulated by heat.

e. Protamines : These are simplest of the proteins and contain about 8 amino acids. Soluble in water and ammonium hydroxide. They are not coagulated by heat.

(ii) Conjugated Protein :- These are proteins composed of simple proteins combined with some non-protein substances known as prosthetic groups.

a) Nucleoproteins :- These are compounds of protein with nucleic acid. They are found in protoplasm and nuclei. Eg :- Nuclein, nucleohistone etc

b) Phosphoprotein :- These are protein containing phosphoric acid.

eg :- Cassin of milk, Vitellin of egg

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c) Glycoprotein and Mucoprotein – These proteins are composed of simple protein and are combined with carbohydrates. Eg: egg albumin, Serum albumin, Serum globulin

d) Chromoproteins: These proteins contain heterocyclic compounds like porphyrins as the prosthetic group.

eg: Hemoglobin

e) Lipoproteins: These proteins conjugated with lipids such as neutral fat, Phospholipids and cholesterol.

Derived proteins

These are proteins derived from the simple proteins or conjugated proteins by the action of acids, alkalies or enzymes.

1. Primary derivative: These are derivatives like metaproteins which are the denaturation products of protein resulting from the action of heat, acids and alkalies on proteins.
   Eg: Proteans, Metaproteins, coagulated proteins
2. Secondary derivatives: These are obtained at a later stage of hydrolysis
   Eg:- peptones, proteoses, peptides.

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~~Proties~~

Properties of Protein

Protein molecules are composed of chains of α-aminoacids, united to one another by peptide linkages. The general properties of proteins are as follows.

1. Proteins are complex substances of high molecular weight molecules contain C, H, O and N and sometimes P and S.

2. The molecular weight of proteins vary from 6000 to many millions. Because of this, most proteins are not diffusible through membrane.

3. Proteins are generally soluble in water, dilute acids and alkalies. Due to their large size they form colloidal solution and exhibit the properties associated with the colloidal state of the matter.

4. Proteins possess give ionic or electrically charged groups so that they can migrate in an electric field. Here, they combine with ionic reagents giving rise to insoluble compounds.

5. Proteins are precipitated by salts of heavy metals like silver, mercury and lead in an alkaline medium.

6. Proteins are precipitated by certain alkaloidal reagents. The various reagents are picric acid, trichloroacetic acid, phosphotungstic acid, phosphomolybdic acid and sulphosalicylic acid.

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7. Some proteins in solution, coagulate on heating. They are called heat coagulable proteins.
eg:- Albumin and globulin

8. All proteins give colour reaction when treated with certain reagents.

9. proteins are hydrolysed to their constituent acids by boiling with acid or alkali or even by the action of appropriate proteolytic enzyme.

The sequence of events and the various products obtained during hydrolysis are as follows

Protein ——hydrolysis——> Metaprotein + aminoacids

Metaprotein ——hydrolysis——> proteoses + aminoacids

proteoses ——hydrolysis——> peptones + aminoacids

peptones ——hydrolysis——> peptides

peptides ——hydrolysis——> Aminoacids

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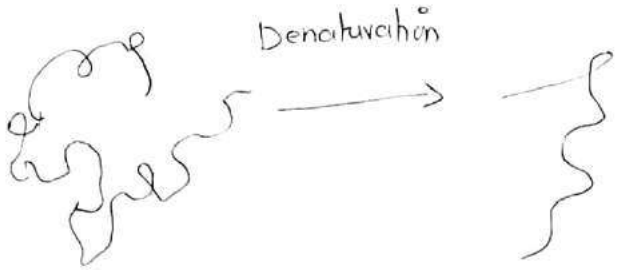
Denaturation

Denaturation means disorganisation of the native protein molecule by which the specific configuration or regular arrangement of the protein molecule is altered to an irregular diffuse arrangement. This causes a change in physical and chemical properties of protein.

Denaturation may involve a mere unfolding of the peptide chain or splitting of the protein into smaller units.



Denaturing can happen in two different ways.

Physical agents: Heat ultraviolet light, X-rays, high pressure and violet shaking of the protein solution.

Chemical agents

They are acids, alkalies, enzymes, organic solvents, strong urea solution and high concentration of salts or heavy metals.

The following physical changes takes place in denaturation

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i) Solubility decreases (ii) Surface tension altered (c) Viscosity increases (iv) it cannot be crystallized

Chemical changes

The structure of the peptide chains of some proteins are held in coiled form by three types of cross linkages namely hydrogen bonds, disulphide and salt linkages. Denaturation cause splitting of one or more of these linkages resulting in unfolding and uncoiling of peptide chains which inturn produce certain chemical changes.

Biological changes

The altered proteins are digested more easily and quickly by proteolytic enzymes. The hormonal and enzymatic activities of harmones and enzymes are destroyed when denatured.

Coagulation

Applying heat to the precipitate of meta protein is called coagulation.

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Amino acids

Amino acids are compounds containing amino group(NH_2) and Carboxyl (COOH) groups. So amino acids are also called aminocarboxylic acids

Amino acids are the essential component of all living cells. They are the building blocks of proteins.

Animals and plants contain about 200 amino acid. But human body contain only about 60 amino acids. of these only 20 amino acids are used as building blocks for the synthesis of proteins

Types of Amino acid

1) Essential Amino acid

The body cannot synthesize essential amino acids. so they must be included in the diet. The essential amino acids are also called indispensable amino acid

10 aminoacids are essential amino acids

1) Phenylalanine
2) Valine
3) Threonine
4) Tryptophan
5) Isoleucine
6) Methionine
7) Histidine
8) Arginine
9) Leucine
10) Lysine

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2) Non-essential Amino acid

Amino-acids which need not be included in the diet are called non-essential amino acids. They can be synthesized in the cells from essential amino acids or other compounds. Hence these amino acids need not be included in the diet.

1) Alanine
2) Asparagine
3) Aspartic acid
4) Cystine
5) Glutamic acid
6) Glutamine
7) Hydroxyglutamic acid
8) Glycine
9) Hydroxyproline
10) proline
11) Serine

Functions

1) Alanine helps the metabolism of glucose.
2) Aspartic acid increases stamina
3) cysteine helps the production of collagen
4) Glutamic acid helps to correct personality disorders.
5) proline improves skin texture

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Classification of Amino acid

1) Classification of amino acids based on their incorporation of proteins

a) Proteogenic Amino acids

-> used for the synthesis of proteins
-> components of proteins
-> Two types, major amino acids and rare amino acids

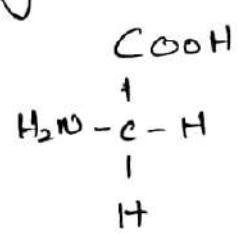
The major amino acids are widely involved in the synthesis of proteins. The rare amino acids are the derivatives of major amino acids

b) Non-Proteogenic amino acid

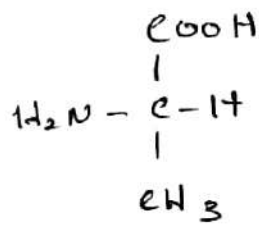
A majority of amino acids are not incorporated into the proteins. They donot participate in protein synthesis

2) Classification based on the structure of side chain

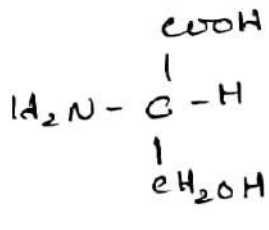
a) Monoamino mono carboxylic acid -> It contains one amino group and one carboxylic group. They are neutral amino acids.



Glycine



Alalanine



Serine

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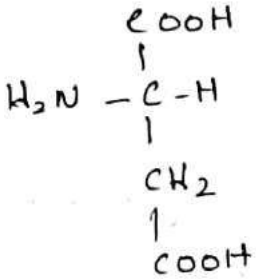
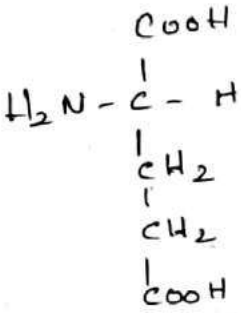
b) Monoamino dicarboxylic acid - It contains one amino and two carboxylic groups. They are acidic amino acids

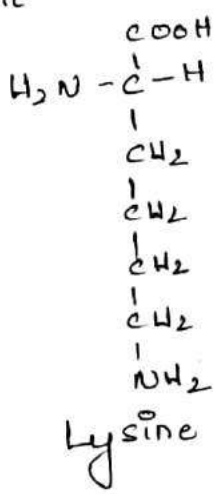


Aspartic acid

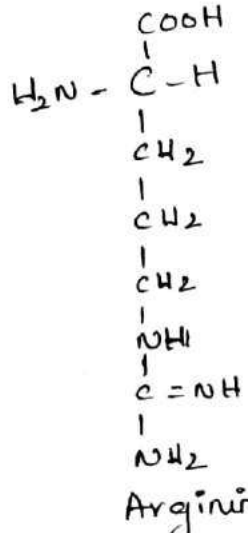


Glutamic acid

c) Diamino monocarboxylic acid :- It contains two amino groups and one carboxyl group. They are also called basic amino acids.

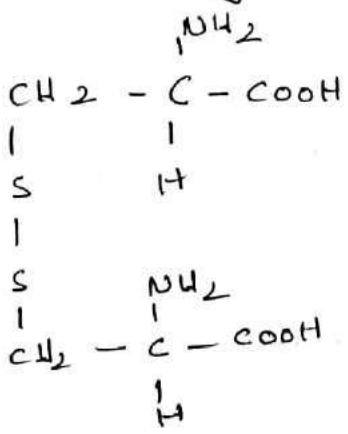


Lysine



Arginine

d) Diamino Dicarboxylic acid : It contains two amino groups and two carboxyl groups.



Cystine (or) Dicystine

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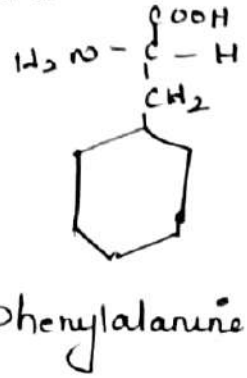
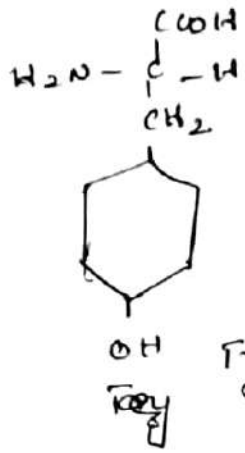
e) Aromatic amino acids:- It contains aromatic rings. They are monoamino monocarboxylic acids and are neutral in reaction



Phenylalanine

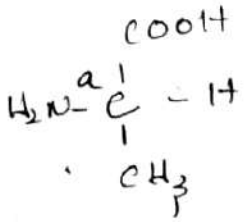


Tyrosine

3) Classification based on the position of the NH_2 group.

a) α-amino acid

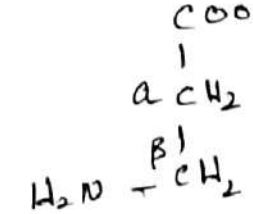
The carbon atom next to the acid group is called α-carbon atom. If the amino group is attached to the α-carbon atom, the amino acid is called α-amino acid.



Alanine

b) β-amino acids

The second carbon atom from the acid group is called β-carbon atom. When the amino group is attached to the β-carbon atom, the amino acid is called β-amino acid.



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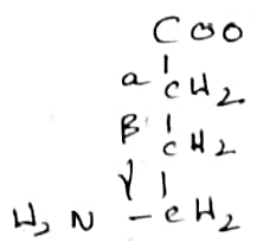
c) γ - amino acids

The third carbon atom from the acid group is called γ - carbon atom. When the amino group is attached to the γ - carbon atom, the amino acid is called γ - amino acid.



4) Classification based on reaction in solution

a) Neutral amino acids - The amino acids which do not contain any amino group (or) carboxyl group in the side chain (R) are called neutral amino acids. They contain one carboxyl group and one amino group and they are neutral in character.

eg:- Glycine, Alanine, serine

b) Acidic amino acids - The amino acids containing additional carboxylic groups in the side chain are called acidic amino acids. As they contain an additional carboxyl group, they impart acidic properties.

Eg:- Aspartic acid, glutamic acid.

c) Basic amino acids - The amino acids carrying an additional amino group in the side chain are called basic amino acids. They impart basic properties.

Eg. Lysine, arginine.

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5) Classification of amino acids based on the polarity of side chain

a) Hydrophilic amino acids - The side chains have high affinity for water. Hydrophilic aminoacids are either electrically charged (or uncharged. The charged side chain attract water dipoles.

Eg:- Aspartic acid, Glutamic acid.

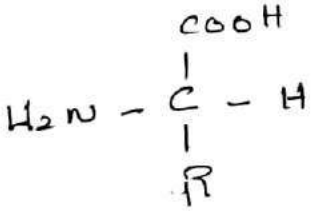
b) Hydrophobic amino acids - The side chains donot interact with water.

Eg:- proline.

Structure of Amino acid

Amino acid is an aminocarboxylic acid. They are the building blocks of proteins. An amino acid is made up of five components, namely.

1) A carbon atom - C
2) A carboxyl group - COOH
3) A hydrogen atom - H
4) An amino group - NH_2
5) A side chain (or residue - R



R is the side chain (or residue. It may be a hydrogen

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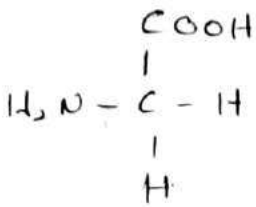
It may be a hydrogen atom (H) or a Methyl group (CH_3), or an aliphatic group (or an aromatic group or a heterocyclic group. In glycine, the simplest amino acid 'R' represents a H atom. In alanine, it is a methyl (CH_3) group. In serine, it is CH_2OH.



Glycine

Physical properties of Amino acids

1. Solids

Most of the naturally occuring amino acids are solids. They are in the form of crystals. The crystal forms vary from slender needles to thick hexagonal plates.

2. Colour

The aminoacids are colourless.

3. Taste

Amino acids may be tasteless (or sweet in taste (alanine) or bitter in taste (arginine)

4) Solubility

The aminoacids are readily soluble in water, slightly soluble in alcohol and insoluble in ether.

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5. Melting point

The amino acids are outstanding among organic compounds in possessing high melting points. In general melting points are above 200°C. certain amino acids melt above 300°C.

6. All aminoacids except glycine are optically active

7. Aminoacids contain both carboxylic and aminogroup and hence they are amphoteric in nature. The $-NH_2$ and -COOH group of amino acids are ionizable in nature. Depending upon the PH of the solution, these groups acts as proton donor (acids) or proton acceptors (base). This property is called amphoteric property.

8. At a specific pH the amino acid carries both charges in equal number and exist as dipolar (or) Zwitter ion. At this pH, the net charge on the amino acid becomes zero. This is called a Zwitter ion.

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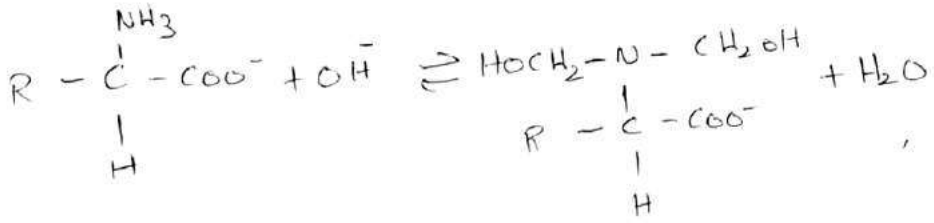
Chemical properties of aminoacids

1. Reaction with formaldehyde

If aminoacid solutions are treated with large excess of neutralised formaldehyde solution, the mixture becomes acidic and can be titrated sharply with standard alkali using phenolphthalein as indicator. This reaction is called Sorensen's formol titration.



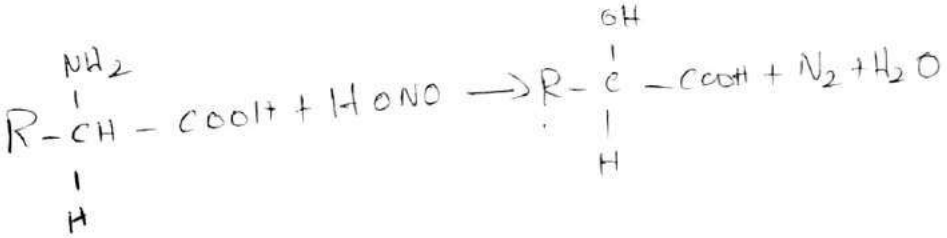
2. Reactions of glycine with benzoic acid

Amino acids act as bases towards acids and form salts. Eg: When glycine react with benzoic acid to form benzoyl glycine

$$C_6H_5-COOH + HNH-CH_2-COOH \rightarrow C_6H_5-CONH-CH_2-COOH + H_2$$

3. Reaction with nitrous acid

Nitrous acid reacts with the amino acid group to form the corresponding hydroxy acid and liberate nitrogen gas



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4. Reaction with Ninhydrin.

The α-amino acid react with ninhydrin to form a purple, blue or pink colour complex (Ruhemann's purple)

Amino acid + Ninhydrin → ketoacid + NH_3 + CO_2 + Hydrindantin

Hydrindantin + NH_3 + Ninhydrin → Ruhemann's purple

Ninhydrin's reaction is effectively used for the quantitative determination of aminoacid and proteins.

(proline gives yellow colour with ninhydrin)

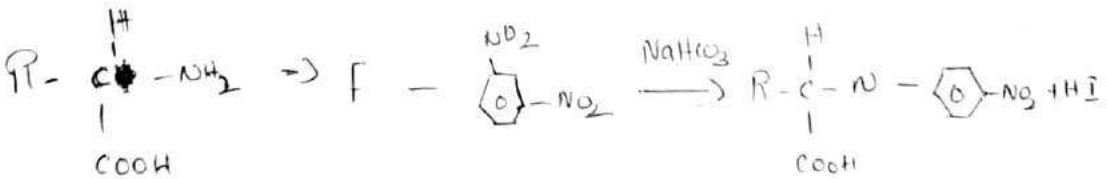
5. Reaction with ammonia. The carboxyl group of dicarboxylic aminoacids reacts with NH_3 to form amide.

Aspartic acid + NH_3^+ → Asparagine

Glutamic acid + NH_3^+ → Glutamine

6. Reaction with 1-Fluoro-2,4-dinitrobenzene

Amino acids react with 1-Fluoro-2,4-dinitrobenzene (Sanger's Reagent) in cold mild alkaline (bicarbonate) to give dinitrophenyl aminoacid



dinitrobenzene Dinitrophenylaminoacid.

7. Chelation with metal ion

Heavy metals like Cu^{+2}, Hg^{+2}, Mn^{+2}, Fe^{2+} form chelated complexes with amino acids in which both carboxyl and amino groups are involved.

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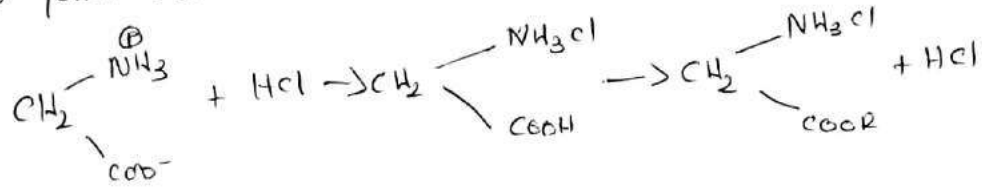
8) Reactions due to carboxyl group

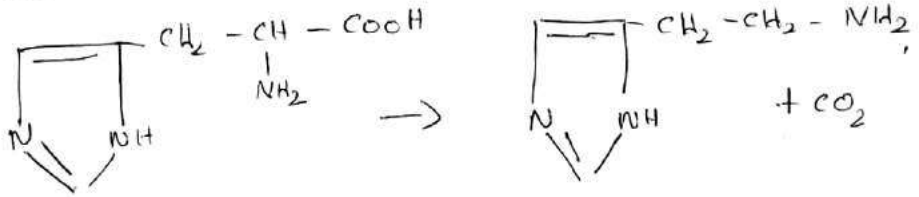
(i) Formation of esters

Aminoacids react with alcohols in the presence of Hcl to form ester.



(ii) De-carboxylation - Formation of amine

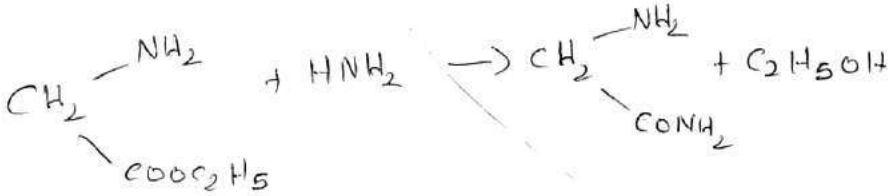
When aminoacids are treated (heated) in the presence of $Ba(OH)_2$, CO_2 is lost and corresponding amine is formed.



Histidine Histamine

(iii) Amide formation

Amino acid esters form amides when treated with anhydrous (or alcoholic ammonia.



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Seperation of aminoacid

Electrophoresis:

Electrophoresis is a technique which is used to seperate charge molecules based on their mobility, in an electric field. Electric mobility depends on the net charge of the molecule, size of the molecule, shape of the molecule and the electric field strength.

polyacrylamide gel electrophoresis

This technique is widely used to seperate proteins and nucleic acids. Successful Performance of electrophoresis requires placing sample molecule in a stable medium. The stable medium decreases (or) eliminates convenctions and does not react with the given sample and stops its movement.

Acrylamide in solution is activated by free radicals formed by ammenium presulphate. This activated acryl amide reacts with successive acetyl amide molecule to produce long polymer chains gelatin and linking is brought about by further polymerisation

An example of such polyacrylamide gel is Agarose gel. It is widely used because of its large size. Many Proteins that differ in size and shape have merely identical charge.

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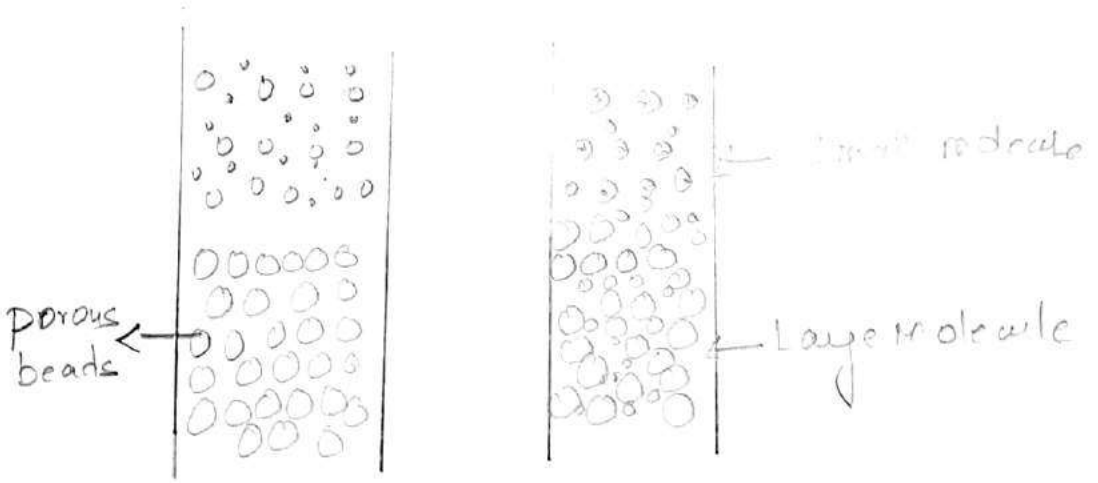
Electrophoresis of such molecules in solution results in no seperation. This limitation is thus overcome by exposing the protein to an ionic detergent such as sodium dodecyl Sulphate (SDS). The protein is exposed to SDS before it is treating for polyacrylamide gel electrophoresis (PAGE). SDS denatures the protein causing multimeric protein to dissociate into their subunits. After this all polypeptide chains are forced into extended conformation with similar charge mass ratio. And the molecules thus seperate only on the basis of their masses.

Net charge on protein molecule depends on the relative proportion of positively charged and negtively charged amino acid side chains at a given pH. Smaller molecules move faster and larger ones move slower.

Gel is the supporting medium and all the proteins move towards the positive end of the gel since SDS with protein is negatively charged.



Gel filtration chromatography

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Gel filtration

In gel filtration chromatography, the seperation of molecules is based on their size, shape and their molecular weight. This technique is also called as molecular seive (or) molecular exclusion chromatography. The apparatus consists of a column packed with sponge like gel beads containing pores. The gels serves as molecular seives for the seperation of smaller and bigger molecules.

The solution mixture containing molecules of different sizes (say proteins) is applied to column and eluted with a buffer. The larger molecules cannot pass through the pores of gel and therefore, move faster. on the otherhand, the small molecules enter the gel beads and are left behind which come out slowly. By selecting the gel beads of different porosity, the molecule can be seperate. The commercially available gels include Sephadex (G-10, G-25, G-100), Biogel (p-10, p-30, p-100) and Sepharose (6B, 4B, 2B)

The gel filtration chromatography can be used for an approximate determination of molecular weight. This is done by using a calibrated column with substance of known molecular weight.

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Ultracentrifugation

The ultracentrifuge was developed by a Swedish biochemist Svedberg (1923). The principle is based on the generation of centrifugal force to as high as 600,000g that allows the sedimentation of particles or macromolecules. Ultracentrifugation is an indispensable tool for the isolation of subcellular organelles, proteins and nucleic acids. Also, this technique is employed in the determination of molecular weight of macromolecules.

The rate at which the sedimentation occurs in ultracentrifugation primarily depends on the size and shape of the particles (or Macromolecules. It is expressed in terms of Sedimentation coefficient(s) and is given by the formula

$$S = \frac{V}{\omega^2 r}$$

V = migration (sedimentation) of the molecule
ω = Rotation of the centrifuge rotor in radian/sec
r = Distance in cm from the centre of rotor.

Isolation of subcellular organelles by centrifugation:

The cells are subjected to disruption by Sonication or osmotic shock (or by use of homogenizer. This is usually carried out in an isotonic (0.25 M) Sucrose. The advantage with Sucrose medium is that it does not cause the organelles to swell.

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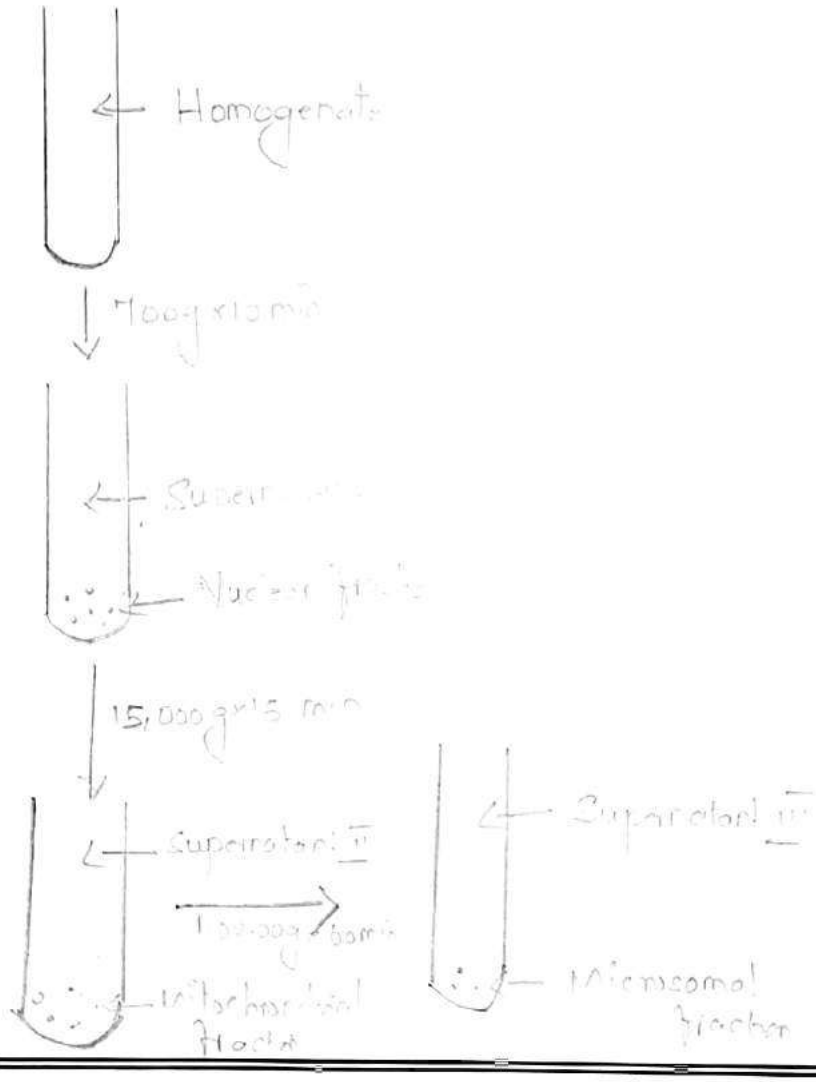
When the homogenate is centrifuged at 700g for about 10 min, the nuclear fraction (including plasma membrane) gets sedimented. On centrifuging the Supernatent (I) at 15000g for about 20 min, mitochondria fraction (that includes lysosomes, peroxisome) is pelleted. Further centrifugation of the Supernatant(II) at 100,000g for about 60 min seperates microsomal (Ribosomes & endoplasmic reticulum). The Supernatant(III) then obtained corresponds to the cytosol. The purity of the subcellular fractionation can be checked by the use of marker enzymes.



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Reg - 2017 (III - Sem)

UNIT - I

CELL DEGENERATION, REPAIR AND NEOPLASIA

TOPIC : Cell Injury

When the cell is exposed to an injurious agent (or) Stress, it encounter sequence of event resulting in changes in its internal and external environment termed as **cell injury**.

* Cell injury is reversible upto a certain point. When the stress is mild to moderate, the injury may be recover called **reversible cell injury**.

* If the stimulus persist (or) if it is severe enough, then the cell reaches a point of no return and suffers **irreversible cell injury**, and ultimately it end up in **cell death** (Result of cell injury).

* If cell adapt to changes morphologically then it revert back to normal called **Cellular adaptation**.

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Broadly it classified into 2 types :

① Reversible cell injury - Pathologic Changes can be reversed

② Irreversible cell injury

↳ Pathological changes that are Permenent and cause cell death, they cannot be reversed to normal.

Causes of cell injury

(i) Hypoxia - Deficiency of oxygen supply in blood

(ii) Ischemia - Loss of blood supply

(iii) Physical agent - eg., Mechanical trauma (Road accidents), extremes of temperature (Hot / cold), Change in atm., Pressure, Increased Radiation dosage, Electric Shock

(iv) Chemical agent - eg., Poisons, toxic agents, air pollutant strong acids & Alkalis, therapeutic administration of drugs, Alcohol, insecticides etc..

(v) Infectious agents - eg., Virus, bacteria, fungi etc...

(vi) Immunologic reactions - defense against biological agents

Causes autoimmune diseases.

(vii) Genetic derangements

(viii) Nutritional disbalance

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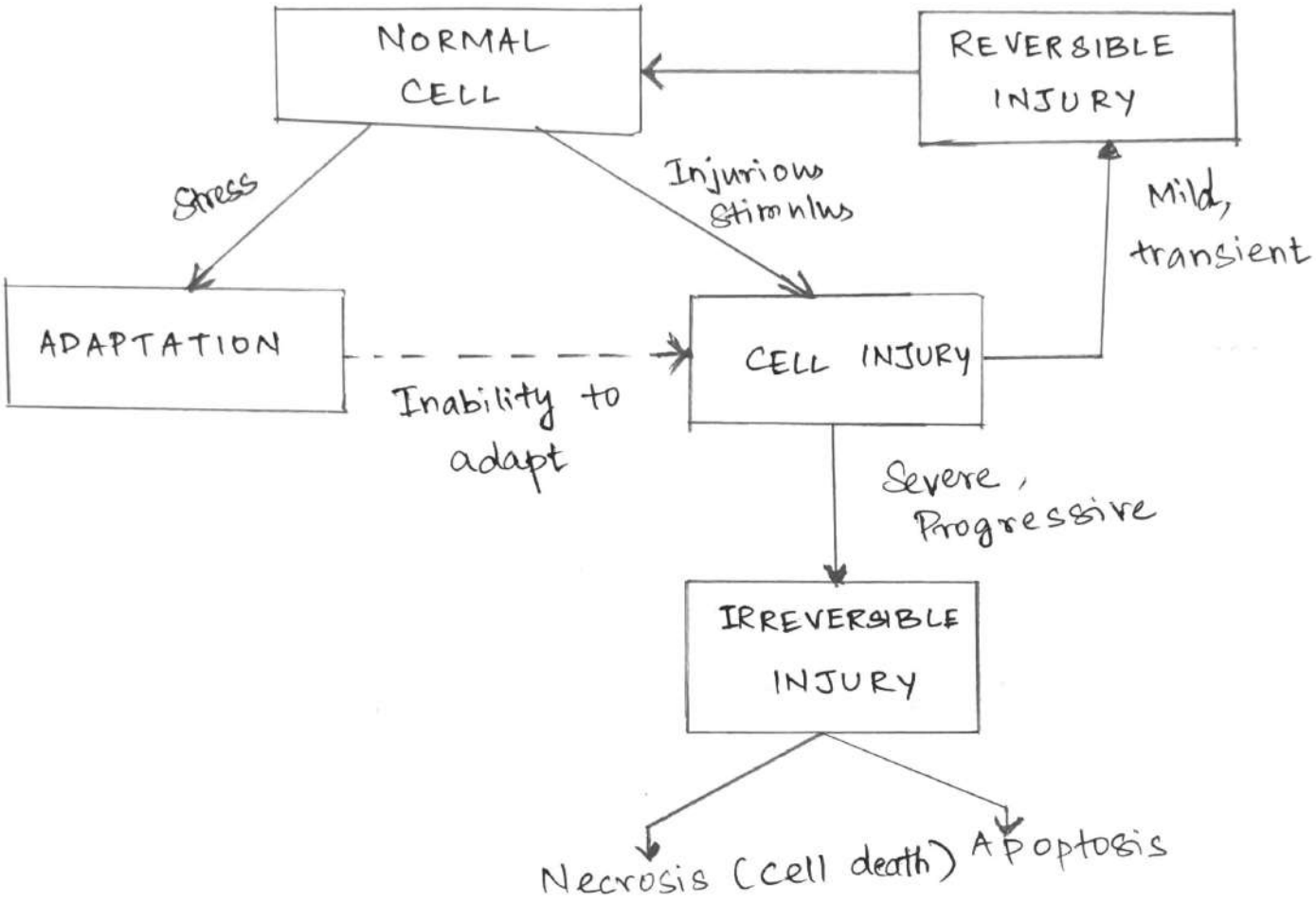

Fig: Cellular Response to Cell Injury

Patterns of Cell death

Two Principle pattern of cell death are,

① Necrosis

② Apoptosis

Apoptosis: (Programmed cell death)

* Apoptosis occurs when cell dies through activation of an internally controlled suicide Program.

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* Apoptosis designed to eliminate Unwanted cells during embryogenesis (Formation of embryo), in various Physiologic process and certain pathologic conditions.

Necrosis:

Necrosis is a type of cell death that occurs after ischemia and chemical injury.

Topic: Mechanism Of Cell Injury

① Loss of energy (ATP depletion, O_2 depletion)

② Mitochondrial damage

③ Loss of calcium homeostasis

④ Plasma Membrane damage & Permeability changes

⑤ Free Radicals

⑥ DNA and Protein structral damage.

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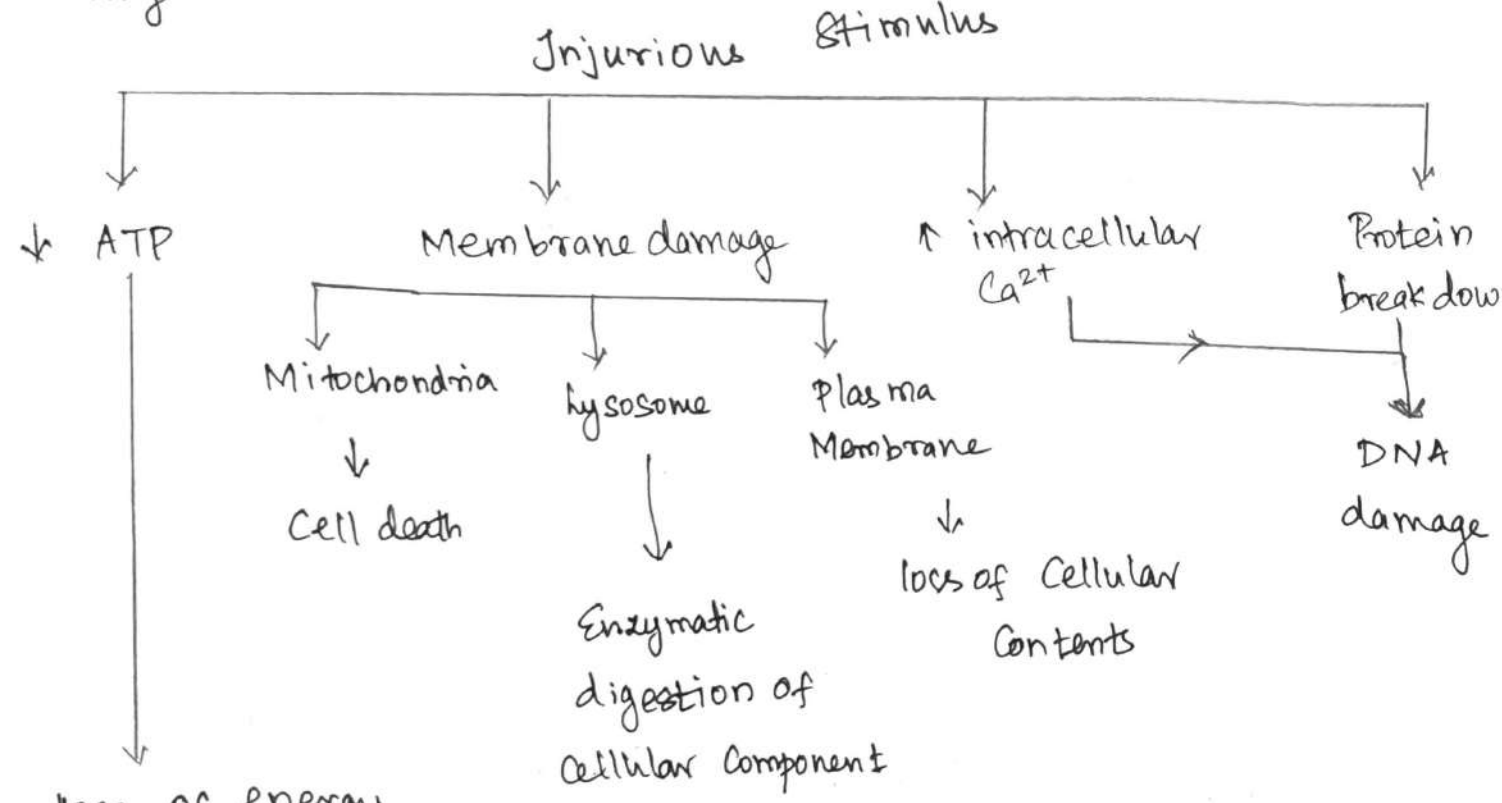
(1) Loss of Energy (ATP depletion)

* ATP depletion and decreased ATP synthesis are associated with both hypoxic and chemical Injury

* Due to reduced Oxygen supply in Mitochondria the Oxidative phosphorylation occurs - leads to loss of energy

* Cell relay on glycolysis for energy production, resulting in depletion of glycogen storage, it reduces the intracellular PH.

* Collectevily it results in decreased activity of many cellular enzymes



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* Decrease in ATP reduces Sodium Pump at cell membrane - It result in Sodium and water enter the cell and Potassium exist.

* As a result Endoplasmic reticulum dilates, the cell starts to swells, and blebs appear.

② Mitochondrian damage

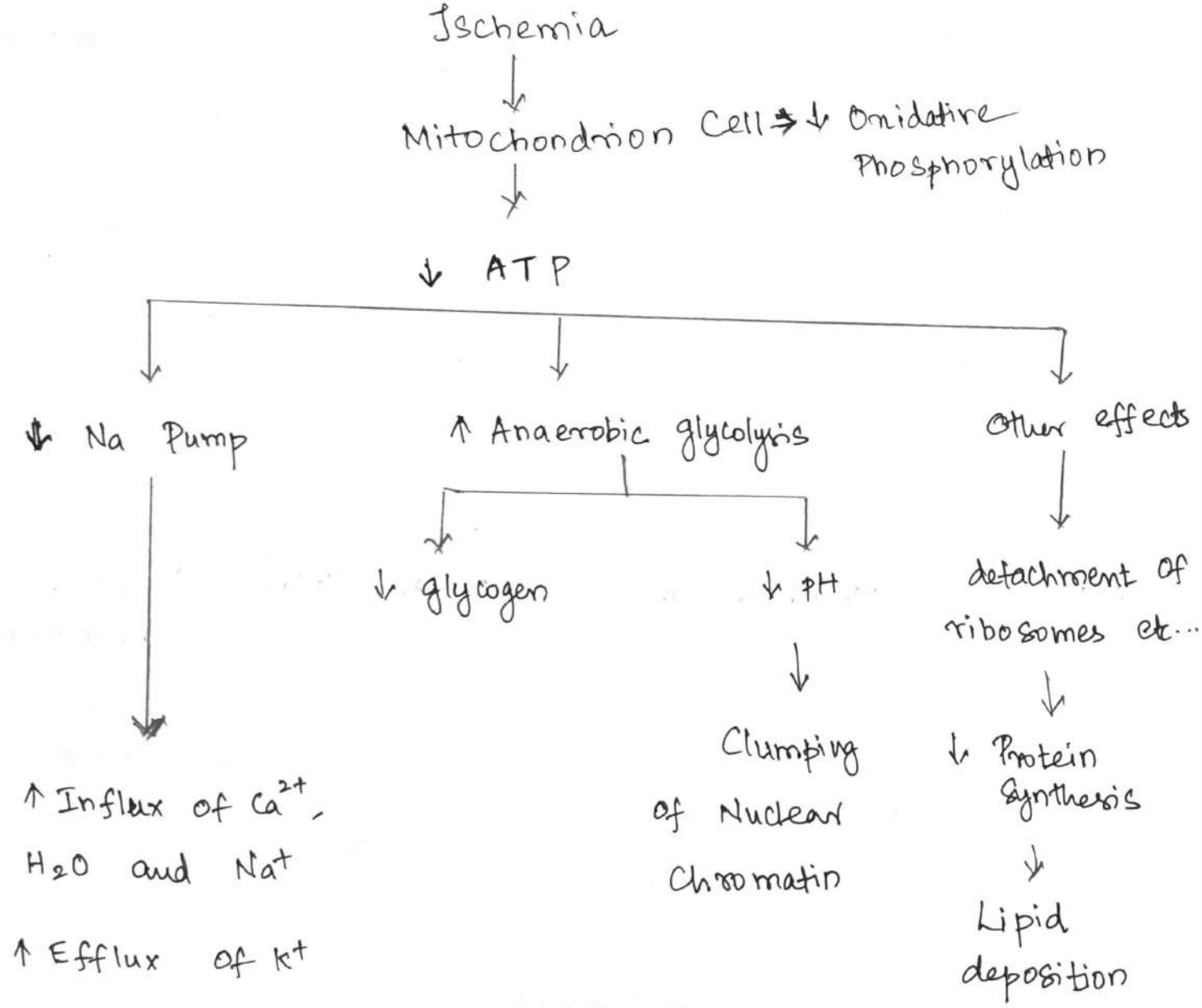

Fig: Proces of Mitochondrian damage

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(3) Importance of Calcium [loss of Calcium]

* Influx of Calcium (Ca^{2+}) to cell comes from the extracellular fluid and stores in mitochondria and Endoplasmic recticulum

* Calcium activates phospholipases and damage the Cell membrane and cytoskeleton.

* Severe damage to membrane of lysosomes and leakage of lysosomal enzymes causes cell death.

* It occurs particularly in hypoxia and Ischaemia and with certain toxins.

* preventing rise of Ca^{++} (or) restoring to normal levels prevents cell death.

(4) Plasma Membrane damage :

Plasma Membrane :-

* Due to hypoxia plasma membrane damage occurs.
* Immune mechanism cells gets infected with virus
* Causes damage to lysosome lead to cell death.

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Mitochondria :-

* Mitochondrial Permeability transition.

* If this process is permanent it leads to cell death and leakage of ATP causes apoptosis.

⑤ Free Radicals :

* Free radicals have single unpaired electron in outer orbit. They are highly reactive with adjacent molecules.

* When radicals are produced in excess, they react with cell then it damages protein, lipids, carbohydrates and Nucleic acid

* These damages converted to chain reaction causes widespread of cell damage.

⑥ DNA and Protein Structural Damage :

All membranes of cell may be damaged and ruptured by mechanical force as in trauma. When damage is severe in any structural membrane lead to cell death by necrosis.

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Topic: Reversible and Irreversible Cell Injury (5)

Reversible Cell injury:

Earliest Changes associated with Cell injury are,

(i) Decreased generation of ATP

(ii) Loss of Cell membrane integrity

(iii) Defects in protein Synthesis, cytoskeletal damage

(iv) DNA damage

Within the limits, the cell can Compensate for these dearrangements. If the injurious stimulus is removed the damage can be reversed called reversible cell injury.

Morphological changes in reversible cell injury are,

⇒ Cellular Swelling - due to accumulation of water in intracellular membrane

⇒ loss of Microvilli (Increased Cell surface area)

⇒ Blebs - Swelling of Endoplasmic reticulam due to energy dependent interaction b/w membranes.

These changes can be seen in microscopes results in,

- Cellular Swelling and
- Fatty changes

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Irreversible Cell injury :

If Ischemia persist for longer period of time the irreversible cell injury occurs. It marked by

⇒ Severe Mitochondrial vacuolization

⇒ Extensive damage to plasma Membrane

⇒ Swelling of ribosomes

⇒ Injury to lysosomal membrane leads to leakage of lysosomal enzymes into cytoplasm

Critical Events of Irreversible cell injury

(i) ATP depletion

Inability to reverse mitochondrial damage

(ii) Cell membrane damage

Functional and structural defects in cell membrane Cause cell death. Ultimate result of irreversible cell injury is cell death.

If irreversible cell injury happen in any cell leads to cell death which cannot be reverse back to normal cell. Causes Severe damage to human Immune System.

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Topic : Cell death

Cell death is an irreversible changes in the Cell associated with its end. Two types are,

① Apoptosis
② Necrosis

① Apoptosis :

Apoptosis is a type of cell death occur in Physiological and embryological processes and whereby Controls Cell population numbers.

* Apoptosis also occurs in pathological processes such as inflammation and Cancer, in an attempt by the body to arrest cell proliferation and tissue damage.

Sequence of events in apoptosis are,

Apoptosis is an energy - dependent Cascade of Molecular events which include protein by a group of enzymes (caspases), protein cross - linking, DNA breakdown.

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* Pro - apoptotic protein found in cytosol where they act as Sensor of Cellular damage (or) Stress.

* Following Cellular Stress, Protein relocate to the Surface Of Mitochondria where it disrupts the Normal function and lead to formation of Pores in mitochondria

* It releases cytochrome c from intramembrane Space. This leads to activation of Caspase.

* Caspase plays an important role in the process of activating DNA and breaking down Structural Protein in Nucleus

* Fragmentation of DNA and nucleus leads to formation of apoptotic bodies.

↳ Small apoptic bodies composes of a fragments of Nuclei

* The apoptotic bodies are rapidly damages the adjacent healthy cells and Causes Cell death.

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Morphological changes in Apoptosis :

① Rapid Volume reduction and Formation of cytoplasmic blebs, Cell Shrinkage and Chromatin Condensation.

② Loss of Cell - Cell Contacts

③ Formation of apoptotic bodies

④ Phagocytosis of macrophages, undergo Cell death

Significance of apoptosis

① Programmed Cell death - require activation of enzyme

② Apoptosis responsible for cell destruction in physiological events

③ Apoptosis leads to removal of Unwanted cells.

Physiological apoptosis, occurs in,

⇒ Normal tissue turnover

⇒ In hormone - induced atrophy

⇒ In developing tissues

⇒ In embryogenesis

Pathological apoptosis occurs in,

⇒ viral Infection

⇒ Tumor regression induced by Chemotherapy

⇒ Spontaneous occurrence of Solid tumor.

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Topic : ② NECROSIS :

Necrosis is most common pattern of cell death.

Defn: Necrosis defined as morphologic changes that following the cell death in a living tissue (or) Organ resulting from progressive degradative activity of Catalytic Enzymes. (Increased Proces in biological molecule)

* Enzymes derived from dying cells themselves Called autolysis

* Necrosis caused by tissue reactivity such as active increase in blood supply of tissue surrounding necross, followed by inflammatory cells.

Two principle proces of Necrosis are.,

(i) Enzymatic digestion of cell
(ii) Denaturation of Proteins

Dead cell morphology of Necrosis happens in Cytoplasm, Nucleus, DNAs, Lysosomes.

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Types of Necrosis

① Coagulative Necrosis
② Liquefactive Necrosis
③ Fat Necrosis
④ Caseous Necrosis
⑤ Fibrinoid Necrosis
⑥ Gangrenous Necrosis

1. Coagulative Necrosis :

* Most Common pattern of Necrosis caused by hypoxia
* Appearance – Firm Consistency, Yellowish Color
* Pathogenesis – Coagulative Necrosis implies Preservation of basic outline of coagulated cells for several days.
* Nucleus usually disappears, but Shape of Cell is Preserved in this type.
* Finally necrotic Cell breaks into fragments by Phagocytosis of cellular debris (death cell)

Example Myocardial Infarction

2. Liquefactive Necrosis :

A dead cells undergo disintegrant and affected tissue is called liquefactive Necrosis.

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* Result from rapid action of hydrolytic enzymes.
* Characteristics of ischemia necrosis of brain, Pancreas.

Morphology :

Necrotic area becomes very soft and fluidy. Visually associated with Cellular - destruction and Pus formation. example : Pneumonia, Brain Infarction

③ Fat Necrosis

* Enzymatic digestion of fat
* Example : Necrosis of fat by Pancreatic enzyme.
* Fat Necrosis refers to Necrosis in adipose tissue - due to action of activated lipases.
* Released Fattyacids Complex with calcium to create calcium soaps to the naked eye.
* Necrotic foci appear opaque and Chalky white (or) yellowish.

④ Caseous Necrosis

It is the Combination of Coagulative and liquefactive Necrosis.

It is encountered in tuberculosis caused by Mycobacterium.

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* Caseous Necrosis appears grossly as soft, whitish - gray debris resembling cheesy material.

Example - Tuberculosis lesions

(5) Fibrinoid Necrosis:

It is a type of connective tissue necrosis seen particularly in autoimmune disease.

* Collagen and smooth muscle are affected.

* Fibrinoid Necrosis is characterized by loss of Normal structure of Collogen fibres.

Example: Polyarteriitis Nodosa - Affecting Blood vessel walls.

(6) Gangrenous Necrosis

Gangrenous Necrosis is a term used by Surgeons.

* It usually applied to limb, generally lower leg, that has lost its blood supply and has undergone Coagulation Necrosis

When bacterial infection is superimposed, then Coagulative necrosis is modified by the liquefactive action of bacteria affected leukocyte leads to Gangrenous Necrosis

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3 types of Gangrene: They are,

① Dry Gangrene
② Wet Gangrene
③ Gas Gangrene

Dry Gangrene:

Necrotic tissue appears black and dry and is sharply demarcated from viable tissue.

* Most commonly it occurs in extremities as a result of ischemia.
* When coagulative pattern Prevails - Dry gangrene develop
* When liquefaction is more pronounced - Wet Gangrene develops.

Wet Gangrene:

* Result from Severe bacterial infection of necrotic area.
* Most Commonly occurs in extremities due to arterial obstruction, also in internal organs such as intestine.
* Tissue is swollen, reddish - black with extensive liquefaction

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Gas Gangrene :

Due to bacterial Infection that produce tissue Gas in gangrene, also due to wound infection.

* Characterized by extensive necrosis and tissue destruction and Production of gas by the Fermentation action of bacteria.

Topic : INTRACELLULAR ACCUMULATION

Cells Can accumulate Pigments (or) other substances as a result of Pathological and various Physiological Process, and is usually an early indicator of cell stress (or) reversible injury

3 Catogory of Intracellular accumulations are,

(i) Accumulation in Normal cell as excess lipids, Proteins, Carbohydrates, Fats.

(ii) Accumulation of Abnormal Cell Substance either exogenous (or) Endogenous

(iii) Accumulation of a Pigment Coloured Substance

Causes

⇒ Inadequate Metabolism (eg., Fatty Change in liver)

⇒ Because of Genetic defects (eg., lysosomal storage disease

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⇒ Cell has inability to degrade substance nor the ability to transport it to other sites

eg:, accumulation of Carbon Particles in Lungs & lymph nodes.

Types of Accumulation

1. Accumulation of Lipids
2. Accumulation of Proteins
3. Accumulation of Glycogen
4. Accumulation of Pigments

1. Accumulation of Lipids:

(a) Fatty change (Steatosis)

Abnormal accumulation of triacylglycerides within Parenchymal Cells of liver. It seen in Liver.

(eg) Liver in alcoholic disease.

(b) Cholesterol and cholesterol esters

Cells use cholesterol for Synthesis of Cell membranes Abnormal accumulation of Cholesterol occurs in several Pathological Process

(eg) 1. Atherosclerosis → Smooth muscle Cells of Arteries filled with cholesterol & become foamy cells

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(eg) 2. Xathomas → Cholesterol - rich material in connective tissues of tendons and other Body parts

2. Accumulation of Proteins

(a) Proteinuria in Renal disease

- Protein loss in Urine
- Accumulation of Protein in epithelial cells of Proximal tubules.

(b) Plasma cells

- Accumulation of Immunoglobin (Ig) in Rough Endoplasmic Recticulum, resulting in formation of Russell bodies occurs in several Pathological Process.

3. Accumulation Of Glycogen

- occurs in Glucose (or) glycogen metabolism disorder.
- Appear as Clear vacuole within cytoplasm.

(eg) 1. Diabetes mellitus → disorder of Glucose metabolism

It happens due to accumulation of glycogen in a epithelial cells of Proximal tubule & Henles loop.

2. Glycogenoses

Defect in Glucose Synthesis Cause accumulation of glycogen (VON GIERKE DISEASE)

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4. Accumulation of Pigments

Pigments - Coloured substance which represents either normal cell (or) in abnormal cell

Two types :

⇒ Exogenous - Coming from outside to the body

⇒ Endogenous - Synthesized within the body itself.

(a) Exogenous Pigments

(eg) 1. Coal dust - Air pollutants inhaled & Picked by alveoli and transport to lymph nodes

(eg) 2. Anthracosis - Accumulation of carbon Particles in Lungs and cause serious lung disease

(eg) 3. Tattooing - form of exogenous pigmentation of skin. Injected Pigment is taken by macrophages and stay in cell. It cause dermal macrophages & fibroblast

(b) Endogenous Pigments

It includes lipofuscin, melanin, bilirubin

(eg) 1. Lipofuscin - Free radical injury, found in liver & Heart

2. Melanin - Brown - Black Pigment in skin lesions. Gives raise to dermal skin layer (Mole)

3. Bilirubin - Yellowish skin decoloration of skin (Jaundice) It is a bile Pigment from hemoglobin.

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PATHOLOGICAL CALCIFICATION

* Abnormal deposition of calcium salts causes Pathological Calcification. Two types :

① Metastatic Calcification

② Dystrophic Calcification

Metastatic Calcification :

- Abnormal deposition of calcium salts in normal tissue is called metastatic calcification
- occurs due to increased level in serum calcium

Causes :

* Hyperparathyroidism - Increase bone resorption
* Vitamin - D intoxication
* Destruction of bone tissue

Sources :

* Deposition of Calcium occurs in arterial walls of kidney, lungs etc....

Dystrophic Calcification :

Abnormal Calcium deposition in dead, dying and injured cells and tissues called dystrophic Calcification.

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Occurs in

→ Atherosclerosis (Deposition in inner walls)

→ Dying (or) damaged heart Valves.

Deposits appears macroscopically as fine, while granules.

Topic:

INFLAMMATION

Inflammation is a complex Protective response involving host cells, blood vessels and Proteins.

- Caused by various endo and exogenous stimuli
- Infectious agents are destroyed and diluted off.

Functions:

* Eliminate the initial causes of cell injury.
* Remove necrotic cells and tissue.
* Initiate the Process of Repair.

Symptoms:

Color - heat, Tumor - Swelling, Loss of function

Pathogenesis:

* Increased blood flow (redness & warmth)
* Increased vascular Permeability (Pain, loss of function Swelling)
* Leukocytic infiltration.

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Types of Inflammation

* Acute Inflammation
* Chronic Inflammation

Acute Inflammation	Chronic Inflammation
(i) It occurs at faster rate - In minutes (or) hours	(i) It occurs slowly - In Days
(ii) Tissue injury usually mild and Self Limited	(ii) often Severe and Progressive
(iii) Prominent signs	(iii) Less prominent, may be subtle (Precise - Difficult to analyze

Stimuli for acute inflammation are,

- Infections - Bacteria, Viral, fungal & toxins
- Tissue Necrosis
- Foreign bodies (dirt)
- Immune reactions (hypersensitivity reactions)

Mechanism of Inflammation

Two types of events

1. Vascular events
   ↳ Event that occur within blood Vessels
2. Cellular events
   ↳ Events that occur within Cells.

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Vascular Events

① Vasodilation

Increased Permeability of blood vessels due to widened intercell junctions and Contraction of a endothelial cells.

② Vascular leakage and edema

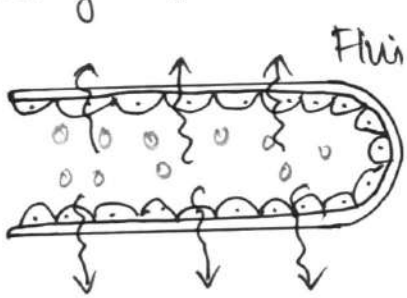
Increased Permeability causes transudate and later exudate into extracellular tissue.

Transudate - Protein - Poor filtrate of Plasma

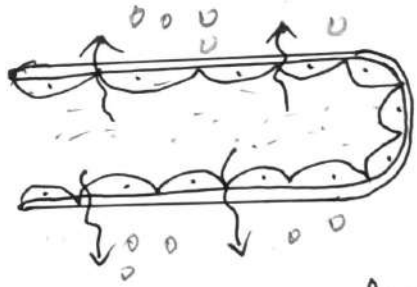
Exudate - Protein - rich filtrate of Plasma

③ Stasis

Slowing of blood flow due to hyperviscocity of blood



Transudate
(Low Protein Content)



Fluid & Protein leakage
Exudate

Cellular Events

Sequence of events taking Place Outside the blood vessels and within the Cell is Called Cellular events.

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① Margination and Rolling

- Fluid leaves the blood vessels, leukocytes marginate along the endothelial surface.
- Cell marginate in rows along the endothelial surface.

② Activation and Adhesion

- After margination, cells are attached to the surface with help of adhesion molecules and bind with leucocyte.

③ Transmigration (Diapedesis)

Leucocyte migrate across the endothelial space through a Process called diapedesis.

④ Chemotaxis

Migration of cells towards chemotactic stimuli from the source of tissue injury eg) Chemokines.

⑤ Phagocytosis

- Finally leucocytes reaches the injured site and Phagocytosis occurs
- Recognition and attachment of injured cell
- Engulfment
- Killing (or) degradation of damaged Cell

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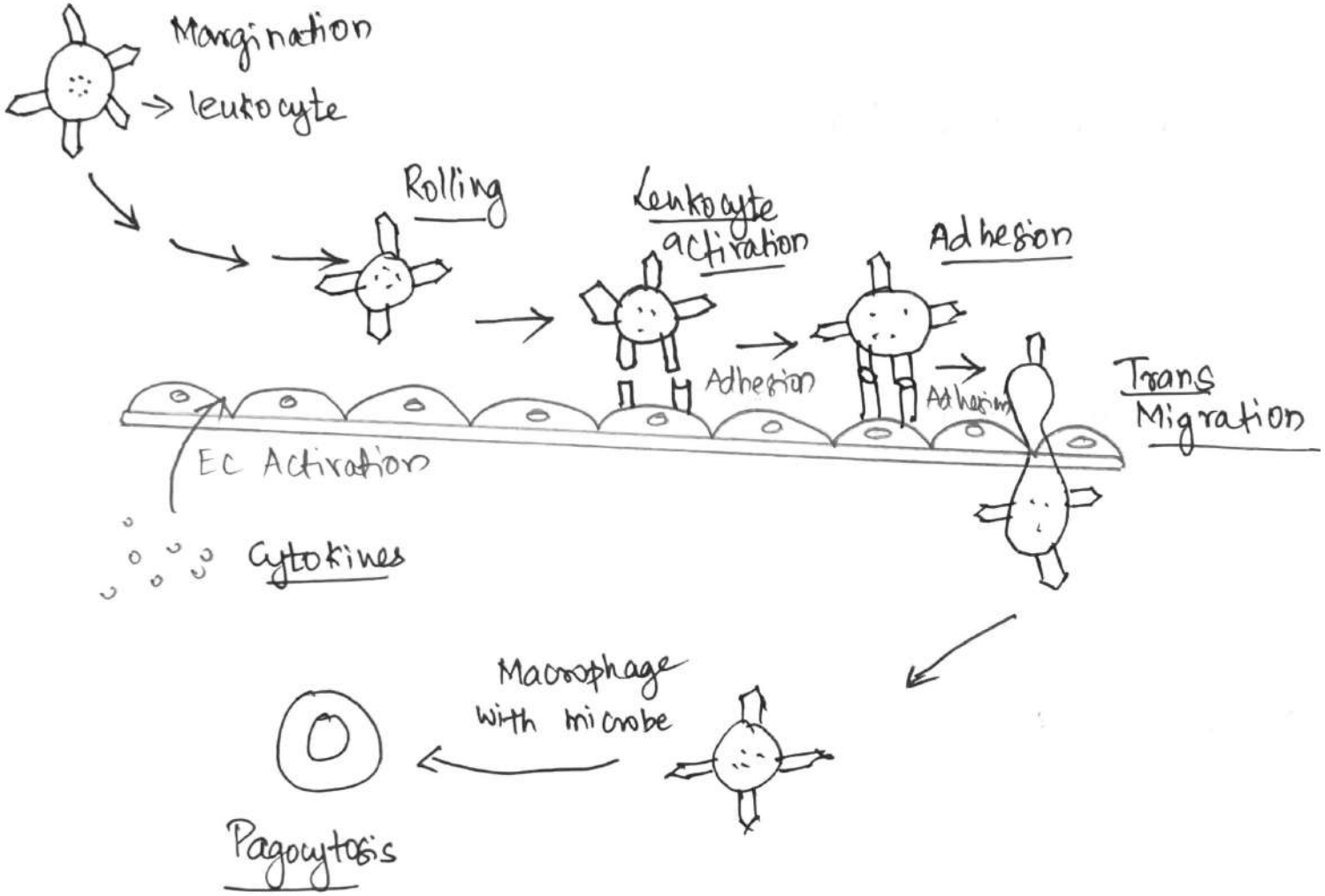
⑥ Chemical mediators

Chemical substance synthesized (or) released and mediate the changes in inflammation



Cellular Events

Outcomes of Acute Inflammation

⇒ Abscess formation - Collection of Pus in tissue and Swelling

⇒ Progressive to Chronic inflammation stage

⇒ Resolution - tissue goes back to Normal Stage

⇒ Repair - healing by scarring

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Outcomes of Chronic Inflammation

⇒ Prolonged exposure to irritants
⇒ Repeated acute inflammation
⇒ Autoimmune reactions

} leads to Chronic Inflammation

Cells involved in inflammations are,

* Lymphocytes - Plasma cells, NK cells
* Plasma cells - Production of Ig
* Monocytes / Macrophages

REPAIR MECHANISM

Wound Healing

Wound heal by Primary intention (or) Secondary intention depending upon whether the wound may be Closed with sutures (or) left to repair. Automatic Natural repair happens, whereby damaged tissue is restored by formation of connective tissue and a regrowth of epithelium.

3 phases of wound healing :

They are,
1. Inflammatory phase
2. Proliferation phase
3. Maturation phase

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Inflammatory phase :

- This phase is body's natural response to injury
- Wound healing cycle starts
- It last from 4 - 6 days.

Sequence of event : ⇒ vessels forms clots to prevent excessive loss of blood and fluids at injured site

⇒ Platelets release growth factors that triggers the healing Process

⇒ WBC go to injured area and Clean/Remove unwanted cell

Proliferation Phase :

- Last for 4 - 24 days

Sequence of event : ⇒ Granulation tissue fills in wound

⇒ Granulation Composed of Collagen + extracellular matrix and network of blood vessels develop - Called angiogenesis

⇒ Fibroblast lay network of Collagen in wound bed which given Strength to tissue

⇒ Epithelial Cells from wound margin migrate inward to cover wound.

Maturation Phase

⇒ It last for 21 days to 2 Years

⇒ Begin when wound has filled & Resurfaced. Remodel happens and form scar tissue (80% Strong than orginal)

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Factors affecting wound healing are, (16)

① Age
② Dehydration
③ Infection
④ Nutrition
⑤ Medication
⑥ tissue Perfusion & Oxygenation

Bone fracture Healing

Bone fracture healing occurs through 4 Phases:

* Inflammatory Stage
* Soft Callus formation Stage
* Hard Callus formation Stage
* Bone Remodelling

1. Inflammatory Stage [Hematoma formation]

⇒ Inflammatory Stage begins the moment the bone is broken and last for around 5 days

⇒ Blood vessels in the broken bone tear and loss of blood occurs, resulting in formation of Blood clot. This is Called Hematoma (Bleeding within tissue)

⇒ Healing Process initiated

⇒ Osteoclast Cells work to remove dead bone cells

⇒ Formation of Soft Callus begin in 4 to 10 days

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2. Soft Callus formation Stage

⇒ Callus formation begins after few days of fracture

⇒ Fibroblast Cells in granulation tissue begin to form Cartilage and fibrocartilage.

⇒ Cartilage is a Spongy material that fills gap blw fracture ends.

⇒ After 2 weaks, soft Callus provide Sufficient Stability at fractme Site for new blood veesels formation and for osteoblast Cells in woven bone

⇒ Woven bone at margin of fractme is little Soft.

3. Hard tissue Callus formation

⇒ It takes 2 to Six weeks, in Some Case 12 weaks. This process begin where Cartilage material of Callus is transformed Completly into woven bone

⇒ Time duration depends on location & type of fracture

⇒ Hard Callus formation occurs by release of mineral Compounds Such as Calcium and Phosphate into Cartilage tissue.

⇒ Fracture union takes place as a outcome.

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4. Bone Remodelling

⇒ Remodelling begins when fracture has united and continue for several years

⇒ Normal shape of bone is restored at this Process

⇒ osteoclast and osteoblast cells helps in Remodelling

⇒ Loosely organized woven bone is gradually replaced by Lamellar bone, which is highly organized along lines of Stress.

⇒ Lamellar bone is stronger than woven bone.

NEOPLASIA

Neoplasia defined as new, uncontrolled growth of cells that is not under physiological control

The term tumor, nodule and mass are nonspecific terms that refer to abnormal proliferation of cells.

General Catogories of neoplasms are:

(*) Adenoma : ⊙ Adenoma is Benign neoplasm.
⊙ Derived from granular cells. Neutrophils, eosinophils Basophils

(*) Carcinoma : ⊙ Malignant neoplasm
⊙ Derived from epithelial cells (Present in Surface of body)

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(*) Sarcoma : ⊙ Malignant neoplasm

⊙ Derived from mesenchymal cells (Fat, Muscle)

(*) Lymphoma : ⊙ Malignant neoplasm

⊙ Derived from lymphocytes [WBC]

(*) Melanoma : ⊙ Malignant neoplasm.

⊙ Derived from melanocytes (Melanin - Pigment formation in skin & eye)

(*) Germ cell tumor : ⊙ Malignant neoplasm.

⊙ Derived from germ cell.

Generally, Cancer → Malignant

Neoplasia → either benign (or) malignant

Tumor → Simply grow which may not be neoplastic

Carcinogenesis :

Carcinogenesis is also called oncogenesis. It is the formation of cancer, whereby normal cells are transformed into cancer cells.

This process is characterized by changes at cellular, genetic and epigenetic levels of abnormal celldivision. According to carcinogeness, somatic mutation in DNA lead to cancer disrupt & cell death.

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Process of Carcinogenesis : (18)

Mutation inactivates tumor Suppressor gene → Cell Proliferate

↓

Mutation inactivates DNA Repair Gene → Creates Oncogene → Several More tumor Suppressor Gene

↓

CANCER

Squamous cell carcinoma is occuring within many organs including mouth, upper respiratory tract and Lungs.

Causes of Neoplasia

(i) Environmental causes

Chemicals → includes dyes, alcohols, Smoking etc... Cause Cancer

Oncogenic virus → Such as Human papilloma virus [HPV] occurs in Squamous Cell Carcinoma of Cervix.

Epstein-Barr virus [EBV] - in lymphoma

Radiation → Including UV light induces pyrimidine dimer in DNA and promotes Skin Cancer.

Ionizing radiations induces mutation in DNA and promotes malagrancies Such as leukemia, thyroid, lung, breast Cancer.

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(ii) Hereditary Causes : Due to defects in Chromosome

(iii) Altered DNA : DNA Repair mechanism affected

Transforming growth factor also promotes tumor growth

CLASSIFICATION OF TUMOR

Based on Behavioural, two types of tumor are,

- BENIGN TUMOR
- MALIGNANT TUMOR

Benign Tumor (Non-Cancerous)

- Slow growing and rarely spread to other areas of body
- They have well-defined border
- Non-invasive
- Donot metastasis
- Can be removed through Surgical treatment

Malignant Tumor (Cancerous)

- Grow faster
- No well-defined border (boundary less tumors)
- More invasive (invade/spread nearby tissues)
- Metastasis (Spread to other parts of the body)
- Life threatening.

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Spread of Tumor

Spread of Tumor is explained using 4 phases.

1. Initiation Phase
2. Tumor Progression phase
3. Local Invasion Phase
4. Metastasis phase

1. Initiation (or) Transformation Phase :

Mutation (change) Causes transformation of normal cells to Cancer cells. If the factors causing tumor Persist for longtime, transformed cells give rise to clones that grows continously. Mutation Causes alteration in gene.

Sequence of event :

Mutation inactivates tumor suppressor gene

↓

Cell Proliferation happens

↓

Mutation inactivates DNA Repair gene

↓

Oncogene created and mutation inactivates several more tumor suppressor gene

↓

CANCER

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2. Growth of Transformed Cells

* Tumor Progression Stage. It indicates Cell Proliferation which leads to outgrowth of Population of clonally derived tumor cells.

* Subclones may arise overtime from original malignant clone

* Subclone diffres from orginal Clone in characteristics such as invasiveness, metastatic & response to therapy.

3. Local Invasion

* Cancer cells starts inrading nearby surrounding tissues. Invasion occurs through following steps:

⇒ Cancerous cells attached to basement membrane

⇒ Local Proteolysis occurs which cause degradation of matrix region

⇒ Locomotion – Cancer Cell enter into nearby tisue through forming diffrent Shapes.

4. Metastasis

Tumor cell get detached from one Part and enter into blood Circulation and transported to other Parts of body for metastasis Proces

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* During the transport it gets attached to basement membrane and start invading that tissue.

The process of spreading from one Part of the body to other is called Metastasis.

Sequence are,

Tumor Cell → Small blood vessel → tumor emboli

↓

Invasion of wall of vessel ← Adheres to the endothelium of vessel ← distant Parts

↓

Proliferate in adjacent tissue → estabish New metastatic tumor.

Factors contribute to Cancer Progression are,

- Age
- Alcohol
- Chronic inflammation
- Diet
- Hormones
- Infectious agents.

"CANCER IS A WORD NOT A SENTENCE"

WORLD FIGHT AGAINST CANCER

Never give up ! Never give in !

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UNIT - I

BIOPSY

To decide whether a tumor is malignant or not, a sample must be taken by a surgeon or an interventional radiologist and sent to the laboratory and examined under a microscope by a pathologist - the sample is called a biopsy.

Introduction:

Many medical conditions, including all cases of cancer, must be diagnosed by removing a sample of tissue from the patient and sending it to a pathologist for examination. This procedure is called a biopsy, a Greek-derived word that may be loosely translated as "view of the living." Any organ in the body can be biopsied using a variety of techniques, some of which require major surgery (e.g., staging splenectomy for Hodgkin's disease), while others do not even require local anesthesia (e.g., fine needle aspiration biopsy of thyroid, breast, lung, liver, etc). After the biopsy specimen is obtained by the doctor, it is sent for examination to another doctor, the anatomical pathologist, who prepares a written report with information designed to help the primary doctor manage the patient's condition properly.

TYPES OF BIOPSIES

1. Excisional biopsy

A whole organ or a whole lump is removed (excised). These are less common now, since the development of fine needle aspiration (see below). Some types of tumors (such as lymphoma, a cancer of the lymphocyte blood cells) have to be examined whole to allow an accurate diagnosis, so enlarged lymph nodes are good candidates for excisional biopsies. Some surgeons prefer excisional biopsies of most breast lumps to ensure the greatest diagnostic accuracy. Some organs, such as the spleen, are dangerous to cut into without removing the whole organ, so excisional biopsies are preferred for these.

2. Incisional biopsy Only a portion of the lump is removed surgically. This type of biopsy is most commonly used for tumors of the soft tissues (muscle, fat, connective tissue) to distinguish benign conditions from malignant soft tissue tumors, called sarcomas.

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3. Endoscopic biopsy

This is probably the most commonly performed type of biopsy. It is done through a fiberoptic endoscope the doctor inserts into the gastrointestinal tract (alimentary tract endoscopy), urinary bladder (cystoscopy), abdominal cavity (laparoscopy), joint cavity (arthroscopy), mid-portion of the chest (mediastinoscopy), or trachea and bronchial system (laryngoscopy and bronchoscopy), either through a natural body orifice or a small surgical incision. The endoscopist can directly visualize an abnormal area on the lining of the organ in question and pinch off tiny bits of tissue with forceps attached to a long cable that runs inside the endoscope.

4 .Bone marrow biopsy

In cases of abnormal blood counts, such as unexplained anemia, high white cell count, and low underlying the "bikini dimples" on the lower back/upper buttocks. Hematologists do bone marrow biopsies all the time, but most internists and pathologists and many family practitioners are also trained to perform this procedure.

SPECIMEN PROCESSING FOR BIOPSY

1. Histologic sections

This involves preparation of stained, thin (less than 5 micrometers, or 0.005 millimeters) slices mounted on a glass slide, under a very thin pane of glass called a coverslip. There are two major techniques for preparation of histologic sections:

a. Permanent sections This technique gives the best quality of specimen for examination, at the expense of time. The fresh specimen is immersed in a fluid called a fixative for several hours (the necessary time dependent on the size of the specimen). The fixative, typically formalin (a 10% solution of formaldehyde gas in buffered water), causes the proteins in the cells to denature and become hard and "fixed." Adequate fixation is probably the most important technical aspect of biopsy processing. The fixed specimen is then placed in a machine that automatically goes through an elaborate overnight cycle that removes all the water from the specimen and replaces it with paraffin wax. The next morning, a technical professional, called a histologic technician, or "histotech," removes the paraffin-impregnated specimen and "embeds" it in a larger bloc of

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molten paraffin. This is allowed to solidify by chilling and is set in a cutting machine, called a microtome. The histotech uses the microtome to cut thin sections of the paraffin block containing the biopsy specimen. These delicate sections are floated out on a water bath and picked up on a glass slide. The paraffin is dissolved from the tissue on the slide. With a series of solvents, water is restored to the sections, and they are stained in a mixture of dyes. The most common dyes used are hematoxylin a natural product of the heartwood of the logwood tree, Haematoxylon campechianum, which is native to Central America, and eosin, an artifcial aniline dye. The stain combination, casually referred to by pathologists as "H and E" yields pink, orange, and blue sections that make it easier for us to distinguish different parts of cells. Typically, the nucleus of cells stains dark blue, while the cytoplasm stains pink or orange.

b. Frozen sections This technique allows one to examine histologic sections within a few minutes of removing the specimen from the patient, but the price paid is that the quality of the sections is not nearly as good as those of the permanent section. Still, a skilled pathologist and a knowledgeable surgeon can work together to use the frozen section's rapid availability to the patient's great benefit.

2. Smears

The specimen is a liquid, or small solid chunks suspended in liquid. This material is smeared on a microscope slide and is either allowed to dry in air or is "fixed" by spraying or immersion in a liquid. The fixed smears are then stained, coverslipped, and examined under the microscope.

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TYPES OF TUMORS

Neoplasms may be benign, pre-malignant (carcinoma in situ) or malignant (cancer).

Benign Tumor

A benign tumor (benign neoplasm) cannot metastasize - it cannot spread. Most benign tumors are not harmful to human health. Even though they are not cancerous, some may press against nerves or blood vessels and cause pain or other negative effects. Benign tumors of endocrine tissues may result in the excessive production of some hormones. Examples of benign tumors include:

- **Adenomas** - tumors that arise from glandular epithelial tissue - epithelial tissue is the thin membrane that covers glands, organs and other structures in the body. A polyp in the colon is a type of adenoma.
- **Fibroids (Fibromas)** - benign tumors that grow on fibrous or connective tissue of any organ in the body. Uterine fibroids are common.
- **Hemangiomas** - benign tumors which consists of a collection of too many blood cells. They can sometimes be seen on the surface of the skin and are colloquially called strawberry marks.
- **Lipomas** - the most common form of soft-tissue tumor. Lipomas consist of adipose tissue (fat cells). Most of them are very small, painless, soft to the touch, and generally movable.

Premalignant Tumor

A premalignant tumor is one that is not yet malignant, but is about to become so. Examples of premalignant growths include: Actinic keratosis (senile keratosis), Dysplasia of the cervix, Metaplasia of the lung and Leukoplakia.

Malignant Tumor

Malignant tumors are cancerous tumors; they tend to become progressively worse, and can potentially result in death. Unlike benign tumors, malignant ones grow fast, they are ambitious, they seek out new territory, and they spread (metastasize).

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Metastasis - malignant tumors invade nearby cells, and then the cells near those spread to various parts of the body through the bloodstream or the lymphatic system. Metastasis is the process by which cancer cells spread from their primary site to distant locations in the human body. For example, a patient may have started off with melanoma (skin cancer) which metastasized in their brain. Lung cancer spreads to the liver, and invade other organs.

There are different types of tumors, which are made up of specific types of cancer cells:

- **Carcinoma** - these tumors are derived from the skin or tissues that line body organs (epithelial cells).
- **Sarcoma** - these are tumors that start off in connective tissue, such as cartilage, bones, fat and nerves.
- **Lymphoma/Leukemia** - cancer arises from the blood forming (hematopoietic) cells that originate in the marrow and generally mature in the blood or lymph nodes.
- **Germ cell tumor** - these are tumors that arise from a germ cell, pluripotent cells (cells than can turn into any kind of cell).

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Autopsy

Definition:

An autopsy (post-mortem examination, obduction, necropsy, or autopsia cadaverum) is a surgical procedure that consists of a thorough examination of a corpse by dissection to determine the cause, mode and manner of death or to evaluate any disease or injury that may be present for research or educational purposes. (The term "necropsy" is generally reserved for non-human animals; see below). Autopsies are usually performed by a specialized medical doctor called a pathologist. In most cases, a medical examiner or coroner can determine cause of death and only a small portion of deaths require an autopsy..

Purposes

Autopsies are performed for either legal or medical purposes. Autopsies can be performed when any of the following information is desired:

- Determine if death was natural or unnatural
- Injury source and extent on the corpse
- Manner of death must be determined
- Time since death
- Establish identity of deceased
- Retain relevant organs
- If infant, determine live birth and viability

For example, a forensic autopsy is carried out when the cause of death may be a criminal matter, while a clinical or academic autopsy is performed to find the medical cause of death and is used in cases of unknown or uncertain death, or for research purposes. Autopsies can be further classified into cases where external examination suffices, and those where the body is dissected and internal examination is conducted. Permission from next of kin may be required for internal autopsy in some cases. Once an internal autopsy is complete the body is reconstituted by sewing it back together.

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Types

Four main types of autopsies:

- Medico-Legal Autopsy or Forensic or coroner's autopsies seek to find the cause and manner of death and to identify the decedent. They are generally performed, as prescribed by applicable law, in cases of violent, suspicious or sudden deaths, deaths without medical assistance or during surgical procedures.
- Clinical or Pathological autopsies are performed to diagnose a particular disease or for research purposes. They aim to determine, clarify, or confirm medical diagnoses that remained unknown or unclear prior to the patient's death.
- Anatomical or academic autopsies are performed by students of anatomy for study purpose only.
- Virtual or medical imaging autopsies are performed utilizing imaging technology only, primarily magnetic resonance imaging (MRI) and computed tomography (CT).

Forensic autopsy

A forensic autopsy is used to determine the cause, mode and manner of death.

Forensic science involves the application of the sciences to answer questions of interest to the legal system.

Medical examiners attempt to determine the time of death, the exact cause of death, and what, if anything, preceded the death, such as a struggle. A forensic autopsy may include obtaining biological specimens from the deceased for toxicological testing, including stomach contents. Toxicology tests may reveal the presence of one or more chemical "poisons" (all chemicals, in sufficient quantities, can be classified as a poison) and their quantity. Because post-mortem deterioration of the body, together with the gravitational pooling of bodily fluids, will necessarily alter the bodily environment, toxicology tests may overestimate, rather than underestimate, the quantity of the suspected chemical.[12]

Following an in-depth examination of all the evidence, a medical examiner or coroner will assign a manner of death from the choices proscribed by the fact-finder's jurisdiction and will detail the evidence on the mechanism of the death.

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Clinical autopsy

Pathologist performing a human dissection of the abdominal and thoracic organs in an autopsy room.

Clinical autopsies serve two major purposes. They are performed to gain more insight into pathological processes and determine what factors contributed to a patient's death. Autopsies are also performed to ensure the standard of care at hospitals. Autopsies can yield insight into how patient deaths can be prevented in the future.

Over time, autopsies have not only been able to determine the cause of death, but also lead to discoveries of various diseases such as fetal alcohol syndrome, Legionnaire's disease, and even viral hepatitis

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What Is a Bleeding Disorder?

A bleeding disorder is a condition that affects the way blood normally clots. The clotting process, also known as coagulation, changes blood from a liquid to a solid. When injured, blood normally begins to clot to prevent a massive loss of blood. Sometimes, certain conditions prevent blood from clotting properly, which can result in heavy or prolonged bleeding.

Bleeding disorders can cause abnormal bleeding both outside and inside the body. Some disorders can drastically increase the amount of blood leaving your body. Others cause bleeding to occur under the skin or in vital organs, such as the brain.

What Causes a Bleeding Disorder?

Bleeding disorders often develop when the blood can't clot properly. For blood to clot, body needs blood proteins called clotting factors and blood cells called platelets. Normally, platelets clump together to form a plug at the site of a damaged or injured blood vessel. The clotting factors then come together to form a fibrin clot. This keeps the platelets in place and prevents blood from flowing out of the blood vessel.

In people with bleeding disorders, however, the clotting factors or platelets don't work the way they should or are in short supply. When the blood doesn't clot, excessive or prolonged bleeding can occur. It can also lead to spontaneous or sudden bleeding in your muscles, joints, or other parts of your body.

The majority of bleeding disorders are inherited, which means they're passed from a parent to their child. However, some disorders may develop as a result of other medical conditions, such as liver disease.

Bleeding disorders may also be caused by:

- a low red blood cell count
- a vitamin K deficiency
- side effects from certain medications

Medications that can interfere with the clotting of the blood are called anticoagulants.

Symptoms

Symptoms of a bleeding disorder include:

- Bleeding into joints, muscles and soft tissues
- Excessive bruising
- Prolonged, heavy menstrual periods (menorrhagia)
- Unexplained nosebleeds
- Extended bleeding after minor cuts, blood draws or vaccinations, minor surgery or dental procedures

Types of bleeding disorders

1) **Hemophilia** is an inherited bleeding disorder in which a person lacks or has low levels of certain proteins called "clotting factors" and the blood doesn't clot properly as a result. This leads to excessive bleeding. There are 13 types of clotting factors, and these work with platelets to help the blood clot.

The three forms of hemophilia are hemophilia A, B, and C.

- Hemophilia A is the most common type of hemophilia, and it's caused by a deficiency in factor VIII.
- Hemophilia B, which is also called Christmas disease, is caused by a deficiency of factor IX.
- Hemophilia C is a mild form of the disease that's caused by a deficiency of factor XI. People with this rare type of hemophilia often don't experience spontaneous bleeding.

Hemophilia is an inherited genetic condition. This condition isn't curable, but it can be treated to minimize symptoms and prevent future health complications.

In extremely rare cases, hemophilia can develop after birth. This is called "acquired hemophilia." This is the case in people whose immune system forms antibodies that attack factors VIII or IX.

2) **Von Willebrand disease** is a bleeding disorder. It's caused by a deficiency of von Willebrand factor (VWF). This is a type of protein that helps your blood to clot.

Bleeding happens when one of blood vessels breaks. Platelets are a type of cell that circulates in your blood and clumps together to plug broken blood vessels and stop bleeding. VWF is a protein that helps platelets clump together, or clot. If your levels of functional VWF are low, your platelets won't be able to clot properly. This leads to prolonged bleeding.

Treatment: no cure for bleeding disorders, these conditions can be successfully managed. A hematologist (a physician with special training in blood disorders) handles a bleeding disorder patient's care and identifies the best treatment options.

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UNIT - II

FLUID AND HEMODYNAMIC DERANGEMENTS

Topic:

THROMBOSIS

⇒ Hemostasis is the physiological Process of maintaining blood in fluid state and formation of hemostatic Plug at site of vessel injury.

⇒ Thrombosis is the physiological Process of maintaining blood in ~~irregular~~ flow by activating blood clotting in uninjured site of blood vessel.

It happens in vessel wall, Platelets & Coagulation Path

Steps are,

* Primary hemostasis
* Secondary hemostasis
* Fibrinolysis.

Factors Predisposing thrombosis are,

* Endothelial injury
* Blood Stasis (or) turbulence of flow
* Blood hypercoagulability

Thrombus - Hemodynamic disorder in blood

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Endothelial Injury :

⇒ Important factor in arterial thrombosis

⇒ Occurs in myocardial infraction, atherosclerosis, trauma, inflammatory disease of vessels.

⇒ Endothelial dysfunction happens and leads to loss of endothelium.

Blood Stasis and turbulence of flow

⇒ Turbulence enhances endothelial injury.

⇒ Stasis enhances venous thrombosis.

Both result in

- Bringing Platelets close to endothelium
- Prerent clotting factor inhibitors
- Endothelial activation.

eg: Myocardial Infraction, Valve Stenosis, Sickle Cell disease

Hypercoagulability

⇒ It is an alteration in coagulation leading to thrombosis. It is due to,

* Primary causes [Genetic]
  - Factor V (mutation)
  - Antithrombin III deficiency

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* Secondary Causes
  - Prolonged immobilization
  - Cancer
  - Smoking

Types :

Arterial Thrombosis :

* Occurs in large vessels (Aorta, heart) and smaller vessels (Coronary arteries, leg arteries)
* Classically have altering white and red layers called lines of Zahn (eg) damaged heart valves, infracted Left Vent

Consequences are,
- Ischemia in tissue distal to thrombus with Possible necrosis
- may embolize due to rapid flow

Venous Thrombi

* occurs at the site of stasis commonly veins of lower extremity

Consequences are
- Rarely occur & cause ischemia if affects arterial supply
- More common embolize.

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Fat Thrombus

* Dissolution - by fibrinolysis
* Propagation - along length of vessel occlusion
* Embolization - Solid mass detached from thrombus
* Organization - Inflammation + Fibrosis

↓

Replaced by scar.

EMBOLISM

Embolism is defined as any intravascular mass (solid, liquid (or) gas) carried by blood to site distant from point of origin.

Types of Embolism

- Thrombo embolism
- Fat embolism
- Air embolism
- Cholesterol embolism

Pulmonary Thromboembolism

* Occlude (mass formation) occurs in pulmonary artery (Saddle embolus) (or) in small branches of vessels
* Embolus from veins to arterial blood system results in hemorrhage and rarely infraction.

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* obstruction of small vessels lead to infraction (5)
* Multiple emboli may lead to pulmonary hypertension

Fat embolism :

* It is a type of embolism caused by physical trauma such as fracture of long bones, soft tissue trauma and burns.
* Release of fatty acids from fat globules causes local toxic injury to endothelium.

The vascular damage is aggravated by platelet activation and granulocytes.

Air embolism

* Caused by gas bubble in vascular system
* Air embolism occurs in xylem of vascular plants especially when suffering from water stress
* Bubbles in blood that can block arterial blood flow.
* Change in pressure can cause nitrogen bubbles to develop in their bloodstream causes serious vascular disorders.

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Amniotic Fluid Embolism

* Amniotic fluid is used to protect a baby inside the mothers womb by surrounding the baby.

* During Labour [child birth] there may be the chance that it may leak into mothers blood vessel and results in blockage.

* This type of embolism is dangerous and leads to breathing Problem, drop/increase in blood Pressure and even loss of conciousness.

Topic: HEMOSTASIS

⇒ Hemostasis is a lympatic disorder in human body. It refers to the arrest of bleeding.

⇒ It keeps blood fluid within normal vessel by rapid clot formation when vessel injured.

⇒ A hemostasis clot is normal in case of the vessel injury. But in normal condition its dangerous

⇒ Thrombosis - Refers to inappropriate activation of hemostatic Process.

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Mechanism of Hemostasis

① Damage to the blood vessel cause arteriolar Vasoconstriction

* Exposure of endothelial nerve fibre causes reflex.
* Endothelial cells lining blood vessels gets damaged causing endothelin secretion.

② Primary hemostasis

* Endothelium damage cause release of Von Willebrand factor [VWF] that binds to exposed collagen.
* Platelets bind to VWF (Platelet adhesion)
* Platelet activated on contact with VWF and it release granule contents like ADP & Thromboxane (TXA_2)
* Platelet aggregated and stimulated by ADP & TXA_2

③ Secondary hemostasis

(i) Activation of Coagulation Cascade

* Tissue factor released from damaged endothelium
* Tissue factor and secreted Platelet factor activate Coagulation Cascade.

(ii) Conversion of fibrinogen to fibrin.

* Cause firin deposition and leads to autolytic activation of Coagulation Cascade.

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(iii) Binding to Platelet surface receptor causes further Platelet aggregation and activation. (5)

- Fibrin deposition stabilized and anchors aggregated Platelets.

(4) Counter - Regulatory Mechanism :

(i) Fibrinolytic Pathway

* Plasminogen activation → Plasmin formation

* Coagulation cascade cause release tissue - type Plasminogen activator (t-pa) from endothelium.

* t-Pa activates plasminogen into plasmin which degrade the fibrin & fibrinogen. Blood clot is dissolved

(ii) Anticoagulant Pathway

Activates thrombomodulin and blocks the coagulation cascade.

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UNIT 2

Hyperemia

Hyperemia is an increased amount of blood in the vessels of an organ or tissue in the body. It can affect many different organs, including the:

- liver
- heart
- skin
- eyes
- brain

Types of hyperemia

There are two types of hyperemia:

- **Active hyperemia** happens when there's an increase in the blood supply to an organ. This is usually in response to a greater demand for blood — for example, if you're exercising.
- **Passive hyperemia** is when blood can't properly exit an organ, so it builds up in the blood vessels. This type of hyperemia is also known as congestion.

Causes of hyperemia

Each type of hyperemia has a different cause.

Active hyperemia is caused by an increased flow of blood into your organs. It usually happens when organs need more blood than usual. Your blood vessels widen to increase the supply of blood flowing in.

Causes of active hyperemia include:

- **Exercise.** Your heart and muscles need more oxygen when you're active. Blood rushes to these organs to supply extra oxygen. Your muscles need up to 20 times their normal supply of blood during a workout.
- **Heat.** When you're running a high fever or it's hot outside, extra blood flows to your skin to help your body release heat.
- **Digestion.** After you eat, your stomach and intestines need more blood to help them break down foods and absorb nutrients.
- **Inflammation.** During an injury or infection, blood flow to the site increases.
- **Menopause.** Women who are in menopause often have hot flashes, which causes a rush of blood to the skin — especially of the face, neck, and chest. Blushing is a similar response.
- **Release of a blockage.** Hyperemia can happen following ischemia, which is poor blood flow to an organ. Once ischemia is treated, blood rushes to the area.

Passive hyperemia happens when blood can't properly drain from an organ and begins to build up in the blood vessels.

Causes of passive hyperemia include:

- **Heart failure or ventricular failure.** The left and right ventricles are the two main pumping chambers of the heart. The right ventricle pumps blood to the lungs, and the left ventricle pumps oxygen-rich blood to the body. When the heart can't beat well enough to push blood through the body, blood begins to back up. This backup causes swelling, or congestion, in organs like the liver, lungs, spleen, and kidneys.

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- **Deep vein thrombosis (DVT)**. DVT is caused by a clot in one of the deep veins — often in your lower legs. The clot can break free and get lodged in a vein in your lung, called a pulmonary embolism.
- **Hepatic vein thrombosis (HVT), also called Budd-Chiari syndrome**. HVT is a blockage in the veins of the liver caused by a blood clot.

Symptoms

The main symptoms of hyperemia are

- redness
- warmth

Other symptoms depend on the cause of the problem.

Heart failure symptoms include

- shortness of breath
- coughing or wheezing
- swelling in the belly, legs, ankles, or feet caused by fluid buildup
- fatigue
- loss of appetite
- nausea
- confusion
- fast heartbeat

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Disseminated Intravascular Coagulation

Disseminated Intravascular Coagulation is a rare, life-threatening condition that Prevents a Person blood from Clotting in a normal Condition [Healthy Person]

* DIC may Cause excessive clotting [thrombosis] (or) bleeding [hemorrhage] throughout body and lead to shock, organ failure and death.

* In DIC bod's natural ability to regulate blood clotting doesnot function Properly.

* This Cause blood's clotting Cells [Platelets] to clump together and clog small blood vessel throughout.

* This excessive clotting damages organs, destroys blood Cells and depletes supply of Platelets so that blood is no longer able to clot normally.

Causes:

⇒ Bacterial, viral (or) fungal infection

⇒ Specific type of Cancer

⇒ complications during Pregnancy

⇒ Snakebite

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Disseminated Intravascular Coagulation

Disseminated Intravascular Coagulation is a rare, life-threatening condition that Prevents a Person blood from clotting in a normal condition [Healthy Person]

* DIC may cause excessive clotting [thrombosis] (or) bleeding [hemorrhage] throughout body and lead to shock, organ failure and death.

* In DIC bod's natural ability to regulate blood clotting doesnot function Properly.

* This cause blood's clotting cells [Platelets] to clump together and clog small blood vessel throughout.

* This excessive clotting damages organs, destroys blood cells and depletes supply of Platelets so that blood is no longer able to clot normally.

Causes:

⇒ Bacterial, viral (or) fungal infection

⇒ Specific type of cancer

⇒ complications during Pregnancy

⇒ Snakebite

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Symptoms

* Blood Platelets and clotting factors depleted Causing excessive bleeding [hemorrhages]

* Organ damage – Shortness of breath, Lung damage, low Urine output from kidney damage.

Pathophysiology

DIC is mediated by widespread release of thrombin and Plasmin into circulation. It leeds to damaged tissue and formation of unregulated thrombin.

Depletion of clotting factors and Platelets - results in bleeding Problem.

INFARCTION

* Infarction is an area of tissue/organ necrosis Caused by ischemia.
* Result from sudden reduction of arterial flow by thrombosis (or) embolism.
* Infarction also Caused by Compression of blood vessels.

Example:

Myocardial infraction, Cerebral infarction and Pulmonary infarction.

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⇒ 99% infarction due to thrombosis mostly in artery. (13)

⇒ Venous infarct occurs in organs with single venous outflow. eg: ovary, testis

Morphological Classification

1. Red infarct
2. White infarct

① Red Infarct

- due to venous occulsion
- Common in loose tissue eg) Lung
- Seen in organs with dual circulation

② White Infarct

- As a result of arterial occlusion
- Common in solid organs eg) Heart, kidney, Spleen
- Seen in organs with end-arterial circulation (heart)

Infarction is usually wedge-shaped, surrounded by rim of hyperemia, leead to hemorrhage

Necrosis is a coagulative type of infarction cause inflammation within few hours and after that repair mechanism occurs.

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Factors influencing development of infarct: (14)

⇒ Nature of blood supply

- ✱ dual : Lung, liver, hands
- ✱ end-arterial : Spleen, kidney.

⇒ Rate of occlusion

⇒ Valnerability to hypoxia

eg: Neuron : 3-4 minutes

Heart : 20-30 minutes

⇒ Oxygen content of blood [hypoxia in blood].

SHOCK

Shock is the condition of decreased tissue Perfusion and impaired Cellular metabolism. As a result of imbalance between the supply and demand for Oxygen and nutrients.

Symptoms of Shock:

* Restless, confused state of mind
* Pale Cold Sweaty
* Low blood Pressure
* Drowsiness
* Coma.

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Cardiogenic Shock

- Condition occurs in Heart
- As a result of systolic or diastolic dysfunction
- Compromised Cardiac output.

Pathophysiology

Decreased Stroke Volume

↓

Decreased Cardiac Output

↓

Decreased Cellular Oxygen Supply

↓

Decreased tissue Perfusion

↓

Impaired cellular metabolism

↓

Increased in acid volume (↓sed PH)

↓

Cell Damage occurs

↓

Cell Death.

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Topic: HEMATOLOGICAL DISORDER (16)

Blood Components :

Red Blood Cells : [Erthrocytes]

* Makeup 40 % of Blood volume and Produced in Bone marrow
* Contain Hemoglobin, a Protein that gives blood its red Color and Carry oxygen.

White Blood Cells : [Leukocytes]

* Fewer in number Compare to RBC [1 : 660]
* Primary responsibility : Defend the body against infection.

Platelets : [Thrombocyte]

* Cell - like Particles Smaller than RBC and WBC.
* Help with Clotting Process by gathering at bleeding Site and Clumping together to form Plug that helps Seal the blood vessel.

Plasma

* Liquid Part of blood. All blood Cells are Suspended here.
* Contain dissolved Salts & Proteins
* Prevents blood Vessels from Collapsing & Clotting.
* Plays a role in warming and Cooling the body.

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Hematology:

Hematology is the study of blood in healthy and diseased person. It includes problems with RBCs, WBCs, Platelets, Blood vessels, bonemarrow, lymphnodes, Spleen and Proteins involved in bleeding and clotting.

RBC DISORDER

- Enythrocyte disorder

RBC is important component of blood [contains 45% in blood]. The various disorders due to RBC are:

Anemia

It is a condition in which concentration of the Hemoglobin (or) erthrocyte in blood is below normal, thus impairing the ability of RBCs to transport Oxygen and CO_2.

→ how Oxygen Carrying Capacity Causes inadequate support to metabolism

⇒ Anemic individuals are fatigue, often pale, short of breath and feel chilly.

Causes of anemia are:

(*) An insufficient number of RBC due to

- Blood Loss - Hemorrhagic Anemia
- Excessive RBC destruction - Hemolytic Anemia.

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- Inadequate Production of RBC due to Bonemarrow failure - Aplastic anemia.

Hemorrhagic Anemia :

* Results because of increased blood loss due to Severe injury / wound.

Hemolytic Anemia :

* In this condition, erythrocyte rupture (or) lyse Prematurely.
* This is due to hemoglobin abnormality of the mismatched blood and bacteria injection.

Aplastic Anemia :

* It result from destruction (or) inhibition of red marrow by bacterial toxins, drugs and ionizing radiations.
* Causing inadequate Production of RBC

② Low Hemoglobin Content

when hemoglobin molecules are normal but RBC contain fewer Hb than normal number, nutritional anemia is always suspected.

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Iron - deficiency Anemia

* It occurs due to inadequate intake of iron-containing foods and impared iron absorption.
* Iron is essential for production of Hb in RBC

Prenicious Anemia

* It is due to deficiency of vitamin B_{12}.
* Intrinsic factor Produced by stomach mucosa must be present for vit B_{12} to absorbed by integtinal cells
* Deficiency in intrinsic factor causes Prenicious anemia.

(3) Causes due to Abnormal Hemoglobin

Production of abnormal Hb due to genetic abnormalities. Common abnormalities are,

- Thalasemia -
- Sickle cell anemia

} Globin Part of Hb is abnormal and RBC Produced are fragile & Rupture Prematurely.

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Thalassemia :

- It is seen in people of mediterranean ancestry
- One of the globin chain is absent (or) the erythrocyte are thin, delicate and deficient in Hemoglobin.

Sickle - cell anemia

- Caused by abnormalities in Hemoglobin gene (Hb A)
- Result from change in aminoacids in B-chain of globin molecules
- Hb becomes Spicky & Sharp - Deform RBC rupture.

Polycythemia

- Abnormal excess of RBC that increses blood viscosity causing it to sludge in blood vessel.
- occurs as a result of bone marrow cancer.
- Dare to this there is less O_2 available in RBC leads to polycythemia.

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WBC DISORDERS

White Blood Cells are part of body's immune system.

Types of WBC are, Granulocytes : * Neutrophils
* Eosinophils
* Basophils

Agranulocytes : * Monocyte
* Lymphocyte [T cell + B cell]

Normal Range of WBC in Blood is, 4,500 to 11,500 WBCs Per microliter (4.5 to 11.0×10^9 L)

More common WBC disorder are,

* Lymphoma
* Leukemia

Patient has increased risk of infection due to the malfunction (or) absence of certain types of WBC.

LEUKEMIA :

(*) Leukemia is a cancer of blood-forming cells, (or) Stem cells, located in the bone marrow.

(*) These Cancer cells have exaggerated Proliferation (or) development Problem Causing immature cells to be released from bone marrow

(*) Overproduction of abnormal leukocytes occur in leukemia.

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* leukemia cell donot die normally causing an increase in number of WBCs.

Based on type of cell line, leukemia classified into,

1. Myeloid leukemia
2. Lymphoid leukemia

- Myeloid Stem cells differentiate into "RBC, Platelets, granulocytes, and Monocytes".

- Lymphoid Stem cells differentiate into T-Lymphocyte and B-Lymphocyte and NK Cells.

Cancer condition occurs in lymphoid Stem cell. It further classified into ⇒ Acute and ⇒ Chronic

Acute form of leukemia have cells that Proliferate quickly and donot develop properly

Chronic forms of leukemia have cells that donot die normally and exist for long time.

⇒ Acute leukemia is Common in Children

⇒ Chronic leukemia is Common in elderly People

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Four types:

① Acute myeloblastic leukemia – Common in Adults & Infants

② Acute lymphoblastic leukemia - Common in Young childrens

③ Chronic myeloblastic leukemia - Common in Adults

④ Chronic lymphoblastic leukemia – affects adult age of 55

* All Leukemias, bone marrow becomes almost totally occupied by Cancerous leukocytes and immature WBC flood into blood stream.

Symptoms include,

Fever, weight loss, bone Pain, Frequent infection

Causes,

- Irradiation and drugs to destroy rapid dividing cells have successfully induced remission.

- Bonemarrow (or) Umblical Cord blood transplant are used in selected patients when donos are available.

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LYMPHOMA :

* lymphoma is Cancer of lymphatic System where T and B lymphocytes are getting affected.

* Group of blood cells [Tumor] dereloped from the lymphatic cells.

* Cause of Cancer in children and young adults aged 15 to 24 Years.

Signs and Symptoms :

- Enlarged lymph nodes
- Ferer
- drenching sweats
- weight loss
- Itchiley
- Feeling tired, Fatigue.

Two Catogories :

① Hodgkin lymphoma [HL] eg: EB Virus

② Non - Hodgkin Lymphoma [NHL] eg : AIDS / HIV disease.

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Cancer of lymphocytes consisting of about 25 different subtypes.

Hodgkin lymphoma affects a specific subtype of B-lymphocytes

Non-Hodgkin lymphoma affects other B-lymphocytes (or) T-lymphocytes.

* There are 5 subtype of Hodgkin lymphoma (HL) and 30 subtype of Non-Hodgkin lymphoma [NHL]

- HL is marked by Presence of type of cell called Reed-Sternberg Cell (RS cell)
- RS cells are large cancerous cell.
- HL is one of the curable form of cancer.
- NHL donot have RS Cell.
- Fast growing NHL can be cured
- Slow growing NHL can be cured slowly.

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BLEEDING DISORDER

Anything abnormal happens in 14 clotting factor mechanism results in abnormal bleeding disorder.

* Bleeding disorder occurs due to platelet deficiency (or) deficits of some procoagulants which can result from impaired liver function.

* Blood lacks certain clotting factor.

* Bleeding disorder may occurs when a patient donot have enough platelets (or) clotting factor, Also due to inherited.

1. Thrombocytopenia

Thrombocytopenia is a decrease in the number of Platelets Caused by decreased platelet production.

* Sequestration of platelets in spleen
* Destruction of platelets by immune system

⇒ Decrease in number of platelets causes spontaneous bleeding from small blood vessels all over the body.

⇒ It arises from condition that suppress (or) destroy bone marrow, exposure to ionizing radiation (or) Certain drug.

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2. Impaired liver Function

* When liver unable to synthesize its usual supply of Procoagulants, its results in excess bleeding.
* Vitamin K deficiency causes impaired liver function
* Vitamin - K is required by liver cell for Production of clotting factors
* Vitamin - K deficiency can occur if fat absorption is impaired, because vit. K is a fat soluble vitamin that is absorbed into blood along with fats.
* In liver disease, liver cell fail to Produce bile which is required for fat and Vit - K absorption

3. Hemophilia

* Hemophilia is a type of bleeding disorder due to deficiency of clotting factor responsible for Clotting.

Types of Hemophilia:

Hemophilia A → occurs due to deficiency of clotting Factor VIII [Antihemophilic Factor]

Hemophilia B → due to deficiency of clotting Factor IX [Christmas Factor]

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Hemophilia C → Due to deficiency of Factor XI
[Plasma Thromboplastin] (28)

* Minor tissue trauma causes prolonged bleeding into tissue that is life-threatening

Other results of bleeding disorders such as -

Hemophilia : Symptoms :

- Blood in Urine
- Internal Bleeding
- Blood in Stool

Von willebrand disease Symptoms

- Excessive Bleeding
- Bleeding tendency
- Oral Bleeding

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Hemorrhage :

↳ Bleeding disorder from damaged blood vessel.

→ Different types of it range from minor, such as bruise, to major bleeding in brain.

↳ The common causes of hemorrhage can be blood clotting disorder, cancer, Hemophilia, bone fracture, viral hemorrhagic fever.

↳ The major types of hemorrhage are Subarachnoid hemorrhage, Subconjunctival hemorrhage Subdural hematoma.

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↳ Subarachnoid hemorrhage - A type of stroke that can be caused by head trauma.

↳ Subconjuctival hemorrhage - broken blood vessel in eye.

↳ Subdural hematoma - blood leaking into the duramater, that is the membrane between brain & skull.

↳ The common ~~Sayth~~ symptoms of hemorrhage are Dizziness, Tired, Nauseous, short of breath, weak, shock, seizures.

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it is degraded
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hemosiderin and biliverdin.

→ The hemosiderin accumulates in form of golden braon pigment as more & more rbc's are lysed.

→ since these pigment laden macrophages are seen in settling of heart failure, there are sometimes called as "Heart failure" cells.

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chronic venous congestion:

→ It is a passive process which results due to impaired outflow of venous blood from a tissue.

→ The lungs are heavy and is rusty brown colored on cut section as a result of hemosiderin laden macrophages.

→ Due to fibrosis, the lungs are firm in consistency. The combination of brown color & firmness.

→ The alveolar wall show dilated and congested capillaries & it is markedly thickened due to increase in fibrous connective tissue.

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↳ The alveolar spaces contain numerous hemosiderin laden macrophages which are also referred to as heart failure cells.

↳ The heart failure cells are basically hemosiderin laden macrophages/ siderophages.

↳ Due to passive congestion with dilated capillaries, the rbc's leak into the alveolar spaces and are broken down resulting in release of hemoglobin.

↳ This hemoglobin is phagocytosed by alveolar macrophages where it is degraded to release hemosiderin and biliverdin.

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UNIT III

MICROBIOLOGY

STRUCTURE OF BACTERIA

The cell is the basic structural and functional unit of all known living organisms. It is the smallest unit of life. Cells are mainly of two types: **Prokaryotic cell** (e.g. bacteria, virus) and **eukaryotic cell** (e.g. plant cell and animal cell).

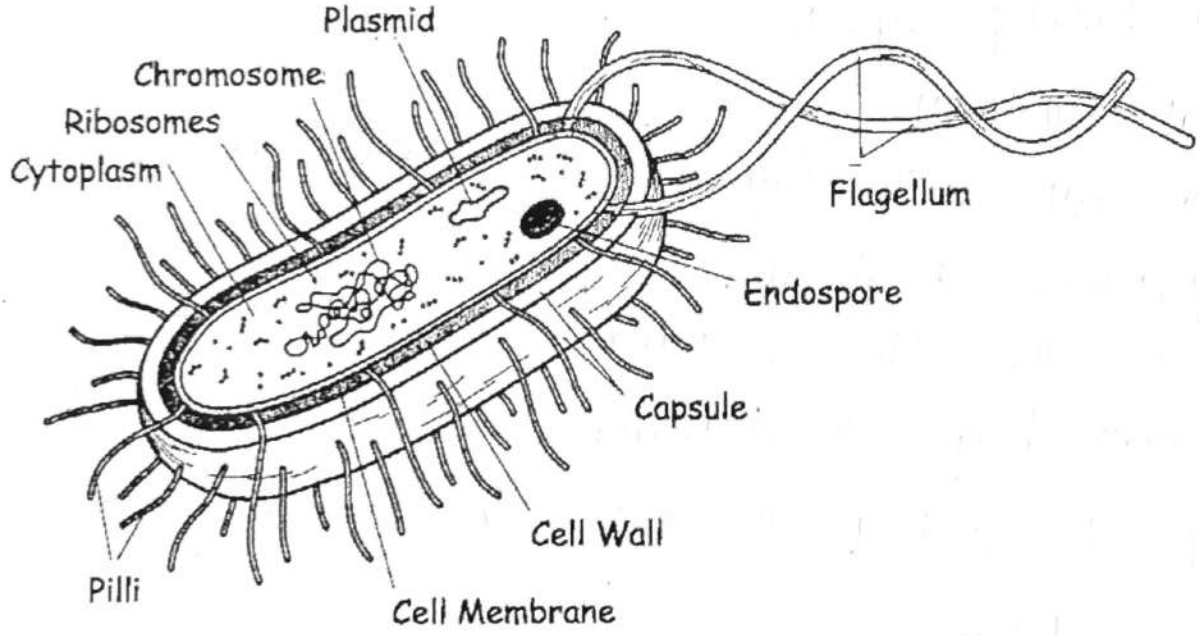
All biological systems have the following characteristics:

(a) The ability to reproduce.

(b) The ability to ingest and metabolize them for energy and growth.

(c) The ability to excrete waste products.

(d) The ability to react to change in their environment.



Morphological features and structural organization of bacteria

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Size of Bacteria - 2 to 4 μm & only 0.25 μm thick (2)

E-Coli is Rod Shaped with average size of 1.1 to 1.5 μm wide by 2 to 6 μm long.

Structure of Bacteria:

Cell Envelop:

Cell membrane seperates cell internal environment from external environment. It is a protective layer of the bacteria. Consist of:

- Cell wall
- Plasma Membrane
- Envelop Capsule

Bacterial Cell Wall

→ Cell wall is the layer, fairly rigid that lies outside the plasma membrane.
→ It is the site of action of several antibodies
→ cell wall helps to determine the shape of the cell.
→ Helps to protect the cell from osmotic lysis and from toxic substances.

Gram Positive Bacteria

Cell wall Consist of single 20 to 80nm thick homogenous layer of Peptidoglycan

Gram Negative Bacteria

It has 2 to 7 nm layer Covered by 7 to 8 nm thick Outer membrane

→ Space between Plasma membrane and Cell wall is Called Periplasmic Space

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→ Gram Staining technique used to diffrentiate Gram Positive and Gram Negative Bacteria based on cell wall Property.

→ Components external to cell wall :

Capsule, Slime layer, S-layer - helps in Protection, attachment of objects and cell movements.

Capsule - Layer is well organized & Not easily washed off

Slime layer - Unorganized zone of diffuse - it easily washed away

Plasma Membrane

→ Encompasses the cytoplasm of both Prokaryotic & Eucaryotic cells

→ Point of Contact with Cell's environment

→ Serves as selectively Permeable barrier, allows Particular ions & molecules to pass

→ Prevent loss of essential Components through leakage

→ Responsible for Crucial metabolic Process, respiration, Photosynthesis and Synthesis of Cellwall Constituents.

Cytoplasmic Matrix :

Cytoplasmic matrix is a substance in which nucleoid, ribosomes & inclusion bodies are suspended. It lacks Organelles bound by lipid bilayer.

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Inclusion Bodies

→ Granules of Organic (or) inorganic material that clearly visible in light Microscope Present in cytoplasm are Called inclusion bodies.

* Used for storage and also reduce osmotic pressure by tying up molecules in particular form.

Two types
- Free inclusion bodies - lie free in cytoplasm
- Enclosed inclusion bodies - Enclosed by shell with 2 to 4 nm thick.

Ribosomes

* Very complex structure made up of both Protein and Ribo-nucleic acid [RNA]
* Site of Protein Synthesis
* Cytoplasmic ribosomes synthesize Proteins destined to remain within the cell.
* Plasma Membrane Ribosomes make Proteins for transport to outside the cell.
* Molecular weight : Approx 2.7 million

Endospore

* Bacteria form a special resistant, dormant structure Called endospore.
* It is resistant to environmental Stress.
* Spores may be central, terminal (or) subterminal.

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Nucleoid

* Procaryotes lack a membrane-delimited Nucleus
* Chromosome located in irregular shape region called Nucleoid.
* Single Circle of double-stranded DNA.
* Nucleoid Composed of 60% DNA
  30% RNA
  10% Protein
* DNA is looped and coiled extensively with help of RNA and Nucleoid Protein.

Flagella - Motility

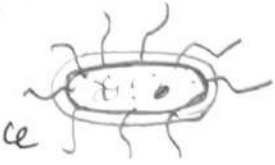
* Flagella are thread-like locomotor appendages extending outward from the Plasma membrane and Cell wall.
* Slender, rigid Structures about 20 nm diameter and 15 to 20 µm long.
* Bacteria differ in their flagella distribution

Monotrichous Bacteria - One Flagella at one end
↳ Meaning: Hair.

Amphitrichous Bacteria - Flagella at each pole of Bacteria

Lophotrichous Bacteria - Have Cluster of flagella at one or both ends

Peritrichous Bacteria - Flagella spread Fairly Evenly over the whole surface



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Structure of Flagella

Composed of three Parts.

(i) Flagellar filament - longest Portion extends from the cell surface to the tip.

(ii) Basal Body - Embedded in the cell

(iii) Flagellar hook - a short, curved segment that links the filament to its basal body and acts as a flexible coupling.

Pili :

Many Bacteria have 1 - 10 Pili per cell. It is hair like structure that diffres from fimbriae.

* Pili are larger than fimbriae (9 to 10 nm diameter)
* Determined by Conjugative Plasmid and required for Conjugation.

Pili and Fimbriae

* Procaryotes have short, fine, hair like appendage that are thinner than flagella - Called fimbriae.
* It can be visible only in Electron Microscope.
* Fibriae + Pili helps in attachment to objects and also required for twitching motility.
* Composed of helically arranged Protein - 3 to 10 nm.

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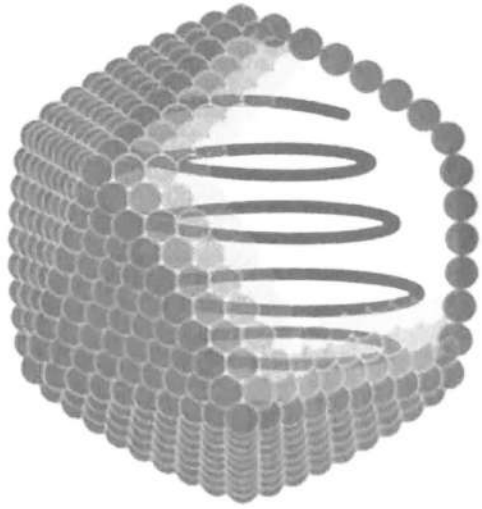
Virus Structure

Viruses come in an amazing variety of shapes and sizes. They are very small and are measured in nanometers, which is one-billionth of a meter. Viruses can range in the size between 20 to 750nm, which is 45,000 times smaller than the width of a human hair. The majority of viruses cannot be seen with a light microscope because the resolution of a light microscope is limited to about 200nm, so a scanning electron microscope is required to view most viruses.

The basic structure of a virus is made up of a genetic information molecule and a protein layer that protects that information molecule. The arrangement of the protein layer and the genetic information comes in a variety of presentations. The core of the virus is made up of nucleic acids, which then make up the genetic information in the form of RNA or DNA. The protein layer that surrounds and protects the nucleic acids is called the capsid. When a single virus is in its complete form and has reached full infectivity outside of the cell, it is known as a virion. A virus structure can be one of the following: icosahedral, enveloped, complex or helical.

Icosahedral

These viruses appear spherical in shape, but a closer look actually reveals they are icosahedral. The icosahedron is made up of equilateral triangles fused together in a spherical shape. This is the most optimal way of forming a closed shell using identical protein sub-units. The genetic material is fully enclosed inside of the capsid. Viruses with icosahedral structures are released into the environment when the cell dies, breaks down and lyses, thus releasing the virions. Examples of viruses with an icosahedral structure are the poliovirus, rhinovirus, and adenovirus.



Icosahedral
Rhinovirus

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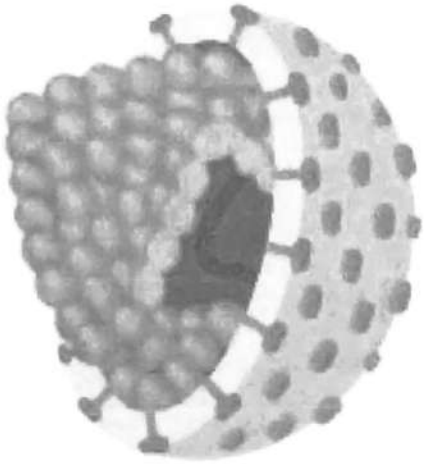

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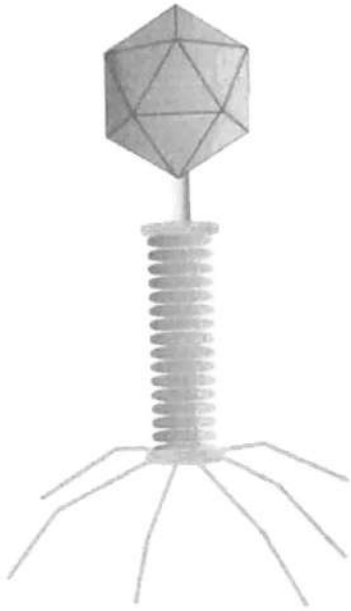
Envelope

This virus structure is a conventional icosahedral or helical structure that is surrounded by a lipid bilayer membrane, meaning the virus is encased or enveloped.

Envelope

The envelope of the virus is formed when the virus is exiting the cell via budding, and the infectivity of theseviruses is mostly dependent on the envelope. The most wellknown examples of enveloped viruses are the **Hepatitis C** influenza virus, Hepatitis C and HIV.



Complex

These virus structures have a combination of icosahedral and helical shape and may have a complex outer wall or head-tail morphology. The head-tail morphology structure is unique to viruses that only infect bacteria and are known as bacteriophages. The head of the virus has an icosahedral shape with a helical shaped tail. The bacteriophage uses its tail to attach to the bacterium, creates a hole in the cell wall, and then inserts its DNA into the cell using the tail as a channel. The Poxvirus is one of the largest viruses in size and has a complex structure with a unique outer wall and capsid. One of the most famous types of poxviruses is the variola virus which causes smallpox.

Complex

Bacteriophage Helical

This virus structure has a capsid with a central cavity or hollow tube that is made by proteins arranged in a circular fashion, creating a disc like shape. The disc shapes are attached helically (like a toy slinky) creating a tube with room for the nucleic acid in the middle. All filamentous viruses are helical in shape. They are usually 15-19nm wide and range in length from

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UNIT - IV

MICROSCOPES

Microbiology :- Study of organisms so small they cannot be seen distinctly with the eye.

Microscopes :-

* Very small Microorganisms are seen with help of Microscopes.

* Variety of Microscopes Available.

- Light Microscope → Bright Field, Dark Field, Phase contrast, Fluorescence

[Maximum resolution - 0.2 μm]

- Electron Microscope → TEM, SEM

[Maximum resolution - 0.5 nm]

Light Microscope :-

* Use glass lenses to bend and focus light rays to produce enlarged images of small objects.

* Maximum resolution of light Microscope is about 0.2 μm.

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* Modern Microscopes all are compound Microscopes.

* Magnification of Light Microscope is limited by its resolving power.

* Resolving power is limited by the wavelength of illuminating beam.

Microscope Resolution :-

* Resolution is the ability of a lens to separate on distinguish between small objects that are close together.

* Resolution is described Mathematically by Ernst Abbe 1870's a german physicist.

* Abbe equation states that the Minimal distance [d] between two objects that reveals them as separate entities depends on the Wavelength of light [λ] used to illuminate the specimen and on the Numerical aperture of the lens [$n \sin\theta$], which is

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ne ability of the lens to gather light.

$$d = \frac{1}{2} \frac{\lambda}{n \sin\theta}$$

* As "d" becomes smaller, resolution increases and finer detail can be discerned in specimen.

* "d" becomes smaller as the wavelength of light used decreases and Numerical aperture increases.

* Greatest resolution with Largest NA and Light of shortest wavelength.

(1) NUMERICAL APERTURE [$n \sin\theta$] of lens is defined by two components:

n - refractive index of Medium in which lens works.

θ - $\frac{1}{2}$ the angle of cone of light entering an objective lens.

(2) NA related to another characteristics of

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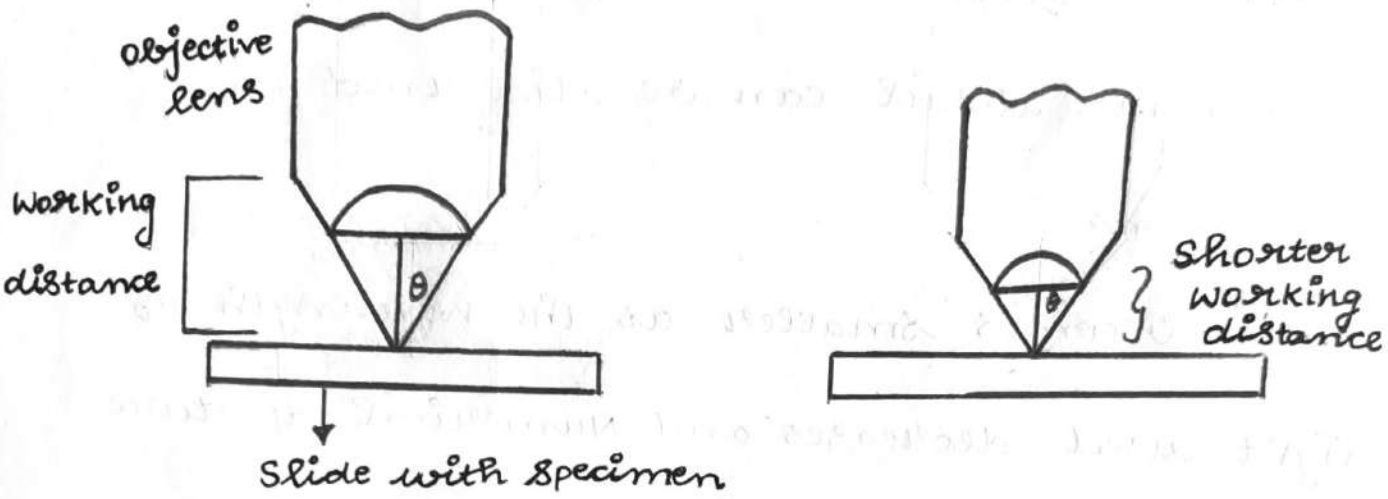
Objective Lens - Working distance.

→ Objectives with large Numerical apertures and great resolving power have short working distances.



→ When angle is narrow and tapers to sharp point - does not spread out much after leaving the slide and do not adequately separate images of closely packed.

Magnification power:-

→ Product of objective lens power and eyepiece lens power

(i.e) 10X X 40X

⇒ 400 X Magnification.

→ If cone of light is wide and spread out

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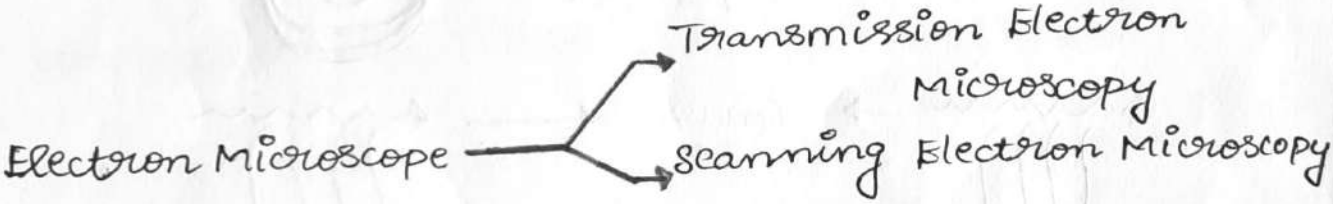
Electron Microscopy :-

→ Light Microscope have a resolution limit of about 0.2 µm.

→ Viruses are too small to be seen with Light Microscope.

→ Detailed internal structures of larger Mo's cannot be effectively studied by Light Microscopy.

→ Electron Microscope have much greater resolution.



→ Electron Microscope use beam of electrons to illuminate and create magnified images of specimens.

→ Electron Microscope have resolution roughly 1,000 times better than Light Microscope.

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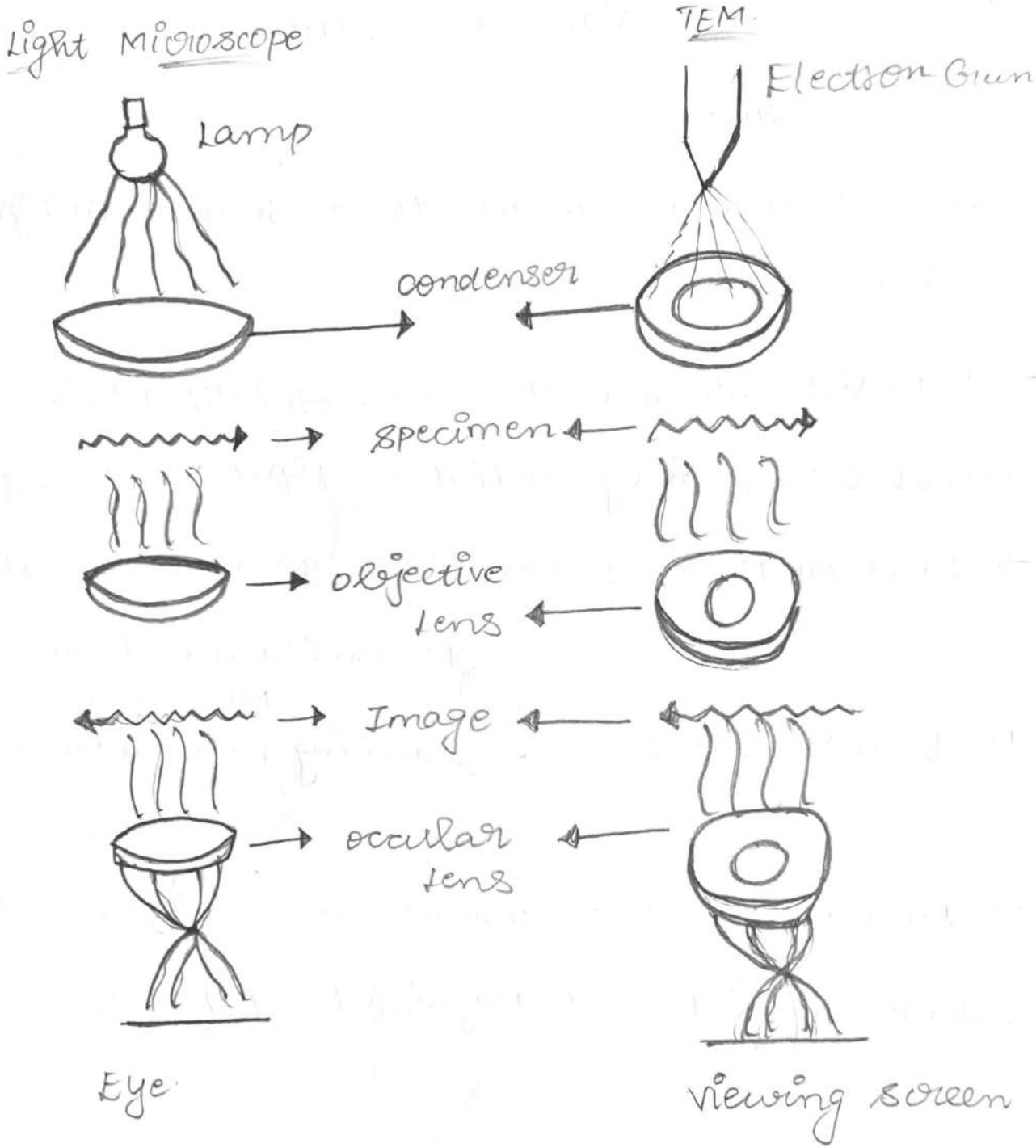
TRANSMISSION ELECTRON MICROSCOPE:-



Principle:-

Electrons transmitted by the specimen are used to form image. Denser specimen - darker Image. Lighter specimen brighter Image.

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Working:-

* Beam of electrons produced from the electron gun is focused on the object by a magnetic condenser.

* whole set up is placed in vaccum to get clear image since electrons are deflected by collision with air Molecules.

* when electron beam strikes the object, it scatters electrons. Those electrons that pass [transmitted] through are used to form enlarged image on fluorescent screen.

* Denser region scatters more electrons and transmit fewer electrons, so that it appears darker in image.

* Thinner region scatters less electrons and transmit More electrons, so that it appears brighter in image.

* Image is recorded on photographic film.

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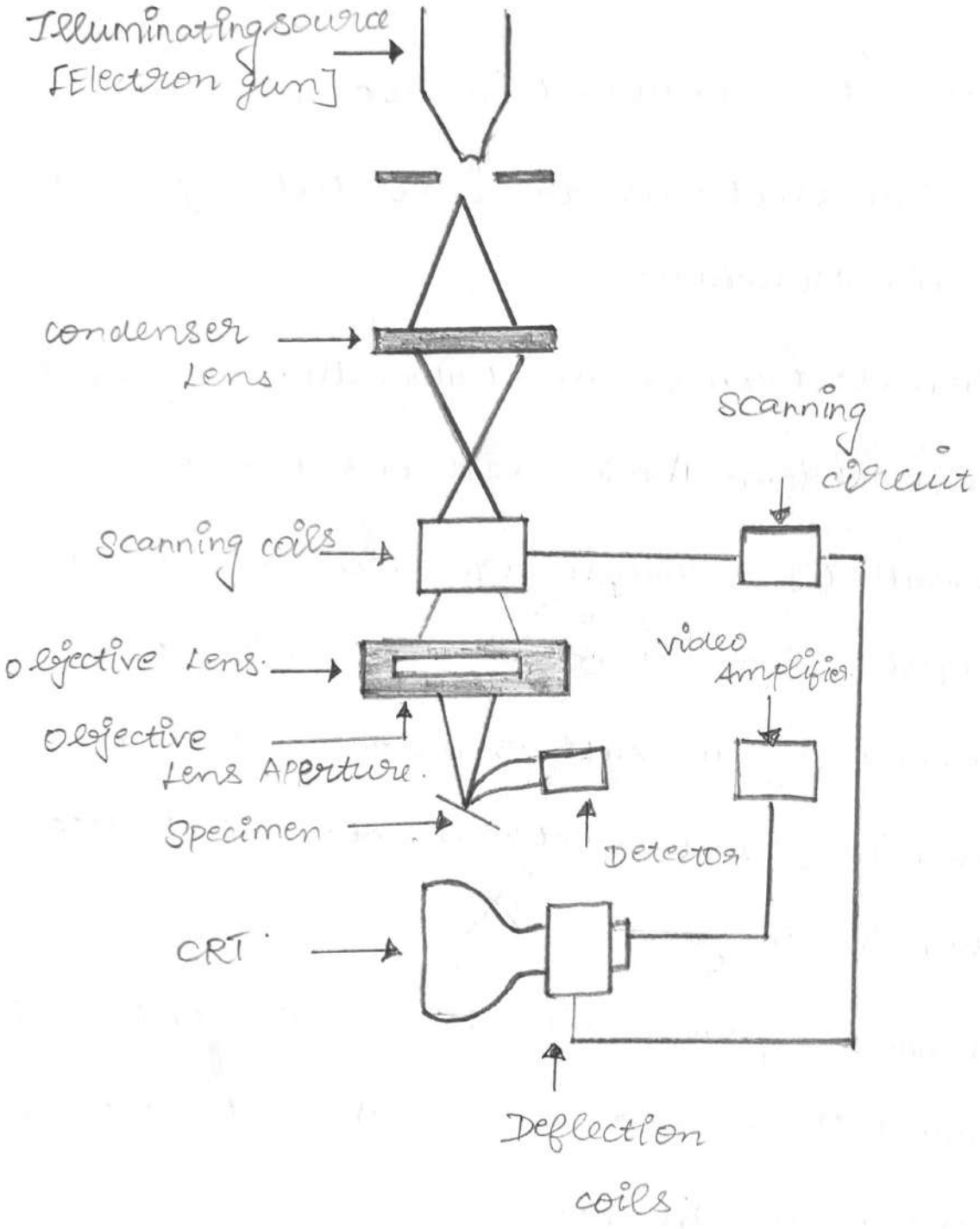
Application:- To examine internal structure microorganism in detail.

SCANNING ELECTRON MICROSCOPY:-



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Principle:- produce an image from electrons released from atoms on an object surface. So that raised area appears lighter and depression appears darker. used to examine surface of Microbes in detail.

Working:-

* Beams of electrons produced from the electron gun is focused on the object by a magnetic condenser.
* Whole set up placed in vaccum to get clear image since electrons are deflected by collision with air Molecules.
* When electron beam strikes the object, surface atom discharge a tiny shower of electrons called secondary electrons and is trapped by special detector.
* Secondary electrons entering the detector strike the scintillator causing it to emit

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Light flashes that a photomultiplier converts it to an electrical current and amplifies.

* cathode ray tube collects it and produces an image.

* No. of electrons produced by specimen depends on nature of sample.

* Raised area produce more secondary electrons and appears lighter.

* Depressed area produce less secondary electrons and appears darker.

Applications:

* Used to examine surface of Microbes in detail.

* Location of Microbes in Human skin, Gut can be examined.

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Innate (Natural) Immunity:

It is the natural resistance components such as intact skin, salivary enzymes, and neutrophils, natural killer cells, which provide an initial response against infection that is present in an individual at birth prior to exposure to a pathogen or antigen

Adaptive (Acquired) Immune System:

It is that which develops antibodies after an attack of an infectious disease or by a pregnant mother passing through the placenta to a fetus or by vaccination.

3. Active Immunity:

It refers to the method of exposing the body to an antigen for generating an adaptive immune response. The response takes days/ weeks to develop but may be long- lasting. For example recovery from Hepatitis-A virus gives a natural active immune response that usually leading lifelong protection. In a similar manner, administration of two doses of Hepatitis-A vaccine generates an acquired active immune response which leading to long lasting defense.

4. Passive Immunity:

It refers to the process of imparting IgG antibodies to keep safe against infection. It gives immediate, but short- lived protection such as several weeks to 3 or 4 months at most. It is occurs during pregnancy. The transfer of maternal tetanus antibody (mainly IgG) across the placenta provides passive immune to newborn baby for several weeks/ months until such antibody is degraded and lost

Naturally acquired active immunity occurs when a person is exposed to a live pathogen, develops the disease, and then develops immunity.

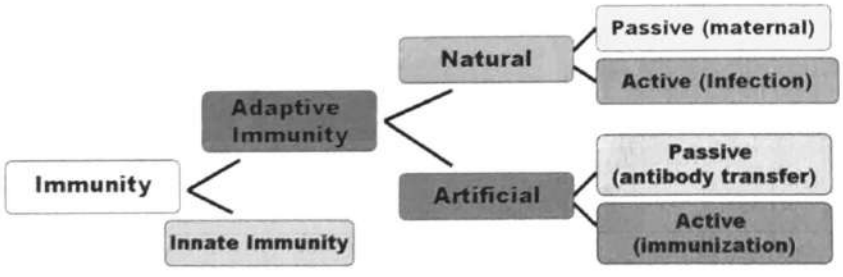
Naturally acquired passive immunity occurs during pregnancy, when antibodies are passed from the maternal blood into the fetal bloodstream.

- Immunity is transferred through the placenta in the form of antibodies, mainly IgG and IgA.
- Natural passive immunity can also be transferred through breast milk.
- Natural passive immunity is short-lived after the birth of the child.

Artificial Immunity

Artificial immunity is a mean by which the body is given immunity to a disease by intentional exposure to small quantities of it.

- The most common form of artificial immunity is classified as active and comes in the form of vaccinations, typically given to children and young adults.
- The passive form of artificial immunity involves introducing an antibody into the system once a person has already been infected with a disease, ultimately relieving the present symptoms of the sickness and preventing re-occurrence.
- Once the body has successfully rid itself of a disease caused by a certain pathogen, a second infection with the same pathogen would prove harmless.



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UNIT-5

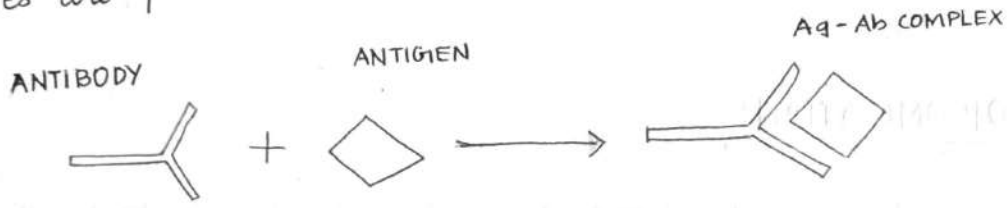
IMMUNOPATHOLOGY

Immunological techniques :

Immunology - study of immune system

Immunological techniques devised by immeunologist for inducing, measuring and characterizing immune responses.

- As a result of reaction of the body to antigen, antibodies are produced.



ANTIGEN - ANTIBODY REACTIONS

→ Ag-Ab reactions are performed to determine the presence of either the antigen or antibody.

→ one of the two components has to be known

Agglutination

→ In this test, antigen is particulate (eg: bacteria, RBC) or inert particle.

→ Ag-Ab cross links to form a lattice network or clumps (agglutination).

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Immuno Diffusion :

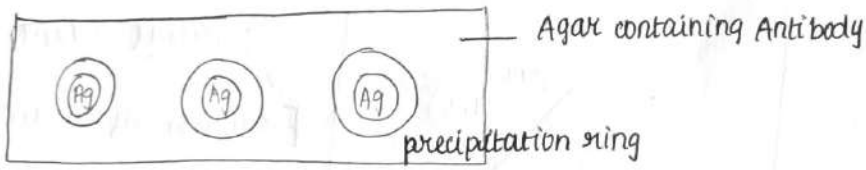
Immuno Diffusion is a diagnostic test which involves diffusion through a substance such as agar.

Types :

Single Radial Diffusion :

* Antibody is incorporated into agar & antigen introduced into the well.

* As antigen diffuses into agar, precipitation rings form depending on the concentration of the antigen.



- Ab in gel
- Ag in a well

Double Immuno diffusion : [ouchterlony Method]

(Both Ag and Ab diffuse from wells)

* Antigen & Antibody are placed in different wells in agar & allowed to diffuse and form precipitation lines at the points of optimal concentrations.

* This method is used to determine whether

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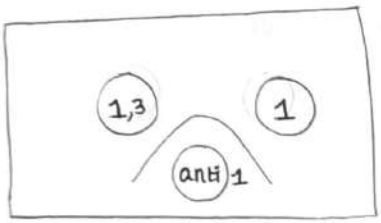
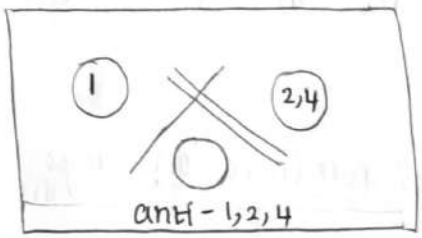
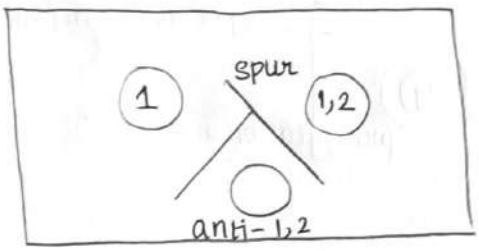
antigens are related, identical or non-identical.



Identity [Fusion of lines at their junction to form an arc]
- presence of common epitope.



Non-identity [crossed lines demostrades 2 separate reactions
- compared Antigens shared no common epitope.



partial identity
[Fusion of 2 lines with spur - Partial identity]

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Immuno electrophoresis:-

→ Some Ag mixtures are too complex to be resolved by simple diffusion and precipitation.

→ Immunoelectrophoresis in which antigens are first separated based on their electrical charge, then visualized by the precipitation reaction.

→ In this, Ag are separated by electrophoresis in an agar gel.

Positively charged proteins move to the negative electrode & negatively charged proteins move to the positive electrode.

* A trough is then cut next to the wells and filled with Antibody.

* Plate is incubated, Ab and Ag will diffuse & form precipitation band or arc.

* This assay is used to separate the major blood proteins in serum for certain diagnostic tests.

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Radio Immuno Assay (RIA)

RIA is an important tool in biomedical research and clinical practice (eg: diagnosis of allergies, blood banking etc.,)

principle:

RIA uses a purified antigen that is radio isotope labeled and competes for antibody with unlabeled standard antigen or test antigen in experimental sample.

Radioactivity associated with antibody is then detected by using radioisotype analyzers and autoradiography.

If there is much antigen in an experimental sample, it will compete with radioisotope-labeled antigen for antigen-binding sites on the antibody, and little radioactivity will be bound.

A large amount of bound radioactivity indicates that there is little antigen present in the experimental sample.

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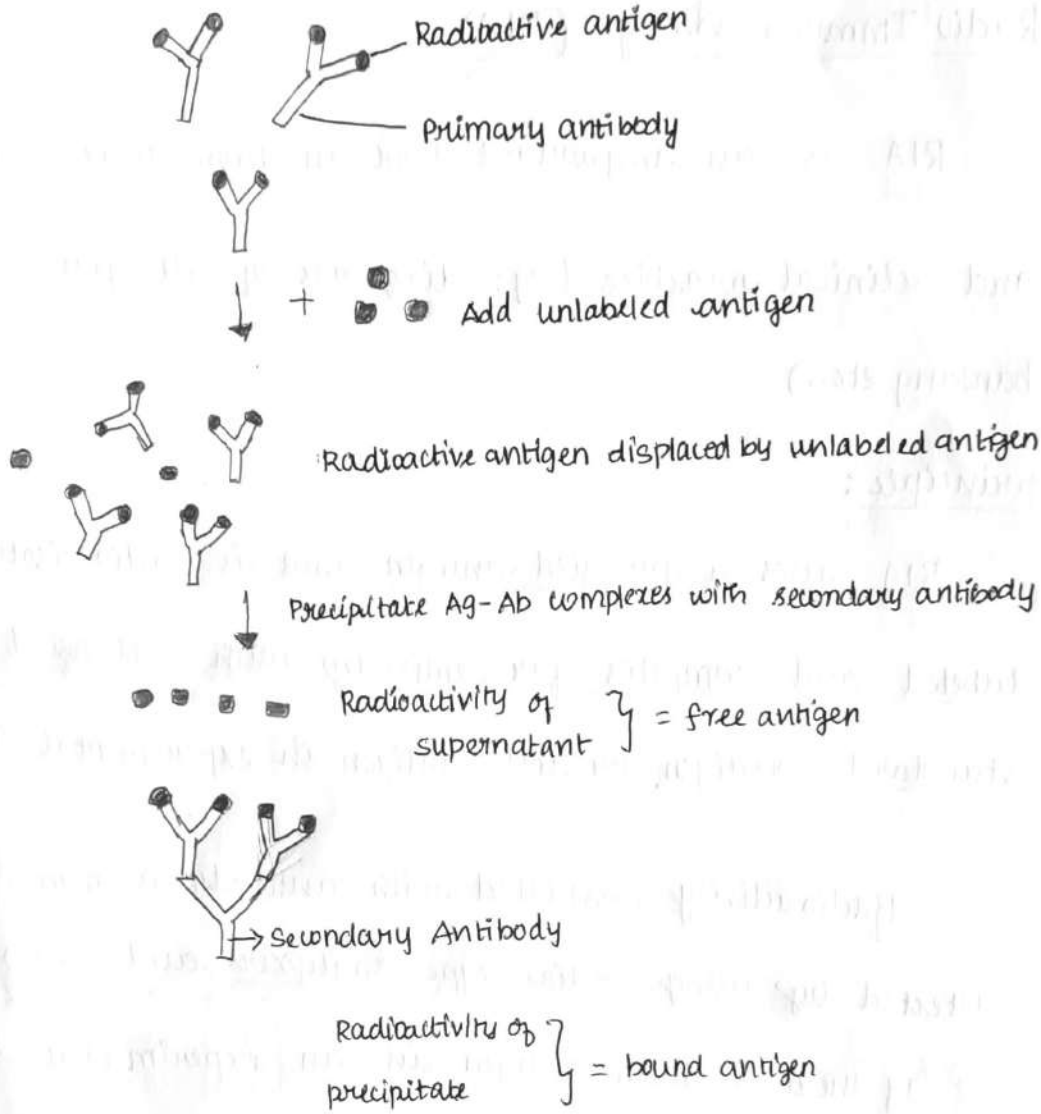

Application:

* used in assay drugs like morphin, digitoxin etc.,
* Analysis of Vitamins like riboflavin, folic acid
* Analysis of hormones like aldosterone, insulin, Growth hormone, thyroxine

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ELISA [Enzyme - linked Immunosorbent Assay]

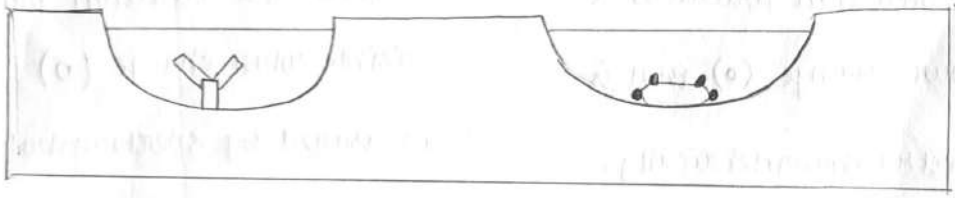
ELISA commonly used in serological test for antigen or antibody detection.

This test enables linking of various "label" enzymes to either antigens or antibodies

Two basic methods:

* Direct Immunosorbent assay
* Indirect Immunosorbent assay

a) Direct Immunosorbent Assay (Ag detection)



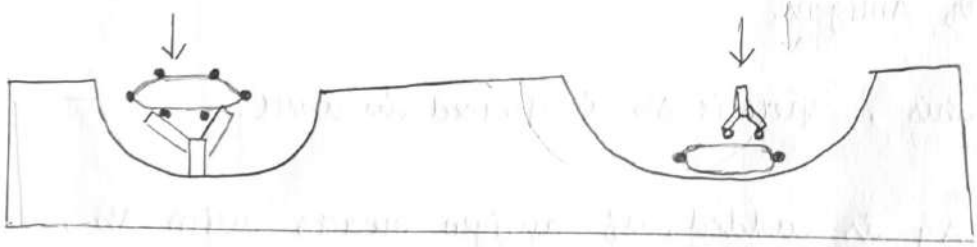
Antibody is absorbed onto the well and sensitizes the plate

Test Antigen is added; if complementary, antigen binds to the antibody

↓ Wash

b) Indirect Immunosorbent Assay (Ab detection)

Antigen is absorbed onto the well and sensitizes the plate.



Test antiserum is added, if antibody is complementary it binds to the Antigen

↓ Wash

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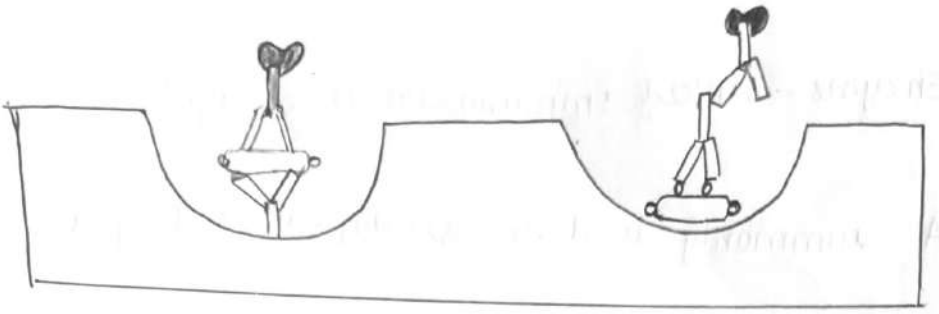
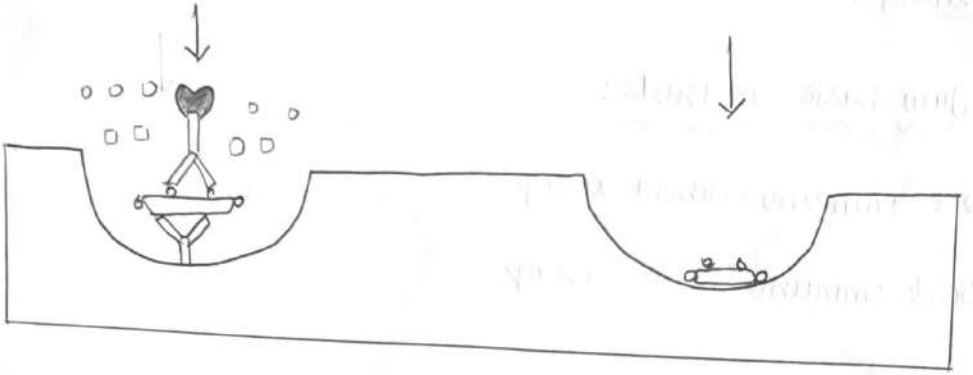


Enzyme - linked antibody Specific for test antigen then binds to antigen, forming a double antibody sandwich.

Enzyme - linked anti-antibody binds to bound antibody



Enzyme's substrate [■] is added and reaction produces a visible colour change (•) that is measured spectrophotometrically.

Enzyme substrate [□] is added and reaction produces visible color change (○) that is measured by spectrophotometry.

* Double Antibody Sandwich assay is used for detection of Antigens.

* In this, specific Ab is placed in well.

* Test Ag is added, if antigen reacts with Ab, Ag retained in well.

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→ Ab labeled with enzyme is added to the well. Eg Ab-Ag-Ab sandwich complex is formed.

→ Substrate is added which is converted to a colored product by the enzyme.

→ Indirect Assay detects antibodies rather than antigens.

Monoclonal Antibody :

* Antibodies used for locating or identifying antigens.
* Monoclonal Ab technology involves hybridizing cancer cells and activated B cells mitro.
* Tumors isolated from multiple myelomas in mice consist of identical plasma cells.
* Monoclonal plasma cells secrete a strikingly pure form of Ab with a single specificity and continue to divide indefinitely.
* Fusion of myeloma cell with normal plasma cell from a mouse spleen to create an immortal cell that secretes a supply of functional Ab with a single specificity.

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* A mouse is inoculated with an antigen having the desired specificity & activated cells are isolated from its spleen. A special strain of mouse provides myeloma cells.

* Two cell populations are mixed with Polyethylene glycol, which cause some cells in the mixture to fuse and form hybridomas.

* surviving cells are cultured and separated into individual wells.

* Test are performed on each hybridoma to determine specificity of the Ab it secretes.

* A hybridoma with the desired specificity is grown in tissue culture; antibody is then isolated and purified.

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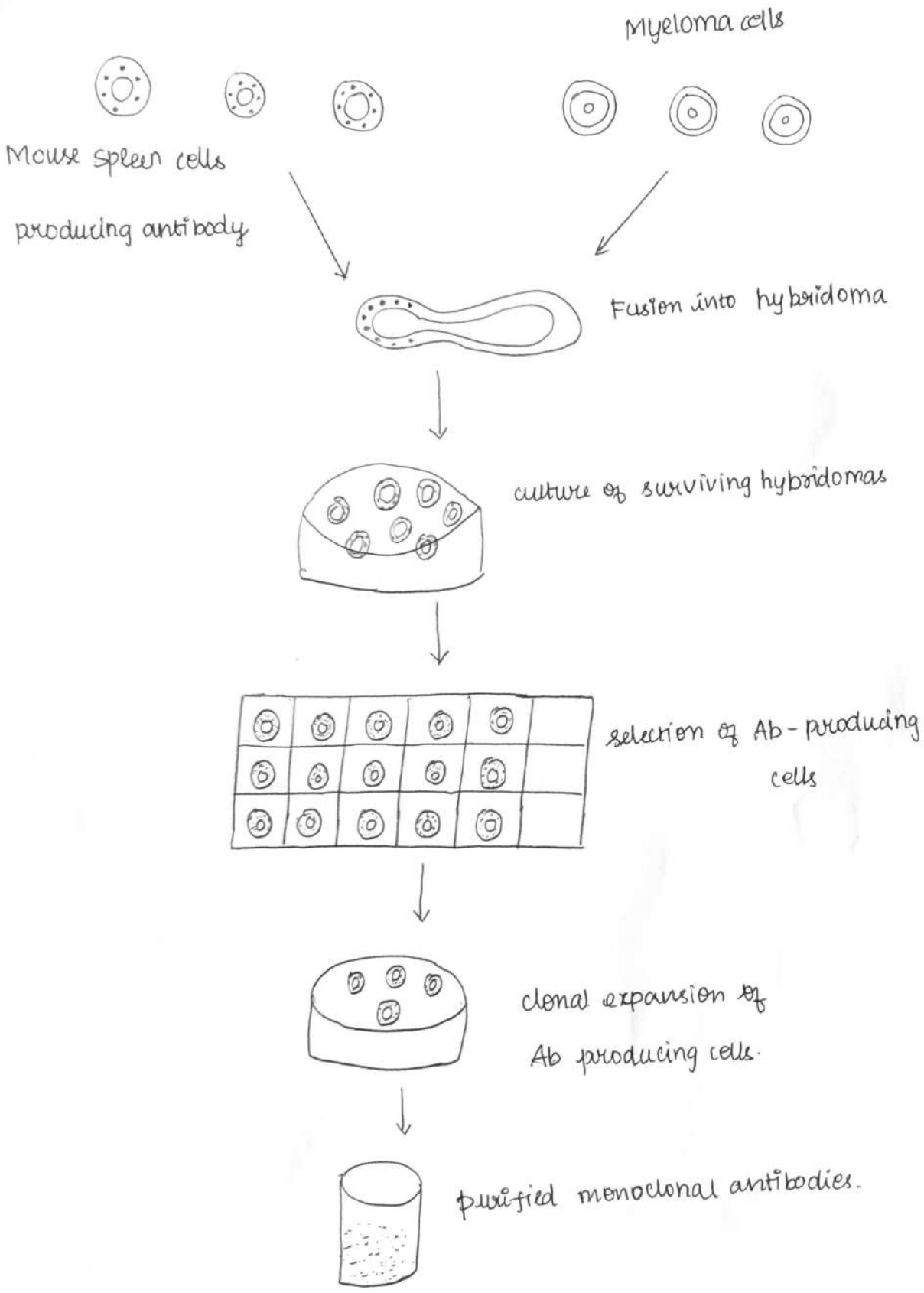

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