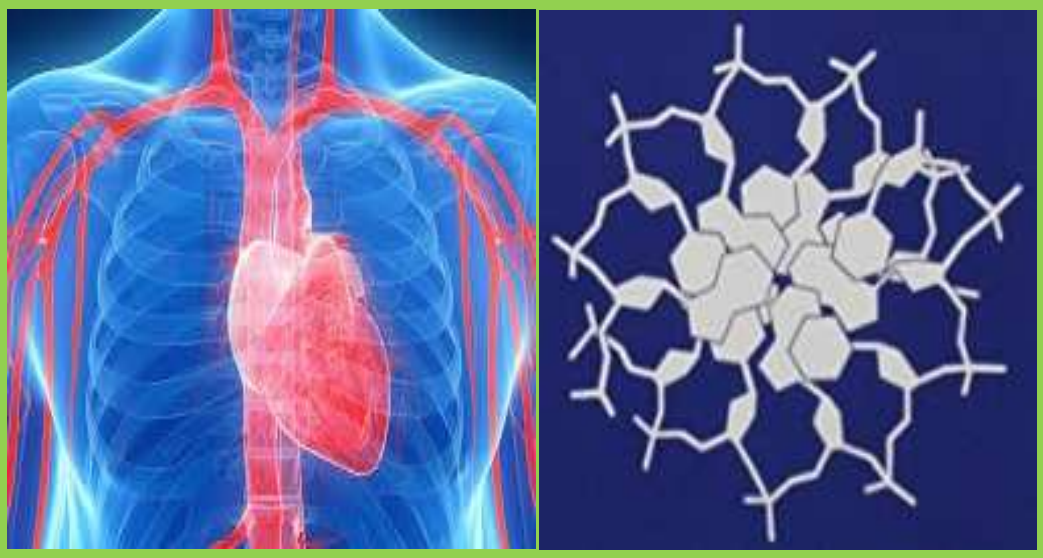
BSCZO- 301

B. Sc. III YEAR

PHYSIOLOGY AND BIOCHEMISTRY



DEPARTMENT OF ZOOLOGY
SCHOOL OF SCIENCES
UTTARAKHAND OPEN UNIVERSITY

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CONTENTS

COURSE 9: PHYSIOLOGY AND BIOCHEMISTRY
COURSE CODE: BSCZO301 **CREDIT: 3**

UNIT 1: DIGESTIVE SYSTEM

CONTENT:

1.1 OBJECTIVES

Study of this unit will let the students to:

- Define Digestive system;
- Explain the general organs of digestive system;
- Write about Digestive enzymes;
- Elucidate the mechanism of digestion and absorption;

1.2 INTRODUCTION

Digestion is a process in which carbohydrates, fats, proteins and minerals are break down into simpler molecules which can be readily used by the animal through absorption and assimilation human diet. Many enzymes secreted by alimentary canal and associated glands help in digestion of food. Once the body has absorbed the required nutrient molecules form food, the rest of the non-utilized component is excreted out. The process of digestion requires precision secretions of many glands and hormones without which it is not possible to harness the energy of the food. Consequent to the feeding habits of the organism, the digestive system and the mechanism has been modified. Overall the purpose of the digestion for all the organisms is to provide energy to body so that it can complete other major physiological functions.

1.3 *DIGESTIVE SYSTEM*

The human digestive system consists of the **gastrointestinal tract** along with accessory organs like **salivary glands**, **pancreas**, **liver** and **gall bladder**. Mouth is followed by a muscular tube called **pharynx** which is continued into another long tube like structure known as food pipe or **oesophagus**. Oesophagus opens up into a relatively large chamber called **stomach**. The opening of oesophagus into the stomach is guarded by a **cardiac sphincter valve**. The **stomach** is followed by **small intestine** which can be further categorized into three parts i.e. **duodenum** (starting part), **jejunum** (middle part) and **ileum** (the last part). Small intestine leads to last

section of alimentary canal called **large intestine**. The initial part of the large intestine is called **colon** and is followed by the **rectum** which has a distal opening called **anus**.

The alimentary canal is made up of four layers from inside known as the **mucosa**, **submucosa**, **muscularis** and the **serosa**. The innermost layer mucosa is made up of single layer of stratified or columnar epithelial cells. The epithelium is followed by submucosa which contains blood and lymph vessels. The muscular is consists of an inner circular and an outer longitudinal layer of muscles. The serosa is the outer most layer which contains blood and lymph vessels and may contain adipose tissues.

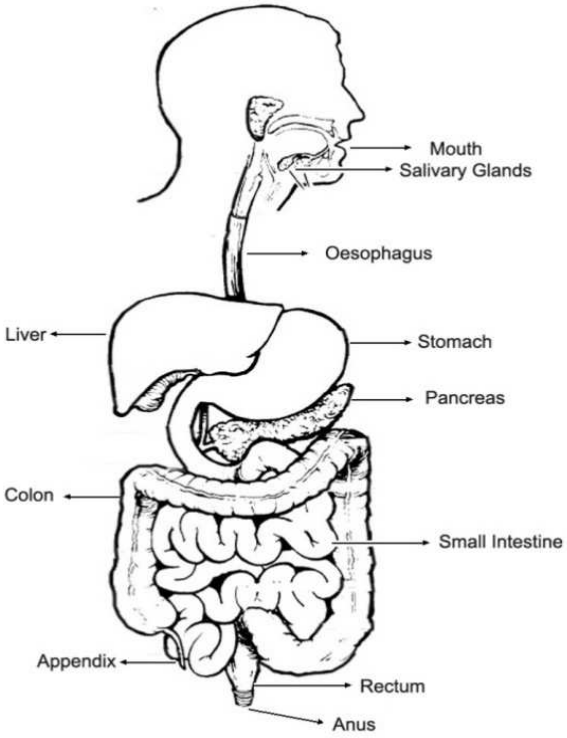

Fig.1.1 Digestive system of Humans

1.3.1 *INTRACELLULAR AND EXTRACELLULAR DIGESTION*

The food is propelled along the digestive tract by muscular movement called **peristalsis** in vertebrates. The main functions of the digestive system include Ingestion of food, b) Digestion of

food, c) Secretion of digestive juices and enzymes, d) facilitating mixing of food and digestive juices, e) absorption of the water, salts and nutrients and finally f) excretion or expulsion of undigested remains.

Intracellular Digestion:

Intracellular digestion is the breakdown of substances within the cytoplasm of a cell. The complex nutrients taken up by cell are further broken down in the different locations inside cell. This mode of digestion is typical of lower animals like protozoans and sponges. However it is also employed by the coelenterates which have both extracellular as well as intracellular digestion. Glucose for example is taken up for oxidative breakdown releasing energy in form of ATP. Many platyhelminths and *limulus* are also considered as examples of animals in which occurrence of intracellular digestion represents as primitive condition.

Extracellular digestion:

'Extra' means outside, hence extracellular digestion occurs outside the cell. During extracellular digestion, food is broken down outside the cell either mechanically or with the help of enzymes. Cells which are in near vicinity absorb the broken down nutrients. Our teeth grind the food, enzymes and acid in the stomach liquefy it, and additional enzymes in the small intestine break the food down into parts our cells can use. The main components of extracellular digestion are salivary, gastric, pancreatic and intestinal enzymes. All of these are secreted directly into the digestive cavity.

1.3.2 *SALIVARY SECRETIONS*

The digestion of food starts as soon it enters the mouth. The first enzyme it encounters comes from salivary secretions. Saliva is secreted by **submaxillary** or **submandibular** glands situated under jaw bone, **sublingual** glands situated under tongue and **parotid** glands found in front of internal opening of ears. Saliva has a pH of about 6.6 and is nearly 99.5 % water. The enzymes that are found in salivary secretions include **amylase**, **acid phosphatase**, **aldolase** and **cholinesterase**. Salivary amylase is the most important of these which helps in conversion of starch to glucose and maltose. Salivary amylase occurs as the mixture of the closely related

isoenzymes, each with a molecular weight of about 56 KDa. About 1-1.5 litres of saliva is secreted every day in an average male adult. Salivary secretions are controlled by neural activities.

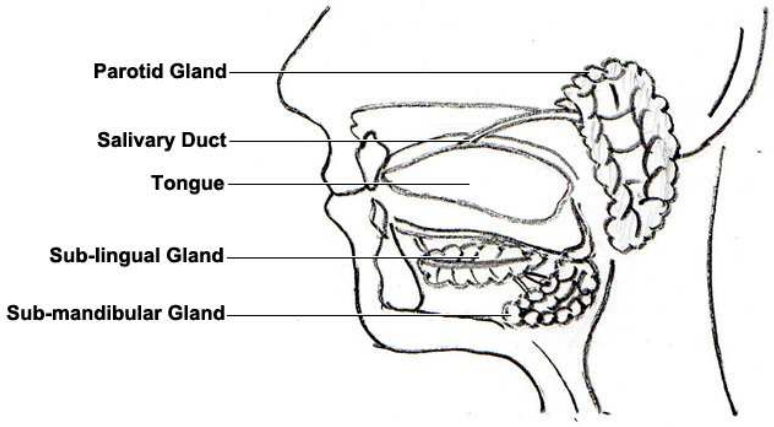

Fig. 1.2 Image showing the position of all the glands presents in mouth that aid in salivary secretions

1.3.3 *GASTRIC SECRETIONS*

Stomach secretes a large amount (more than 2 litres/day) of gastric juices. As soon as the food enters the stomach from mouth, secretion starts. Even if the stomach is empty, small amount of gastric juices continue to secrete. In the human digestive system, a bolus (a small rounded mass of chewed up food) enters the stomach through the oesophagus. **Proteases** (protein-digesting enzymes such as pepsin) and **hydrochloric acid (HCl) released by stomach** either kills or inhibits bacteria and also provides the acidic pH of two for the proper functioning of proteases. Food is churned by the stomach through muscular contractions of the wall called peristalsis – reducing the volume and the boluses are converted into **chyme** (partially digested food). The stomach take about few hours to completely digest the food into chyme depending upoin the contents of meal. Gastric juice is highly acidic and is secreted by three different cells types present in stomach lining which are: 1) **Parietal cells** (also known as oxyntic cells), which secrete hydrochloric acid, 2) **Mucus cells**, which produce mucus and 3) **Chief cells** which produce pepsin and other digestive enzymes. The HCl secreted has a pH of about 1 and strength

of about 0.10 N. The other secretions of the gastric cells increase the pH of the gastric juice to about 2.0. **Gastrin hormone** is released by G cells in the antrum of the stomach in response to a specific neurotransmitter released from the enteric nerve endings, known as gastrin releasing peptide and also in response to the presence of oligopeptides in the gastric lumen. Parietal and chief cells can also be stimulated by acetylcholine, released from enteric nerve ending in the **fundus**.

Food is subjected to both muscular movements and chemical reactions in the stomach. Gastric juices have three major enzymes **pepsin**, **rennin** and **gastric lipase** in them. Pepsin is secreted as zymogen pepsinogen and rennin as prorennin. Pepsinogen is activated by HCl. Pepsin is a proteolytic enzymes with a molecular weight of about 35 kDa. The pH optimum of pepsin is about 2 and thus works best in gastric environment. Rennin acts specifically on milk proteins and helps in their digestion. Gastric lipase helps in the breaking down of the emulsified fats.

1.4 INTESTINAL *DIGESTION*

After the stomach food enters the small intestine and further digestion and absorption takes place here. Intestinal digestion is aided by secretions of pancreas, liver and gall bladder.

1.4.1 *PANCREATIC SECRETION*

The pancreas has a dual role to play in the body. It acts as an endocrine gland that produces several important hormones including **insulin**, **glucagon**, **somatostatin**, and **pancreatic polypeptide.** It also acts as a digestive organ that secretes pancreatic juice important for absorption of nutrients and digestion in the small intestine. The pancreas is situated between the duodenum (small intestine) and stomach and is connected via a pancreatic duct to the bile duct coming from the liver forming a common bile duct which opens up in duodenum. Pancreatic juice contains about 99% water, 0.5% inorganic salts and 0.5% organic matter. Pancreas secretes about 1-1.5 liters of juice every day. Pancreatic fluid has high pH because of the presence of bicarbonate ions that neutralize the gastric acid and allow effective enzymatic action.

Pancreatic secretions are regulated by the hormones **secretin** and **cholecystokinin**. It is produced by the walls of the duodenum upon detection of acid food, proteins, fats, and vitamins. HCl

present in the acidic chyme from stomach stimulates the duodenal mucosa to produce secretin. Secretin is absorbed into the blood stream and activates pancreas to produce pancreatic juice containing salt and water but no enzyme. Another hormone cholecystokinin or pancreozymin produced also by intestinal mucosa stimulates the secretion of pancreatic juice supplemented with enzymes. Major enzymes produced are **trypsin**, **chymotrypsin**, **carboxypetidase**, **Elastase**, **amylase** and **lipases**. Most of these are secreted in the form of inactivated precursors or zymogens which is a strategy employed by the cells to prevent self digestion of cells. As soon as these precursors are secreted they are activated either by self digestion or by the action of other enzyme. Pancreas also produces some additional enzymes like **maltase**, **ribonuclease** and **esterase**. The detailed action of these enzymes is given in Table 1.

1.4.2 *BILE JUICES*

Bile juice is secreted by liver and concentrated and stored in gall bladder. It is collected by hepatic ducts which join together to form a **common hepatic duct**. It joins the duct from gall bladder to form the cystic duct and opens up in intestine as bile duct. The opening of the bile duct in small intestine is guarded by a **Sphincter of Oddi**.

Adult humans produce about 400-800 mL of bile daily which is approximately 97 % water and 3 % salts. The salts are composed mainly of sodium salts of glycocholic and taurocholic acid, bile pigments (bilirubin), mucin, cholesterol, lipids and inorganic salts. Bile is dark green to yellowish brown in color. It is controlled by the action of the hormone cholecystokinin which is released by the mucosa of the small intestine. Cholecystokinin then enters the blood stream and causes the simultaneous contraction and relaxation of sphincter of Oddi which results in secretion of bile into duodenum. Bile salts help in emulsification and absorption of fats. Apart from the digestive role, bile also helps in excretion of bilirubin, a byproduct of red blood cells recycled by the liver.

The composition of human liver bile is as follows:

Water	97.0%
Bile Salts	0.7 %

Inorganic Salts	0.7 %
Bile Pigments	0.2 %
Phosphatidylcholine	0.2 %
Cholesterol	0.06 %
Fatty Acid	0.15 %
Alkaline phosphatase	0.1 %

Bile salts emulsify fat into small particles so that enzymes could act upon them. The bile not only assists in digestion of the fats but also in the absorption of the digested fats by intestinal villi. They speed up the absorption by increasing the permeability of fatty acids in the absorbing epithelium. In the absence of bile the absorption of the fat-digested products is reduced drastically. The bile pigments **biliverdin** and **bilirubin** are derived from the degradation of hemoglobin in liver cells and give bile its color.

1.4.3 *DIGESTION IN SMALL INTESTINE*

Most of the enzymes that act in the small intestine are secreted by the pancreas and liver and enter the small intestine via the pancreatic duct. Pancreatic enzymes and bile from the gallbladder enter the small intestine in response to the hormone cholecystokinin, which is produced in the small intestine in response to the presence of food. Small intestine produces another hormone called **Secretin** which promotes the pancreas to release bicarbonate into the duodenum in order to neutralize the potentially harmful acid coming from the stomach.

The three major classes of nutrients that undergo digestion are proteins, lipids (fats) and carbohydrates:

Proteins are broken down into small peptides and amino acids before absorption. Chemical breakdown begins in the stomach and continues in the small intestine. Proteolytic enzymes, including **trypsin** and **chymotrypsin**, are secreted by the pancreas and cleave proteins into

smaller peptides. **Carboxypeptidase** proteolytically removes amino acids from C-teminal end of protein. **Aminopeptidase** and **dipeptidase** generate free amino acids.

Lipids (fats) are degraded into fatty acids and glycerol. **Pancreatic lipase** breaks down triglycerides into free fatty acids and monoglycerides. Pancreatic lipase works with the help of the salts from the bile secreted by the liver and stored in the gall bladder. Bile salts bind and emulsify triglycerides making it easier for pancreatic lipase to act on them. The lipase is water-soluble while the fatty triglycerides are hydrophobic. They tend to aggregate with each other to avoid the water present in intestinal surroundings. The bile salts emulsify the triglycerides and lipase break them into the smaller components that are able to enter the villi for absorption.

Few carbohydrates are broken down into simple sugars, or monosaccharides (e.g., glucose). Pancreatic amylase breaks down some carbohydrates (notably starch) into oligosaccharides. Other carbohydrates pass undigested into the large intestine and are further digested by intestinal microflora. Brush border enzymes take over from there. The most important brush border enzymes are **dextrinase** and **glucoamylase**, which further break down oligosaccharides. Other brush border enzymes are **maltase**, **sucrase** and **lactase**. Lactase is absent in some adult humans and, for them, lactose, like most polysaccharides, is not digested in the small intestine. Some carbohydrates, such as cellulose, are not digested at all, despite being made of multiple glucose units. This is because the cellulose is made out of beta-glucose, making the inter-monosaccharidal bindings different from the ones present in starch, which consists of alpha-glucose. Humans lack the enzyme for splitting the beta-glucose-bonds, an action that is reserved for herbivores and bacteria from the large intestine.

1.4.4 *DIGESTION AND ABSORPTION IN LARGE INTESTINE*

The large intestine or colon is shorter in length than small intestine. It harbors some bacteria which secrete enzymes for the digestion of the plant cellulose. This process is important in herbivorous animals. Most of the food that enters the large intestine is already digested and the nutrient depleted. A the food makes its way through the large intestine, some remaining nutrient molecules are absorbed along with water that further concentrates the material to be excreted. Undigested materials are eliminated from the lower part of the intestine in the form

of feces. The characteristic brown color of the feces is due to the excretion of bile pigments from the liver.

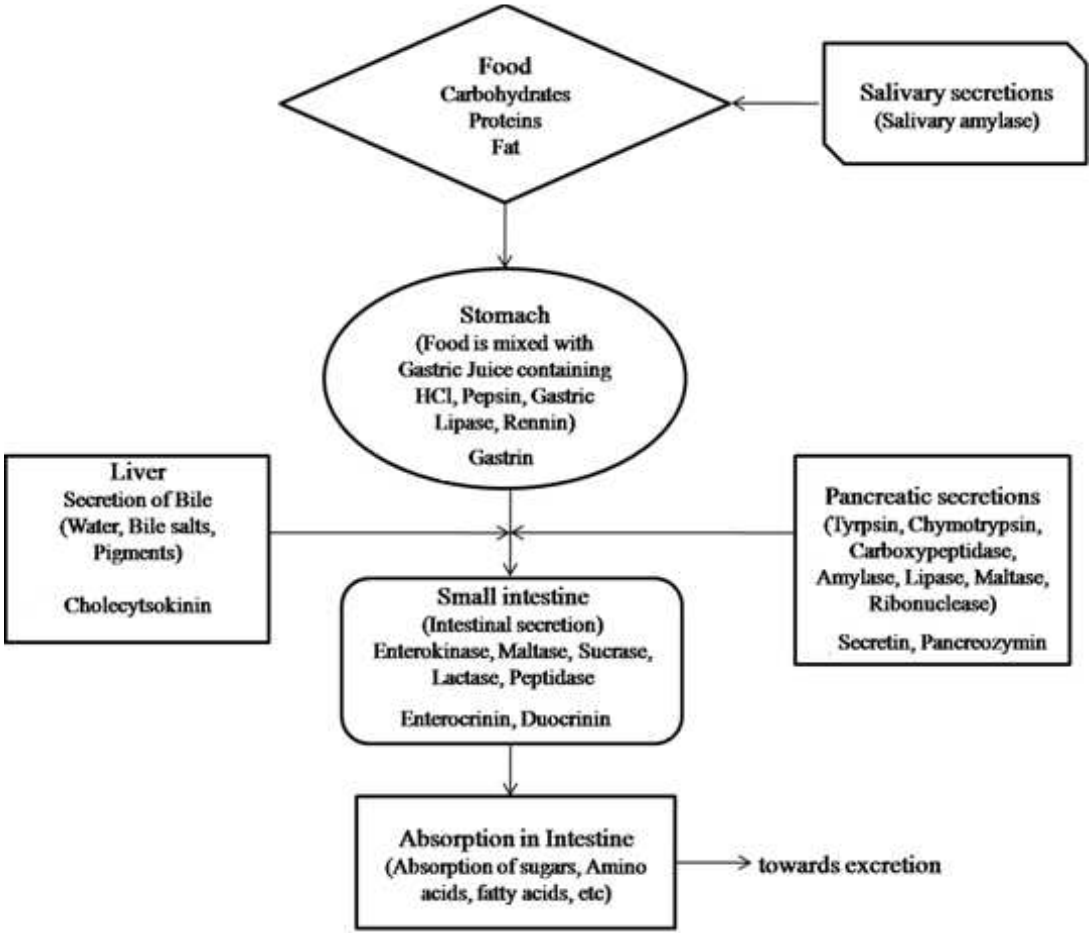

Fig. 1.3 Overview of digestion process

1.4.5 *INTESTINAL MICROFLORA*

The human large intestine contains a rich amount of microflora consisting of many hundreds of different types of bacteria. They have a diverse physiology and biochemical characters and exist in a different microhabitats like the lumen of the large gut, the mucin layer and on mucosal surfaces. (Cummings, J.H. and Macfarlane G.T., 1997). Their primary function is to reclaim energy from carbohydrates not digested in the upper digestive tract. This is achieved through fermentation and absorption of short chain fatty acids, which represent 40-50% of the available

energy of the carbohydrate. Intestinal bacteria also aids in vitamins B and K synthesis as well as the metabolism of bile acids and other sterols (Kong F. and Singh R.P., 2008).

1.5 DIGESTION *AND ABSORPTION*

1.5.1 *DIGESTION AND ABSORPTION OF CARBOHYDRATE.*

Digestion of carbohydrates implies breaking down of large complex molecules into the monosaccharides by the process of hydrolysis. One molecule of a disaccharide when hydrolyzed with one molecule of water in the presence of enzyme gives following reaction:

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{sucrase} \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6}$$

When polysaccharides are hydrolyzed they break up into many simple sugars. Out of the common diet carbohydrates like starch, glycogen, lactose, sucrose, maltose, glucose etc., glucose is directly absorbed in the blood. Cellulose remains undigested as humans do not have necessary enzymes for its breakdown.

The chemical digestion or hydrolysis of carbohydrates occurs in the presence of different enzymes called **glycosidases**. Digestion of carbohydrates starts in the mouth itself where salivary amylase converts the starch into maltose. When the chyme reached stomach the partially digested starch is hydrolyzed by the action of gastric HCl. Starch is completely digested further in intestine with the help of pancreatic and intestinal amylases.

Disaccharides like sucrose, lactose, maltose are digested in intestine by the action of enzymes **invertase, β-glucosidase** and **α-galactosidase** respectively converting sucrose to glucose and fructose, lactose to glucose and galactose and maltose to glucose. Glucose thus makes a fairly large portion of the carbohydrates available for absorption followed by galactose and fructose.

Absorption of the digested carbohydrates takes place through intestinal villi either via diffusion or active absorption. Fructose for example is taken up by the simple diffusion along a

concentration gradient by the cells of villi. While glucose and galactose needs to be actively transported coupled with ATP hydrolysis. Glucose of absorption is immediately transported throughout the body fluids while fructose and galactose are taken to liver for conversion to glucose and then transported to other body parts. The concentration of glucose in body is about 90 mg/100 ml of blood. If excess glucose is taken up in diet, most of it is transported to muscle and liver for storage as glycogen aided by **insulin** hormone. When blood glucose levels are decreased the glycogen is converted back to glucose by hormone **glucagon** and supplied to blood. In extreme fasting or starving conditions when the reserve glycogen stores are also depleted and there is shortfall of glucose, proteins and fats can also be used to generate glucose by a process called as **gluconeogenesis**. This helps in maintaining the minimum level of the glucose in blood and proper functioning of brain.

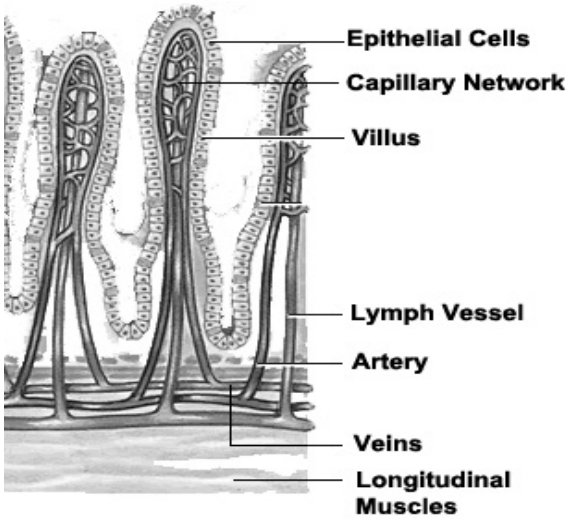

Fig.1. 4. Microscopic structure of Intestinal villi showing the internal organization of absorptive cells and blood capillaries

1.5.2 DIGESTION AND ABSORPTION OF FAT

Digestion of fats or lipids is a hydrolytic process, similar to carbohydrates. Complex lipid molecules are hydrolyzed into fatty acids, glycerol and glyceride in presence of enzyme lipase secreted by stomach, pancreas and intestine. Only a small portion of the fats is digested in stomach with help of gastric lipase. Majority of the fat digestion takes place in small intestine.

When fat enters the small intestine, it stimulates the release of the gastrointestinal hormone cholecystokinin. Cholecystokinin stimulates lipase secretion by the pancreas and contraction of gall bladder to release bile into the small intestine. Bile salts emulsify the fat into small globules called micelles. Formation of these small globules or micelles increases the surface area of action of intestinal pancreatic lipase which converts them into their end products. Only about 25 - 60 % of the fat is completely digested into fatty acids and glycerol.

$$\underset{\text{Stearin}}{C_3H_5O_3\,(C_{17}H_{35}CO)_3} + 3H_2O \xrightarrow{lipase} \underset{\text{Glycerol}}{C_3H_5(OH)_3} + \underset{\text{Stearic acid}}{3HC_{18}H_{35}O_2}$$

The mechanism of the fat absorption is still not fully understood. Some of short chain fatty acid molecules are believed to be directly absorbed by the absorptive cells of the small intestine. Rest of the fatty acids, in the form of micelles is absorbed through the crypts between the microvilli of the intestinal absorptive cells. From the crypts the micelles of the size ranging from 0.5 – 1.0 μ in diameter are transferred to cytoplasm of cell by the process of **pinocytosis**. Inside the cells the long chain fatty acids recombine with glycerides to form triglycerides which are in the form of small globules called **chylomicrons**. Chylomicrons are finally transferred to lymphatic vessels known as lacteals. Here they absorb proteins on their surface and becomes **chyle** which prevents their adhesion to each other.

Recent experiments have shown that 25-60% of the fat appears to be fully hydrolyzed to free fatty acids before absorption. The greater part of the remaining fat seems to be absorbed as monoglycerides. Bile salts play a crucial role as also discussed above. Water insoluble fatty acids and monoglycerols are solublized within the micelles formed by bile emulsification. They also contain lipid soluble vitamins (A, D, E and K) along with cholesterol. Since the concentration of these constituents is larger in micelles than in aqueous solutions they increase the rate of the delivery of fatty acids to intestinal absorptive surface. The fatty acids, monoglycerols and fat soluble vitamins leave the micelles and passively diffuse along the membrane of the epithelial cells along their concentration gradient. The digestion products of the medium chain triacylglycerols (C8 to C12) are water soluble and therefore, don't require micelles for solublization. They are absorbed directly by the intestine.

1.5.3 *DIGESTION AND ABSORPTION OF PROTEIN*

Proteins are hydrolyzed to their constituent amino acids during digestion with the help of many proteolytic enzymes. The digestion of the protein by a proteolytic enzyme occurs by the hydrolysis of peptide bonds and the protein is digested according to the following sequence:

Protein → proteoses → peptones → polypeptides → amino acids

Digestion of dietary proteins starts with the entry of food into the stomach where enzyme **pepsin** is present. **Pepsin** is secreted as **Pepsinogen** which is its inactive form or zymogen by chief cells of stomach. It is auto-catalytically converted into **pepsin** due to acidic environment in stomach. **Pepsin** acts upon the peptide bonds adjacent to the aromatic amino acids and breaks the protein into oligopeptides and polypeptides. Milk present in diet is coagulated by a specialized gastric enzyme **rennin** which is present in high quantities in infants. The coagulated casein protein of milk is then hydrolyzed by **pepsin**.

After stomach the partially hydrolyzed proteins reach the small intestine where they are further processed by the enzymes **trypsin** and **chymotrypsin** which are secreted by pancreas. **Trypsin** is secreted as **trypsinogen**, a zymogen which needs to be activated by the action of another enzyme **enterokinase**. **Trypsin** recognizes and cleaves the peptide bonds adjacent to carboxyl terminal of argine and lysine (Cantarrow and Schepartz, 1967). pH optimum for trypsin is 7.0-9.0. **Chymotrypsin** is also secreted as **chymotrypsinogen** and is activated at pH 7.5 – 9.0 in presence of **trypsin**. **Chymotrypsin** cleaves the peptide bonds adjacent to aromatic amino acids. **Carboxypeptidases** are another class of the enzymes which hydrolyzes (cleaves) a peptide bond at the carboxy-terminal (C-terminal) end of a protein or peptide and releases the free amino acids.

In the complete hydrolysis of a polypeptide, only amide bonds (-CO-NH-) are broken. A protein molecule is first cleaved by endopeptidases (e.g. pepsin, chymotrypsin and trypsin) into smaller fragments from the ends of which amino acids are hydrolyzed by exopeptidases (e.g. carboxypeptidases etc.).

In addition to the dietary proteins, lots of cellular proteins are also pushed into alimentary canal for digestion. These are metabolic products or intermediates produced during various cellular pathways and need to be recycled.

The free amino acids generated during gastric and intestinal digestion are absorbed both by diffusion and active transport. The major portion is absorbed by the absorptive cells of the small intestine from where they enter the blood and move to liver. The movement of digested protein products is aided by four different transport systems. First is the transport system for neutral amino acids like alanine, glycine, Serine, threonine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, aspartate, histidine, cysteine, methionine and citrulline. Second is transport system for dibasic amino acids e.g. Lysine, arginine, ornithine and cysteine. There seems to exist an alternate pathway for dibasic amino acids transport which carries all of the above mentioned amino acids except cysteine. Third transport system carries aspartic acid and glutamic acid. Fourth system is specific for glycine, proline and hydroxyproline.

Some of the amino acids are moved along by coupling to Na^+ ion symport into the mucosal cells against their concentration gradient. Besides amino acids, oligopeptides can also be transported across the intestinal epithelial cells by a Na^+ symport. Large polypeptides which remain unhydrolyzed in intestine are excreted from body. A sufficient amount of the proteins is maintained by the body in blood for metabolic pathways and cellular processes. Amino acids which are not utilized are then marked for degradation to form nitrogenous waste.

Table 1.1: Summary of Enzymes produced by organs of digestive system and their action on different food materials.

Sr. No.	Name of the Gland	Enzyme secreted	Place of action	Substrate	End Products
1.	Salivary Gland	Salivary Amylase	Buccal cavity	Starch	Maltose
2.	Gastric Gland	i) Pepsin ii) Rennin iii) Gastric Lipase	Stomach	Proteins Caseinogen Fat	Peptides Casein Glycerol & Fatty Acids
3.	Pancreas	i) Trypsin ii) Chymotrypsin iii) Carboxypeptidase iv) Amylase v) Pancreatic Lipase vi) Maltase vii) Ribonuclease	Small Intestine	Proteins Peptides Starch Fats maltose RNA	Peptides Free Amino acids Maltose Glycerol & fatty acids Glucose Nucleotides
4.	Small Intestine	i) Enteropeptidase ii) Maltase iii) Sucrase iv) Lactase v) Lipase	Small Intestine	Peptides Sucrose Lactose Fats	Free amino acids Glucose Galactose Fructose Glycerol & fatty acids

1.6 REGULATION *OF ENZYME ACTION*

All the enzymes that are secreted during digestion are under the control of various hormones. These hormones are secreted in response to nerve stimuli which can originate in the head, stomach, or small intestine. Based on these sites, there are three phases of digestive regulation:

1. **The cephalic phase** consists of those stimuli that originate from the head: sight, smell, taste, or thoughts of food, as well as emotional states. In response to these stimuli the following reflexes are initiated: **Neural response**: Stimuli that arouse digestion are relayed to the hypothalamus, which in turn initiates nerve impulses in the parasympathetic vagus nerve. These impulses activate enteric nervous system of the GI tract, which promote contraction of smooth muscle causing peristalsis and secretion of gastric juice. Stimuli that limit digestion (feeling of fear, anger or anxiety) activates sympathetic nerves that suppress muscle contraction and secretion of the enzymes. In response to the cephalic phase stomach prepares for the digestion of proteins.

2. **The gastric phase** comprises of stimuli that originate from the stomach. These stimuli include distention of the stomach (which activates stretch receptors), low acidity (high pH), and the presence of peptides. In response to this phase gastric juice secretion and smooth muscle contraction are promoted. Secretion of Gastrin hormone is increased which prepares stomach and small intestine for the digestion of chyme.

3. **The intestinal phase** carries stimuli which originate in the small intestine. These include enlargement (distension) of the duodenum, high acidity (low pH), and the presence of chyme (especially fatty acids and carbohydrates). The inhibition of gastric secretion and gastric motility(enterogastric reflex) follows. As a result, intestinal secretions, smooth muscle contraction, and bile and pancreatic juice production are increased. Stomach emptying is retarded to allow adequate time for digestion (especially fats) in the small intestine. Intestinal digestion and motility are promoted.

1.7 SUMMARY

Digestion is a step wise process which is crucial for the survival of any organism. It is a conversion of solid complex food in to the absorbable simpler molecules and is competed at different location in alimentary canal. Digestion starts with mouth with the secretion of salivary amylase that digests carbohydrates during chewing. As the food is ingested through oesophagus to the stomach a very low pH environment greets the food which is maintained due to the secretion of HCl. Bile salts are mixed at this stage and the emulsification of the fats takes place.

Further progression of the food into small intestine triggers the release of bicarbonate that neutralizes the acidic chyme and prepares the food for further enzymic action. Enzymes like pepsin, trypsin and chymotryspin are here released for efficient breakdown of the proteins.
Liver, pancreas, gall balder etc play an important role in digestion and absorption of the nutrients. The internal cell lining of stomach and small intestine allows the efficient absorption of the digested material.

1.8 *GLOSSARY*

Digestive system: The combination of organs and various secretions which aids in conversion of complex food materials into simpler absorbable nutrients.

Digestion: Processing of food to smaller and simpler molecules.

Intracellular digestion: Breakdown of food within the cytoplasm of a cell.

Extracellular digestion: Digestion which occurs outside the cell aided by several secretions by different organs.

Saliva: Secretion of glands situated in mouth cavity. Contains salivary amylase.

Chyme: Partially digested food.

Zymogen: Inactivated precursor of gastric and intestinal enzymes.

Pancreas: Glandular organs situated near intestine. It secretes several important hormones including insulin, glucagon, somatostatin, pancreatic polypeptide and enzymes like trypsin, chymotrypsin, carboxypetidase, Elastase, amylase and pancreatic lipases.

Bile: Secreted by liver and stored in gall bladder. Helps in emulsification of fats and lipids and facilitate their digestion.

Bilirubin: Yellowish pigment found in bile; produced by degradation of hemoglobin.

Insulin: Hormone secreted by pancreas; regulates the glucose level in blood.

Glucagon: Peptide hormone secreted by pancreas; converts glycogen to glucose.

Gluconeogenesis: Metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as lactate, glycerol, and proteins.

1.9 SELF *ASSESSMENT QUESTION*

Multiple Choice questions:

1 . In humans the digestion is:

a) Intracellular b) Extracelluar

c) Both intra as well as extracellular d) None of these

2. Which of the following enzyme helps in digestion of milk?

a) Pepsin b) Trypsin c) Rennin d) Lipase

3. Which of the following is stimulated by cholecystokinin?

a) Secretion of HCl b) Secretion of mucous

c) Secretion of bicarbonate ion d) Release of bile

4. Bile is secreted by:

a) Salivary glands b) Gall Bladder c) Liver d) Stomach

5. The digestive enzymes of the small intestine

a) Are all secreted by pancreas b) Do not function best at low pH

c) Are Produced and released under neural control d) Are all activated by an acidic environment.

6. Round spheres of fat molecules generated after emulsification with bile are called:

a) Micelles b) Bolus c) Droplets d) None of these

7. Bilirubin is:

a) Enzyme b) Hormone c) Degradation product of hemoglobin
d) All of the above

8. Conversion of glycogen to glucose is under the control of which hormone:
a) Insulin b) Glucagon c) Gastrin d) None of the above

9. Pepsin is secreted by:
a) Stomach b) Small Intestine c) Pancreas d) Liver

10. Enzyme that cleaves the peptide bonds adjacent to carboxyl terminal of argine and lysine is:
a) Pepsin b) Trypsin c) Chymotryspin d) Rennin

11. Which of the following has the highest pH?
a) Saliva b) gastric juice c) pancreatic Juice d) Bile Juice

12. Digestive organs protect themselves from self digestion by:
a) secreting digestive enzymes as inactivated precursors
b) lining the digestive tract with mucous
c) Both a) and b)
d) None of the above.

13. Most of the digestive enzymes are:
a) Hydrolases b) Transferases c) Mutases d) Oxidoreducatses

14. In small intestine bicarbonate is released to neutralize the acidic chyme from stomach. It is secreted in response to which hormone:
a) Gastrin b) Insulin c) Glucagon d) Secretin

15. Most of the absorption of digested food takes place in:
a) Mouth b) Stomach c) Small Intestine d) Large Intestine

M.C.Q:- ANSWERS

1. (c) 2. (c) 3. (d) 4. (c) 5. (b) 6. (a) 7. (c) 8. (b) 9. (a) 10.(b) 11. (c) 12. (c) 13. (a) 14 (d) 15. (c)

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1.11 SUGGESTED *READINGS*

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1.12 TERMINAL *QUESTIONS/ANSWER*

1.12.1 *VERY SHORT ANSWER TYPE QUESTIONS*

1. Where is sub-lingual gland located?
2. Which enzyme is secreted by salivary glands for carbohydrate digestion?
3. Entry of food from oesophagus to stomach is guarded by which valve?
4. From where the HCl is secreted in stomach?
5. What is the pH of chyme in stomach?

6. Name the four layers that make up alimentary canal?
7. What is the main source of bilirubin and biliverdin?
8. Which enzyme converts Trypsinogen to trypsin?
9. Which is the specialized enzyme for digestion of milk in infants?
10. What is the range of pH if intestinal juices?

Answers:

1. Under the tongue
2. Salivary Amylase
3. Cardiac Sphincter valve
4. Parietal cells
5. ~ 2.0
6. Mucosa, Submucosa, Muscularis and the Serosa.
7. Degradation of hemoglobin
8. Enterokinase (Enteropeptidase)
9. Rennin
10. pH 7.0 – 8.5

1.12.2 *SHORT ANSWER TYPE QUESTIONS*

1. Define Digestion?
2. Differentiate between intracellular and extracellular digestion?

3. Mention the organs of Digestive system?
4. What are the functions of the saliva?
5. What is the composition of gastric juices?
6. Name all the enzymes secreted by pancreas?
7. What is the composition of Bile? What is its function?
8. Discuss the regulation of intestinal secretions?
9. Name all the hormones involved in digestion?
10. How does insulin maintain sugar level in blood?

1.12.3 *LONG ANSWER TYPE QUESTIONS*

1. Discuss the movement of food starting from mouth and the changes it undergo?
2. Discuss the role of pancreas in the digestion of proteins?
3. Discuss the process of digestion and absorption of fats?
4. Explain the mechanism of protein digestion?
5. Discuss the mechanism of carbohydrate digestion?
6. Explain in detail the regulation of hormones involved in digestion?
7. Explain the structure of intestinal villi? Discuss the absorption of digested food in small intestine?
8. Discuss the formation and role of Bile in digestion of lipids?
9. Explain zymogens? Discuss the activation and function of various zymogens involved in digestion?
10. Write a detailed essay on Digestive glands and their importance?

UNIT 2: RESPIRATORY SYSTEM

CONTENTS

2.1 *OBJECTIVE*

Study of this unit will let the students to:

i. Explain Respiration
ii. Describe organs of respiratory system and respiratory pigments
iii. Understand the mechanism of Gaseous transport
iv. Explain the Gaseous exchange
v. Understand the effect of pH, temperature etc. on respiration

2.2 *INTRODUCTION*

Respiration is essential for the survival of the organisms. It is an involuntary process that is common to almost all the organisms. Although the mode of the respiration can be different for different groups of organisms depending on their habitat and other factors, it remains the central process for sustaining the life. All life forms have optimized their way of respiration and have evolved as such. They have the specialized organs to achieve that. In mammals the inhaled oxygen helps in the oxidation of the digested food products and thus in turn helps in production of energy for the other metabolic processes. It is a co-ordinated effort of many organ systems of the body which work tirelessly to maintain the required level of the oxygen in the body. Our bodies have been evolved in such a way that they need oxygen (O_2) for cellular oxidation and in turn release carbon dioxide (CO_2). Any interference in this routine is not good for the body and may prove fatal.

2.3 *BASIC CONCEPT & INTRODUCTION OF RESPIRATORY SYSTEM*

Respiration in simplest of definitions means taking up oxygen and giving back carbon-dioxide in exchange. Animal cells require oxygen from the environment to produce energy that drives their metabolism. Oxygen is utilized for oxidation of food or carbon and hydrogen in cells.

Respiratory organs in humans:

The respiratory system consists of **respiratory passage, lungs and associated organs**. The respiratory passage consists of **nostrils (external nares), nasal chambers, internal nares, pharynx, epiglottis, larynx (Voice box), trachea** or **wind pipe**, and two **bronchi**. Air enters through the external nares or nostrils and enters into pharynx through internal nares. Larynx is a cartilaginous tissue that is situated between pharynx and trachea. It is called sound box as it is associated with voice production. Epiglottis is part of larynx that serves as lid for closing of larynx during swallowing of food. Larynx is followed by the trachea which is a tubular structure about 4.5 inches long and a diameter of about 1 inch. It further extends through the neck region into the thorax where it divides into two branches called the right and the left bronchi. The trachea is provided with a series of 15-20 C-shaped cartilage rings which prevent the trachea from collapsing. The right and the left bronchi enter the respective lungs and further subdivide into smaller tubes. Right lung has three primary lobes while left has two, thus the right bronchus divides initially into three while left bronchus divides into two main bronchial branches. The bronchial tubes have the same cartilaginous rings as that of trachea.

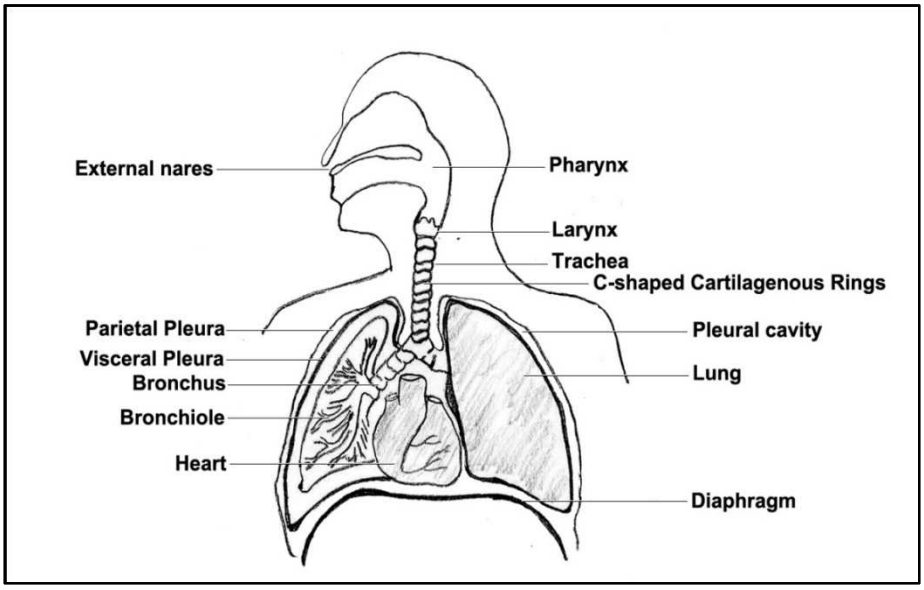

Fig. 2.1. Respiratory System of Humans

Lungs: The bronchioles further divide and subdivide and terminate into a balloon like air sac called alveoli. Alveoli are the physiological unit of the lungs. There are numerous (about 7.5 x 10^8) alveoli in human lungs which makes the total surface area of about 100 square meters. The respiratory tract is lined by the ciliated columnar epithelial cells. The cilia beat synchronously propelling mucus and foreign bodies outwards for expulsion through trachea. The walls of the pulmonary or lung alveoli on the other hand are simply composed of a single layer of cuboidal non-ciliated cells. The alveoli are surrounded by an extensive network of capillaries. These capillaries are fed blood by pulmonary artery. The exchange of the gases takes place in the alveoli.

Each lung consists of its bronchial tree with many air sacs and alveoli unit together associated with blood vessels, nerves and pleura. All of these structures are associated supported and attached to each other by the connective tissue. They are covered externally by the visceral pleura and surrounded by the similar layer called parietal pleura forming a thin lining of the wall of the chest. Pleural cavity is filled with small amount of pleural fluid which prevents the friction of the lungs during breathing movements. The diaphragm is a dome shaped structure that forms the floor of the chest cavity and separates it from abdomen.

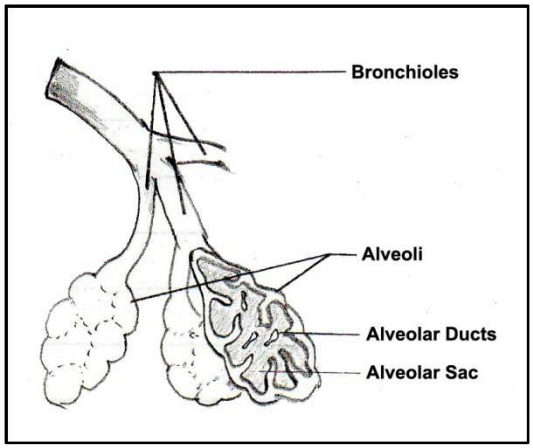

Fig. 2.2. Structural organization of Alveoli

2.4 *DEFINITION & TYPES OF RESPIRATION*

Respiration can also be defined as a catabolic process in which the respired oxygen is used in the oxidation of food resulting in the release of energy. Respiration thus includes the exchange of the gases in the lungs. Lungs only serve as the point of gaseous exchange and the actual oxidation occurs at the cellular level. Respiration can be of two types*:* ***external respiration*** that includes exchange of gases (oxygen and CO_2) in lungs and ***internal respiration*** consisting of energy yielding oxidative reactions inside cells. Respiration is completed by different mechanisms in different organisms. For example in invertebrates like protozoa and porifera oxygen is directly obtained by diffusion from surrounding water. In annelids, gaseous exchange is done thorough a moist skin.

Respiration in vertebrates is carried out by three modes:

Direct surfaces like skin are used as respiratory organs by most amphibians. Some salamanders use skin and highly vascular pharyngeal region while some fishes like eel absorb oxygen through skin.

Gills are choice of respiratory organs of many aquatic vertebrates like fishes.

Lungs are used mainly by land vertebrates. The complexity of the lungs increases from amphibians to higher mammals. As the size of the lings increase the available surface for gaseous exchange also increase and make them more efficient.

2.4.1 *BREATHING MECHANISM*

Breathing is a step wise process where first step is inhalation or inspiration and second is exhalation or expiration. Inspiration is the intake of air in which the ribs are elevated and the diaphragm contracted and flattened, the chest cavity is enlarged. This increases the volume of the chest cavity and lungs and results in the dropping of air pressure inside lungs below atmospheric pressure. The negative pressure created forces the air to enter lungs to equalize the pressure.

In expiration, the ribs and the diaphragm return to the original position, the chest cavity volume is decreased. The lungs contract and force the air out, which is then exhaled.

In inspiration, the expansion of the thorax, supported by the movement of diaphragm, decreases the intrathoracic pressure from -4 to -10 mm Hg resulting in entry of air into lungs. In this process the lungs are extended passively in response to the increase in thoracic cavity volume. In expiration as the volume of thorax decreases, the intrathoracic pressure is raised to -2 mm Hg, lungs contract to accommodate in decreased space and air is forced out.

Diaphragm is the main muscle for the inspiration because it s movement leads to the intake or expulsion of the air from lungs. If the diaphragm descends 10 mm, it increases the volume of thoracic cavity by about 250 ml. As it relaxes and returns to original position, passive expiration occurs. This contraction and relaxation of the diaphragm is controlled by the phrenic nerves arising in the neck from the third, fourth and fifth cervical nerves and passing down through the thorax to the diaphragm. External intercostal muscles help the ribs in upward movement during inspiration.

2.4.2 *PULMONARY VENTILATION*

The maximum amount of air that can be expired after a forceful maximum inspiration is called the vital capacity. Normal rate of breathing is sufficient to properly oxygenate the blood and remove carbon dioxide. If the breathing is insufficient to maintain normal pressure of the gases in blood, the person is said to be hypoventillating. If breathing is excessive so that the blood P_{CO2} is lowered, the person is said to be hyperventilating.

Some of the terms used to describe the respiration are ***Eupnea*** or normal respiration, ***Hypernea*** refers to increase in the respiratory rate and depth, ***Dyspnea*** refers to irregularities of respiration and **apnea** means cessation or stopping of respiration. The normal rate of respiration in adults is 14 breathes per minute. There is about 2500 ml air present in lungs at any given time. Each successive breathe adds about 350 ml of fresh air. The quantity of new air that enters the lungs per minute is known as the **minute respiratory volume**.

Tidal volume is the volume of the air inspired or expired with each normal breath. It is about 500 ml. About 150 ml of this air remains in tubular passages like trachea, bronchi and bronchioles where no gaseous exchange occurs. **The inspiratory reserve volume** is the

maximum extra volume of air that can be inspired over and above the normal tidal volume. It is about 3000 ml in adult human male.

The expiratory reserve volume is the extra volume of air that can be expired by forceful expiration after the end of a normal tidal expiration. It is about 1100 ml. **The residual volume** is the volume of the air that remains in lungs even after most forceful expiration. This volume is about 1200 ml.

Alveolar ventilation:

The gaseous exchange in the lungs includes the alveoli, alveolar sacs, alveolar ducts and respiratory bronchioles. The rate at which new air reaches these areas per minute is called **alveolar ventilation.**

It can be calculated by using following formula:

$$V_A = f\,(V_T - V_D);$$

V_A is the volume of alveolar ventilation per minute, f is the frequency of respiration per minute, V_T is the tidal volume and V_D is the physiological dead space.

With a normal tidal volume of 500 ml, a dead space of about 150 ml and the respiratory rate of 12 breaths per minute, the alveolar ventilation will be 4200 ml per minute. This is the major factor to determine the concentration of oxygen and carbon dioxide in the alveoli.

2.4.3 *RESPIRATORY PIGMENTS*

A respiratory pigment is a molecule that increases the oxygen carrying capacity of the blood or in other words they are the substances which combine with oxygen reversibly and acts as the carrier or storage units for it. These pigments are generally carried by the blood. Their oxygen affinity determines the efficiency of these pigments and their use as the oxygen carrier. The oxygen affinity of these pigments is indicated by their *p*50 or partial pressure of oxygen at which they are half saturated with oxygen. The value of p50 is inversely proportional to the oxygen affinity of the pigment. Some of the common respiratory pigments are as follows:

i) Hemoglobin

Hemoglobin is the iron containing oxygen transport metalloprotein found in red blood cells (RBC) of all vertebrates as well as tissues of some invertebrates. With some very rare exception of leptocephalus (larvae of eel), all classes of vertebrates possess hemoglobin in their RBCs and their muscle contain myoglobin.

Structure: Hemoglobin is a conjugated protein made up of four subunits. Each subunit is made up of a protein part called globin and an iron containing protoporphyrin (heme) ring. Heme attaches to the polypeptide chain by a nitrogen atom to form one subunit of hemoglobin. Each of the four heme portions of the hemoglobin molecule contains an atom of Iron (Fe) which binds oxygen. The iron in the heme is in ferrous state (Fe^{2+}). The protein moiety or globin varies considerably in size, amino acid composition, solubility and other physical properties from animal to animal. The iron content of mammalian hemoglobin is 0.336 % and the heme content is 4 %.

In each unit of heme, the iron atom is joined by four of its co-ordination bonds to the four nitrogen atoms of protoporphyrin and one of the remaining six co-ordination bond is joined to the molecule of globin.

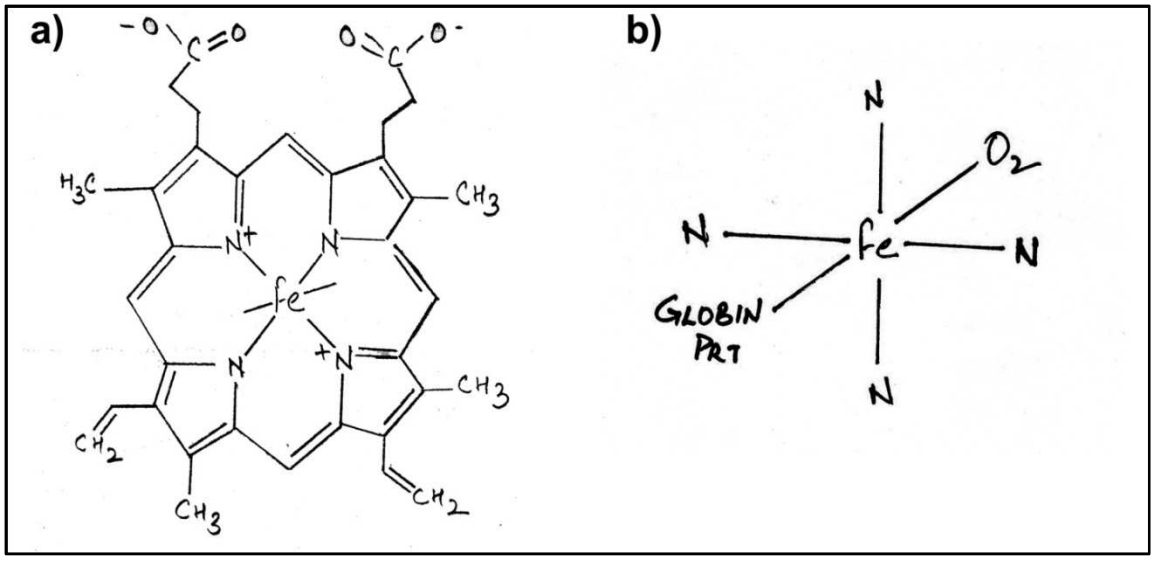

Fig. 2.3 a) Structure of protoporphyrin (Heme) ring; b) Schematic co-ordination of Ferrous ion with oxygen and protein part of hemoglobin.

Hemoglobin devoid of oxygen is called **deoxyhemoglobin**. Adult blood contains two types of hemoglobin: 98 % **hemoglobin A** (HbA) and 20 % **hemoglobin A_2** (HbA_2). HbA contains two two α chains and two β chains ($\alpha_2\beta_2$) and HbA_2 contain a pair of α chain and a pair of δ chains ($\alpha_2\delta_2$). Each heme can combine with two atoms of oxygen to form **oxyhemoglobin** (HbO_2). Oxygen carrying capacity of the hemoglobin is the function of the iron atom. This co-ordination of heme iron with oxygen is reversible and it depends on the partial pressure of oxygen (PO_2). Oxyhemeoglobin is formed when PO_2 is high i.e. oxygen is present in excess and deoxyhemoglobin is formed when PO_2 is low i.e. oxygen concentration is low.

Since each hemoglobin molecule has four heme rings, it carries a total of 4 oxygen molecules. Hemoglobin is beautifully adapted to the task of oxygen carrier as it can pick up or release oxygen readily in response to the change in partial pressure of the oxygen in lungs, blood or tissues. Also, its affinity for oxygen changes with variations in pH and CO_2 concentrations. This is called **Bohr Effect**.

Hemoglobin can also combine with CO_2 or other gases. It can carry a small amount of the CO_2 out of tissues. Hemoglobin can combine with 4 molecules of carbon monoxide (CO) and form **carboxyhemoglobin**. The affinity of hemoglobin is about 200 times high for CO than oxygen. It can therefore cause CO poisoning if inhaled in even in small quantities and result in death by anorexia (lack of oxygen).

Fetal blood contains a distinct form of hemoglobin called **fetal hemoglobin**. Its structure is similar to hemoglobin A except that the β chains are replaced by γ chains making it $\alpha_2\gamma_2$.

ii) Myoglobin: Myoglobin (Mb) is found in vertebrate muscles and is an oxygen storage protein. It stores oxygen in resting skeletal muscles as oxymyoglobin (MbO_2). Myoglobin protein sequence has low similarity with the hemoglobin monomeric chains but it shares a striking similarity with the quaternary structure of Hemoglobin with the same kind of helical and other secondary structures.

In muscles, myoglobin binds O_2 reversibly. It does not exhibit Bohr effect and its oxygen dissociation curve is hyperbolic. During muscle contraction when the demand for the oxygen is highest, oxygen dissociates from the Myoglobin and is available for oxidation. Humans have a

large quantity of myoglobin in cardiac muscles only. In birds the flight muscles are rich in myoglobin. Even at low partial pressure of oxygen, when the Hb is only partially saturated, myoglobin can be fully saturated. Myoglobin can even accept O_2 from hemoglobin and store it in muscle cells for later release.

iii) Haemerythrin: It is another respiratory pigment which is present in the blood of all sipunculid worms, a few polychaete forms and the branchiopod *Lingula*. It is a rare, reddish-violet, iron containing pigment. *Sipunculus* haemerythrin has a molecular weight of 105 KDa and contains 16 Fe^{2+} atoms. It binds one molecule of oxygen per iron atom.

iv) Haemocyanin: Haemocyanin or the blue pigment found mostly in mollusks and arthropods is a copper containing pigment. Squid haemocyanin is a decamer and each monomer contains two Cu^{+} atoms and they bind one O_2 molecule per two Cu^{+} ions.

5. Erythrocruorin: It is a large molecule consisting of multiple subunits and is found in many annelid worms and mollusks. *Limnodrillus* erythrocruorin consists of 108 subunits each having on heme group.

6. Chlorocruorin: It is green in color and is found in certain annelids like *Spirographis*. It is present in coelomic cavity and serves to store rather than transport oxygen.

2.4.4 *GASEOUS TRANSPORT*

Oxygen has very low solubility in plasma (~0.3 ml/100ml). Most of the oxygen is carried by hemoglobin in the form of oxyhemoglobin. Most of the CO_2 on the other hand is transported by plasma in the form of soluble bicarbonates.

i) **Transport of Oxygen:** Oxygen is carried by hemoglobin which is found in red blood cells. The maximum amount of O_2 that normal human blood can absorb is 20 ml per 100 ml of blood. During the passage of oxygen from lung alveoli to lung capillaries, diffusion of oxygen occurs in to the blood and it is captured by the hemoglobin to form oxyhemoglobin.

$$Hb_4 + 4O_2 \rightleftharpoons Hb_4(O_2)_4$$

(Oxyhemoglobin)

Each molecule of hemoglobin has 4 iron atoms; therefore it can carry 4 molecules of oxygen.

Normally, the arterial blood which has been exposed to the alveoli of the lungs is not completely oxidized. At the O_2 pressure of 100 mm Hg, blood is about 98 % saturated and therefore contain 19.6 ml of O_2 per 100 ml. About 0.2 -0.3 ml of O_2 is dissolved in plasma. The arterial blood and the alveoli has the same O_2 pressure (100 mm of Hg). But in the cells and tissues of the body the O_2 tension is quite low (1 to 40 mm of Hg). Now since the co-ordination of the oxygen with the hemoglobin is reversible, it dissociates from the oxyhemoglobin and diffuses into the cells. This phase is important to supply oxygen to cells as well as regenerate hemoglobin for further transport cycle.

$$Hb_4(O_2)_4 \longrightarrow 4Hb + 4O_2$$

(Oxyhemoglobin)

The reduced hemoglobin is transported back via blood to the lungs.

Oxygen hemoglobin dissociation curve:

The binding of oxygen to hemoglobin is influences by four factors viz. partial pressure of oxygen and carbon–dioxide, temperature, H^+ concentration and 2,3-diphosphoglyceraldehyde (2,3-DPG). The quantity of oxygen that can be held by hemoglobin depends on the partial pressure of oxygen (PO_2). The relationship between the two can be graphically represented by a curve called oxygen-hemoglobin dissociation curve. The curve is sigmoid (S-shaped) in nature.

The important feature of the oxygen-hemoglobin dissociation curve is that hemoglobin takes up oxygen when the partial pressure of the latter is high; oxyhemoglobin dissociates when the PO_2 is low. The hemoglobin is almost completely saturated at O_2 tension of 100 mm of Hg. But as the oxygen pressure drops below 60 mm of Hg, it dissociated rapidly thus forming the

steep slope of the curve. When PO_2 is zero, all of the oxyhemoglobin is dissociates into hemoglobin. The actual relationship between the partial pressure of O_2 and the degree of saturation of the hemoglobin with O_2 is shown by Oxygen-hemoglobin dissociation curve (Fig. 2.4).

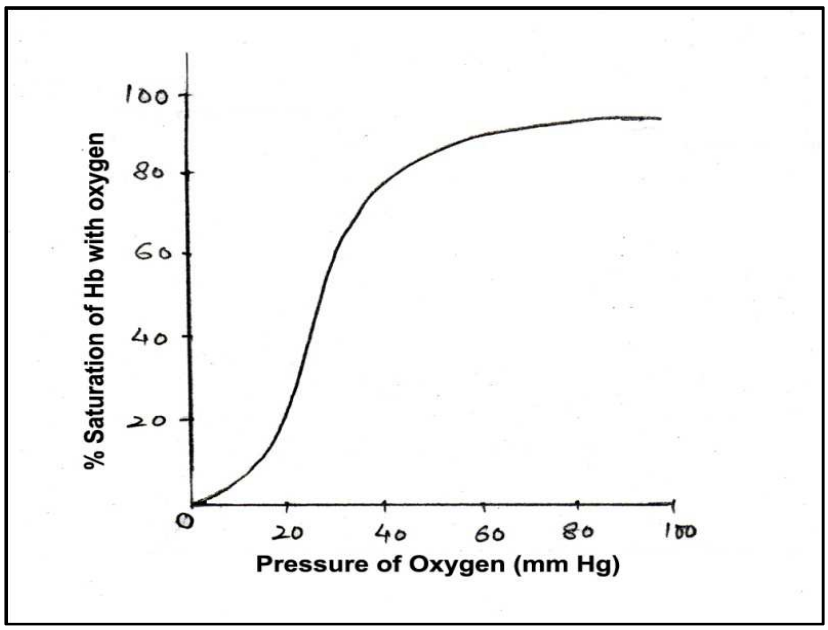

Fig 2.4. Oxygen-hemoglobin dissociation curve: shows the relationship between the partial pressure of the oxygen and the formation of oxyhemoglobin.

Hemoglobin gets saturated at about 100 mm Hg pressure and no more oxygen can be taken up even if the pressure is increased. Inside tissues where the partial pressure of oxygen is less the oxygen is rapidly dissociated from the oxyhemoglobin, thus yielding the larger quantities of the O_2 to the surrounding tissues and cells where it is needed most.

During exercise or hard physical work, the partial pressure of oxygen falls in tissues accompanied by the increase in pH, local temperature and increase in 2,3-DPG concentration. All these factors combined promote the dissociation of oxyhemoglobin to release more oxygen.

Factors that affect the oxygen hemoglobin binding:

a) Effect of Partial pressure of CO_2: Partial pressure of CO_2 (PCO_2) also determines the equilibrium of Hb and O_2. An increase in the CO_2 shifts the curve to the right known as Bohr

effect. A higher CO_2 concentration causes more oxygen to be dissociates at any given O_2 pressure. This phenomenon is important in tissues where the concentration of CO2 is relatively higher and they need oxygen. As soon as the oxygenated blood reached these cells the higher pressure of CO_2 from tissues forces the oxyhemoglobin to dissociate and thus supply the bound oxygen into the cell. Bohr effect therefore results in delivery of additional oxygen into the tissues. Similarly when the blood is again oxygenated in the alveoli of the lungs, it reduces the CO_2 carrying capacity of the blood, thus facilitating the release of CO_2 to the alveoli.

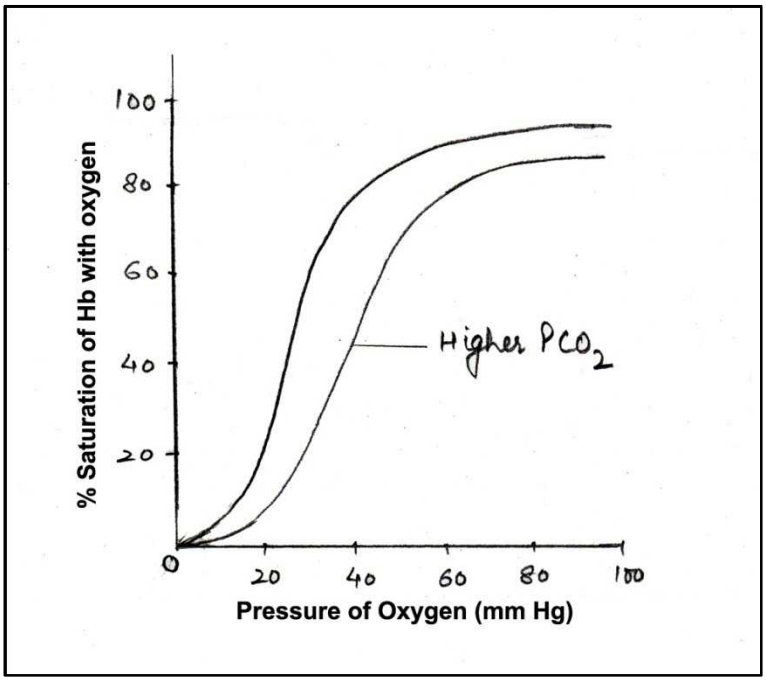

Fig.2. 5 Oxygen-hemoglobin dissociation curve: Increase in concentration/ pressure of CO_2 shifts the curve to the right

b) Effect of temperature: A rise in temperature decreases the oxygen carrying capacity of lungs. When muscle activity is increased during exercise or physical work, the rise in local temperature results in splitting of oxyhemoglobin to hemoglobin and thus in turn releasing of higher amount of oxygen in tissues. Therefore, temperature affects the dissociation curve of O_2 by affecting the partial pressure of oxygen.

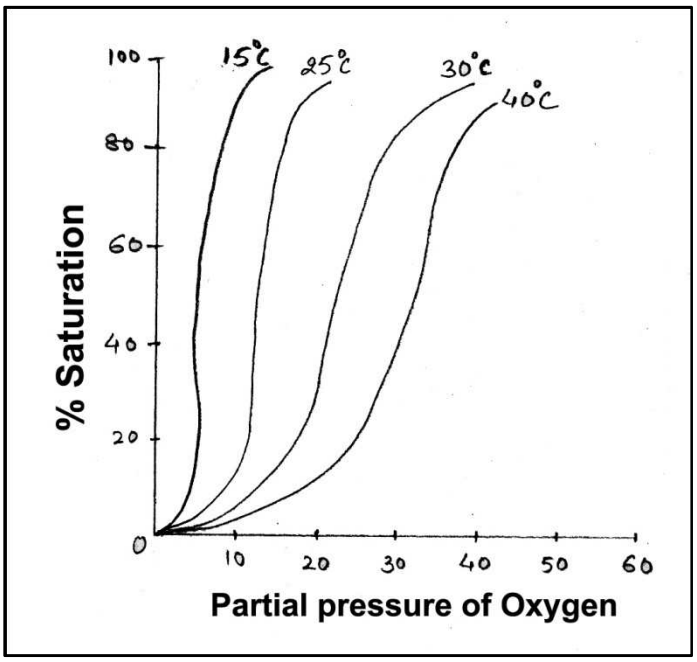

Fig. 2.6 Effect of increasing temperature on the Oxygen-hemoglobin dissociation curve.

c) Effect of hemoglobin: Since the quantity of oxygen transported by blood is directly proportional to the amount of the hemoglobin present, it is obvious that the blood containing less hemoglobin will carry less oxygen.

d) Effect of pH: The pH of blood affects the degree of saturation of hemoglobin with oxygen. An increase in the metabolic rate in the issues increases the CO_2 and the acid metabolites. The partial pressure of oxygen is thus lowered and more oxygen is dissociated.

e) Effect of 2, 3 Diphosphoglycerate (DPG): DPG carries most of the phosphate in the erythrocytes and play an important role in the release of the oxygen to the tissues. An increase in the DPG concentration is associated with decrease in oxygen levels. This type of condition is found in persons suffering from anemia or cardiac inefficiency. DPG combines with hemoglobin and reduces its affinity for oxygen.

ii) Transport of Carbon dioxide:

CO_2 is formed in the body as a result of various metabolic activities of the cell and diffuse into the blood. CO_2 concentration in venous blood is about 60 ml/100 ml of blood while in arterial blood it is about 50 ml/100 ml of blood. CO_2 is transported in three ways which are as follows:

a) Transport as carbonic acid: From the body tissues as the CO_2 enters the blood; it reacts with water present in the plasma to form carbonic acid (H_2CO_3). About 5% of the total CO_2 dissolved in blood is carried as carbonic acid.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

b) Transport as carbamino compounds: In Red Blood Cells (RBCs), CO_2 combines directly with the amino groups ($-NH_2$) of the hemoglobin to form the carbaminohemoglobin. About 10 % of the total CO_2 is transported as this complex.

$$CO_2 + Hb.NH2 \rightleftharpoons Hb.NH.COOH$$

(Carbaminohemoglobin)

c) Transport as bicarbonates: The remaining about 85 % of the CO_2 is carried in the form of bicarbonates both in plasma and RBCs. As CO_2 diffuses into the blood, it forms carbonic acid as discussed earlier which then dissociates to give bicarbonate ions (HCO_3^-) and hydrogen ion (H^+). The bicarbonate ion then combines with either sodium or potassium to form respective bicarbonates.

$$Na^+ + HCO_3^- \longrightarrow NaHCO_3$$

(Sodium bicarbonate)

$$K^+ + HCO_3 \longrightarrow KHCO_3$$

(Potassium bicarbonate)

In normal conditions, most of the carbon-dioxide is present as bicarbonate ions. According to Henderson Hasselbach equation, at pH of 7.4, the ratio of carbonic acid to bicarbonate ions is 1:20. The hydrogen ion formed as a result of dissociation of carbonic acid is neutralized by various buffering agents present in blood.

The RBCs contains an enzyme carbonic anhydrase which catalyzes the reversible reaction between CO_2 and H_2O resulting in the formation of carbonic acid (H_2CO_3) and subsequently bicarbonates.

$$CO_2 + H_2O \underset{\text{(in lung capillaries)}}{\overset{\text{carbonic anhydrase (in tissue capillaries)}}{\rightleftharpoons}} H_2CO_3$$

The enzyme also facilitates the rapid release of CO_2 from the blood during its passage through the lungs. Thus despite the low concentration gradient (partial pressure of CO_2 in tissues is 45 mm of Hg while in arteriolar capillaries it is 40 mm of Hg) carbon dioxide diffuses rapidly from the tissues into the blood.

Isohydric Shift: This is an important phenomenon that occurs during the exchange of oxygen and CO_2 in tissues. During the gaseous exchange the carbonic acid formation tends to lower the pH of the blood while the dissociation of HbO_2 (oxyhemoglobin) to Hb (Hemoglobin) involves a change of pK from 6.2 to 6.6 which raises the pH within the RBCs. Due to these opposing phenomena, the protons formed in the dissociation of H_2CO_3 are accepted by the groups in Hb. The net result is that the overall pH remains unchanged and K^+ ions previously neutralized by HBO_2 are now neutralized by bicarbonate ion. This set of transformation sis termed as *isohydric shift*.

Chloride shift: Chloride shift involves the passage of chloride ions from the plasma into the RBCs to balance the bicarbonate ions that have passed from the RBCs to plasma. This is

important to maintain the acid base equilibrium for the blood and the electrical neutrality of the RBCs.

Release of CO_2 in the lungs: Carbonic acid, bicarbonates and carbamino compounds are the major species that carry CO_2 to the lungs via blood. Due to higher pressure of CO_2 in lung capillaries than in the lung alveoli, CO_2 is released from the blood. The reciprocal effect of the oxygenation on the acid strength of the hemoglobin also known as Haldane effect; accounts for the CO_2 exchange. In the lungs chloride ions move out of the RBCs and bicarbonate ions move back in. Carbonic anhydrase rapidly generates the free CO_2 form the bicarbonates and the gas diffuses from blood into the lung alveoli.

2.4.5 *RESPIRATORY QUOTIENT*

The ratio of the CO_2 volume given off to the volume of O_2 consumed during same time is called respiratory quotient (R.Q.). The R.Q. can be calculated for the oxidation of various food materials. For example, in the following reaction:

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

$$\text{R.Q.} = 6 \text{ volumes of } CO_2/6 \text{ volumes of } O_2 = 1.0$$

It is different for different foods e.g. fats have a R.Q. of about 0.7, proteins have 0.8 and for the average mixed diet it is about 0.85.

2.5 *SUMMARY*

Respiration is the process of gaseous exchange in which oxygen is taken in and carbon-dioxide is exhaled out. The inhaled oxygen is utilized by the cells for oxidation of the food materials to liberate energy by a cascade of reactions also known as aerobic breakdown of digested food. Major organs that facilitate this process are nasal chambers, trachea, lungs and blood. In most of the vertebrates, blood carries respiratory pigment hemoglobin present in the RBCs which has the high affinity for oxygen. It is a multi-subunit protein that has a metallic group containing Fe^{2+} atom. Oxygen when inhaled is carried by hemoglobin in the form of oxyhemoglobin to the

different tissues of the body where the oxygen is releases for the utilization by the cells. The CO_2 in return is taken up by the blood in the form of Carbonic acid, bicarbonates and carbamino compounds and is transported back to the lungs where it is again converted into the free CO_2 and diffused to the lung alveoli for expiration.

2.6 *GLOSSARY*

Respiration: catabolic process in which the respired oxygen is used in the oxidation of food resulting in the release of energy.

External respiration: includes exchange of gases (oxygen and CO_2) in lungs.

Internal respiration: consisting of energy yielding oxidative reactions inside cells.

Larynx: cartilaginous tissue that is situated between pharynx and trachea.

Epiglottis: part of larynx that serves as lid for closing of larynx during swallowing of food.

Lungs: Two balloon like structures that are present in the upper thoracic cavity; expands on inhalation and constricts on exhalation.

Alveoli: Physiological unit of the lungs; balloon like air sac present at the end of bronchioles.

Eupnea: normal respiration.

Hypernea: increase in the respiratory rate and depth.

Dyspnea: irregularities in respiration.

Apnea: cessation or stopping of respiration.

Minute respiratory volume: the quantity of new air that enters the lungs per minute.

Tidal volume: volume of the air inspired or expired with each normal breath.

The inspiratory reserve volume: maximum extra volume of air that can be inspired over and above the normal tidal volume.

The expiratory reserve volume: extra volume of air that can be expired by forceful expiration after the end of a normal tidal expiration.

The residual volume: volume of the air that remains in lungs even after most forceful expiration.

Alveolar ventilation: the rate at which new air reaches alveoli, alveolar sacs, alveolar ducts and respiratory bronchioles per minute**.**

Respiratory pigment: molecule that increases the oxygen carrying capacity of the blood.

Hemoglobin: iron containing oxygen transport metalloprotein found in red blood cells (RBC) of all vertebrates as well as tissues of some invertebrates.

Oxyhemoglobin: hemoglobin carrying oxygen (HbO_2).

Deoxyhemoglobin: Hemoglobin devoid of oxygen.

Carboxyhemoglobin: Hemoglobin can combine with 4 molecules of carbon monoxide (CO).

Bohr effect: Effect of pH and temperature on the oxygen affinity of hemoglobin.

Myoglobin (Mb): is found in vertebrate muscles and is an oxygen storage protein.

Carbonic anhydrase: enzyme that catalyzes the reversible reaction between CO_2 and H_2O resulting in the formation of carbonic acid (H_2CO_3) and subsequently bicarbonates.

Respiratory quotient: ratio of the CO_2 volume given off to the volume of O_2 consumed during same time.

2.7 *SELF ASSESSMENT QUESTION*

i. In Respiration, the gas that is exhaled is:

a) Oxygen
b) Carbon dioxide
c) Nitrogen
d) none of the above

ii) The organ that is also known as sound box is:

a) Larynx
b) Trachea
c) Lungs
d) Alveoli

iii) Physiological unit of lungs is known as:

a) Trachea
b) Bronchioles
c) Alveoli
d) None of the above

iv) The volume of the air inspired or expired with each normal breath is called:

a) Tidal Volume
b) Residual Volume
c) Reservoir volume
d) None of the above

v) The metal ion present in heme group of hemoglobin is:

a) Cu
b) Zn
c) Fe
d) Mg

vi) Haemocyanin, as copper containing pigment is of which color:

a) Red
b) White
c) Black
d) Blue

vii) Each molecule of hemoglobin can carry …….. oxygen molecules:

a) 2 b) 4

c) 6 d) 8

viii) The reversible reaction between CO_2 and H_2O resulting in the formation of carbonic acid (H_2CO_3) is catalyzed by which enzyme:

a) Carbonic anhydrase b) Lysozyme

c) MAP kinase d) none of the above

ix) The ratio of the CO_2 volume given off to the volume of O_2 consumed during same time is called:

a) Exhalation coefficient b) Respiratory quotient

c) Lung Volume d) None of the above

x) Volume of the air that remains in lungs even after most forceful expiration is called:

a) Tidal Volume b) Minute Respiratory volume

c) Residual Volume d) None of the above

MCQ answers:

i) b; ii) a; iii) c; iv) a; v) c; vi) d; vii) b; viii) a; ix) b; x) c

2.8 *REFERENCES*

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2.9 *SUGGESTED READINGS*

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2.10 *TERMINAL QUESTIONS/ANSWER*

i. Which organ is known as ‘voice’ box?
ii. What is the physiological unit of lungs?
iii. Write the formula for respiratory quotient (R.Q.)?
iv. What is the major mode of respiration in annelids?
v. What is Eupnea?
vi. What is the formula for alveolar ventilation?
vii. Which animals have Haemerythrin as respiratory pigment?
viii. In normal conditions, how many molecules of oxygen can a molecule of hemoglobin carry?
ix. Which metal ion is present in Haemocyanin?
x. What is the R.Q. for proteins?

Answers:

i. Larynx
ii. Alveoli
iii. ratio of the CO_2 volume given off to the volume of O_2 consumed
iv. Moist Skin
v. Normal Respiration
vi. VA = Freq. (VT-VD); VA is the volume of alveolar ventilation per minute, Freq. is the frequency of respiration per minute, VT is the tidal volume and VD is the physiological dead space.
vii. Sipunculid worms, *Lingula, Sipunculus*
viii. 4
ix. Cu
x. 0.7

2.10.2 *SHORT ANSWER TYPE QUESTIONS*

i. Define Respiration?
ii. What is residual Volume?
iii. What is Tidal Volume?
iv. What is oxygen dissociation curve?
v. In what forms the carbon-dioxide transported by blood?
vi. What is myoglobin?
vii. Define Alveolar ventilation?
viii. What is Bohr Effect?
ix. What is Isohydric Shift?
x. What is Chloride Shift?

2.10.3 *LONG ANSWER TYPE QUESTIONS*

i. What is respiration? Differentiate between external and internal respiration?

ii. What are respiratory pigments? Discuss various respiratory pigments?

iii. Discuss the process of gaseous exchange in respiratory organs and tissues?

iv. Discuss the structure of Hemoglobin? Explain oxygen transport by hemoglobin?

v. Discuss the transport of CO_2 by blood?

UNIT 3: BLOOD AND CIRCULATORY SYSTEM

CONTENTS

3.1 *OBJECTIVES*

After reading this unit the reader will be able to -

- Describe the biochemical composition of blood
- Describe the functions of blood in vertebrates.
- Explain the mechanism and various theories of blood clotting.
- Distinguish between R.B.C and W.B.C
- Write an essay on Blood groups in man
- Differentiate between myogenic and neurogenic hearts.
- Explain the physiology of heart beat
- Describe the origin, conduction of impulses through mammalian heart with chemical and mechanical regulation.
- Writs short note on Heart sounds
- Describe the medico-legal importance of ABO blood groups
- Explain Rh factor and its importance in blood transfusion.
- Describe the structure of human heart and how the heart pumps blood throughout the body and the facts about the human heart.
- Compare and contrast the oxygenated and deoxygenated blood.
- Explainhow the circulatory system works with other system to maintain homeostasis.
- Describe the stages in the cardiac cycle and the conduction system of the heartbeat
- Write note on ECG.

3.2 *INTRODUCTION*

In single celled animals and lower animals each cell of the body is in direct contact of the environment and can get nourishment and oxygen. In the multi-cellular animals, body is large and complex and each cell of the body cannot come in direct contact of the environment for obtaining food and exchange of respiratory gases. For carrying out the functions of nourishment, oxygen supply and removal of metabolic wastes, some extra cellular fluid is needed. The blood lymph and coelomic fluids forms the internal circulating medium of the body of animals and it is regulated in a precise way ,with respect to its ionic contents, osmotic pressure, gas content and temperature. The maintenance of this constant internal environment through the circulating fluid (homeostasis) is one of the striking features of a living animal.

3.3 *BLOOD*

Blood is a fluid connective tissue that flows in circulatory system. It is a red-coloured fluid that is 1.7 to 2.2 times more viscous than water. Blood is some what salty in taste, slightly alkaline in pH (7.4), with a specific gravity of 1.025 to 1.034. Study of blood is called **hematology.** Study of blood circulation in the body is called **angiology.**

In body of a normal adult human about 15 litres of extra cellular fluid (**ECF**) is present. Of the total body water about 45% is in the form of extracellular fluid and about 55% occurs in the cells as intracellular fluid. Extracellular fluids of body include blood, lymph, cerebrospinal fluid, interstitial fluid, aqueous humor etc. of the total extracellular fluid, Blood forms 30 to 35%. in a healthy adult human being. The volume of blood is about 5 liters and it forms about 7% to 8% of the total body weight. In blood, matrix is plasma into which float many types of cells. In blood 55% plasma and 45% cells (formed elements) are present. The cells of blood are red blood corpuscles or erythrocytes, white blood corpuscles or leucocytes and thrombocytes or blood platelets collectively all types of cells are also called formed elements. Formed elements (blood cells) of blood are also called **haematocrit** volume. The fluid part of blood left after removal of blood corpuscles is plasma. While, serum is liquid part of blood left after clotting of blood.

3.3.1 *COMPOSITION OF BLOO*

The blood contains a fluid matrix called plasma in which different types of blood corpuscles are suspended.

- **Plasma**

Plasma is fluid part of blood. The chief constituent of plasma is water (9-92%) into which remain dissolved organic and inorganic constituents. In the plasma, proteins, salts, nutrients, excretory substances, dissolved gases, enzymes, hormones and antibodies are present in fixed quantities.

- **Organic Constituents of Plasma**

  The organic constituents of plasma include proteins, lipids, glucose, amino acids, fatty acids, vitamins, hormones, urea, uric acid, dissolved gases such as oxygen and carbon di oxide etc.

- **Proteins** are main constituents of plasma. Plasma proteins are albumin, globulin, and fibrinogen. The viscosity of blood is due to plasma proteins, which remain in colloidal state. Albumin and globulins retain water in blood plasma by their osmotic effects. About 75% of total plasma proteins are albumins and constitutes 4.5 to 4.8 gm % of plasma. Main osmotic pressure of the blood is due to the presence of albumins. The synthesis of albumins takes place in liver.
- **Globulin** is other class of important plasma proteins. They constitute about 20% of plasma proteins and are 2.3 to 2.5 gm % of plasma. Globulins are also synthesized in liver except one class of globulins, i.e immunoglobulins that are synthesized in reticulo-endothelial tissue. Globulins are α1 – globulins, α2 – globulins. They are highly mobile in alkaline or electrically charged solutions. They inhibit certain blood proteases and show significant inhibitor activity.
- **Prothrombin** is essential for the clotting of blood. **γ – globulins** are immunoglobulins, have a vital role in natural and acquired immunity to infections. They are produced by lymphocytes which act as antibodies.

- **Fibrinogen** is soluble plasma protein that is synthesized in liver. It is 0.3gm % of plasma proteins. Fibrinogen is essential for clotting of blood. Due to fall in plasma proteins oedema (swelling) occurs.
- **Carbohydrates** occur mainly in the form of glucose. Little amount of lactose and other polysaccharides are also present.
- **A lipid** constitutes (0.2gm %), are mainly cholesterol, its esters, neutral fats, lecithin, phospholipids and some fatty acids.
- In the plasma **urea** and **uric** acid are also present, which are excretory products.
- Dissolved gases: The main gases found dissolved in plasma are oxygen, and carbon-di-oxide. The oxygen content of arterial and venous blood plasma at various pressure ranges between 0.3-0.12%, while that of CO_2 varies from 1.6-1.8%.

➢ Inorganic Constituents of Plasma

Inorganic constituents of plasma are chlorides, phosphates, carbonates, sulphates, iodides of sodium, potassium, calcium, magnesium and iron. Salts present in plasma are sodium chloride and sodium bicarbonate.

➢ Erythrocytes or Red Blood Corpuscles

Red blood corpuscles or erythrocytes (gr. Erythro – red, cytos –cell) are found in blood of all vertebrates and are red in color due to the presence of oxygen carrying pigment haemoglobin. RBCs are formed in liver during embryonic life and in red bone marrow during adult life. The process of formation blood is called **haemopoiesis**(erythropoiesis = RBC formation, Leucoiesis = WBC formation). Fe and proteins are necessary for RBC formation. Kidney secretes hormone erythropoietin, which stimulates bone marrow to produce more RBCs .Immature RBC is called **reticulocyte.** Vitamin B_{12} and folic acid are necessary for maturation of erythrocytes.

a) **Shape.** Red blood corpuscles are nucleated and oval in shape in all vertebrates except in mammals. In mammals, RBCs are circular in shape and without nucleus. In mammalian RBCs nucleus is initially present, but disappears during maturation of RBCs.

b) **Size.** Mammalian RBCs measure about 6 – 7 μ in diameter and 1 – 2 thick. The surface area of the RBC of man is about 135.2 μ .In the animal kingdom, largest RBCs are found in class Mammalia.

c) Number. The number of RBCs in blood of man is 4.5 to 5 million/cubic mm of blood. RBC count is higher in new born babies (60, 00,000 –70, 00,000/Cu mm). Number of RBCs increases by 30% in people living at higher altitudes. RBC count is higher during physical activity, pregnancy and emotional upsets. Abnormal fall in number of RBCs is called a**naemia**. Abnormal rise in RBCs number is called **polycythemia.**

e) Life Span. Normal life span of erythrocytes of man is 120 ± 10 days. In rat it is 55± 5 days. 60±10 days in rabbit, 100± 10 days in frog and 32± 6 days in chicken. Old and worn out RBCs are destroyed in spleen and liver. About 1% RBCs is destroyed daily in spleen and liver. Spleen is called **"graveyard of RBCs".**

- **Structure**

The mammalian RBC is unique in having lost its nuclei and many cytoplasmic organalles at an early stage of development, leaving a homogenous cytoplasm enveloped in a stroma.. Enucleated RBCs can carry more haemoglobin for transportation of oxygen. Oxygen consumption is very low in non-nucleated RBCs that are without mitochondria. RBCs take energy from anaerobic respiration and lactic acid is produced. Mammalian RBCs are circular and biconcave.

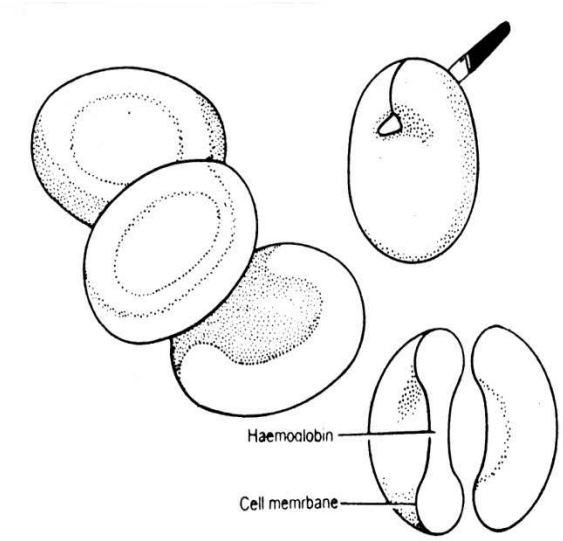


Fig: 3.1 Erythrocytes *of Mammalia showing surface area and section of an erythrocyte*

Human RBC consists of approx. 64%water and 28% Hb,7% lipid and remaining 3% sugars, salts enzyme and other proteins. The red colour of blood is due to **haeme** of haemoglobin. The surface of RBC of man can carry up to 280 million haemoglobin molecules.Haemoglobin is a conjugated protein. Hemoglobin is formed of an iron- porphyrin complex, called **haeme** and a protein called **globin**. Iron containing porphyrin ring—Haeme is conjugated with protein globin by covalent bonds. Haeme is iron containing tetrapyrrole porphyrin ring that is synthesized from iron, acetic acid, glycine and succinyl CoA. Iron in our blood is transported by transferin, a β – globulin. While, Globin protein is formed of two β chains and two α chains. Each β chain of haemoglobin is formed of 141 amino acids. Each α chain of haemoglobin is formed of 146 amino acids.

Life span: The average life span of RBC is approx. 120days in humans,90-135days in dogs,50days in rat,50-70days in rabbit,100days in frog,28-38days in chicken and about 11months in turtle**.**

- **Leucocytes or White Blood Corpuscles**

White blood corpuscles or leucocytes (Gr. **Leuco white, cytos** – cell) are colorless blood cells found in the blood of all vertebrates and many invertebrates. They are metabolically more active.

i)Shape. White blood corpuscles are amoeboid, nucleated and of irregular shape blood cells.

ii)Size. WBCs are comparatively larger in size than RBCs. The size of different types of WBCs varies between 8 to 20 μ.

iii)Number. The number of WBCs is 6,000 to 10,000/cubic mm of blood of man. Ratio between RBC and WBC is 600:1. WBCs produce antibodies and help in phagocytosis. Abnormal fall in number of WBCs is called **leucopenia**. While Leucocytosis is abnormal rise in number of WBCs. Number of WBCs also increases during blood cancer, also called **leukemia**. Number of WBCs also increases during infections. T.L.C. is total leucocyte count. D.L.C. is differential leucocyte count (counting of different types of leucocytes).

iv) Life Span. Average life span of leucocytes is 10—12 days. Daily about 30% WBCs are destroyed. No division occurs is mature WBCs.

- **Structure**

Structure and fine details differ in different types of leucocytes. Leucocytes are of two types, granulocytes and agranulocytes.

A. Granulocytes

. Granulocytes are with 2 to 5 lobed nucleus and granules in cytoplasm. Granulocytes are of three types:

(i) Eosinophils
(ii) Basophils
(iii) Neutrophils or Heterophils

In the cytoplasm of eosinophils, granules measuring 0.7 to 1.3 μ are present which are stained red to purple by acidic dyes such as eosin. Because of these staining properties these blood corpuscles are, called eosinophils or acidophils. The granules of eosinophils are modified lysosomes. In eosinophils nucleus is bilobed. The diameter is 10-12 μ. Eosinophils are about 2.3% of total WBCs with a cell count of 150 to 400/cub mm. Number of

eosinophils increases during allergy or worm infections. Eosinophils are weak phagocytes but their number increase enormously whenever there is an invasion of antigen, a condition called **Eosinophila**

(ii) Basophils. The basophils are the leucocytes with coarse granules. They are circular with a diameter of about 1 μare present, which are stained by basic dyes, such as methylene blue. Because of their staining properties these cells are. called basophils. In the granules, histamines, serotonin and heparin are stored. Basophils secrete histamines, serotonin and heparin. Histamines are released during allergic conditions. Serotonin is a vasoconstrictor. Heparin, present in basophils is a natural anticoagulant. Nucleus of basophils is with 2 or 3 lobes. The diameter of basophils is 8-10 μ. The normal number of basophils is about 0.4% of total WBCs.

(iii)**Neutrophils or heterophils or polymorphs.** In the cytoplasm of neutrophils, abundant fine granules with a diameter of about 0.2 μ are present, which are stained by neutral dyes. The granules of neutrophils are of lysosomal origin and are phygocytic in nature. Nucleus of neutrophils is with 2—5 lobes. The diameter of neutrophils varies from 10-15 μ. Most abundant leucocytes of the blood are neutrophils, they constitute 60 to 70% of total leucocytes. In some neutrophils of women, a barr-body like drumstick is present attached to the nucleus. Neutrophils are phagocytic. As neutrophils phagocytosize, they keep on changing their shape due to amoeboid movement.

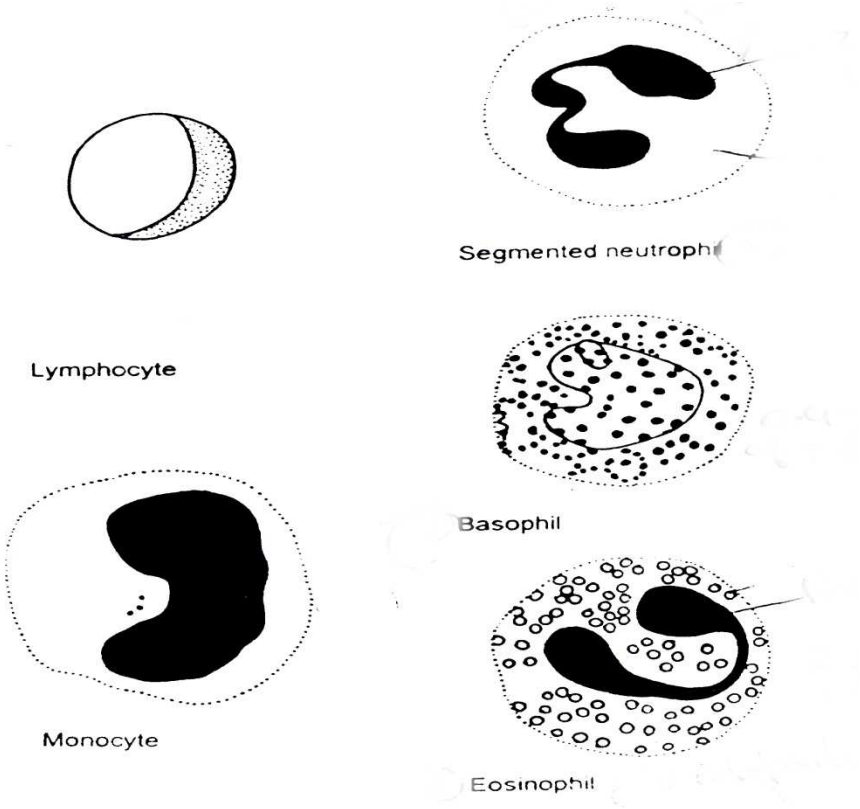


Fig:3.2 *Types of Leucocytes*

B. Agranulocytes

Cytoplasm of agranulocytes is clear. Agranulocytes are with dented nucleus and clear cytoplasm. Agranulocytes are of two types:

(i) Monocytes

(ii) Lymphocytes

(i) **Monocytes**. Monocytes are largest blood cells (12--20 μ) with a prominent horse- shoe-shaped nucleus. These cells are 5 to 10% of the total WBCs. Monocytes are phagocytic in nature and are also called scavenger cells, because they remove dead cells from the site of injury.

When some tissue is injured, ruptured cells secrete chemicals that cause inflammation. Due to inflammation blood capillaries are dilated and WBCs (mainly neutrophils and monocytes) squeeze out of blood capillaries for phagocytosis. Squeezing out of WBC through capillary

walls is called **diapedesis**. Swelling and redness at the site of tissue injury is due to collection of leucocytes. Pus is dead WBCs, bacteria and destroyed tissue cells.

2) Lymphocytes. Lymphocytes are small with comparatively large nucleus. The size ranges from 7 to 16 μ. Lymphocytes are second largest in number of white blood cells. They constitute 25 to 30% of the total leucocytes.Lymphocytes are of two types depending upon their place of maturation: **T lymphocytes** and **B lymphocytes.** T lymphocytes are those cells which mature in thymus and B lymphocytes mature in lymphoid tissue of the body, such as spleen, tonsils, lymph nodes, appendix etc. Lymphocytes play important role in the defense mechanism of body. The production of antibodies is inherent property of lymphocytes. Some lymphocytes are sensitized and then produce specific antibodies. Some lymphocytes stimulate other lymphocytes to produce antibodies.

- **Blood Platelets or Thrombocytes**

Blood platelets occur only in mammals. Blood platelets are non-nucleated, round or oval,biconvex disc like bodies. Their size varies between 1.3 to 3 μ Thrombocytes bud off frommegakaryocyte cells of red bone marrow. Thrombocytes or blood platelets are actually cellsfragments in mammals.Life span of blood platelets is 8 days. The platelets count ranges from 2-4lakhs per cubic mm of blood. An increase in platelet count known as **thrombocytosis.** Physiological increase in platelet is generally observed during exercise and at high altitude whereas pathological increase occurs in trauma and haemorrhage. Decrease in platelet count is known as **thrombocytopenia**and the number of platelet is less than 1 lac per cu.mm. It can be caused by viral infections like chicken pox, dengue fever etc. or by toxic agents like drugs and ionizing agents.

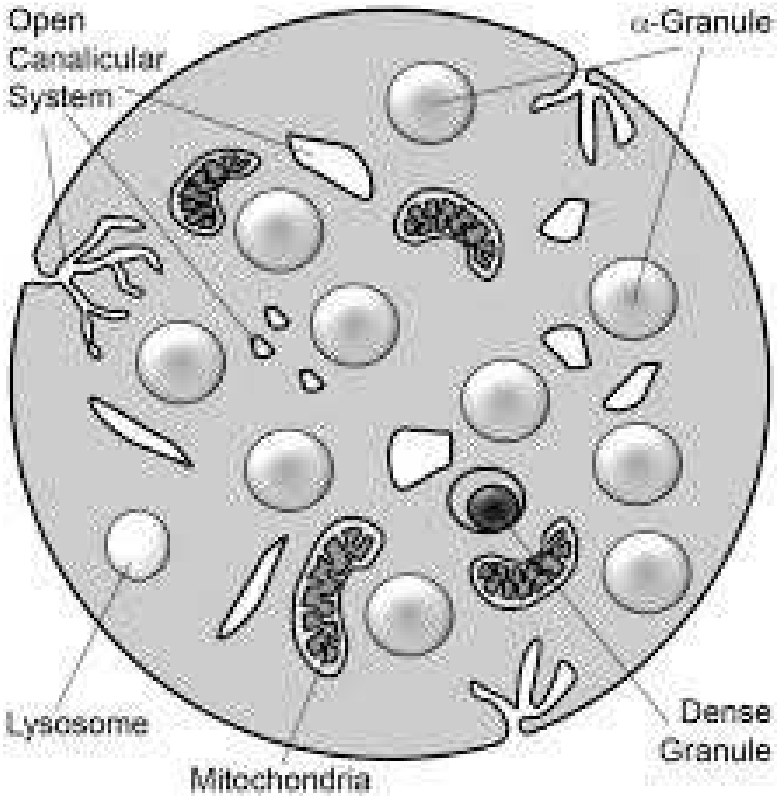


Fig: 3.3 Structure of Platelet (Magnified)

- **Structure:** Blood platelets contains a blue violet granular material in the centre known as **granulomere..**The peripheral part of platelet s is without granules and is called**hyalomere**.Only 60-70% of platelets are found in the circulating blood and rest is stored in spleen. Blood platelets help in blood coagulation. Thrombocytes secrete thromboplastin.that plays an important role in blood coagulation. The main function of platelets is to maintain homeostasis:ie the process of stopping bleeding at the site of interrupted endothelium and coagulation of blood.

Table: 3.1 showing size, number and percentage of various blood cells in ahealthy man

Tyes of blood cell	**size**	**origin**	**% of blood**	**No/cu mm**	**Life span**
Red blood corpuscles	6 – 7 µ	Liver, spleen (embryo) bone marrow(adult)	99% of total blood cells	4.5 to 5 million	113 – 118 days
Neutrophils	10 -15µ	Bone marrow	62% of WBCs	150 to 400	Variable
Eosinophils	10 -12µ	Bone marrow	2.3% WBCs	3000 to 6000	Variable
Basophils	8 -10µ	Bone marrow	0.4 of WBCs	0 to 100	Variable
Lymphocytes	7 -17µ	Lymphoid tissue	30% of WBCs	1500 to 2700	Variable
Monocytes	12- 20µ	Reticuloendothelial	5.3% of WBCs	350 to 800	Variable
Blood Platelets	1.3 - 3µ	Bone marrow	_	250,000	

3.3.2 *FUNCTIONS OF BLOOD*

1. Transportation Blood is main circulatory fluid in the body of chordates. All useful substances are transported by blood to different body organs and tissues and harmful waste materials are collected from the site of their formation and transported to the organs from where these are removed. Blood helps in the transportation of nutrients, respiratory gases, metabolic wastes, hormones etc.

3. Defense mechanism of body: Blood plays important role in defense mechanism by direct phagocytosis and antibody production.

4. Homeostasis: Blood helps in homeostasis by preventing the flow of blood from injured blood vessels by coagulation.

5. Chemical co-ordination: Hormones are chemical messengers of body and help in regulation and co-ordination of all body function. Hormones reach their target organs through blood only. Thus, blood helps in chemical co-ordination of the body.

6.Maintenance of pH: The plasma proteins of blood act as a buffer system and prevent a shift in pH of blood.

7. **Defense (Protection):** Blood provides protection to the body against infection with the help of antibodies and leucocytes.

8. Body movements: Blood help in performing various types of body movements in some lower animals. An earthworm extends its anterior body end by pumping out more blood into it, similarly a newly hatched butterfly expands its wings by forcing blood into them.

9. Functions of mineral ions of plasma: The mineral ions of plasma are essential regulators of the osmotic pressure of the blood. They also function as cellular control agents.

10. Diapedesis and healing: Whenever a part of the body is injured or damaged, the circulatory system reacts promptly by dilating the neighbouring blood vessels under the influence of certain vasodilators.

3.4 *BLOOD GROUPS*

Blood of all persons is not similar. **Karl Landsteiner (1900)** discovered that blood of different persons was mutually incompatible. He observed that when a person require blood transfusion, blood of certain persons will match but blood of some persons will cause agglutination. Plasma membrane of RBCs contains **antigens** A and B and two **antibodies** a and b (or anti B and anti A antibodies) occur in the plasma.

3.4.1 *TYPES OF BLOOD GROUP*

Depending upon the distribution of these antigens and antibodies, four types of blood groups are found in human beings. These blood groups were designated as blood group **A, B, AB** and **O.** In the A blood group, antigen A is present on RBCs and antibody b are present in plasma. In the B blood group, antigen B is present on RBCs and antibody a are present in plasma. In AB blood group both antigens A and B are present but no antibodies are found in plasma and on the other hand in blood group O no antigens are present, but both antibodies a and b are present in plasma.

These four blood groups are found due to the presence of three genes I^A, I^B, i. The personwith blood group A can be of genotype I^AI^A, i^Ai**i.** A person having blood group B can be of genotype I^B I^B**,**or I^Bi. in blood group AB genotype is I^A I^B and in blood group O genotype is ii.

Table: 3.2 Types of different blood groups

Blood type	Antigen on RBC	Antibodies in blood	Safe transfusions	
			to	from
A	A	B	A,AB	A,O
B	B	A	B,AB	B,O
AB	A,B	-	A ,B,	A,B,AB, O
O	-	A,B	A,B,AB,O	O

During blood transfusion, if a person with one kind of blood group (e.g. A blood group), is given the blood of other group (e.g. B blood group) agglutination of RBCs occur, Agglutination occurs due to the reaction between antibodies present in plasma (e.g. antibodies b present in plasma of type A person) and antigens on the RBC (e.g. antigen B present on the RBCs of type B person). Antibody b reacts with antigen B. Blood group O is called **Universal donor** group because no antigen is present on RBCs **Universal recipient** is blood group AB because both antigens are present. (These terms are no longer in use.)

- **Testing of Blood Groups**

Before blood transfusion, matching of blood groups is essential. For blood transfer, blood groups are tested. For testing of blood groups, A anti serum and B anti serum and B anti serum are used Agglutination with A antiserum indicates blood group A. Agglutination with B antiserum indicates blood group B. If agglutination occurs in both A antiserum and B antiserum then blood group is AB. If there is not agglutination with any of the serum then blood group is O.

Table: 3.3 Testing of Blood Groups

Group	Serum A	Serum B
O	–	–
A	–	+
B	+	–
AB	+	+

The sign' __' indicates no agglutination While +sign shows agglutination

3.4.2 *RH FACTOR*

The **Rh blood group system** (including the **Rh factor**) is one of thirty-five known human blood group systems. It is the second most important blood group system, after the **ABO** blood group system. The Rh blood group system consists of 50 defined blood group antigens, among which the five antigens D, C, c, E, and e are the most important. The commonly used terms *Rh factor*, *Rh positive* and *Rh negative* refer to the *D antigen* only. Besides its role in blood transfusion, the D antigen is used to determine the risk of hemolytic disease of the newborn (or ***erythroblastosis foetalis***) for Rh disease management.

The term Rh derives from rhesus that means rhesus blood group system, rhesus factor, rhesus positive and rhesus negative are also used.The rhesus blood type named after the rhesus monkey, discovered in 1937 by **Karl Landsteiner** and **Alexander S. Wiener**. The significance of the discovery was not immediately apparent and was only realized in 1940, after subsequent findings by **Philip Levine** and **Rufus Stetson**. This serum that led to the discovery was produced by immunizing rabbits with red blood cells from a **rhesus macaque**. The antigen that induced this immunization was designated by them as ***Rh factor*** to indicate that *rhesus* blood had been used for the production of the serum.

3.4.3 *BLOOD CLOTTING MECHANISM*

Blood, when comes out of the vessels quickly changes from a fluid state into a thick jelly like material. This is known as **clot** and the process of separation of clot from the plasma (the serum- the liquid part of blood left after clot formation)is known as **blood clotting**. Normally the clotting time of blood is 2—8 minutes. Vitamin K is required for production of prothrombin.

The essence of all theories of blood coagulation is the conversion of soluble plasma protein fibrinogen into insoluble fibrin in presence of thrombin. Thrombin is present as prothrombin in the plasma. After an injury, blood platelets and injured tissue releases thromboplastins which activate prothrombin into thrombin in presence of Ca++ .Heparin and antiprothrombin present in plasma prevents coagulation of blood in the vessels. The formation of prothrombin in the liver however depends on the absorption from the alimentary canal of adequate amounts of vitamin.

Different workers have provided theories of coagulation of blood from time to time. First scientific account of blood clotting was provided by **Alexander Schmidt** in 1861. Subsequently, the other theories have also been proposed-

- **Howell's theory**
- **Quicks theory**
- **Best and Taylor's theory**
- **Fuld and Spiro's theory**

1. **Howell's theory**

This theory was given in 1943 and it is most widely accepted theory of blood coagulation. This theory is best explained in the following steps:

(i) **Formation of thromboplastin:** According to Howell and Ferguson, thromboplastin is released from the injured blood vessel and the tissue. The substance contains a phospholipid-cephalin, in the form of a thromboplastin-cephalin compound.

(ii) **Release of prothrombin:** Prothrombin circulates in the blood in an inactive form under the influence of an inhibitor called antiprothrombin. The antiprothrombin is an anticoagulant from the liver and blood vessels and it does not allow the circulating blood to coagulate in the blood vessels.

(iii)**Thrombin formation:** Prothrombin (unstable protein)is a precursor of thrombin present in the blood plasma. It is synthesized in the liver in presence of vitamin K. Prothrombin is converted into thrombin in the presence of Ca++ ions and another enzyme tryptase.

(iv)**Formation of fibrin from fibrinogen:** in presence of active thrombin, soluble plasma protein fibrinogen is converted into insoluble fibrin. Fibres of fibrin are laid down at the site of injury in which blood corpuscles, ruptured platelets and damaged cells are entangled, forming a clot or thrombus

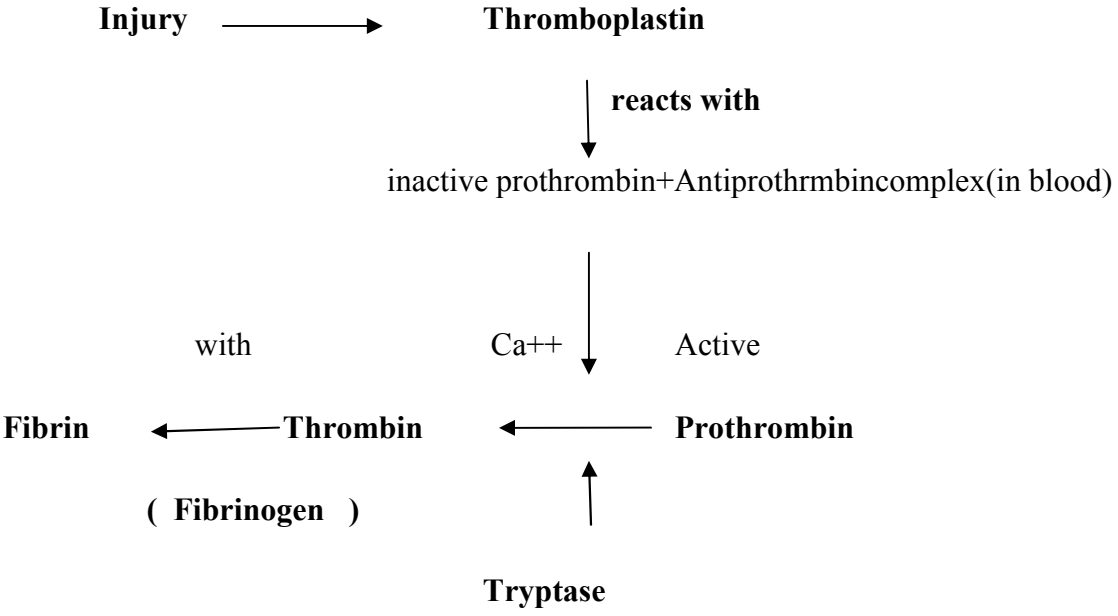


Fig: 3.4 Process of clotting

2) Quicks theory

This theory was proposed in 1949 by J. Arnold Quick and his co-workers. This theory deals mainly with the role of platelets in blood clotting and biochemical aspects of action of thromboplastin and antiprothrombin complex. According to this theory, a thromboplastin activating enzyme is secreted by blood platelets after an injury of a blood vessel. This enzyme is known as thromboplastinogen. The influence of thromboplastinogen makes the process of blood clotting faster. The entire process is completed in three stages-

StageI-Secretion of activating enzyme by the platelets,

StageII -Labellizing influence of the enzyme making the process of blood clotting **faster**

StageIII-finally the conversion of fibrinogen into fibrin under the influence of thrombin.

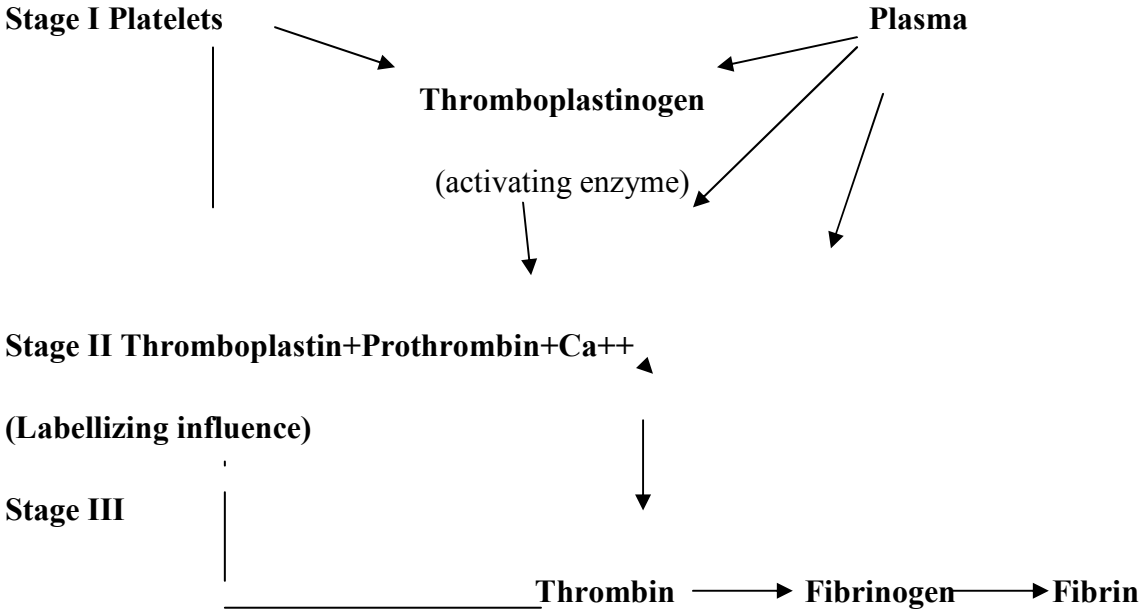


Fig: 3.5 Quick's theory of clotting of blood

3) Best and Taylor's theory

According to Best and Taylor, four substances are needed for the clotting of blood, thromboplastin, thrombin, calcium and fibrinogen. Prothrombin, fibrinogen and calcium are

present in blood plasma. Thromboplastin is derived from injured tissues. After an injury to a blood vessel, from damaged cells thromboplastin is released in the form of thrombokinase. In the presence of calcium ions, thrombokinase converts inactive prothrombin into its active form thrombin. Soluble plasma protein fibrinogen is converted into insoluble fibrin, in presence of active thrombin.

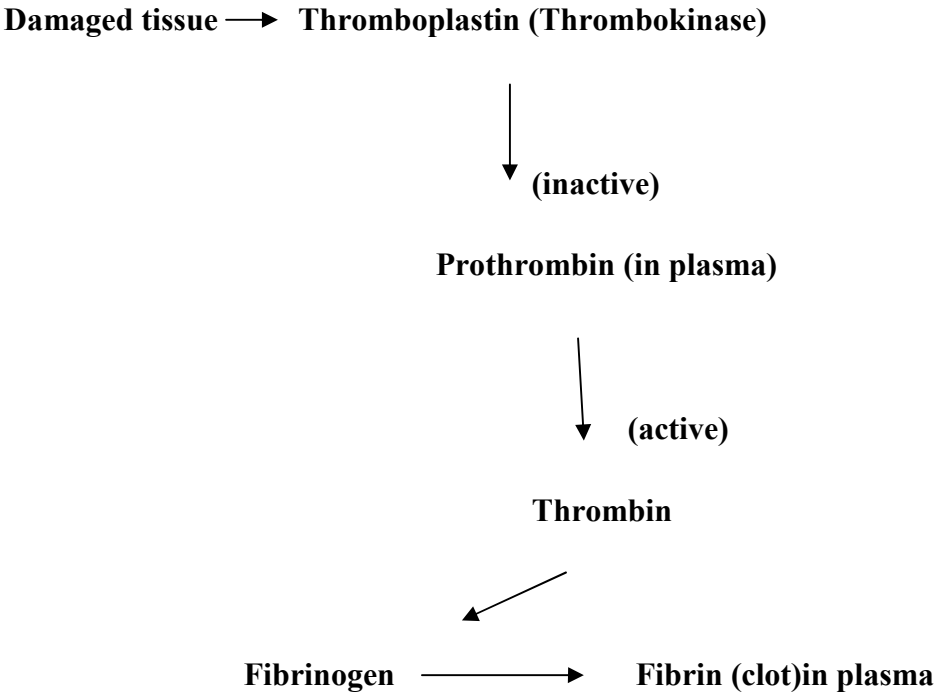


Fig: 3.6 Best and Taylor's theory

4. Fuld and Spiro's theory

The theory of fuld and Spiro's is just like Howells theory, with the only difference that according to fuld and Spiro thrombokinase is released by injured blood platelets instead of tissues. The entire chain of events is expressed as under Fig:

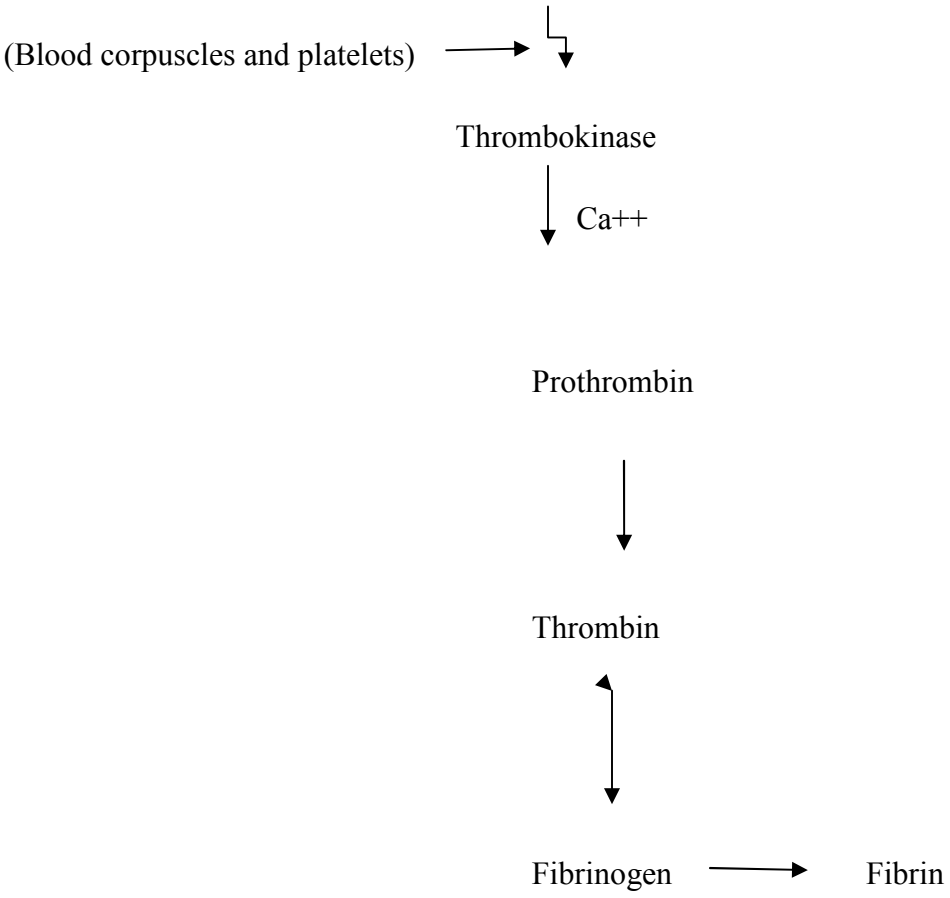


Fig: 3.7 Fuld and Spiro's theory

- **Blood clotting factors**

According to modern views thirteen factors take part in coagulation of blood. Some factors are extrinsic factors and others are intrinsic factors. These factors are given Roman numerals

according to the sequence of their discovery. Thirteen factors involved in blood coagulation are:

1. **Factor I:** Fibrinogen is soluble plasma protein of a high molecular weight. It is intrinsic factor. The deficiency of fibrinogen is a condition, called **afibrinogenaemia**. In this condition blood does not clot at all.
2. **Factor II:** Prothrombin is also an intrinsic factor. Prothrombin occurs in the blood plasma in inactive form. Prothrombin is converted into its active form thrombin in the presence of prothrombin activator. It is a glycoprotein that is synthesized in the liver in presence of vitamin K.
3. **Factor III:** Thromboplastin is also called tissue factor. It is also an intrinsic factor. Thromboplastin is present in blood platelets and tissues. At the time of injury thromboplastin is released in its inactive form **prothromboplastin**.
4. **Factor IV:** Calcium ions are required for synthesis of **thromboplastin** and conversion of prothrombin into thrombin. It is an intrinsic factor.
5. **Factor V:** Labile factor or proaccelerin or accelerator globulin factor is required for the conversion of prothrombin into thrombin.
6. **Factor VI: Accelerin** was previously supposed to be activation product of proaccelerin. Now this factor is not supposed to be present.
7. **Factor VII:** Stable factor or **proconvertin** or serum prothrombin conversion accelerator (SPCA) activates the formation of tissue prothrombin and formation of prothrombin activator by tissues.
8. **Factor VIII:** Antihaemophilic globulin **(AHG)** or antihaemophilic factor **(AHF)** or platelet co-factor I is required for the formation of prothrombin activator from blood constituents. This factor is quite well known, because deficiency of factor VIII or antihaemophilic globulin causes classical **haemophilia**.
9. **Factor IX:** Plasma thromboplastin component (PTC) or **Christmas factor** or **platelet co-factor II** is also required for the formation of prothrombin activator from blood constituents. The deficiency of factor IX or plasma thromboplastin component causes haemophilia or Christmas disease.
10. **Factor X: Stuart factor** or antiprothrombin III is found in blood and is essential for blood clotting. But it is not consume during blood clotting so present in serum also.

11. **Factor XI:** Plasma thromboplastin antecedent is also present in plasma and serum. It is intrinsic factor. This factor is activated by factor XII and initiates the formation of prothrombin activator.
12. **Factor XII:** Hageman factor or surface factor activates factor XI. It is intrinsic factor. This factor itself is activated by surface contact.
13. **Factor XIII:** Fibrin stabilizing factor or fibrinase is an enzyme, which activates polymerization of soluble fibrin into insoluble fibrin.

The modern theories explain the process of blood clotting by taking into consideration the role of all 13 factors (**Table 3.4**)

Roman Numerical	Name	Synonyms
I	Fibrinogen	—
II	Prothrombin	—
III	Tissue factor	Thromboplastin
IV	Calcium ions	Ca^{++}
V	Proaccelerin	Liable factor, Accelerator globulin factor (AGF)
VI	Accelerin	thrombogen
VII	Proconvertin	Stable factor, Auto prothrombin I Serum prothrombin conversion accelerator (S.P.C.A.)
VIII	Antihaemophilic globulin factor (AGF)	Antihaemophilic factor A, platelet cofactor I, thromboplastinogen
IX	Plasma thromoplastin componenet (PTC)	Christmas factor, Antihaemophilic factor B, Platelet cofactor II Autoprothrombin II
X	Stuart factor	Prower factor, Autoprothrombin III, thrombokinase
XI	Plasma thromboplastin antecedent (PTA)	Antihaemophilic factor C.
XII	Hagemen factor	Surface factor, Contact factor
XIII	Fibrin stablizing factor (FSF)	Laki Lorand factor (LLF), Fibrinase, Plasma transglutaminase

Two more theories are given by modern scientists which are under mentioned-:

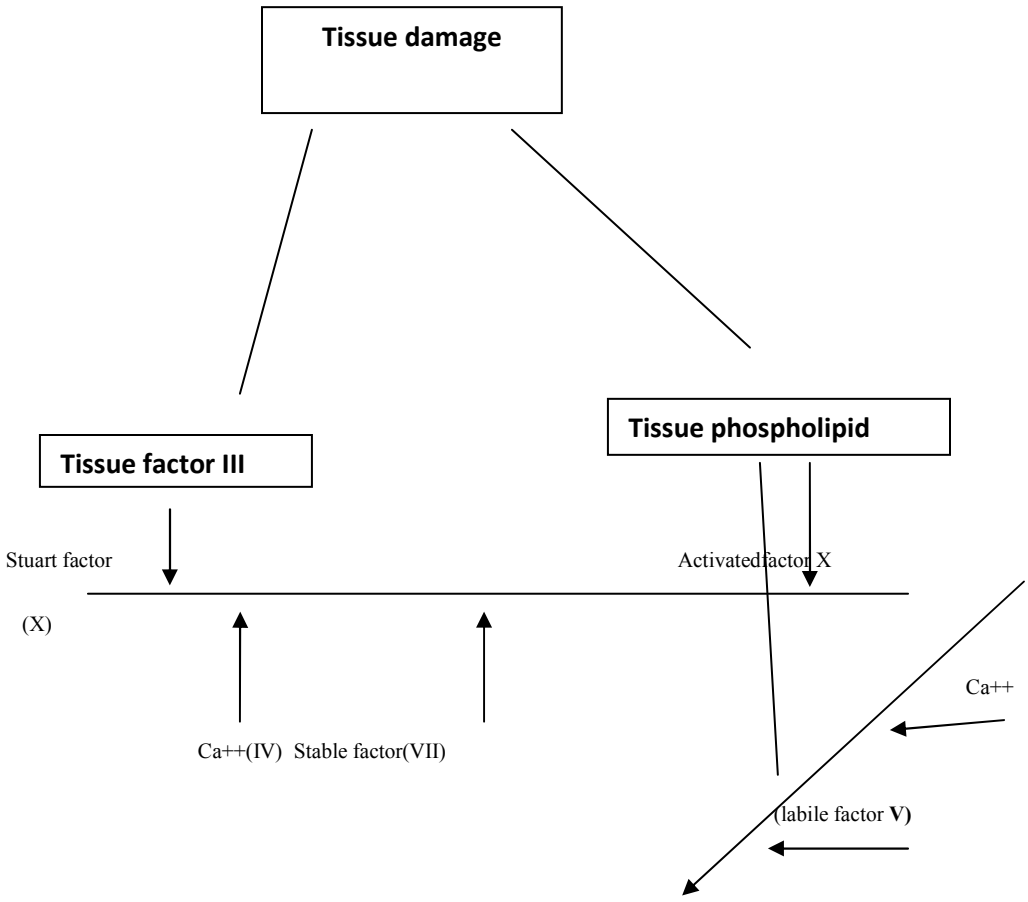
1. Davie and Ratnoffs theory

2. Enzyme Cascade theory of Macfarlane

- ✓ **Davie and Ratnoff's theory**

Davie and Ratnoff tried to explain the process of blood coagulation by explaining the origin and mechanism of action of all thirteen factors involved. This theory is also called '**Waterfall sequence**' hypothesis. According to this theory, thromboplastin is of both blood and tissue origin i.e. extrinsic and intrinsic.This process is summarized in the given flow chart: (**Fig:3.8)**

EXTRINSIC PATHWAY



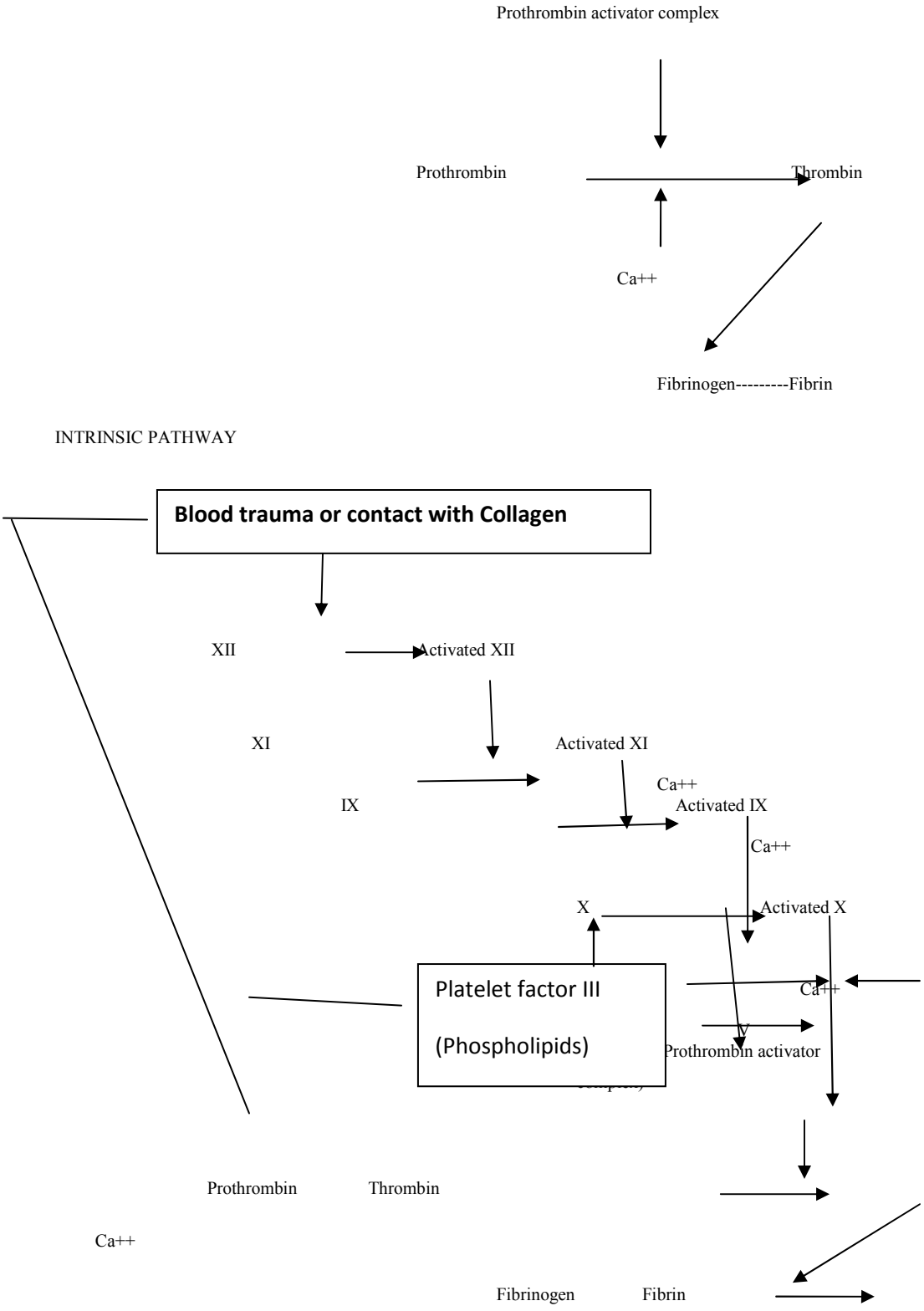


Fig: 3.8 Enzyme cascade hypothesis

Enzyme Cascade theory of Macfarlane

In 1967, Macfarlane explained that during clotting of blood a cascade of enzyme action takes pace,that is why this theory is called enzyme cascade theory. Four main steps of blood clotting are initiation of clotting, conversion of prothrombin into active thrombin, proteolysis of fibrinogen and clot formation. More recently **Guyton**(2003) has proposed a more simplified explanation of Marcfarlane hypothesis as under.

1. **Initiation of clotting.** The clotting of blood starts when blood comes in contact with external environment that occurs due to injury of tissues or injury of a blood vessel.

   Release of prothrombin activator or thromboplastin. Thromboplastin can be released by extrinsic pathway or intrinsic pathway.

   Intrinsic pathway. Intrinsic pathway consists of blood. When blood flows some intrinsic factors are released, which cause conversion of one factor into another. This chain of events occurs as follows:

(i) Activation of factor XII : When blood starts flowing after an injury to a blood vessel, it releases two important factors – platelets phospholipids and Hageman's factor or surface contact factor or factor XII. Factor XII is activated when it comes in contact of some wet surface or collagen fibres.

(ii) Activation of factor XI : In presence of activated factor XII, factor XI is activated. Activated factor XII acts as enzyme in activation of factor XI or plasma thromboplastin antecedent.

(iii)Activation of factor IX : In presence of activated factor XI, plasma thromboplastin or Cristmas factor or factor XI is activated. Activated factor XI acts as enzyme in activation of factor IX.

(iv)Activation of factor X: In presence of factor III or platelet factor and calcium ions, activated factor IX acts with factor VIII or platelet cofactor I and Stuart factor or autoprothrombin III or factor X is activated.

(v) Formation of prothrombin activator: Phospholipids and factor V combine to form prothrombin activator.

Extrinsic pathway

Extrinsic pathway consists of cells of blood vessels and tissues. When blood flows due to injury of some vessel, some factors are extrinsic factors. In the extrinsic pathway, following events occur:

(i) **Release of factor III:** Injured tissues release two factor, platelet factor III or thromboplastin and tissue phospholipids.

(ii) **Activation of factor X:** A complex is formed by interaction of platelet factor III or thromboplastin and autoprothrombin I or factor VII in the presence of factor IV or calcium ions and tissue phospholipids. This complex activates factor X or autoprothrombin III or thrombokinase.

(iii) **Formation of prothrombin activator:** Tissue phospholipids, thrombogen or factor V and activated factor X combine to form a complex, known as prothrombin activator.

The complex prothrombin activator formed by combination of phospholipids, thrombogen or factor V and activator factor X is same both in extrinsic and intrinsic pathways:

2. Formation of Thrombin

Prothrombin activator or prothrombinase splits the prothrombin molecule at two places that results in the formation of thrombin having a molecule that has two unequal chains interconnected by a single disulphide bond. Initially formed thrombin enters into a positive feed back mechanism of an amplifying system that leads to rapid conversion of prothrombin into thrombin.

3. Formation of fibrin

Thrombin acts as a proteolytic enzyme and causes proteolysis of fibrinogen into fibrin by removing two polypeptides fibrinopeptide A and B from the molecule of fibrinogen.

Clot Formation

The monomers of fibrin form fine fibrils due to a specific surface charge aggregation. In presence of factor XIII or fibrinase fibrin monomers are converted into fibrin polymers resulting in clot formation. This raction takes place in the presence of calcium ions. Covalent cross linkages are formed between monomers of fibrin during the formation of fibrin polymers. In this network damaged blood corpuscles and cells are entangled forming a solid clot. Freshly formed clot is jelly like but some fluid oozes out from the clot and it becomes solid.Blood can also coagulate without coming in contact with air e.g. clot formation in blood vessels causing coronary thrombosis.

This complete process can be summarized as follows: (**Fig: 3.9)**

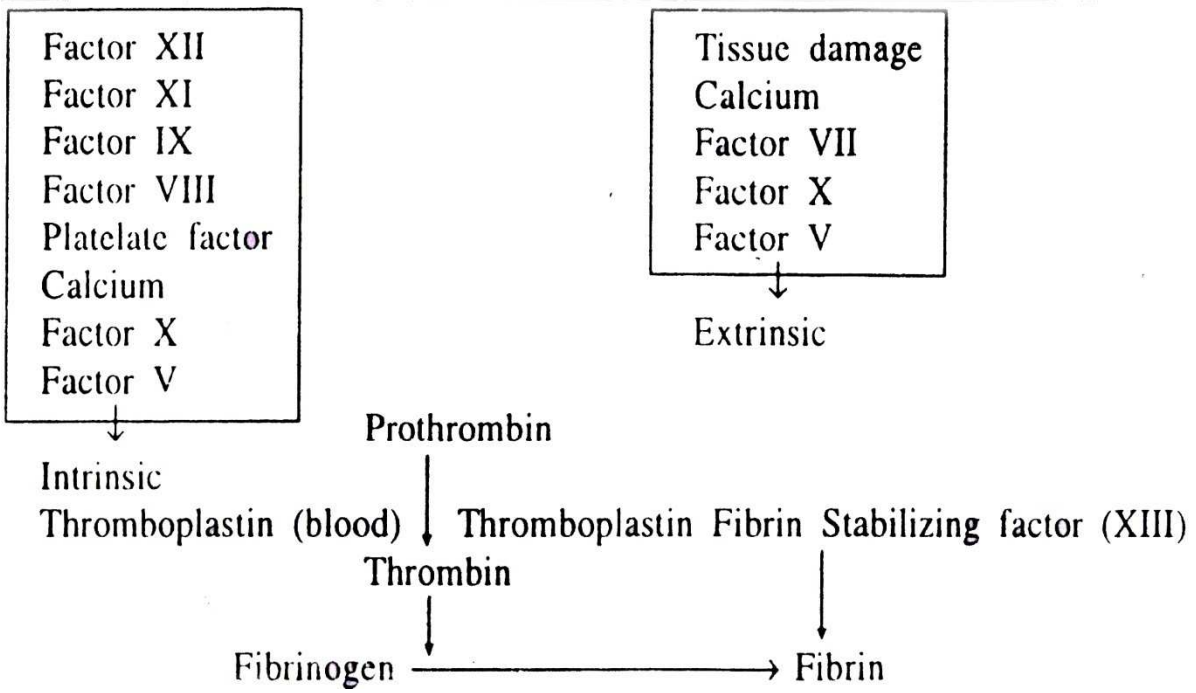


Fig 3.9

3.5 Heart /cardiovascular physiology

All the animals, above the level of helminthes have been gifted with a circulatory system which besides performing subsidiary function s like thermoregulation in higher forms .it is primarily concerned with the translocation of various substances like oxygen, carbohydrates, amino acids, fats, hormones, and vitamins to different tissues and even individual cells of the body and with the removal of metabolic waste from different parts of the body.

- **Types of circulation**

A) **Open type circulation:** In many invertebrates, such as insects, most of the crustaceans, arachnids, and mollusks, the heart pumps the blood through arteries, into the haemocoelomic sinuses and intracellular spaces. Thus, the blood flows more or less freely between the tissues before it eventually returns to the heart.

B) **Closed type circulation**: A closed –type circulatory system is found in vertebrates, cephalopods and annelids. Here the blood is carried in a circuit of closed tubes called arteries, capillariesand veins and the blood returns to the heart without leaving this system of closed and true vessels.

3.5.1 STRUCTURE OF HEART

All vertebrates are gifted with a pulsating heart which receives blood from different parts of the body by means of veins and pumps in into various parts of the body through arteries arising from the anterior end. The wall of the heart is composed of three layers namely ,the endocardium, myocardium, and the epicardium .The endocardium consists of connective tissues lined with a thin layer of endothelium. The myocardium is the principal muscle layer which is thin in the auricles and thick in the ventricle. The epicardium is made up of epithelial cells and connective tissue and remains lodged in a special coelomic chamber known as pericardial cavity. The heart is ventrally placed below the alimentary canal.

The vertebrate (mammalion) heart is four chambered two auricles and two ventricles ensuring complete separation of oxygenated and deoxygenated blood. The blood enters the right auricle from systemic circulation through the vena cavae. The left auricle receives blood from the pulmonary circulation. The blood is then pushed into the two ventricles. The right ventricle receives deoxygenated blood from the right auricle and pumps into the pulmonary circulation. The left ventricle receives oxygenated blood from the left auricle and pumps it into the systemic circulation through aorta. The circulation of blood through the heart is guided by four valves. The left auricle opens into the ventricle guarded by a mitral valve which is bicuspid, and the opening of the right auricle into the ventricle is guarded by tricuspid valve. There is an aortic valve between the left ventricle and the aorta. The opening of the pulmonary artery into the right ventricle is guarded by a pulmonary valve.

- **Functions of heart valves**

The auriculo-ventricular valves (tricuspid or bicuspid) prevent the backward flow of the blood from ventricles to the auricles during ventricular diastole. The semilunar valve (aortic and pulmonary valves) prevents the backflow of the blood from the systemic aorta and pulmonary artery into the ventricles during and after diastole.

The human heart has a volume of about 700ml at the end of each diastolic expansion, whereas the actual volume of heart is roughly 300ml.The total mean weight of the heart is approximately 328gms in male and about 244 gms in female (**Reiner,1959).**

- **Special conductive tissues of heart:**

The heart of all vertebrates is endowed with a special conductive tissue which besides generating rhythmic impulses to make the heart beat non-stop, is also involved in spreading these impulses all through the heart. The excitatory and conductive system in a mammalian heart includes-

1) The sino-auricular node: It is located in the posterior wall of the right auricle just below the opening of the superior vena-cava, Sino-auricular node **(S-A node**) is a small crescent to fusiform node of specialized muscles of about 3mm in width and 1 cm in length. Like all other cardiac muscle fibre, it has the capability of self-excitation and sends out 70-80 rhythmic impulses per minute. The fibres of S.A node normally initiate the heartbeat and due to this reason the S.A node is termed as the **pace-maker.**

2)The internodal pathways and the auriculo-ventricular node: The ending fibre of the S-A node fuse with the surrounding auricular muscle fibres and thus form the intermodal pathways between the S-A node and A-V node.

The Atrio-Ventricular node is a part of the right atrium close to the atrio-ventricular septum.It is innervated by left vagusnerve, and lies close to the tricuspid valve. It generates impulses at a rate of 60/min. It delays and relays cardiac impulses.

3)Bundle of His: It is a thick band of muscle fibres starting from A.V Node. It runs along the intra-ventricular septum and forks into right and left branches. It generates impulses at a rate of 40/min.

4)Purkinje fibres: These fibres arise from the branches of Bundle of His. Eachfibre is about 16μ long, has indistinct cellular outlines, a granular cytoplasm with many nuclei and the impulses travel more faster(1.5 to 4metre per second)in them than in any other cardiac muscle.(fig)

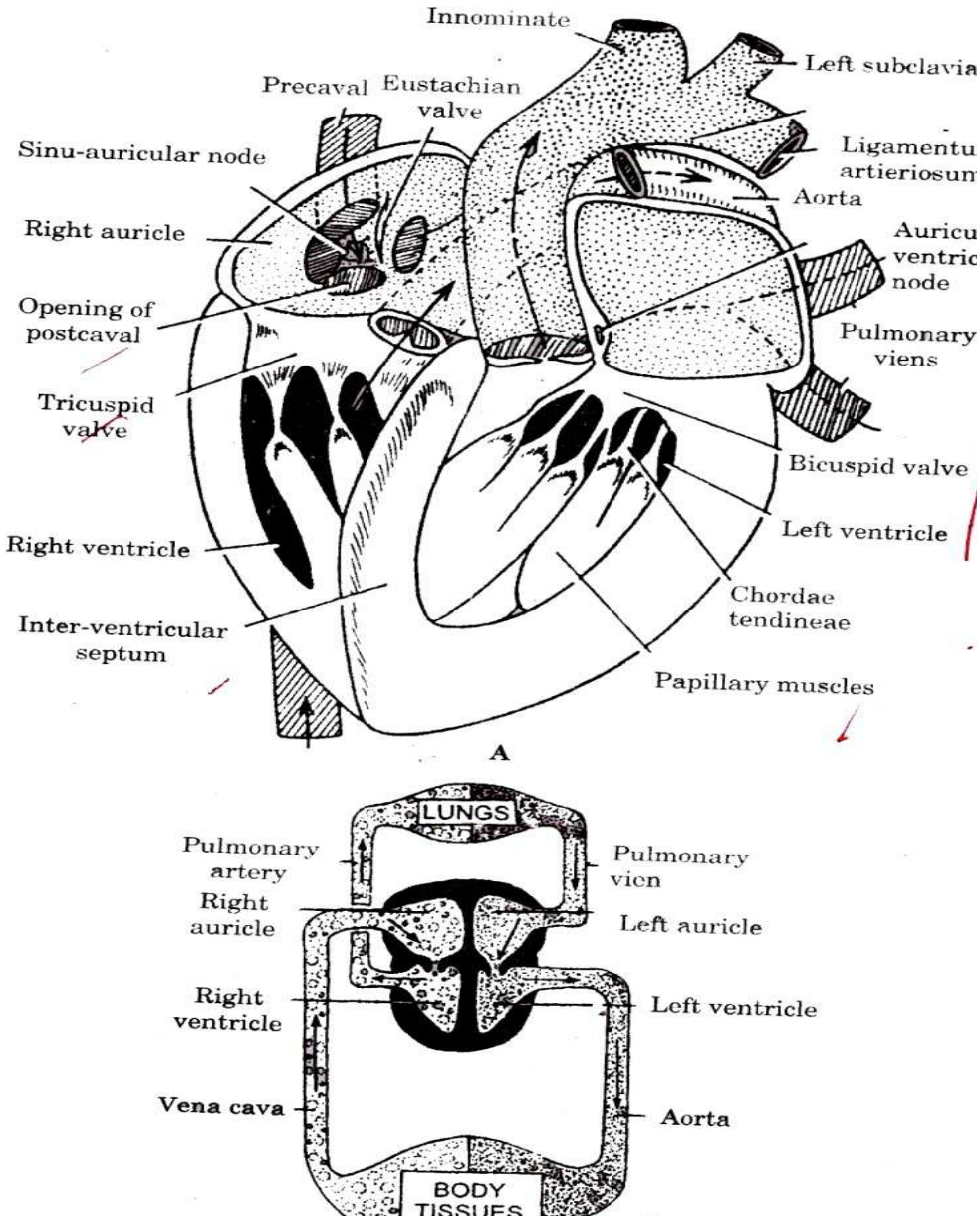


Fig: 3.10 Structure of heart of mammal(L.S) B)Course of circulation

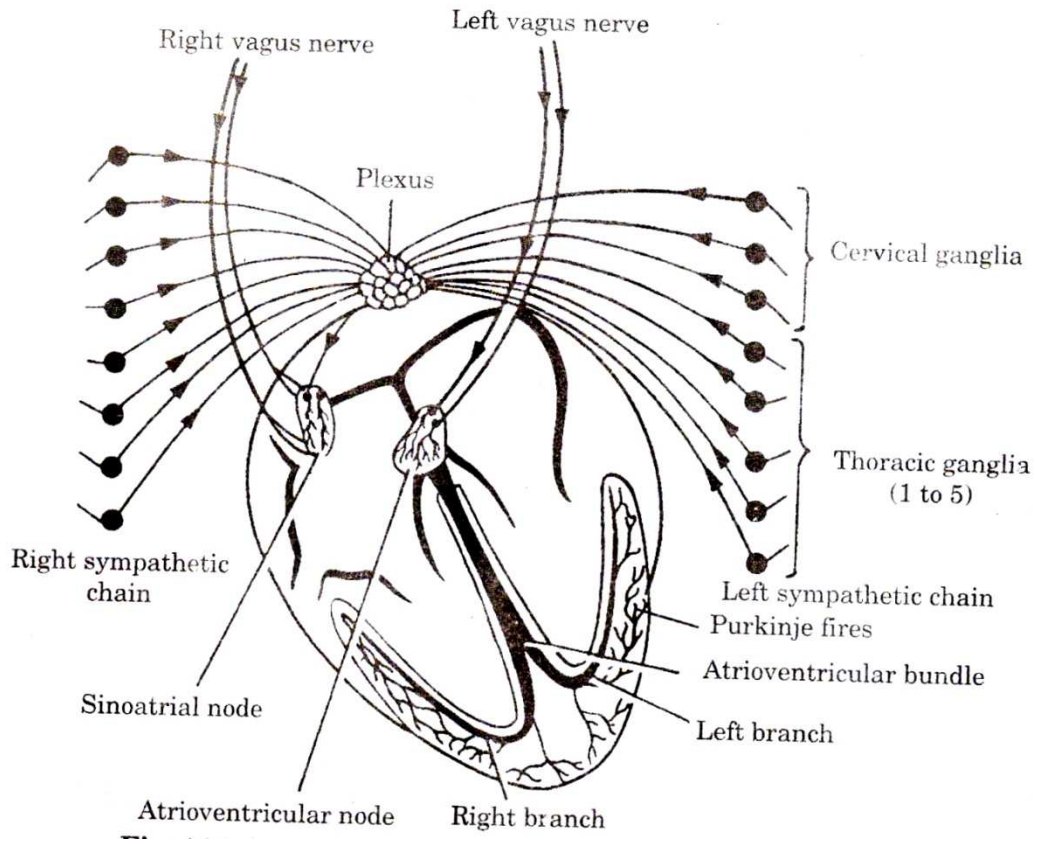


Fig:3.11 Conductive tissues and innervation of the heart

3.5.2 TYPES OF HEART

Morphologically there are four types of heart have been recognized by **Clark (**1927)

1) Pulsating heart

In annelids and Amphioxus, many blood vessels adopt a pulsating function by rhythmic peristalsis and the blood is thus, propelled through various parts of the body. However, in earthworm, the dorsal blood vessel shows about 15-20 peristaltic waves per minute at a speed of some 20mm per second.

2) Tubular heart: In Arhropods, the heart assumes the shape of a contractile tube divided into one to many chambers, each receiving blood from the heamocoel through paired ostia. Tubular hearts are provided with the pericardium and it gives out one or two larger vessels anteriorly .In insects ,the tubular heart is suspended and supported by alary muscles and ligaments, their rhythmic contraction together with closing of ostia forces the blood into the anterior aorta and to the body organs inturn.

3) Ampullar or accessory heart: In some invertebrates the blood vessels are modified to pump the blood through one or other organ or system of the body. In cephalopods such modifications form the branchial heart which serves to pump the blood into the ctenidia.In insects' ampullar heart are located at the base of antennae, wings and legs.

4) Chambered heart: In all vertebrates and in a few molluscs, the heart is made up of two to four chambers termed as auricles and ventricles. To regulate the flow of blood various types of valves are present between auricles and ventricles .and at the opening of various aorta which pour the blood into the auricles or which carry the blood from the ventricles to the lungs or different body organs.

Physiologically, heart in animals has been divided into two types depending upon the nature of control and mode of origin of heart beat.

1) Myogenic heart: Among vertebrates and ,the impulse for contraction of heart originate in the cardiac muscles fiber and is not brought about to the heart by the nerve fibers .Such heart has an auto rhythmic control and the wave of contraction originates at the sino-auricular node. Acetylcholine inhibits the heart beat of a myogenic heart. If the heat is removed from the body it continues to beat for some time. All vertebrates and mollusks have a myogenic heart.

2) Neurogenic heart: It is heart in which the beat originates by nervous stimulation or neurogenically i.e the contraction is initiated by nerve ganglion present on or around the heart. . Acetylcholine accelerates the heart beat of a neurogenic heart. If the heart is removed from the body it stops beating immediately. The crustaceans, insects like cockroaches, arachnids have a neurogenic heart.

3.5:3 CARDIAC CYCLES

The **cardiac cycle** is the performance of the human heart from the beginning of one heartbeat to the beginning of the next. It consists of two periods: one during which the heart muscle relaxes and refills with blood, called diastole (die-ASS-toe-lee), followed by a period of robust contraction and pumping of blood, dubbed systole (SIS-toe-lee). After emptying, the heart immediately relaxes and expands to receive another influx of blood returning from the lungs and other systems of the body—before again contracting to pump blood to the lungs and those systems. A normally performing heart must be fully expanded before it can efficiently pump again. Assuming a healthy heart and a typical rate of 70 to 75 beats per minute, each cardiac cycle, or heartbeat, takes about 0.8 second. Thus, cardiac cycle is inversely proportional to the heart rate. The important changes that are taking place during cardiac cycle are -

1. Mechanical changes: The contraction and relaxation of various chambers of the heart.

2. Electrical changes .in the form of generation and transmission of impulses. It is represented by ECG.

3. Volume changes inside the heart.

4. Pressure changes inside various chambers of the heart.

5. Opening and closing of the valves.

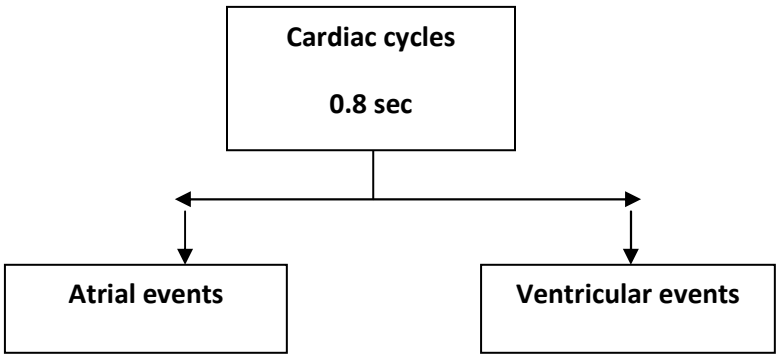
6. Heart sounds.

- **Origin of the heartbeat**

Each cardiac cycle begins by spontaneous generation of an action potential in sino-auricular node or the pace maker. The fibres of the S.A node have a resting potential of -50 to -60 mV while it is approx. -85 to -95mV in the cardiac muscles and -90 and -100 mV, in the special conductive fibres spreading down into the heart. The low resting potential is due to a natural leakage of the sodium ions across the membrane, and it is this leakage of the sodium ions which is solely responsible for the self excitation of the S-A node originating a heartbeat. When an

action potential is over, the process of **hyper polarization** begins in which the membrane potential is at the peak of negativity due to the out flux of positively charged potassium ions. **Depolarization** starts immediately after a brief resting potential in which the membranes becomes highly permeable to sodium ions. At this stage the membrane potential is about +20mV and this generates a new **hyperpolarization –depolarization** cycle. This process continues throughout the life providing a rhythmic excitation to the **S -A** node at a normal resting rate of 72 beats per minute.

Cardiac cycle is broadly divided into atrial event and ventricular events.



Atrial events: Duration: 0.8sec. It is divided into two phases. Atrial systole and atrial diastole **Atrial systole**: With the initiation of the heartbeat in the S.A node, the auricles contract. The pressure inside the auricles increases and volume decreases. The blood from the auricles is forced into the ventricle which is in relaxation at that moment. The duration of atrial systole is 0.1sec. **Atrial diastole**: Duration is 0.7second.During this period the two atria relax. The pressure decreases in the atria and volume increases.

Ventricular events: Duration is 0.8sec.It is divided into two parts. Ventricular systole and ventricular diastole during **ventricular systole** the ventricle contract Its .duration is 0.3 sec. It follows atrial contraction. Ventricular systole is again divided into two parts-

1. Isometric contraction phase (Duration is 0.05sec)

2. Ejection period (Duration is 0.25)

At the beginning of ventricular systole, pressure inside the ventricles increases. This makes the closure of A.V valves. This produces the first heart sound. A.V valves have just closed and semi-lunar valves are not yet opened. The ventricles contract as a closed chamber. No change occurs in the volume and length of the muscle fiber but only pressure increases so, they are called **isometric contraction phase**.

Ejection period: Duration is 0.25 sec.Now pressure inside the ventricles increases. This opens the semilunar valve. Blood from left ventricle is ejected into the aorta and blood from the right ventricle is ejected into the pulmonary arteries. Ejection period is again divided into two parts-

1. Maximum Ejection period (Duration is 0.11sec)

2. Reduced Ejection period (Duration is 0.14sec)

- **Ventricular Diastole:** Duration is 0.5sec**.** During this period the ventricles relax. Ventricular diastole is divided into three parts.

1. **Proto diastolic period** (Duration 0.04sec): During this phase ventricular pressure falls very rapidly. At the end of this phase semilunar valves close. This causes the second heart sound.

2. **Isometric phase or isovolumetric relaxation period** (Duration 0.08sec): As the semilunar valves just closed.The ventricle relaxes as a closed chamber. So no much change occur in the length of the muscle fibre and blood volume. Therefore, it is called **Isometric phase or isovolumetric relaxation period.** Pressure continues to decrease. Isovolumetric relaxation ends when pressure inside the ventricle falls below the atrial pressure and A.V valve opens.

3. Filling phase (Duration 0.38sec):A.V valves open and blood starts flowing from atria to ventricles. The mitral and tricuspid valves, also known as the atrioventricular, or AV valves, open during ventricular diastole to permit filling. Filling phase is again divided into three -

a) First rapid filling phase-0.1sec

b) Slow rapid filling phase or diastasis-0.18sec

c) Last rapid filling phase-0.1sec

During the first rapid filling phase blood rushes from atria through the A.V valves. This causes the vibration of A.V valves. This produces the **third heart sound**. After that flow of blood becomes slow. This phase is called as slow filling phase or **diastasis**. While during last rapid filling phase atria contract. Once again the flow of blood becomes turbulent. This causes vibration of the A.V valves. This produces the **fourth sound**.

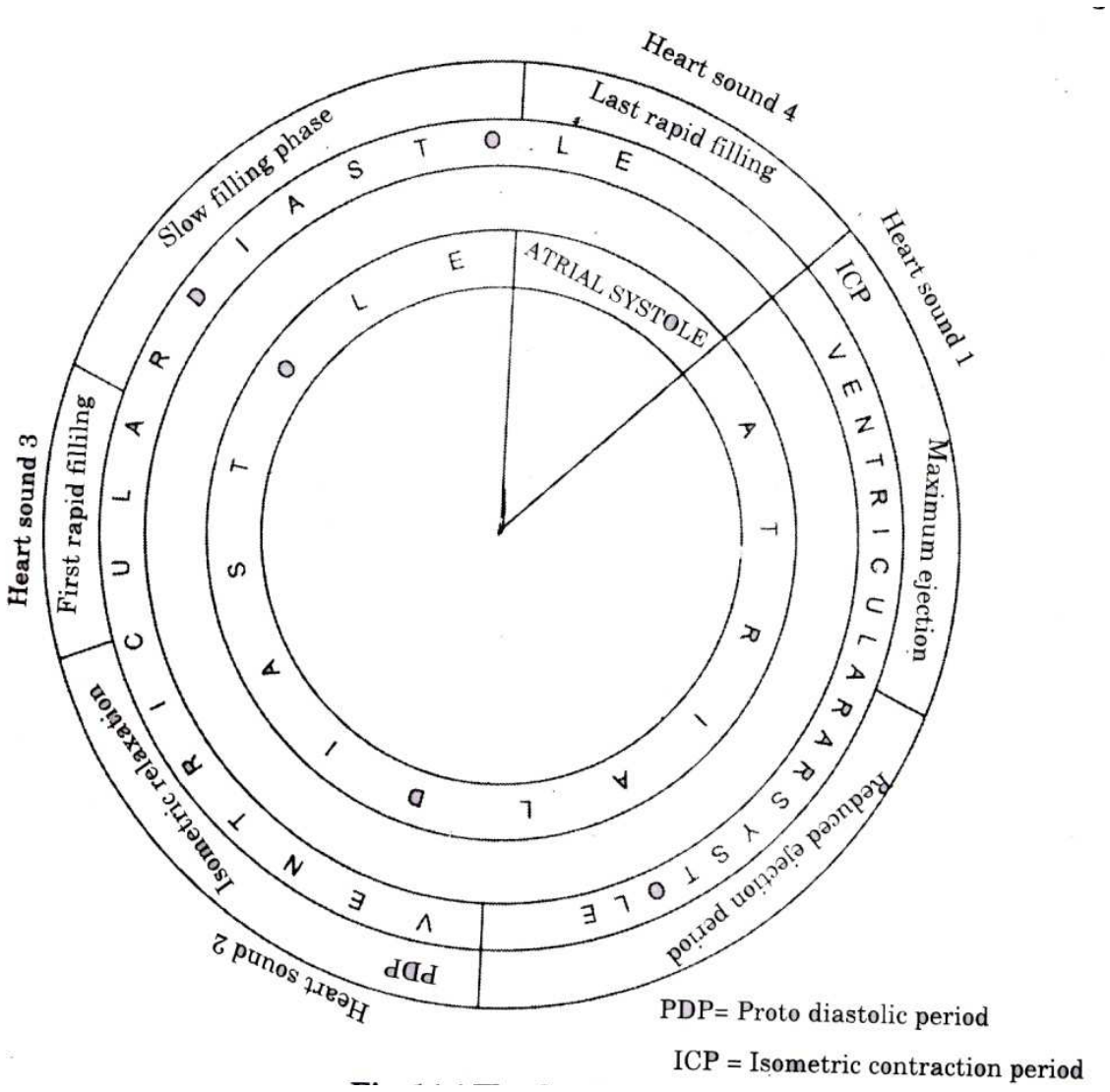


Fig:3.12 The cardiac cycle

- **Spread and conduction of impulses**

Impulses are generated at the S.A node. From the S.A node impulses are transmitted through the muscles of the atria and impulses reach both the right and left atria. The impulses are preferentially transmitted to the A.V node. Across the A.V node the velocity of impulse transmission is very slow. Therefore, it takes time to cross the impulse from atria to the ventricle, from the A.V node to the bundle of His. This delay is known as A.V nodal delay (0.1second).This delay ensures that ventricles start contraction only after atrial contraction. Impulses are transmitted through the branches of Bundle of His to the Purkinje fibres.

Velocity of conduction:

a) S.A node 0.05m/sec

b) A.V node 0.05m/sec

c) Atrium 1.0m/sec

d) Ventricle 1.0m/sec

e) Bundle of His 1.0m/sec

f) Purkinje fibre4.0m/sec

3.5.4 HEARTBEAT AND REGULATION OF HEART BEAT

The rate at which heart beats per minute.It varies among animals and generally depends upon the age, sex, body size, state of development. As the age advances (old age) heart rate increases. It is slightly higher in male than in female. It generally increases after exercise, emotions, after meal, and during pregnancy. It is lower in sluggish animals than in active worms. The body temperature is also influences the heart rate. In poikilothermic animals the rate of heartbeat increases two or three times per 10□ rise in temperature over a normal biological range.

Normal heart rate of man is 72/min. when the tonic effect parasympathetic and sympathetic nervous system are abolished by the administration of drugs heart rate increase to 100/minute

number of heart beats per minute varies among animals. While it decrease during sleep at rest for eg, in athletes at rest heart rate is lower than normal called "**Bradycardia**" around 60/min. But, when it increases about 100/min then it is called as **Tachycardia.**

3.5.5 REGULATION OF HEARTBEAT

The cardiac muscles exhibit the property of rhythmic automaticity and the rate of mammalian heart is ultimately determined by the pace maker. In life ,this inherent rhythm of the heart is under the influence of the integrated action of endocrine nervous and thermal reflexes and many other physical, chemical and physiological factors and so it needed to be corrected and regulated from moment to moment or even second to second. In general the working of heartbeat is regulated by the following mechanism-

1. Nervous regulation: The vertebrate heart is under the influence of two pair of nerves, arising in two different functional sets from the cardiac centers located in medulla oblongata and the spinal cord. These nerves are arranged in the inhibitory and acceleratory components and reach the heart through the parasympathetic and sympathetic nerve supply of the autonomic nervous system. These two sets of nerves affect the working of heart in two ways-

A) By changing the heart rate

b) By changing the strength of contraction of the heart

The inhibitory nerves forming the parasympathetic element are a pair of vagus nerve (X) which originates from the cardio-inhibitory centre located in the dorsal motor nucleus of vagus in the medulla. The nerve fibres from the right vagus end mainly around the S-A node while those from the left branch terminate chiefly near A-V node. Some of the post ganglionic nerve fibre also passes into the coronary plexuses of the auricles and the bundle of His.

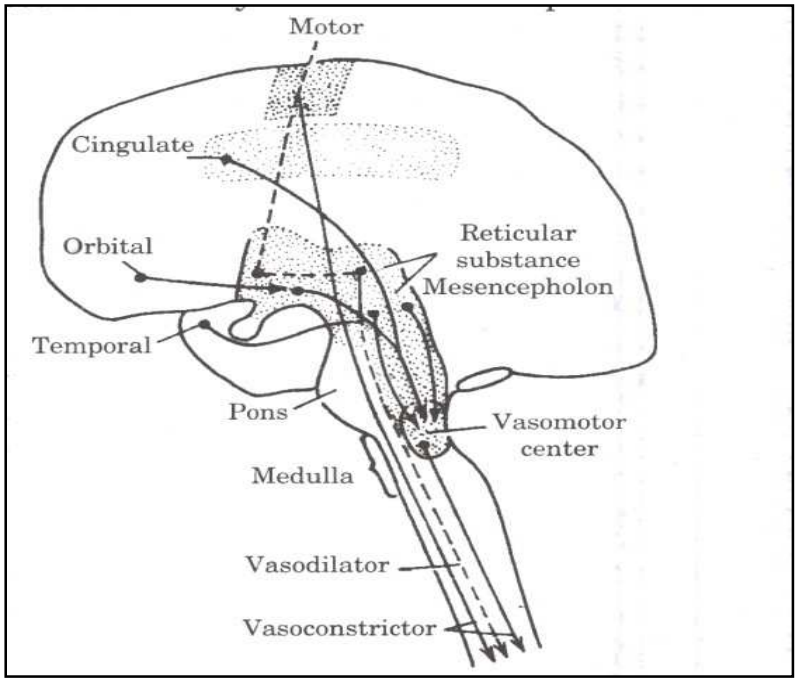


Fig:3.13 areas of the brain that play an important role in the nervous regulation of circulation

ii) The acceleratory nerves consist of sympathetic fibres which arise chiefly from the second and third thoracic segments of the spinal cord and pass into the dorsal roots of spinal nerves as myelinated nerve fibres. These acceleratory nerves form the cardio-accelerator fibres which arise from the lateral grey horns of the thoracic spinal cord and their preganglionic neurons a spinal cardio-accelerator centre. A similar cardio-accelerator centre is also located in the medulla oblongata which remains connected with the cardio-accelerator centre of the spinal cord through internuncial neurons (**Taylor, 1989**). Sympathetic stimulation causes essentially the opposite effects on the heart to those caused by the vagal stimulation as below,

Firstly, it increases the rate of S-A nodal discharge and secondly it increases the rate of conduction and excitation in the cardiac tissue. The accelerator nerves are known as adrenergic nerves as they release a substance sympathin (comprising hormones adrenaline and non-adrenaline) which increases the fibre permeability to sodium and calcium ions. In S-A node, increased sodium permeability causes a tendency for the resting membrane potential to diminish to the threshold limit which inturn leads to the self-excitation of the S-A node and thus increasing the heart rate as a consequence.

iii)The vasomotor centre: Located bilaterally in the reticulum of the **pons of medulla**, the **vasomotorcentre** transmits impulses downward through the spinal cord and sympathetic nerves and can increase the heart rate .The median area of the vasomotor centre transmits impulses through the vagus nerve and can decrease the heart rate through a mechanism described earlier.

2) **Hormonal regulation**: The two hormones involved in the regulation of the heart rate are -

i) Thyroxine: The rate of heart beat increases considerably under the influence of thyroid hormone. An increased rate of cellular metabolism under the influence of thyroxine causes a more rapid utilization of oxygen which inturn increases the cardiac output. Thyroxine also has a direct effect on the excitability of the cardiac muscles and thus increases the heart beat.

ii): **Adrenaline and non adrenaline**: These hormones are liberated into the circulating blood when the adrenal medulla is stimulated by the sympathetic nerve supply. The circulating adrenaline and non-adrenaline almost the same effect on the heart as those created by the direct nervous stimulation, except that these effects last about 10 times as long because they are removed from the blood quite slowly.

3) Other factors influencing the heart rate are-

i) **Inorganic ion**: Three cations- potassium, sodium, and calcium have a marked effect on the action potential transmission. The excess of potassium ions in the extracellular fluids causes the heart to dilate and the heart rate slows down if the potassium concentration elevates in the body fluids. An excess of sodium ions depresses the cardiac rhythm and hear rate but the excess of calcium ions causes effects almost exactly opposite to those of potassium and sodium ions.

ii) **Thermal regulation**: Increase in temperature increases the heart rate. Whenever metabolic rate increases, body temperature increases, heart rate increase, so cardiac output increases.So more O_2 is supplied. For every 1□ rise in temperature the heart rate increases by 10 beats.

iii) **pH and CO_2**: During increased metabolic activities the carbon di oxide level of the blood increases gradually. ThisCO_2 combines with the water of the blood forming carbonic acid and as

a result pH of the blood is lowered thus an acidic pH accelerates the heart rate while the alkaline pH slows down the activity of the heart.

iv) **Exercise:** Putting up heavy exercise over long period of months causes hypertrophy of the cardiac muscles and enlargement of the ventricles. Thus an athlete heart has a greatly enhanced .muscle –power and a more effective pumping strength.

3.5.5 CARDIAC OUTPUT

Cardiac output is defined as amount of blood pumped out of each ventricle perminute. Cardiac output is expressed in two forms

1. **Stroke volume**: It is defined as the amount of blood pumped out of each ventricle per beat or contraction. Normal value is 70ml. It depends largely on the volume of venous blood returning to the heart, size of the heart and its contractile strength.

2. **Minute volumes:** It is defined as the amount of blood pumped out of each ventricle per minute. In man, the heart beats about 70-72times/min. Thus the stroke volume multiplied by the heart rate gives the total amount of blood pumped out by the heart each minute. This volume is termed as the cardiac output and can be represented as follows-

Cardiac output=Heart rate x stroke volume

The cardiac output in a normal young man at resting condition should be C.O=72 x 75=5400ml.

The age of an individual, his posture, metabolism and exercise affect the cardiac output in one way or the other way. One of the major factors determining the amount of blood pumped by the heart each minute is the rate of blood flow into the heart from the veins, which is known as **venous** return. The venous return varies greatly from moment to moment, sometimes the input falls to as low as 2-3litrs/min and other times it rises to as high as 25litres or even more. The heart adapts itself to this widely varying range of venous input and this intrinsic property of the heart is termed as **Frank-Starling law of heart.** It is named after two physiologists, **Otto Frank (1895**) and **Starling (1910**), it states that the greater the heart is filled during diastole, the greater

will be the quantity of blood pumped into the aortas. In other words, as a larger volume of blood flows in to the ventricle, the blood will stretch the walls of the heart causing a greater expansion during diastole, which in turn increases the force of contraction and thus the quantity of blood that is pumps into the aorta during systole. The increased stroke volume stretches the ventricular wall causing the cardiac muscle to contract more forcefully. The heart plays a permissive role in regulation of the cardiac output. Under normal resting conditions the cardiac output ranges from 5 to 5.5litres/minute. The unstimulated heart can pump out a maximum of about 15litres/min., but if more blood flows into the right auricle, the heart cannot pump it without cardiac stimulation.

- ***Heart sounds***

The sound produced by the heart is known as **heart sounds**. These sounds are produced due to the vibration of the leaflets of valves. It may be due to three reasons-such as due to sudden closure of the valves, turbulent flow of the blood, contraction of the chambers of the heart. There are four heart sounds. Of this only I and II sound can be heard by the stethoscope while, III and IV sound are not commonly heard but they can be recorded in a phonocardiogram **Lear (1979**).

I Heartsound or S_1

- **Cause:** Vibration of the A.V valves due to the sudden closure
- **Occurrence**: It takes place at the beginning of ventricular systole.e.,Isovolumetric contraction period
- It coincides with R wave of ECG
- **Nature of the sound**:LUBB-DUBB
- **Duration**:long,0.14sec
- **Frequenncy**:25-45cycle/sec
- It can be best heard at Mitral valve

II sound or S_2

- **Cause:** Vibration of the semilunar valves due to the sudden closure
- **Occurrence:** It takes place at the end of prodiastole

- It coincides with T wave of ECG
- **Nature of the sound**:'DUP'
- **Duration:long**,0.11sec
- **Frequenncy**:50 cycle/sec
- It can be best heard at pulmonary area and tricuspid area.

III heart sound

- **Cause:** Vibration of the A.V valves due to the turbulent flow of blood
- **Occurrence**: It takes place during I^{st} rapid filling phase. 3^{rd} heart sound can only be heard in healthy young adults.
- **Frequency:** very low that cannot be picked up with a stethoscope

IV heart sound

- **Cause:** Vibration of the A.V valves due to the turbulent flow of blood
- **Occurrence:** It happens during the last rapid filling phase or during the systole.
- **Frequenncy**:20 cycle/sec
- **Duration:**0.5sec

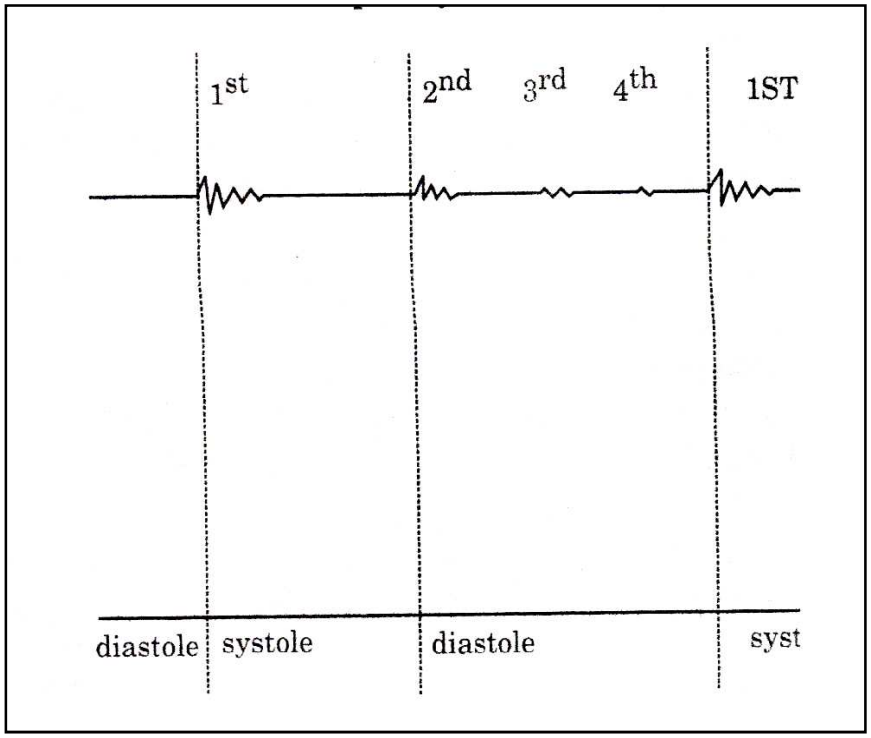


Fig: 3.14 A phonocardiogram of normal heart

3.5.6 ELECTROCARDIOGRAPHY (ECG OR EKG)

It is the process of recording the electrical activity of the heart over a period of time using electrodes placed on the skin. These electrodes detect the tiny electrical changes on the skin that arise from the heart muscle's electrophysiological system depolarizing and repolarizing during each heartbeat. It is a very commonly performed cardiology test.

Electrical activity of heart was first recorded by **Waller (1906). Willem Einthovan (1923)** Nobel prize,1924,first of all designed an instrument for recording the electrical activity of excited cardiac muscles in the form of an **Electrocardiography**. A pen recorder with a cathode ray tube was later added to the instrument for clinical recording of ECG. It may also be connected to an oscilloscope for displaying the ECG on a TV type monitor.

Forms of ECG: Electrocardiogram may be taken in two forms:

Resting ECG: When a patient under observation is in a lying position.

Stress ECG:It is being recorded when the patient under observation is put to a mild form of exercise which may be increased gradually.

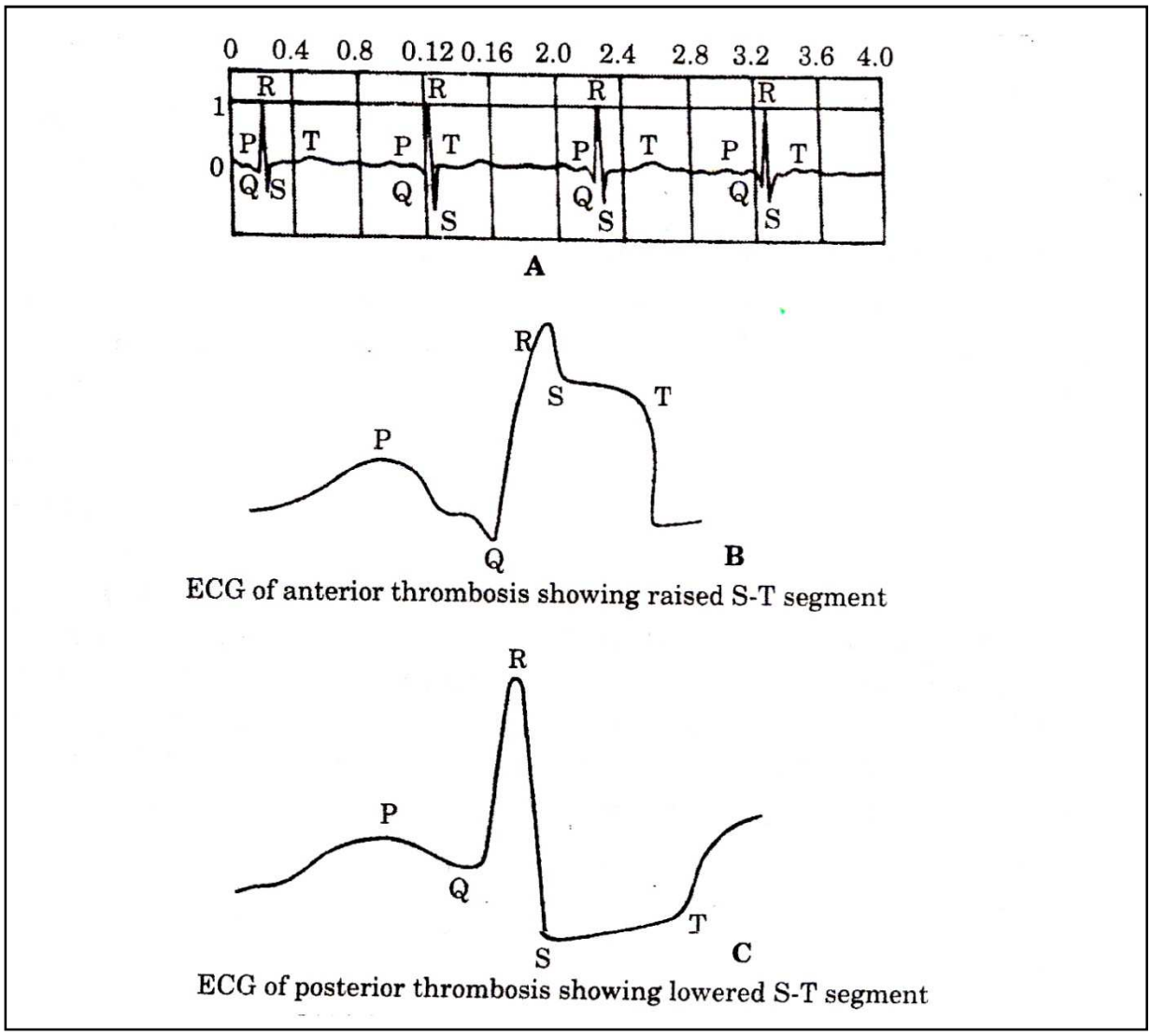


Fig: 3.15 A) Normal ECG B) Abnormal ECG during anterior thrombosis C) Abnormal ECG during posterior thrombosis

- **Stress ECG**

Observations: The rhythmic beating of heart produces some electrical variations across the surroundings tissue spreading to chest, arms and other parts of the body. The ECG of a normal person recorded on a strip of graph paper shows X-axis representing time and Y axis denotes amplitude. It shows the following waves as-

P-wave: It represents the impulses of contraction generated at the SA node causing depolarization of atria. It is Dome shaped and its duration is 0.1sec.

QRS is a complex and represents the depolarization of ventricles.

RS wave and **ST** interval displays ventricular systole lasting for 0.3sec

T-Wave: It represents the ventricles. Diastole involving 0.5sec

In a normal ECG **P, R, T** waves are above the base line of graph and are termed as positive waves. The **Q** and **S** wave of ECG run below the base line are called negative waves.

Intervals

P-P or **P-R** interval between two successive P- waves or two-R waves. Duration is 0.8sec.

P-Q interval or P-R interval: interval between the beginnings of Pwave to the beginning of Q wave. Duration is 0.12 to 0.16sec.

Thus ECG serves as an important diagnostic tool detecting a number of cardiac diseases. It helps in recording almost all forms of abnormal movements of various sections of the heart including the functions of heart valves During each heartbeat, a healthy heart has an orderly progression of depolarization that starts with pacemaker cells in the sinoatrial node, spreads out through the atrium, passes through the atrioventricular node down into the bundle of His and into the Purkinje fibers, spreading down and to the left throughout the ventricles. This orderly pattern of depolarization gives rise to the characteristic ECG tracing.

An ECG conveys a large amount of information about the structure of the heart and the function of its electrical conduction system. ECG can be used to measure the rate and rhythm of heartbeats, the size and position of the heart chambers, the presence of any damage to the heart's muscle cells or conduction system, the effects of cardiac drugs, and the function of implanted pacemaker.

3.5.7 BLOOD PRESSURE (BP)

It may be defined as the pressure that the blood exerts against the walls of its containing vessels. "Blood pressure" usually refers to the pressure in large arteries of the systemic circulation. Blood pressure is usually expressed in terms of the systolic pressure (maximum during one heart beat) over diastolic pressure (minimum in between two heart beats) and is measured in millimeters of mercury (**mmHg**), above the surrounding atmospheric pressure (considered to be zero for convenience).

It is one of the vital signs, along with respiratory rate, heart rate, oxygen saturation, and body temperature. Normal resting blood pressure in an adult is approximately 120 millimetres of mercury (**16 kPa**) systolic, and 80 millimetres of mercury (**11 kPa**) diastolic, abbreviated "120/80 mmHg". Hence, the difference between two pressures is 80mmHg.The difference between two pressures, which is about 40 mm Hg is termed as the **pulse pressure.**

- **Method for measuring blood pressure**

Traditionally, blood pressure was measured non-invasively using a mercury manometer and this is still generally considered the gold standard. More recently other semi-automated methods have become common, largely due to concerns about potential mercury toxicity although cost and ease of use have also influenced this trend Early alternatives to mercury sphygmomanometers were often inaccurate, but more modern validated devices have similar accuracy to mercury devices.

The first attempt to measure the arterial blood pressure was made by Stephen Hales in 1732 in a mare. The arterial blood pressure of man was first measured by **Vaivre (1856**) by Ludwig's mercury manometer. Now-a-days blood pressure is measured by the help of Sphygmomanometer

(**Riva-Rocci, 1896**) using auscultatory method by using blood pressure cuff around the upper arm by locating the **Korotkoff (1905**) sounds.

Sphygmomanomater is an instrument consists of an inflatable cloth, covering a flat rubber bag 12cm wide, which fits around the upper arm and is held in position by wrapping extension of the cloth like a bandage. It is known as **cuff** or **armlet**. The cavity of the rubber bag is connected by a long rubber tube to a mercury manometer and by another tube with a pressure bulb or pump. The mercury manometer is considered as a standard method. By pumping the pressure bulb the rubber bag can be inflated to any desired pressure. On the tube with the pressure bulb there is a needle valve which can be opened gradually to allow the air to escape and the pressure in the bag is reduced. The top panel of the instrument marked in figure to read the pressures from 0-3000mm Hg.

Fig: 3.16 Sphygmomanomater

- **How to use Sphygmomanometer**

Nowadays, BP is measured indirectly by **auscultatory** method. At first wrap the armlet of sphygmomanometer around the upper arm and rest the arm at about the level of the heart.Inflate the armlet and place the chest piece of the stethoscope over the position of the branchial artery

and listen to the sound (auscultate). Open the valve gradually to reduce compression until faint tapping sounds produced by successive pulse –waves, are first heard. This is the systolic blood pressure. Continue to listen the sound which becomes louder and suddenly becomes soft and muffled. This point is the diastolic blood pressure**.**

Blood pressure is influenced by cardiac output, total peripheral resistance and arterial stiffness and varies depending on situation, emotional state, activity, and relative health/disease states. In the short term it is regulated by **baroreceptors** which act via the brain to influence nervous and endocrine systems. BP is more in adults than in children. It is higher in people living at higher altitude. BP increases after a meal. It is maximum in the morning and minimum in late evening. Systolic BP rises during exercise and during emotional expressions.

Blood pressure that is low due to a disease state is called **hypotension**, and pressure that is consistently high is **hypertension**. Both have many causes and may be of sudden onset or of long duration. Long term hypertension is a risk factor for many diseases, including heart disease, stroke and kidney failure. There is an increased incidence of athero-sclerosis and myocardial infarction even when there is no ventricular enlargement. There are chances of thrombosis in cerebral hemorrhage. Hypertension can be treated but not curable risk of long term hypertension is more common than long term hypotension. Long term hypertension often goes undetected because of infrequent monitoring and the absence of symptoms.

3.6 HOMEOSTASIS

The mechanisms within the circulatory system ensure that every cell maintains a constant internal environment. The circulation of blood is vital in maintaining homeostasis, which is the regulation of the internal conditions of the body; Blood carries food to cells and removes waste products.

The circulatory system maintains homeostasis by the controlled and continuous flow of blood that reaches each cell in the body; the circulatory system delivers oxygen and nutrients in the blood so that they can pass into fluids surrounding the cells. There are controlling mechanisms within the system to ensure that specific body areas receive a supply of blood according to their

needs so that they can maintain their internal equilibrium. The circulatory system also facilitates the removal of waste products, carrying them away in plasma. There are so many ways by which our cardiovascular system can maintain homeostasis. To counteract excessive heat, our capillaries dilate to allow heat from the blood to radiate off of the skin and out of the body, by lowering body temperature by preventing overheating.

2) Heart Rates: During exercise our cells use oxygen at a much higher level than if you were simply walking. Therefore, to maintain homeostasis and to allow our body to continue exercising, our heart rate speeds up to compensate. By speeding up our heart rate, our heart beats faster and pumps more blood to the cells that need the oxygen to continue to exercise. That is the way how the homeostasis is maintained.

3) The Heart & the Kidneys: Without the heart, our kidneys wouldn't be able to filter wastes out of the body. The blood pressure provided by the heart allows the kidneys to do their job, eliminating wastes from out body. All in all, our body has to work together to maintain homeostasis.

3.7 SUMMARY:

The circulatory system comprises the heart, veins, capillaries and arteries. The system moves oxygenated blood in a continuous and controlled way from the lungs and heart so that blood reaches every cell. Blood travels through a network of vessels that include capillaries that permeate every tissue of the body. Once depleted of oxygen, the blood returns to the lungs and heart.

The cardiovascular system consists of the heart, which is an anatomical pump, with its intricate conduits (arteries, veins, and capillaries) that traverse the whole human body carrying blood. The blood contains oxygen, nutrients, wastes, and immune and other functional cells that help provide for homeostasis and basic functions of human cells and organs.The pumping action of the heart usually maintains a balance between cardiac output and venous return. Cardiac output (CO) is the amount of blood pumped out by each ventricle in one minute. The normal adult

blood volume is 5 liters (a little over 1 gallon) and it usually passes through the heart once a minute. Note that cardiac output varies with the demands of the body.

The cardiac cycle refers to events that occur during one heart beat and is split into ventricular systole (contraction/ejection phase) and diastole (relaxation/filling phase). A normal heart rate is approximately 72 beats/minute, and the cardiac cycle spreads over 0.8 seconds. The heart sounds transmitted are due to closing of heart valves, and abnormal heart sounds, called murmurs, usually represent valve incompetency or abnormalities.

3.8 GLOSSARY

Aorta: The major blood vessel that carries blood away from the heart to the rest of the body.

Atria: The two chambers at the top of the heart are called the atria.

Atrialsepta: Ahole in the heart wall (called the septum) that separates the left atrium and the right atrium.

Atrium: The two upper chambers of the heart are called the atria.

Blood pressure: It shows how hard the heart is pumping to move blood through the body.

Bundle of His: Conducting tissue within the interventricular septum of the mammalian heart

Blood vessels: The vessels that carry blood

Cardiac impulse: Motion caused by rapid increase in tension of ventricle

Cardiac output: The total volume of the blood pumped by the heart per unit of time.

Coagulation: Curdling or clotting

Diastole: Relaxation of the heart

Electrocardiogram: The test that records the heart's electrical activity.

Endocardium: The internal lining of the heart.

Epicardium: The external covering of the heart wall

Hypertension: The other word for high blood pressure.

Left ventricle: The left ventricle is one of the four chambers of the heart. It pumps oxygen-rich blood out to the rest of the body.

Mitralvalve: The mitral valve lets blood flow from the left atrium to the left ventricles.

Myocardium: Heart muscle

Pulmonary: Lungs or related to breathing.

Purkinje fibres: modified cardiac muscle

Red blood cells: Cells carrying oxygen.

Right ventricle: It pumps deoxygenated blood through the pulmonic valve into the lungs.

Septum: A thick wall of muscle that divides the heart into left and right sides of the heart.

Stethoscope: An instrument to hear the heartbeat and other sounds that the inside of the body makes.

Systole: Contraction of the heart.

Ticuspidvalve: The tricuspid valve lets blood flow from the right atria to the right ventricle.

Valve: A valve lets something in and keeps it there by closing, like a door.

Ventricles: The two chambers at the bottom of the heart are called the ventricles. The heart has a left ventricle and a right ventricle.

White blood cells: White blood cells are part of the germ-fighting immune system. They are like little warriors floating around in your blood waiting to attack invaders, like viruses and

3.9 SELF ASSESSMENT QUESTIONS

Q1.What is coagulation or clotting of blood? What are the various theories of blood coagulation? Describe the process of blood coagulation.

Q2. Describe the structure of blood in detail.

Q3. How many types of WBCs are found in man? Describe the structure and function of each in detail.

Q4.What is the role of blood in the defense mechanism of body? Write in detail.

Q5. Describe the enzyme cascade theory of blood clotting in detail with the help of flow charts.

Q6. List the factors involved in the process of blood clotting along with their sources.

Q7. Write note on the composition of blood plasma.

Q8. Differentiate between myogenic and neurogenic heart.

Q9. Describe the mechanism of conduction and origin of heart beat.

Q10.Describe the regulation and control of heart beat.

Q11. Write note on the special conductive tissue of the heart.

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3.12 TERMINAL QUESTIONS/ANSWERS

1. Main Plasma protein is:
   (a) Albumin
   (b) Gamma globulin
   (c) Globulin
   (d) Fibrinogen

2. Largest blood cells are:
   (a) Erythrocytes
   (b) Neutrophils
   (c) Basophils
   (d) Monocytes

3. Which of the following cells are involved in phagocytosis:
   (a) Erythorocytes
   (b) Neutrophils
   (c) Basophils
   (d) Lymphocytes

4. Which blood cells produce antibodies:
    (a) Erythrocytes
    (b) Neutrophils
    (c) Basophils
    (d) Lymphocytes

5. Which blood cells produce antibodies:
    (a) Ig E
    (b) Ig A
    (c) Ig M
    (d) Ig G

6. Most abundant antibody in our body is:
    (a) Injured tissues
    (b) Blood platelets
    (c) WBCs
    (d) Both a and b

7. Enzyme cascade theory of blood coagulation was given by:
    (a) Howell
    (b) Best and Taylor
    (c) Macfarlane
    (d) Davie and Ratnoff

8. During clotting of blood, clot formation is due to:
    (a) Conversion of prothrombin into thrombin
    (b) Conversion of fibrin into fibrinogen
    (c) Both of these
    (d) None of these

9. Which of the following antibody is also called secretory anti body:
    (a) Ig E
    (b) Ig a
    (c) Ig M
    (d) Ig G

10. Rouleaux formation is:
    (a) Agglutination of blood corpuscles
    (b) Piling up of blood corpuscles
    (c) Piling up of red blood corpuscles
    (d) Clotting of blood platelets

11. Structural protein of erythrocytes is:
    (a) Haemoglobin
    (b) Globulin
    (c) Stromatin
    (d) Globin chains

12. Blood helps in:
    (a) Homeostasis
    (b) Homeostasis
    (c) Temperature regulation
    (d) both a and b

13. In origin, vertebrate heart is:

    (a) Ectodermal
    (b) Mesodermal
    (c) Endodermal
    (d) Mesendodermal

14. Normal human blood pressure is:

    (a)120/100mm/Hg
    (b) 120/80mm/Hg
    (c) 80/120mmHg
    (d)100/100mmHg

15. Pace maker of heart is:

    (a) SA Node
    (b) AV Node
    (c) Bundle of His
    (d) Purkinje fibres

16. The heart sound “dub” is produced when:

    (a) Mitral valve opens
    (b) Mitral valve close
    (c) Semilunar valve at the base of aorta close
    (d) Tricuspids open

17. In ECG, the P-Q interval represents-

(a) Ventricular systole
(b) Ventricular diastole
(c) Auricular systole
(d) Auricular diastole

18. In man, the stroke volume of heart is:

(a) 50ml
(b) 60ml
(c) 70ml
(d) 100ml

19. Sphygmomanometer serves to measure:

(a) Heart rate
(b) Pulse rate
(c) Blood pressure
(d) Blood volume

20. Normal diastolic pressure in man is about

(a) 80mm/Hg
(b)100mm/Hg
(c)120mm/Hg
(d)140mm/Hg

Answers

1.	(a)	2.	(d)	3.	(b)	4.	(d)	5.	(d)
6.	(d)	7.	(c)	8.	(d)	9.	(b)	10.	(c)
11.	(c)	12.	(d)	13.	(b)	14.	(b)	15.	(a)
16.	(c)	17.	(c)	18.	(c)	19.	(c)	20.	(a)

UNIT 4: EXCRETORY SYSTEM

CONTENTS

4.1 OBJECTIVES

After reading this unit you should be able to-

- Describe the bi-products of excretion.
- Give an account of the structure and function of a typical kidney.
- Explain the type of nephrons.
- Describe the mechanism of urine formation.
- Explain the renal physiology of excretory system.
- Explain the Counter- Current mechanism.
- Give an account of homeostasis by kidney.
- List the mode of excretion of nitrogenous waste in animals.
- Give an account of the role of hormones in urine formation.
- Explain the mechanism by which urinary bladder empties itself by micturition.

4.2 INTRODUCTION

A number of byproducts are produced in the animal body as a result of carbohydrates, protein and fat metabolism. Some of these may be fatal .therefore they are either to be eliminated or are to be converted into less toxic substances. Thus, the removal of such waste products becomes necessary.

On the other handsome of the metabolic by product perform useful functions in the body for example water, is a metabolic byproduct and is excreted out in large amount as urine. In some other forms, metabolic water is the only available source of water, so it is rigorously conserved. Similarly carbon dioxide serves as an important component in the synthetic and regulatory mechanism in animals and plants. The same is true with urea which is one of the main excretory end products in a large number of animals but discharges some useful physiological functions in many other forms. In a healthy man the normal blood urea level is approx. 0.01to 0.04percent and if it rises to about 0.05percent, a serious pathological state of uremia develops.

Excretion has an important role in maintaining homeostasis in the body. If excretory system any how fails, waste products will accumulate in the body, causing disturbance in osmoregulation, ionic regulation, and acid- base balance and finally death may occur.

4.3 EXCRETORY SYSTEM

The following steps are involved in the excretory system.

4.3.1 STRUCTURE OF VERTEBRATE KIDNEY

The main excretory organs of vertebrates are kidney (fig 4.1). Kidneys are mesodermal in origin. Each adult kidney is composed of a number of structural and functional units called nephronsor, nephric tubules or the uriniferous tubules. Basically there are three types of kidneys met within the vertebrates.

i) **Pronephros**: It is the embryonic kidney of all vertebrates except *Myxine* and *Belladona*, it is the head kidney that remains functional in the larvae of cyclostomes, fishes and amphibians but later degenerates

ii) **Mesonephros**: It is the opisthonephric kidney of adult cyclostomes,fishes and amphibians and also serves to be the embryonic excretory organ in amniotes.

iii) **Metanephros**: It is the functional kidney of the adult, reptiles, birds, mammals and man. The detailed study of metanephric kidney is discussed below-

In man, the two kidneys (fig 4.1) lie behind the peritoneum on either sides of the vertebral column extending from 12^{th} thoracic to 3^{rd}lumbar vertebrae. In man and most mammals the left kidney is usually a little higher than the right kidney, reaching the level of the eleventh rib. In healthy man, each dark red bean shaped kidney weighs between 120-170 gms and measures from 11-13 cm in length. Each kidney is covered by a layer of fibrous connective tissue, called **renal capsule**.

The physiological anatomy (fig 4.2) reveals that each kidney comprises about 1.2 million **nephric tubules** or **nephrons**, functional unit of kidney. Each nephron is 4–5 cm. long. It is divisible into an outer granular **cortex** and inner paler **medulla**. **Hilus** is the inner medial border of the kidney which is somewhat concave and has an indentation through which passes the renal arteries, veins, nerves and the lymphatics, the **pelvis** or the **ureter**. The funnel shaped upper end

of pelvis is formed by the joining together of about three or four major calyces each of which is inturn made up of several short branches or **minor calyces**. The medulla of kidney comprises of some ten to fifteen pyramids which project into the calyces and separated by the **renal columns of Bertini**.

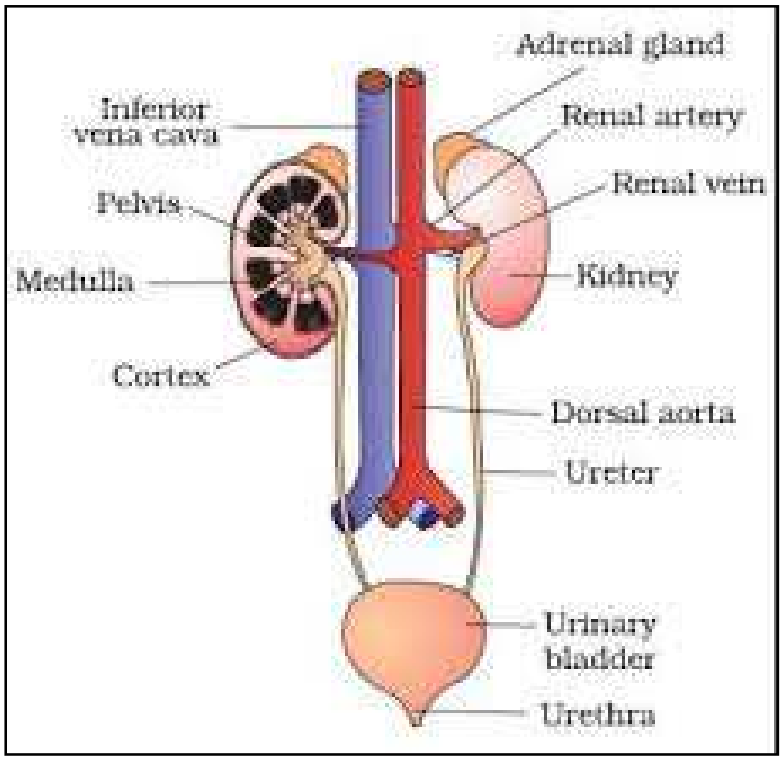

Fig. 4.1: The human urinary system

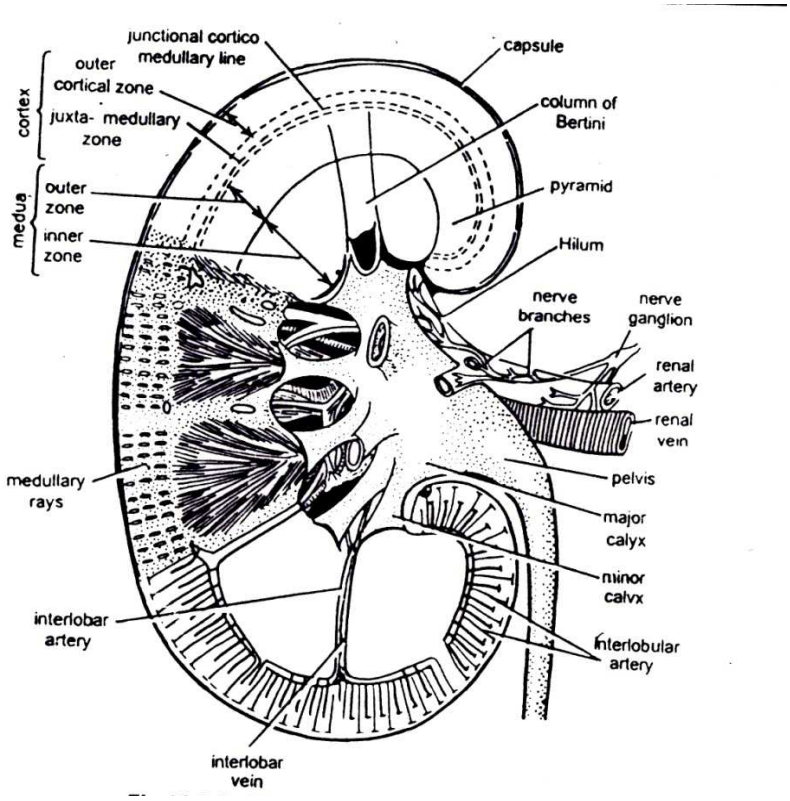

Fig. 4.2: L.S of Mammalian kidney showing internal anatomy

- **Structure of single nephron**

The renal tubule or nephron was first described by **Marcello Maiphigi (1666)** and later by **Bowman (1842).** Approximately 2.2million nephrons are present in both kidneys (i.e., 1.1 million in each kidney). In an adult person, a single nephron measures about 5-6 cm and is divisible into two parts (fig 4.3).

1) Malpighian or renal corpuscle

2) Renal tubule

1) Malpighian or renal corpuscle: Each nephric tubule begins as ablind dilated cup shaped sac called **Bowman's capsule**. It is double layered and situated in the cortex of kidney. The

Bowman's capsule is invaginated by a tuft of some 40-50capillary loops, each covered by special epithelial cells known as **Podocytes**. Podocytes have slit pores through which ultra filtration occurs and forms the **Glomerulus**. The glomerular capillary tuft surrounded by its capsule is termed as **Malpighian corpuscle** .Blood enters the capsule through **afferent renal arteriole** and after passing through glomerular capillary network it is collected by the **efferent renal arteriole**.

2) Renal tubule: The long portion of the nephron following the Bowman's capsule is termed as renal tubule. It is divided into the following regions-

1) The proximal convoluted tubule,
2) The loop of Henle (Descending limb and ascending limb)
3) The distal convoluted tubule
4) Collecting duct

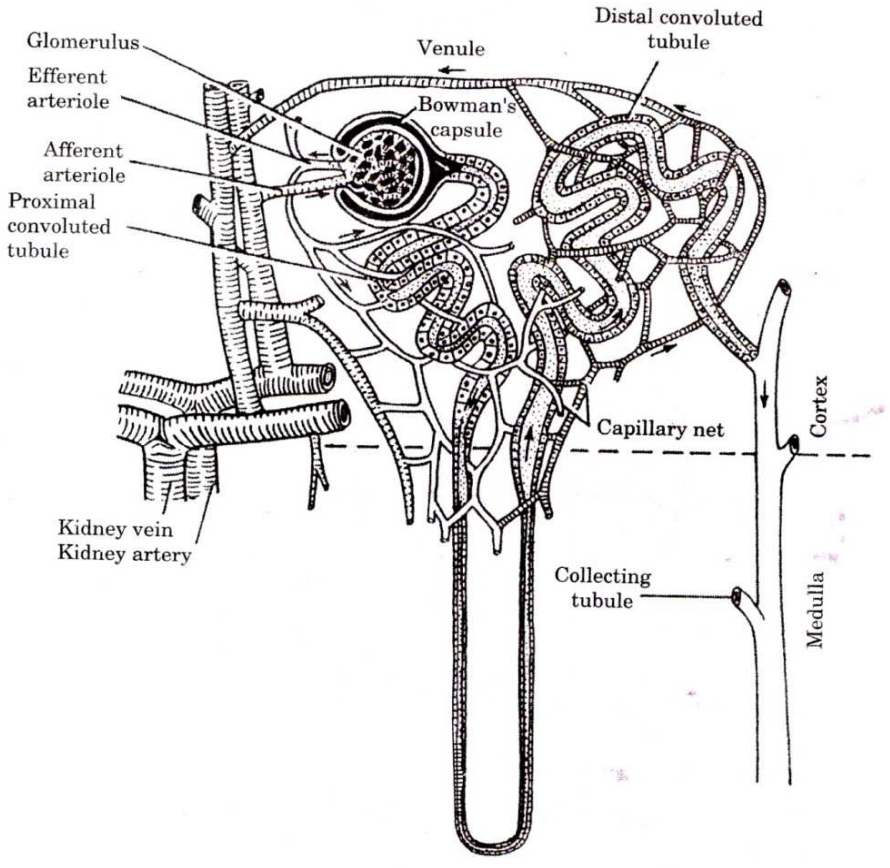

Fig 4.3: Structure of single kidney tubule and its blood vessels

- **PCT:** The proximal convoluted tubule lumen of which is continuous with Bowman's capsule is about 12-24mm in length lies in the cortex of kidney. It consists of large columnar cells with a brush border produced into numerous microvilli of 1 to 4mμ in size.

- **The loop of Henle:** It is a hair pin-shapedpart of nephron after PCT. The loop of Henleconsists of one descending limb and one ascending limb Descending limb of loop of Henle is permeable for water and is impermeable for salts while ascending limb of loop of Henle is impermeable for water. Ascending limb of loop of Henle is called diluting segment of nephron.

- **DCT:** The next part of nephron that is again highly convoluted is, called distal convolutedtubule (DCT). It lies in renal cortex. It is made up of cuboidal epithelial cells but have few scattered microvilli. DCT opens into collecting tubule.

➢ **Collecting tubule** (CT): All collecting tubules receive many functional tubules from other nephrons and finally open into the renal pelvis as the papillary duct of **Bertinii.**

❖ **Types of nephrons in mammalian kidney**

a) Cortical Nephrons

The nephrons situated in cortical region are, called cortical nephrons. In cortical nephrons, loop of Henle is short. In a kidney 15 to 35% nephrons are cortical nephrons.

b) Juxtamedullary nephrons

Nephrons situated near medulla part are, called **juxtamedullary nephrons**It is formed by the DCT and glomerular afferent arteriole.It is so named because it lies next (juxta)to the glomerular. In juxtamedullary nephrons, loop of Henle is long and parallel blood vessels, called **vasa rectae** are present. In a kidney 65 to 85% nephrons are juxtamedullary nephrons. It works only in condition of stress (fig. 4.4).

It consists of three types of cells.

1**) The macula densa**, apart of the distal convulated tubule of the same nephron. It act as a chemoreceptor and are stimulated by decreased NaCl concentration and thereby cause release of rennin.

2) **Juxtaglomerular cells** or granular cells which secrete rennin. These cells are smooth muscle cells of afferent arteriole which supply blood to the glomerulus. They are baroreceptors and respond to changes in the pressure gradient. They are innervated by sympathetic nerves.

3)**. Extraglomerular mesangial cells (Laci's cells)** These cells are located at the junction between afferent and efferent arterioles. They are contractile and play a role in regulation of GFR.

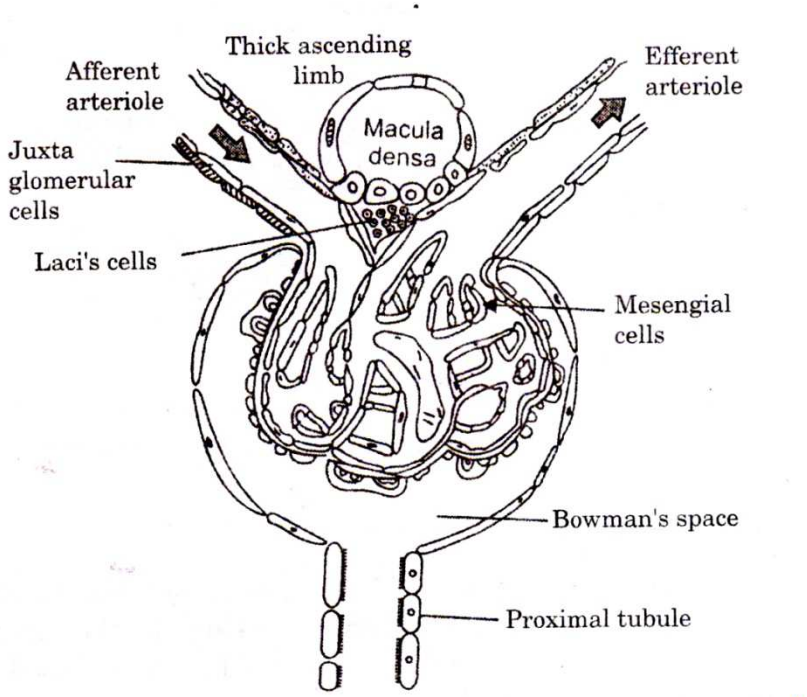

Fig. 4.4: Juxtaglomerular apparatus

4.3.2 MECHANISM OF RENAL EXCRETION (URINE FORMATION)

Asmentioned above, kidneys are the chief organs of nitrogen excretion invertebrates. Each minute, the two kidneys filter approximately 1200 ml ofblood to collect 125 ml of filtrate and form about 1 to 2 ml of urine (per minute) andthus eliminate extra water, nitrogenous wastes and inorganic salts from the body. The entire mechanism of renal excretion involves three main steps (Fig. 4.5).

- Ultra filtration
- Selective reabsorption
- Tubular secretion

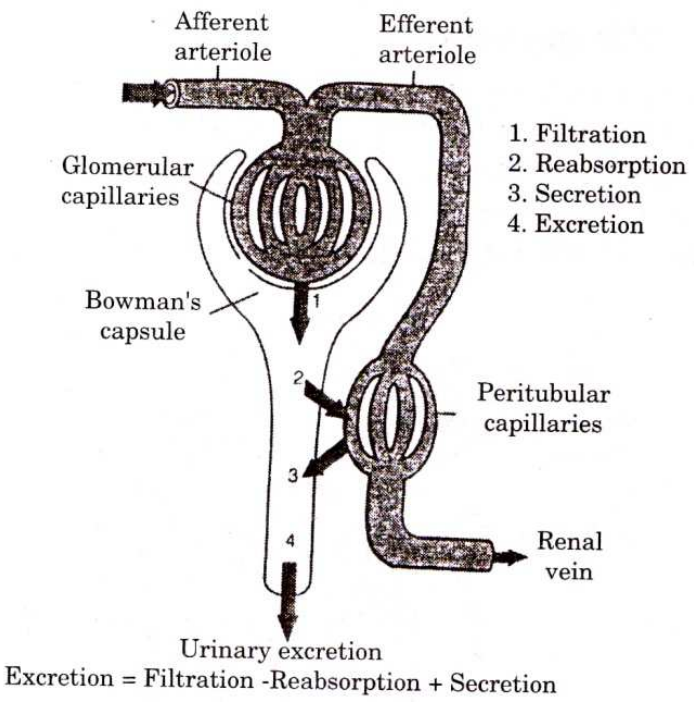

Fig.4.5: Mechanism of urinary excretion (Diagrammatic)

(A) Ultra-Filtration

Described first by **Ludwig** (1844) and later modified by Richards (1942), the **ultra-filtration or glomerular filtration** is the first step in urine formation. Theglomerular capillaries receive the renal blood supply through afferent renal arteriole which form a high pressure bed in glomerulus and it is approximately 60 mm Hg (**Guyton,2003**).Opposing the effects of blood pressure in the glomerulus are-

(**1**) **Colloidal osmotic pressure** of plasma proteins

(2) **Tubular pressure** of the Bowman's capsule itself; which are approximately 32 mm Hg and 18 mm Hg, respectively. Thus, in the Bowman's capsule a blood pressure of about 60 mm Hg is being opposed by an internal pressure of approximately 50 mm Hg (colloidal osmotic pressure plus the tubular pressure) and this spares a filtration pressure of about 10 mm Hg (called the **net filtration pressure**) which is sufficient to cause filtration of the non-protein substances of plasma across the glomerulus (fig. 4.6). Everything except plasma proteins are filtered and the fluid collected is called **ultrafiltrate**. The total amount of glomerular filtrate formed each minute

in all thenephrons of both kidneys is known as the **glomerular filtration rate.** It is approximately 125 ml/min or 180 litres/day.

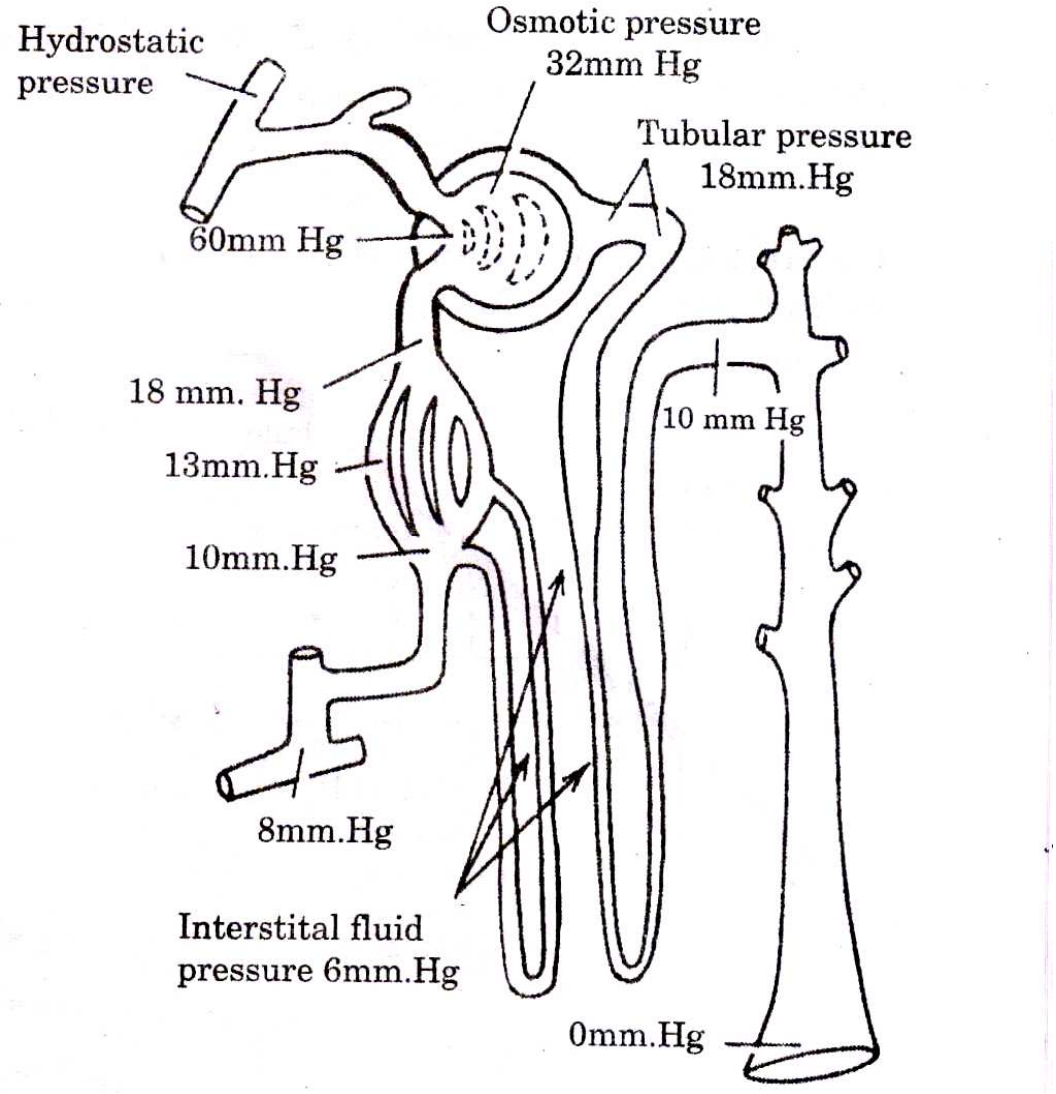

Fig.4.6: Pressure of different points in the vessels and tubules of the functional nephron and in the interstitial fluid

- **Filtration Coefficient** (Kf) is defined as total amount of ultra filtrate formed by all the nephrons of both kidneys per minute per 1mm of Hg of net Filtration pressure.

Kf = 125/10 = 12.5 ml/min/mm of Hg

Another noteworthy feature of ultrafiltration is the tremendous permeability of the glomerular capillary membrane which is several hundred times higher than any capillary membrane in

human body. Histologically it is made up of three layers: **(1) endothelium** of the capillary, (2) **basement membrane**, and (3) a layer of **epithelial cells** that line the surface of Bowman's capsule.The fenestrae and slit-pores **(**fig 4.7) of these layers increase permeability but prevent the filtration of all particles having an average diameter greater than 70 A. Since plasma proteins are slightly larger than 70 Aº diameter, it is possible for the glomerular membrane to prevent the filtration of all those substance with molecular weight equal to or higher than those of the plasma proteins.

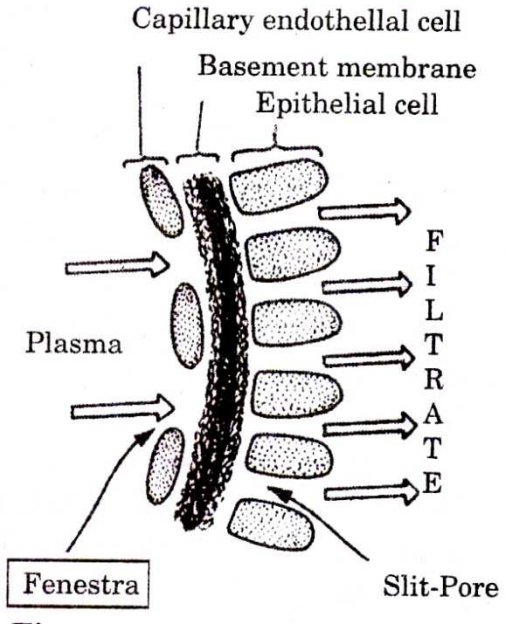

Fig.4.7:Functional structure of the glomerular capillary membrane

- **Factor affecting Glomerular Filtration Rate (GFR)**

Any change in any of these three pressures affects GFR.

1. When the blood pressure in the glomerulus raises GFR increases.
2. When colloidal osmotic pressure (CPO) increases, GFR decreases provided other two factors are constant (COP decreases in starvation and due to under nutrition).
3. When the tubular pressure in the Bowman's capsule increases, GFR decreases.
4. Increase in blood volume increases GFR.
5. Increase in cardiac output increases GFR.

6. Decrease in the number of functional nephrons will decrease GFR (Some nephrons become non-functional due to old age).
7. Sympathetic discharge will produce constriction in afferent arteriole and this decreases GFR.

- **Determination of GFR**

GFR is taken as one of the kidney function test. By quantifying GFR one can understand how the kidney handles various substances. Thus substances with excretory rate less than filtration rate must undergo tubular reabsorption whereas substances with excretion rate more than filtration rate must undergo secretion.

- **Clearance Test**

The clearance of a substance may be defined as the concentration of that substance in plasma same as in glomerular filtration and is neither reabsorb nor secreted by the tubular epithelium. It is a measure of the rate of glomerular filtration. Inulin**,** a polymer of fructose found in the roots of certain plants is easily filtered, it is neither reabsorbed nor secreted and has a clearance equal to GFR.

- **Transport Maximum (Tm)**

Another parameter of renal excretory function is the transport maximum which is the maximum ability of the kidney either to reabsorb or secrete a given material. For example, Tm of glucose is defined as the maximum amount of glucose that can be reabsorbed by all nephrons of both the kidneys per minute

Average TmG = 360 mg/min

(320 mg/min in females and 375 mg/min in males).
If the tubular load of glucose becomes 400 mg then it will be excreted in urine at the rate of 40 mg/min. When tubular load of glucose is less than TmG then no glucose is lost in urine.

Glucose lost in urine = tubular load of glucose - TmG
= 400-360
= 40 mg/min

B). Selective reabsorption

The filtered fluid in the Bowman's capsule and in the tubule is called the **glomerular filtrate**. It has almost the same composition as the fluid that filters from the arterial end of capillaries into the interstitial fluids. The glomerular filtrate has no red blood cells but consists of about 0.03 percent proteins, a great amount of glucose, salts, nitrogenous end-products and water. About 99 percent of the water and almost all other useful substances present in the glomerular filtrate are to be reabsorbed back into the blood as the filtrate passes through various parts of the convoluted tubule (fig 4.8).

(1) Reabsorption in the proximal convoluted tubule

About 65 to 80 percent of the glomerular filtrate is reabsorbed in the proximal convoluted tubule and this fraction is referred to as the **obligatory reabsorption**. The epithelial cells of the proximal tubule have a **brush border** composed of thousands of very minute microvilli which facilitate a rapid and active diffusion of sodium from the **peritubular capillary blood.** Each time a positively charged sodium ion is pumped out of the tubule, a negatively charged ion follows it and it is usually a chloride ion which thus leaves the tubule, since it is the most abundant negatively charged ion in the tubular fluid.

The peritubular capillary bed has a mean blood pressure of about 13 mm Hg which is slightly higher than the filtration pressure. The peritubular fluid now has a higher osmotic pressure because of an increased concentration. Both these factors influence the movement of water from the filtrate which also carries away, passively, substance like glucose, amino acids, and potassium, calcium and phosphate ions from the filtrate.

(2) Reabsorption in the loop of Henle

At the end of proximal tubule the fluid is almost isotonic with blood. In the rest of its course it is converted into urine of the appropriate concentration through a complicated sequence of events termed as the **counter-current mechanism**. The mechanism is basically associated with the anatomy of the loop of Henle which is a hair-pin loop between the proximal and distal convoluted tubules of nephrons. Entire mechanism of the **counter-current system** is as follows:

(a) The ascending limb of loop of Henle with thicker walls is impermeable to water but actively transports sodium and chloride ions passively from the tubular wall into the renal medulla. This causes an increase in the osmolarity of the interstitial fluid in the medulla.

(b) The increase in the osmolarity due to the sodium pump causes water to leave the thin walled descending limb.This makes the filtrate increasingly hypertonic as it proceeds from the renal cortex towards the papilla.

(c) The Na+ secreted by the cells of the ascending limb of the loop of Henle into the interstitial fluid escape passively into the adjacent blood vessels or the descending limb of the loop following a concentration gradient.

(d) Thus, by this retransport of the sodium (and chloride) again and again the **counter-current multiplier mechanism** increases the concentration of sodium chloride in the medulla, and consequently determines the concentration of urine.

(3) Reabsorption in the distal convoluted tubule

The fluid reaching the distal tubule of the nephron is **hypotonic**and it is the **antidiuretic hormone (ADH)** from the **hypothalamic-pituitary** which now controls the concentration of the urine. This **ADH** controlled water reabsorption is the distal and collection tubule is termed as **facultative reabsorption**, and its mechanism is simple.

In water diuresis very little ADH is present in the blood, so the distal and collecting tubules become relatively impermeable to water and a dilute urine is excreted but when the body has little water (or say when the blood is more concentrated) a larger amount of ADH is present in the blood and the kidneys conserve more water because of tubular reabsorption Hence the urine becomes concentrated.

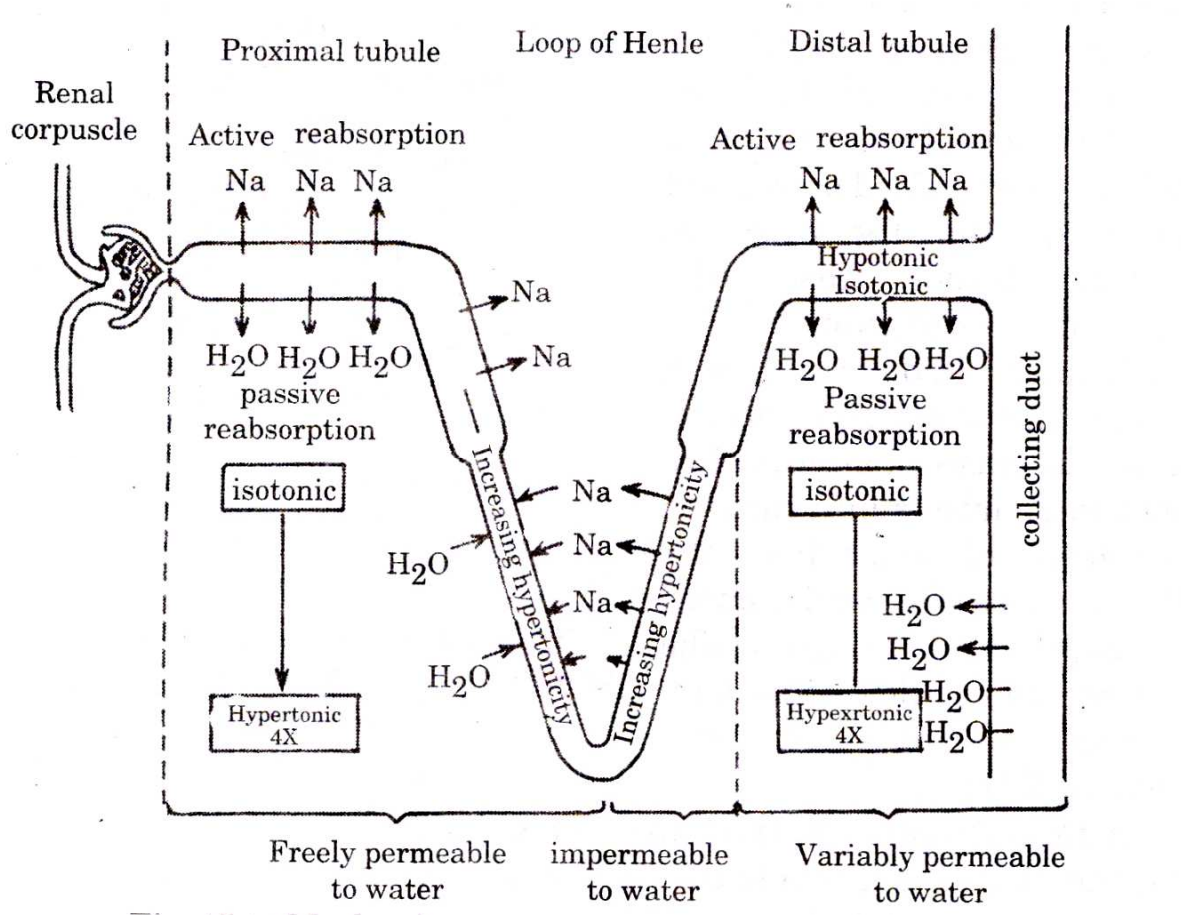

Fig.4.8: Mechanism of urine formation in mammal

C. Tubular Secretion

Tubular secretion is the final step in the urine formation. The epithelial lining of the tubule is able to collect electrolytes and water from the fluid but at the same time many foreign substances are readily secreted by the tubule. It is believed that some creatinine, potassium, phenol red, H ion and penicillin are the main substances secreted by the tubular epithelium in man.

- **Composition and characteristics of urine**

The concentrated fluid entering the **collecting tubule** is called Urine. Through the way of pelvis and ureters, it is usually stored in the urinary bladder and is discharged from the body from time to time. The normal volume and composition of urine varies widely from day to day and is being governed by, among other things, the type of food and fluid taken and the amount of fluid loss

by other agencies, a factor which itself depends upon environmental temperature, humidity, exercise and sweating etc.

Table 4.1 Composition and some of the character of normal urine of man

Colour	Pale yellow to amber (due to pigment and urobilinogen)
Odour	Normally aromatic
Volume	600 to 2,500 ml/24 hrs. (average volume-1)
Specific gravity	1.003 to 1.030
Turbidity	Clear and transparent (when allowed becomes turbid.)
pH	Slightly acidic 5.5 to 7.5 (average pH
Total Solids	30 to 70 gm/l. (average 50 gm/l)
Inorganic constituents (gm/24 hrs)	
Chlorides (NaCl)	10-15 gm (average 12 gm)
Sodium	2.5-6.0 gm (average 4.0 gm)
Phosphorus	1.5-2.5 gm
Potassium	1.5-2.0 gm
Sulphur (as SO_3)	0.7-3.5 gm
Calcium	0.1 to 0.2gm
Magnesium	0.05 to 0.2 gm
Iodine	50 to 50µg
Arsenic	0.05 µg or less
Lead	g or less
Organic constituents	
Urea	20-30 gm
Creatinine	1.0-1.8 gm
Ammonia	0.3-1.0 gm
Uric acid	0.5-0.8
d-Creatine	60-150 mg
Hippuric acid	0.1-1.0 gm

Micturition

Micturition is the mechanism by which the urinary bladder empties itself when it becomes filled with the urine. The urine is basically collected in the pelvis of the kidney where all the nephrons of a kidney open through their collecting tubules. As urine collects in the pelvis, its pressure increases and initiates peristaltic waves occurring every 10 sec or so and travelling through the ureters at a velocity of about 3.0 cm/sec and pushing a little spurt of urine into the bladder, the stretch receptors located in the bladder wall and proximal urethra become stimulated creating a micturition reflex. The detrusor muscles, which make-up the body of urinary bladder, contract

during the micturition reflex; the internal sphincter of the urethral opening relaxes and the urine is evacuated.

4.3.3 MODE OF EXCRETION OF NITROGENOUS WASTE IN ANIMALS

The metabolic waste products which are excreted by the animals may be grouped under two heads:

- **Respiratory waste products**

The catabolic waste products of various types of food stuffs are CO_2 and water. In lower animals CO_2 is eliminated directly into the environment through the general body surface. In higher animals it is excreted with the expired air through the lungs. Excess of water is eliminated in the form of sweat and urine.

- **Nitrogenous waste products**

The nitrogenous waste products are derived from the deamination of the excess of amino acids taken in with the diet and also from the breakdown of animal proteins and nucleic acids. The chief nitrogenous waste products excreted by animals are ammonia, urea, uric acid guanine, trimethylamine oxide.

4.4 AMMONOTELISM

It is a type of excretion in which the main nitrogenous waste material is ammonia and such animals are called ammonotelic.

Occurrence

It is found in aquatic animal groups like sponges coelentrates, crustaceans, echinoderms, bony fishes, tadpole larvae and salamander. Ammonia is produced as result of catabolism of proteins especially in the liver cells by oxidative deamination of excess of amino acids in the presence of oxidase enzyme. Ammonia is highly toxic and must be metabolized or expelled from the body as soon as possible.

4.5 UREOTELISM

It is a type of excretion where urea is the main nitrogenous waste material and the animals showing ureotelism are called ureotelic animals.

Occurrence

Generally found in land animals which can afford to excrete sufficient volume of water or to concentrate urea in considerable quantity in the urine. It is commonly found in man ,whales, seals, desert animals like kangaroo rats, camels, toads, frogs, cartilaginous fishes, aquatic and semi aquatic reptiles, like alligator, terrapins and turtles.

In the liver of the animals, ammonia is detoxified to form urea by the ornithine cycle. The urea cycle (also known as the ornithine cycle) is a cycle of biochemical reactions that produces urea ($(\underline{N}H_2)_2CO$) from ammonia (NH_3).The urea cycle converts highly toxic ammonia to urea for excretion. This cycle was the first metabolic cycle to be discovered (Hans Krebs and Kurt Henseleit, 1932), five years before the discovery of the TCA cycle. The urea cycle takes place primarily in the liver and, to a lesser extent, in the kidneys.

Urea is less far toxic than ammonia and so can remain inside the body for a longer period without causing any ill effects inside the body.1gm of urea needs about 50 ml. of water to be expelled out.

Ureotelism helps in conservation of water. Elasmobranches fishes conserve urea to maintain somore gulation. Some marine bony fishes excrete **trimethylamine oxide** (TMO) along with urea. Aquatic turtles excrete both ammonia and urea. Some tortoises can change their excretory product according to the environment in which they are present.

4.6 URICOTELISM

Elimination of uric acid as the main nitrogenous waste material is called uricotelism. Animals showing uricotelism are called uricotelic animals.

Occurrence

It is commonly seen in birds, land reptiles, insects, land snails and some land crustaceans. Uric acid is formed from ammonia mostly in the liver and to some extent in the kidneys. The process is highly energy dependent, but is less toxic than both ammonia and urea and it is almost insoluble in water and can be eliminated from the body in nearly solid state, saving a lot of water. Since kidneys can handle the nitrogenous wastes only in solution, reptiles and birdspass a dilute solution of uric acid into the cloaca, where water is absorbed and solid uric acid is eliminated along with faeces. The fecal matter of certain birds like commorants, pelicans and gannetcalled guano has been used for the commercial extraction of uric acid. Man also excretes a small amount of uric acid in his urine formed by the catabolism of nucleic acids. $\text{Adenine} - \text{guanine} + \text{xanthine} \rightarrow \text{uric acid}$. Other nitrogenous wastes as Allantoism, creatine, creatinine and hippuric acid are some other nitrogenous products excreted by animals.

4.7 GUANOTELISM

In some arthropods like spiders, unio and birds like penguin, guanine is a predominant excretory product elaborated by the Malpighian tubules and cloacal sacs. However, the formation of guanine from protein nitrogen is still inadequately known.

4.8 HOMEOSTASIS BY KIDNEYS

Homeostasis is maintenance of the constant internal environment of body by regulating the water content of extracellular fluids. Besides removal of excretory products, kidneys also help in homeostasis of the body. ADH or vasopressin from posterior lobe of pituitary regulates absorption of water by DCT and CT thus, help in maintenance of water balance in the body. Mineralocorticoids (aldosterone) from adrenal cortex regulate reabsorption of Na+ and K+. The

amount of water excreted in urine is directly controlled by ADH and indirectly controlled by mineralocorticoids. Kidneys filter all the fluid in the body, removing nutrients for cellular use and also the wastes.

It also uses a number of hormones to regulate homeostasis, i.e., by releasing hormones to regulate blood pressure and by altering sodium and electrolyte balances to maintain proper fluid amounts in the body. The excretory system also helps the body cool down when it is sweating, the kidneys put chemicals inside the blood, the liver puts toxins and acids in the blood, and the colon lets the e solid waste go through the body.

4.9 SUMMARY

The excretory system is a system of organs that removes waste products from the body. This system is a passive biological system that removes excess unnecessary materials from the body fluids of an organism so as to help maintain internal chemical homeostasis and prevent damage to the body .The dual function of this system is the elimination of the waste products of metabolism and to drain the body of used up and the broken down components in a liquid and gaseous state. In humans and other amniotes (mammals, birds and reptiles) most of these substances leave the body as urine and to some degree exhalation, mammals also expel them through sweating.

When cells in the body break down proteins (large molecules that are essential to the structure and functioning of all living cells).They produce waste such as urea (a chemical compound of carbon .hydrogen and oxygen).When cells break down carbohydrates, they produce water and carbon di oxide as waste product. If these useless waste products are allowed to accumulate in the body, they would become dangerous to the body's health. Thus, the kidneys, are considered as the main excretory organs in humans, eliminate water, urea and other waste products from the body in the form of urine.

4.10 GLOSSARY

Ammonotelic: Excreting nitrogen as ammonia

Anti-diuretic: Reducing the volume of urine

Bowman's capsule: Glomerular capsule

C**ollecting tubule:** The portion of the renal tubules in which the final concentration of urine occurs

Creatinine: Nitrogen waste product of muscle creatinine

Deamination: Removal of amino radical from an amino acid

Diuretic: An agent that increases urine secretion

Excretion: Act of eliminating waste material

Glomerulus: A coiled mass of capillaries

Isosmotic: Having the same osmotic pressure

Isotonic: Having equal osmotic pressure

Loop of Henle:A U-shaped bend in the portion of a renal tubule that lies in the renal medulla

Urea: The primary nitrogenous waste product in the urine of mammals

Ureotelic: Excreting nitrogen as urea

Uricotelic: Excreting nitrogen as uric acid

Deamination: Removal of amino acid

Guanotelism: Excreting waste as guanine

Homeostasis: Maintaining standing state

Juxtaglomerular apparatus: A region of tissue found in each nephron in the kidney that is important for regulating blood pressure

Nephron: Unit of kidney

4.11 SELF ASSESSMENT QUESTIONS

1. The basic unit of kidney is:

(a) Loop of Henle (b) Nephron

(c) Glomerulus (d) Pelvis

2. The function of glomelulus is:

(a) Filtration of blood (b) Selective reabsorption

(c) Filtration (d) Regulation of blood pressure

3. Excretion of nitrogenous waste product in semisolid from occurs in:

(a) Ureotelic animals (b) Ammonotelic animals

(c) Uricotelic animals (d) Anmiotes

4. Ornithine cycle refers to the sequence of biochemical reactions taking place in the:

(a) Oral cavity (b) Liver

(c) Pancreas (d) Kidney

5. Which the following is generally not present in the urine of health person:

(a) Uric acid (b) Creatine

(c) Alanine (d) Vitamin B complex

6. Henle's loop is the part of:

(a) Pelvis (b) Nepheron

(c) Brain (d) Lungs

7. Reabsorption of glucose occurs in:

(a) Collecting tubule (b) Proximal convoluted tubule

(c) Loop of Henle (d) Distal convoluted tubule

8. Loop of Henle is meant for absorption of :

(a) Potassium (b) Glucose

(c) Urea (d) Water

9. In which cycle, ammonia is converted into urea:

(a) Carbon cycle (b) Ornithine cycle

(c) Hydrogen cycle (d) Ammonia cycle

10. Urine is concentrated in:

(a) Kidney (b) Malpighian tubule

(c) Loop of Henle (d) Distal convoluted tubule

11. The concentration of inorganic salts in the normal urine of human being is about :

(a) 0.15% (b) 2.5%

(c) 15% (d) 0.25%

12. The phenomenon which helps in maintaining a constant internal environment in living organism is

(a) Entropy (b) Homeolysis

(c) Homeostasis (d) Apoptosis

13. Blood vessels leading into Bowman's capsule are called:

(a) Renal vein (b) Renal artery

(c) Efferent arteriole (d) Afferent arteriole

14. Ornithine cycle producing urea occurs in

(a) Liver (b) Kidneys

(c) Muscles (d) Blood

Answer:

1. (b) 2. (a) 3. (c) 4. (b) 5. (a) 6. (b) 7. (d)
8. (b) 9. (c) 10. (c) 11. (d) 12. (c) 13. (d) 14. (a)

4.12 TERMINAL QUESTIONS/ ANSWER

1. What do you understand by ammonotelism? Write down the names of some ammonotelic animals.
2. What is the advantage of elimination of nitrogenous wastes in the form of urea?
3. What is ureotelism? Name some ureotelic animals.
4. Describe the structure of nephron?
5. Explain the reason of lumen of afferent arteriole being larger than the lumen of efferent arteriole.
6. What is the difference between juxtamedullary nephrons and cortical nephrons?
7. What is Malpighian corpuscle?
8. Why filtration of blood occurs in the glomerulus?
9. What are the excretory products of nucleic acids in different mammals?
10. Show the process of urine formation with the help of a well labelled diagram.
11. The kidney function is regulated by which hormones?
12. Explain the mechanism of regulation and control of renal functions.

13 How urea is synthesized from ammonia? Describe in detail.

14. Describe the physiology of urine formation in detail.
15. What is selective reabsorption? How counter current system helps in concentration of urine?

16. Describe the counter current theory of Urine formation?

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UNIT 5 NERVOUS SYSTEM

CONTENT

5.1 OBJECTIVES

After reading this unit you should be able to

- Explain the mechanism of conduction of nerve impulse
- Describe the structure, classification and properties
- Define Nerve fibre
- Describe the conduction of impulses in myelinated and non-myelinated nerve fibre
- Explain the mechanism of conduction of an impulse across a synapse
- Write a note on neurotransmitter and their physiological significance.
- Differentiate between myelinated and non-myelinated nerve fibre
- Differentiate between resting potential and Action potential

5.2 INTRODUCTION

The diversified activities in multicellular animals depend on a steady flow of information from the environment around it. For this, some system is required for the coordinated activities of numerous cells of the body and for receiving information from external as well as internal environment, such as pain, hunger, thirst temperature, body orientation and blood pressure etc. Thus, these stimuli are coded by specialized cells called receptors, into a series of electrogenic pulses and then they are transmitted through the nervous system to activate muscles, glands, chromatophores and other organ systems, called effectors. Thus, nervous system performs dual function of transmission and integration between receptors and effectors. The neurons have evolved for the purpose of communication between effectors and receptors. These are the chief functional unit of the nervous system. There are other cells called neuroglia, which make half of the nervous system. These cells are derived from the non-nervous spongioblast cells of the neural plate and generally thought to be supporting in function but also plays a vital role in the nutrition of neurons.

The study of structure and functions of nervous system is, called neurology. The nervous system of vertebrates can be divided into three components : Central nervous system (CNS), peripheral nervous system (PNS) and autonomic nervous system (ANS). The central nervous system consists of brain and spinal cord. The peripheral nervous system consists of nerves arising from brain and spinal cord. The autonomic nervous system consists of sympathetic and parasympathetic nervous systems.

- **Structure of nerve tissue**

The nervous system is formed of nervous tissue. In nervous tissue, two types of cells are present:

1. Nerve cells
2. Supporting cells

The cells of nervous tissue, which carry nerve impulse are, called nerve cells or neurons, which are generally adapted for the handling information in the form of nerve impulses. A typical mammalian neuron was first described by **Camillo Golgi** of the University of Pavia in 1873.later **Ramon Y.Cajal** demonstrated that neurons are the functional units of the nervous system, and thus shared the Nobel Prize in 1906.

- **Shape and size**: The neurons vary considerably in shape and size in different animals and even in different parts of the animal body .for eg in man the granule cells of the cerebellum are 5μ in diameter, while the great pyramidal cells are 130 μ.

- **Number:** The no. of nerve cells is almost fixed for a particular species. In man the entire nervous tissue comprises some $3000x10^6$ nerve cells and thus makes up approx.2.5%of the body weight (**Marshall and Hughes, 1972**). The supporting cells inside central nervous system are **neuroglia** or **glials** cells, while in the peripheral nervous system there are **Schwann cells** and **Satellite cells**.

- **Structure of Neuron**

A typical neuron has three parts

A neuron consists of a large cell body, known as **cyton**, perikaryon or soma, which is about 4-25μ in dia. thin protoplasmic processes arising from cell body, called **dendrites**, and a long process called **axon**.

a) Neurocyton: Each neuron has a cell body called, the soma, neurocyton or perikaryon. A large nucleus with prominent nucleolus is also found in the centre of soma. It contains a granular cytoplasm due to the presence of **Nissl's granules**, which are darkly stained and highly basophilic bodies. Nissl's bodies are rich in RNA and are concerned with protein synthesis Mitochondria are also in abundance in a nerve cell.A mature neuron however, doesnot undergo cell division (**Warwick and Williams, 1973)**.

b) **Dendrites**: Dendrites arise from the soma, which are the extension of cell body. Dendrites are afferent processes, and are meant for bringing impulses into the cell body. Information received by the dendrites can influence the cell body to generate action potential that is then conducted along the axon to the target cell. The cell bodies and dendrites aggregate into ganglia thus forms central nervous system (CNS).

c) **Axon**: The axon or neurite is a single but much longer extension that projects from **an axon-hillock** of the cyton of a nerve cell. It forms the nerve fibre that carries away impulses or signals from the cyton to another neuron forming a **synaptic connection** between its terminal buttons or synaptic knobs and the dendrites of the following neuron.(fig 5.1 a).The length of an axon varies at different places in the body .There are two main types of axons in vertebrates ,the larger axons,1-25µ in dia being myelinated and the smaller ones under 1µ are non-myelinated. Almost all vertebrates are equipped with non-myelinated fibres, but some of them differ from vertebrates non-myelinated fibres in being much large,for eg.the giant axons of the squid approx 1000µ in diameter.

- **Axoplasm**: All nerve fibre consist of a long cylinder of cytoplasm, the axoplasm.The axoplasm is a viscous fluid and contains ultramicroscopic and extremely fine neurofibrils that serves to transmit signals. The plasma membrane of axon is called axolemma where axoplasm and neurofibrils are enclosed within.
- **Myelin or Medullary Sheath:** An axon is not uniformly thick throughout its length. It is constricted more or less at regular intervals to form **Nodes of Ranvier;** the part between the two successive nodes is internode. Just outside the axon sheath, there lies another coat of one

to many sheaths of lipoproteins called myelin or **medullary sheath.** The sheath is secreted by large flattened cells the **Schwann cells**, whose cytoplasm bathes the myelin sheath.

- **Neurilemma**: Outside the **Schwann cells** and the myelin sheath lies a tough and inelastic layer of scleroproteins which is known as **neurilemma** or the **sheath of Schwann.** Outside the neurilemma is yet another thicker layer of collagen fibres, the sheath of Henle, which is continous with **endoneurium,** the connective tissue that binds the axons together in a nerve.

- A neuron may have small and delicate side branches called **collaterals,** which may terminate into synapse like an ordinary axon ending.

- **Telodendria**:At its distal end,an axon breaks up into a number of fine branches called telodendria .Each telodendron ends in a tiny knob like structure ,the terminal button which rests on the dendrites and cyton of the following neuron and thus forms a synapse between two adjacent neurons.At a synapse the two cell membranes are usually thickened and the gap between the two is about 20-50nm(nano meter)

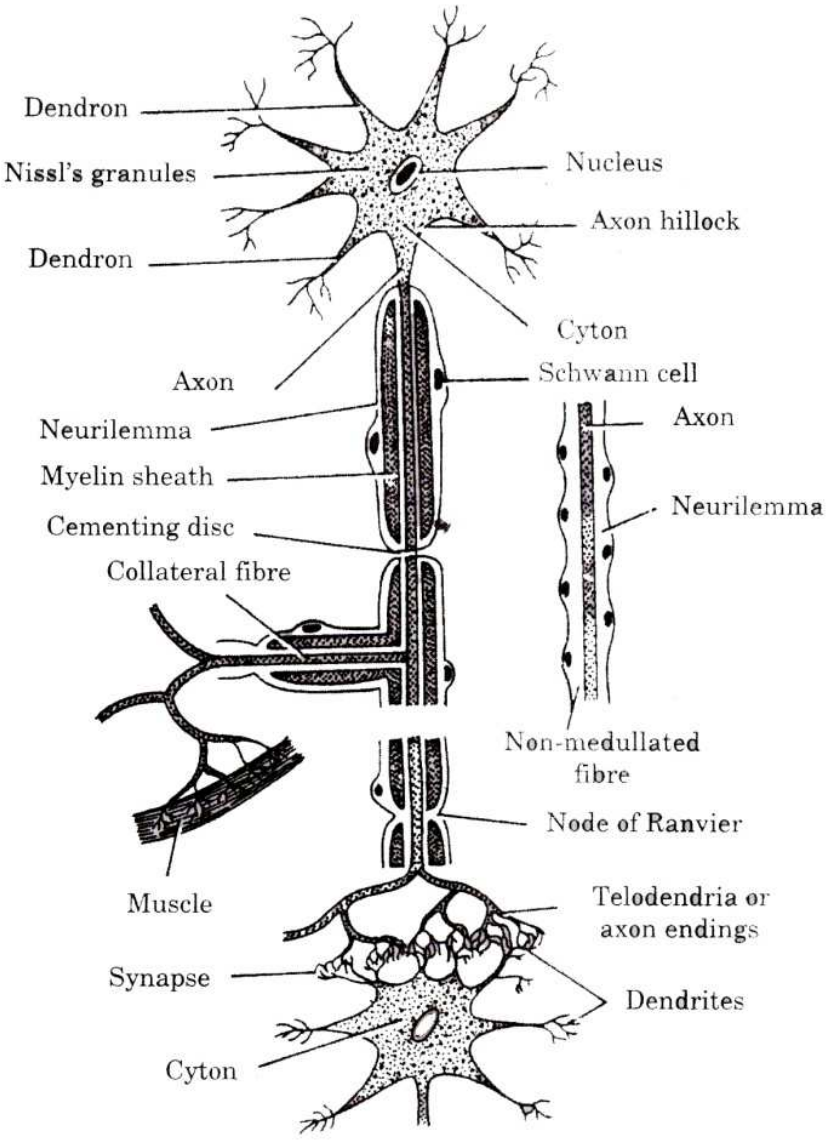


Fig: 5.1 A nerve cell

5.3 NERVE FIBRE

A nerve fibre is a cord like structure containing bundles of axons in the peripheral nervous system. It provides a common pathway for the electrochemical nerve impulses that are transmitted along each of the axons to peripheral organs. Within a nerve,(Fig 5.2..) each axon is surrounded by a layer of connective tissue called **endoneurium**.The axons are bundled together into groupss called **fascicles** and each fascicles is wrapped in layer of connective tissue called **Perineurium**.Finally ,the entire nerve is wrapped in a layer of connective tissue called **epineurium.** Nerves are bundled along with blood vessels since the neurons are of a nerve have fairly high energy requirements.A blood vessel carrying blood to a nerve is known as **vasal nervosa**. Within the endoneurium, the individual nerve fibres are surrounded by a low protein liquid called endoneurial fluid.it constitutes a blood nerve barrier similar to the blood brain barrier. Molecules are thus prevented from crossing the blood into the endoneurial fluid..During the development of **nerve oedema** (swelling)due to nerve injury,the amount of epineural fluid may increase at the site of injury. This can be visualized using **magnetic resonance neurography.**

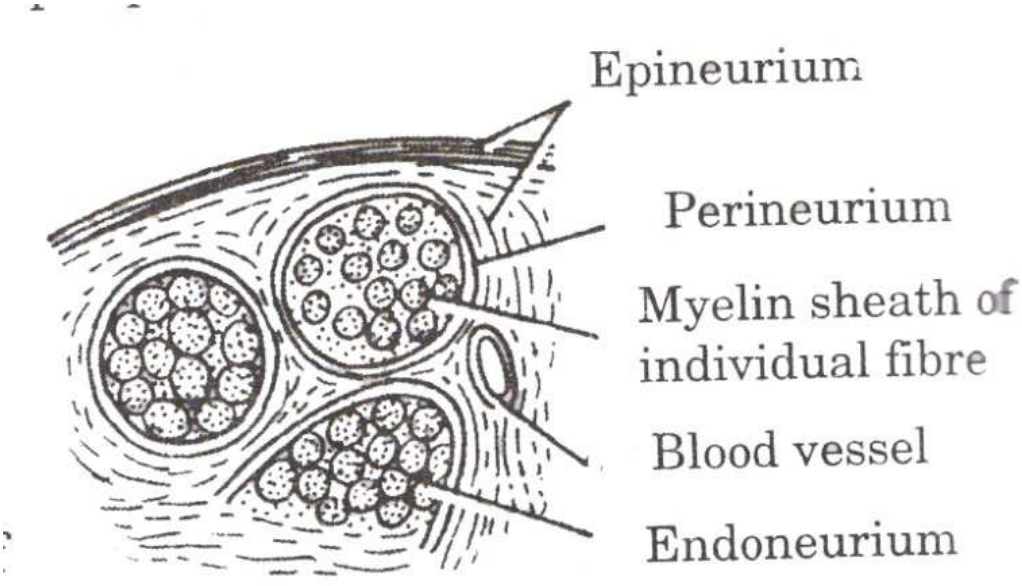


Fig 5.2:T.S of Nerve fibre

Histologically nerves are of two kinds:

5.3.1 MYELINATED NERVE FIBRE

The nerve fibre which are surrounded by a lipid rich insulating layer, so it appear white called myelin sheath. Myelin sheath is an electrical insulator, its purpose is to speed the transmission of nerve impulse .A neuron having myelin sheath means faster conduction, faster transmission and faster transfer of nerve impulses. (Fig 5.3 a)

Myelin sheath is interrupted at some places; these are called nodes of Ranvier, which is always constant in number. The space between two nodes of Ranvier is called internode. Speed of conduction of nerve impulse is faster in myelinated fibre. These nerves are more than 1μ in dia

5.3.2 NON-MYELINATED

Nerve fibres without myelin sheath are, called non-myelinated nerve fibres.In non-myelinated nerve fibres, nodes of Ranvier are absent. They appear grey because of the absence of myelin sheath; post ganglionic sympathetic nerves are non-medulated. The nerve fibres, which carry nerve impulse from receptor organs to the central nervous system are, called afferent nerve fibres or sensory neurons. The nerve fibres, which carry nerve impulse from the central nervous system to effecter organs are, called efferent nerve fibres or motor neurons.The nerve fibre that contain both afferent and efferent axons and thus conduct both incoming sensory information and outgoing muscle commands in the same bundle .

Types of Neurons

The nerve cells have been classified either on the basis of their structure or function as follows

- **Functional type of Neurons** :On the basis of their functional or their physiological properties ,the neurons may be divided into following types:

  a) **Sensory or afferent neurons**: These neurons carry a stimulus from the peripheral or visceral receptors to the central nervous system.

b) **Motor or Efferent Neurons**: These neurons carry impulses away from CNS to the effectors like muscles and glands.
c) **Neurosecretory neurons:** These cells are specialized for producing neurohormones.
d) **Internuncial neurons**: These are generally located in the central nervous system and serve to link the sensory and motor neurons.

- **Structural classification of neurons**

a) Multipolar Neurons

These neurons have an axon and many dendrites.Examples of multipolar neurons are: motor neurons and inter neurons found in brain and spinal cord.

b) Bipolar Neurons

When one axon and one dendrite arise from one soma i.e they have an axon and a dendrite. The bipolar neurons are found in retina and in the ganglia of VIII nerve.

c) Unipolar or Pseudounipolar Neurons

All developing neuroblast cells pass through a stage in which they have only one process the axon. During the later development it may be divided into two processes, one terminating in the central nervous system while another moves out. The unipolar neurons are found in the posterior roots of spinal nerves, in the mesencephalic nucleus of V cranial nerve and in the nerve roots of IX and X cranial nerve.

d) Non-polar Neurons

When many processes arise from the soma and all are equal without any distinction of axon or dendron, the neuron is called **non-polar neuron**. These nerve cells are unpolarized and cannot be differentiated into dendrites and axon. In non-polar neurons, nerve impulse can be conducted in any direction. Non-polar neurons are found in nerve net of coelenterates.

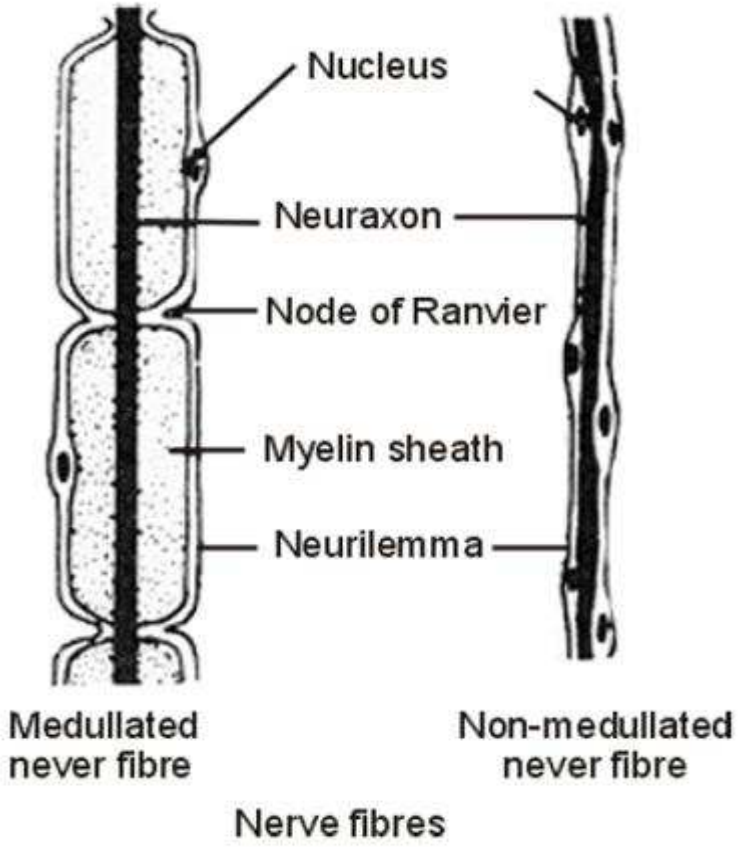


Fig: 5.3.1 a) myelinated nerve fibre

5.3.2 b) Non-myelinated nerve fibre

5.4 NEUROTRANSMITTERS

Neurotransmitters are the endogenous chemical messengers that enable neurotransmission liberated at the nerve ending They transmit signals across a chemical synapse from one neuron (nerve cell) to another target neuron a, muscle cell, or a gland cell. They play an important role in shaping everyday life and functions.

- **Types of neurotransmitters**

There are about 40 neurotransmitters and they have been classified into two types

a) Rapidly acting neurotransmitters

b) Slowly acting neurotransmitters

- **Rapidly acting neurotransmitters** are small molecules and cause acute response.

It includes

i) Acetylcholine
ii) Amines: Nonepinephrine, epinephrine (Catecholamines), Dopamine, Sertonin
iii) Amino acids like GABA(glycine and gamma amino butyric acid),glutamate, asparate

- **Slowly acting neurotransmitters** are neuropeptides having prolonged effect .It includes)

1) Neuroactive peptides which are releasing hormones secreted by hypothalamus, for eg. TRH, LH, somatostatin
2) Pituitary peptideslike ACTH, vasopressin, oxytocin, endorphins
3) Peptides acting on gut and brain like leucin, metionine, cholecystokinin, neurotensin, insulin, and glucagon.
4) Neuropeptides from other tissues like angitensin-II, bradykinin, and bombesin.

5.4.1 SYNAPSE

A synapse is defined as the functional connection between two neurons.(Fig :5.4) It permits a neuron to pass an electrical or chemical signal to another neuron.T he term "**synapse**" was given by **Charles Sherrington** in 1887.The word synapse is derived from the Greek word *synapsis* meaning conjugation.

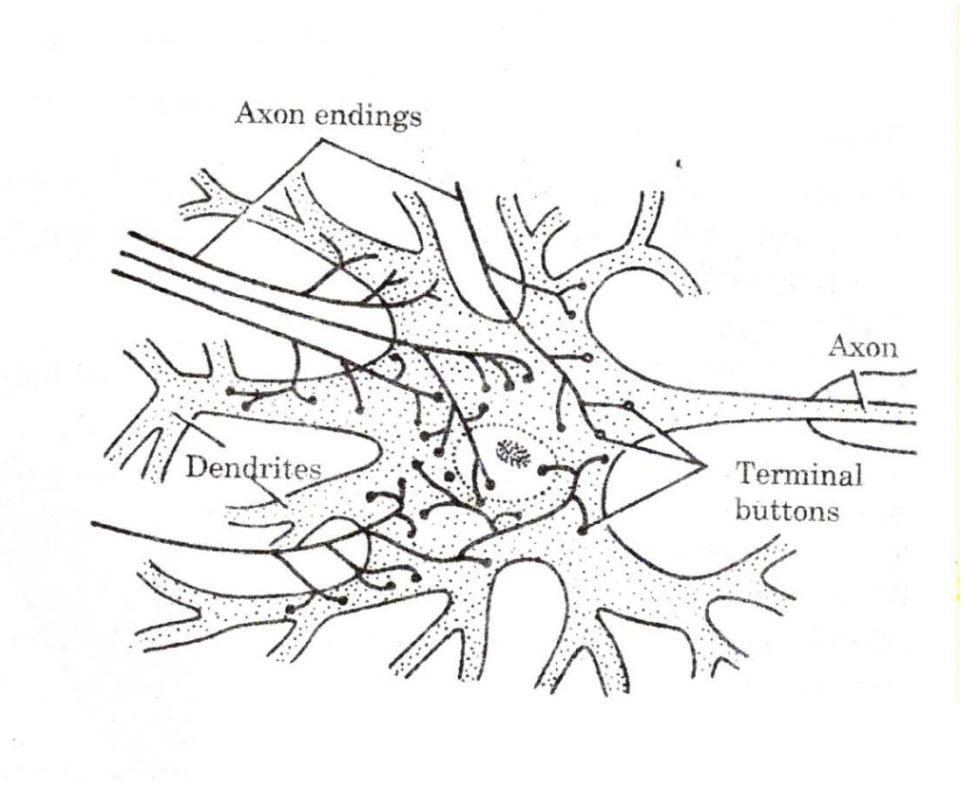


Fig 5.4 :A synapse between two neurons

- **Structure of Synapse**

At a synapse the plasma membrane of the signal passing neuron (presynaptic neuron) comes into close contact with the plasma membrane of the target (postsynaptic neuron) cell but does not fuse with it. Inside the pre-synaptic membrane there are several vesicles filled with neurotransmitters and numerous mitochondria necessary for active synthetic processes occurring in the terminals and at the post-synaptic membrane there are receptor proteins which respond to chemical stimulation and inhibition. The gap between pre-synaptic and post- synaptic membranes is known as synaptic cleft and it is about 20-50nm (fig: 5.5).

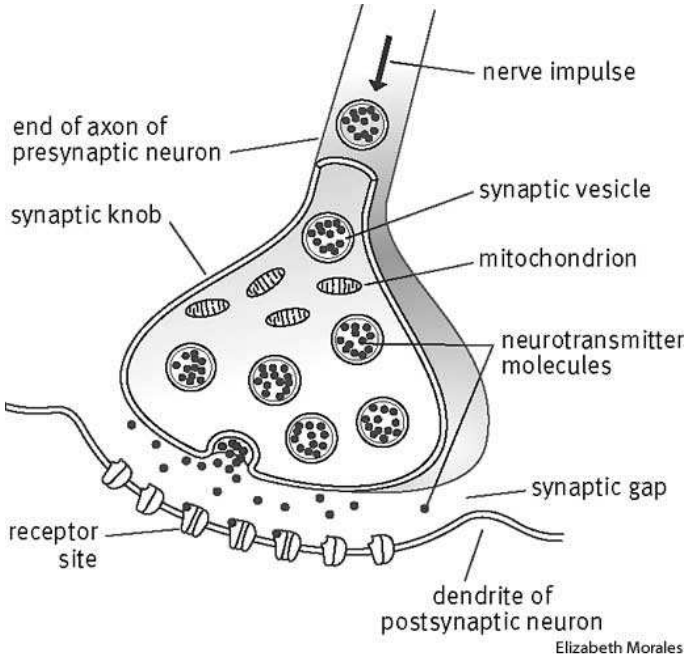


Fig:5.5 Physiological anatomy of the synapse

5.4.2 Types of Synapses

Synapses are of two types: electrical synapses and chemical synapses.Depending upon mode of transmission across the synapse.

- **Electrical synapse**: In electrical synapse, synaptic cleft is only 0.2 mm, so action potential can directly be transmitted to the next neuron. Electric synapse is quite rare and occurs in neural system that requires fastest possible response
- **Chemical synapse**: in chemical synapses, neurotransmitters are present. Each synaptic vesicle is of about 50 mm. diameters and stores about 10,000 molecules of a neurotransmitter. It is the most common type of synapse and allows the nervous system to connect and control other systems of the body. Acetylcholine, adrenaline and nonradrenaline are chemical transmitters, released at synapses. Dopamine, serotonin and sympathin are some other excitatory neuron transmitters. Most synapses are chemical synapse.
- **Conjoint synapse**: the synapses where transmission of nerve impulse is both chemical and electrical are, called conjoint synapses.

- **According to the nature of connections the synapse are of the following types-**

1. **Axo-axonic synapse**: A synapse between axon of one neuron and axon of another neuron is Axo-axonic synapse.
2. **Axo-dendritic synapse:** A synapse between axon of one neuron and dendrite of another neuron is knownas Axo-dendritic Synapse.It is found in the cerebellum where the climbing fibres form connections with dendrite of Purkinje cells.
3. **Axo-dendrsomatic synapse**: A synapse between axon of one neuron anddendrites and cell body of other neuron is known as axo-dendrosomatic synapse.
4. **Axo somatic synapse**: A synapse between axon of one neuron and cell body of another neuron is axosomatic synapse**.**
5. **Dendro-dendritic synapse**: A synapse between dendrites of two different neurons is known as dendro-dendritic synapse.

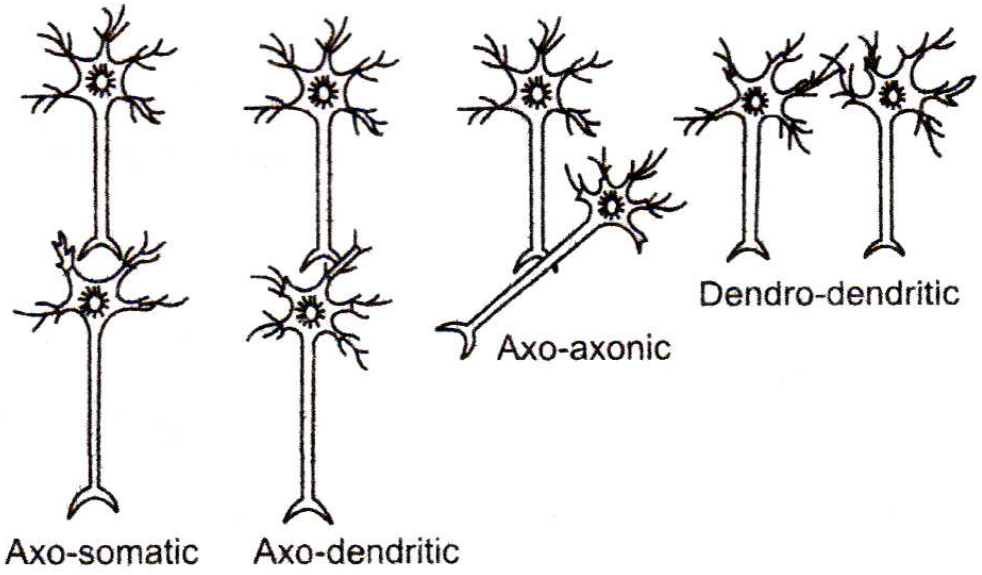


Fig. 5.6: Types of synapses

❖ **Properties of a Synapse**:

1). An impulse can be transmitted only in one direction across the synapse,i.e from presynaptic neuron to post synaptic neuron.This is known as the law of forward conduction.

2). The minimum time required for the transmission of impulse from one neuron to next is known as synaptic delay and it is about 0.5miilisecond.

3). Synapse is a site where impulses are received and discharged, it is therefore, regarded as **relay station**.

4). **Summation** is an important characteristic of synapse.It means adding up of the effects of multiple impulses at the synapses. It is of two types: Spatial and temporal summation.

5). Synapses bring about convergence and divergence of nerve impulses .Suppose many neurons synapse with a common post synaptic membrane, then impulses coming from various directions get converged at the synapse. All the impulses are further transmitted in a single uniform direction .This is known as **convergence (fig: 5.7).**

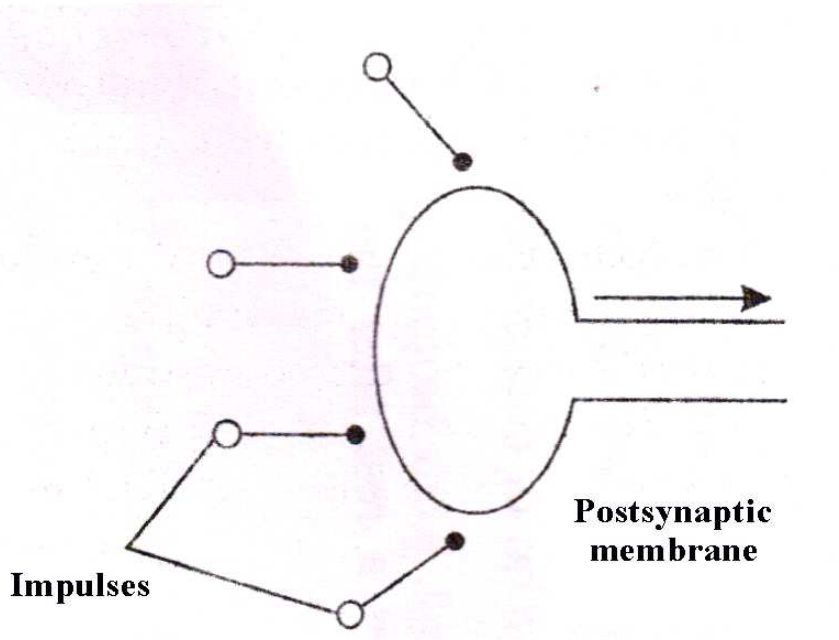


Fig: 5.7 Convergences of Impulses

Suppose a neuron makes synaptic contact with many postsynaptic neurons, the impulse coming through the first neuron in a single direction get diverted at the level of synapse and transmitted further in different directions. This is known as **divergence**. (Fig: 5.8).

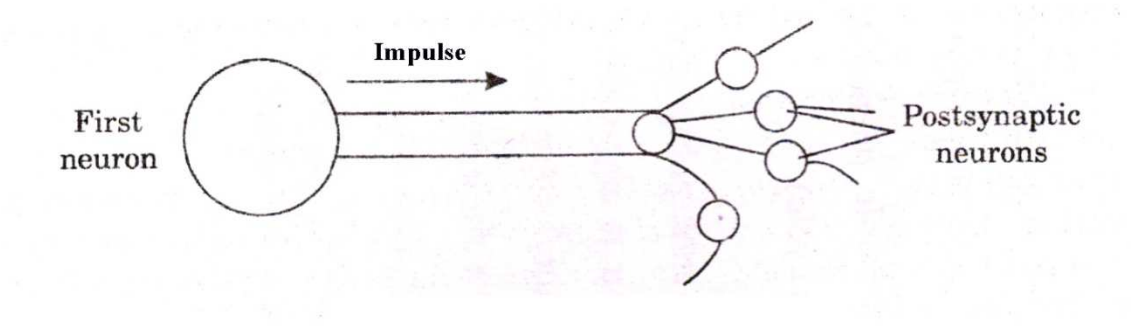


Fig: 5.8 Divergence of impulses

6) Sometimes when impulses are transmitted repeatedly across a synapse it stops transmitting impulses after sometimes. This is fatigue and it is due to neurotransmitter. However, it is a temporary phenomenon.

7) The phenomenon of passage of impulses from presynaptic to post synaptic neuron and back to presynaptic neuron is known as **reverberation** .As a result of continous transmission of impulses, a circuit is maintained.

❖ **Mechanism of synaptic transmission**

When a nerve impulse travelling along an efferent peripheral nerve reaches the synaptic terminals it produces a characterstic response in the effector tissue (increased or decreased activity of smooth muscle or cardiac muscle, secretion of glands, contraction of skeletal muscles). The current in the presynaptic membrane is too weak to excite the post synaptic membrane directly and there are evidences that the activity is now conveyed to the effector organs by the release of chemical transmitters into the synaptic cleft (Fig: 5.9).

The process of chemical transmission across the synapse was first revealed by Loewi in 1921.Later Henry Dale (1936) worked out the chemical nature of these neurotransmitters and their mode of action is mentioned below:

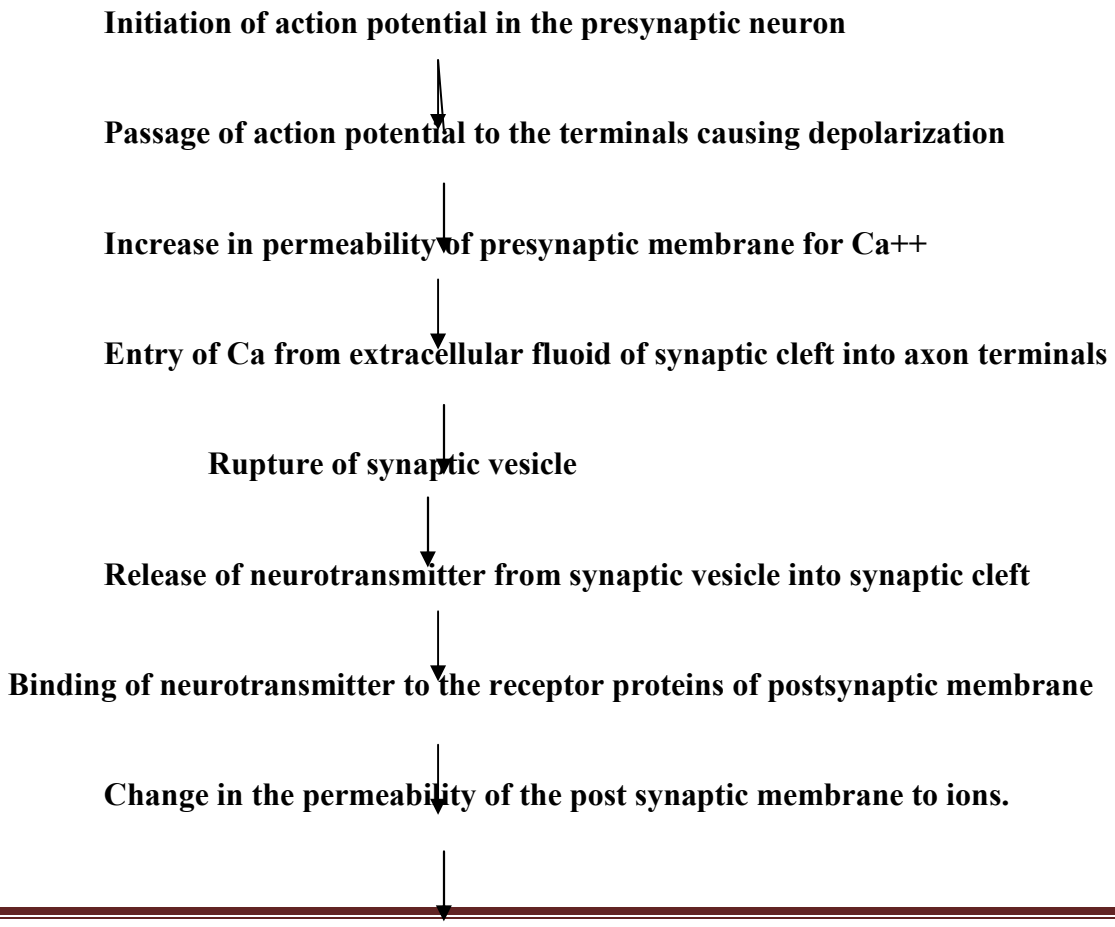
1) When a nerve impulse reaches the interneuronal or the neuromuscular junction, the weak action potential that it has carried to the synapse causes the calcium ions to move from the extracellular fluid into the membranes of the axon terminals.

2) The calcium ions in turn, cause the synaptic vesicle to rupture through the membrane and release the neurotransmitter.

3) Within approx.2-3 milliseconds after the chemical transmitter (which is either excitatory or inhibitory in nature) is released by the axon terminal, it traverses the synaptic gap, combines with a specific receptor on the post synaptic membrane and causes a local depolarization.

4) The local depolarization creates a synaptic potential across the synapse and when this reaches a certain magnitude it fires off an action potential in the next neuron or in the effecter cell.

5) The weak presynaptic potential is thus sufficient to release the transmitter which then greatly lowers the resistance of the post synaptic membrane by increasing the membrane permeability to Na+ and K+.The post-synaptic membrane gets depolarized.A fresh action potential is generated in the post synaptic neuron and is propagated further.Transmission of impulses across the synapse is chemical in nature.



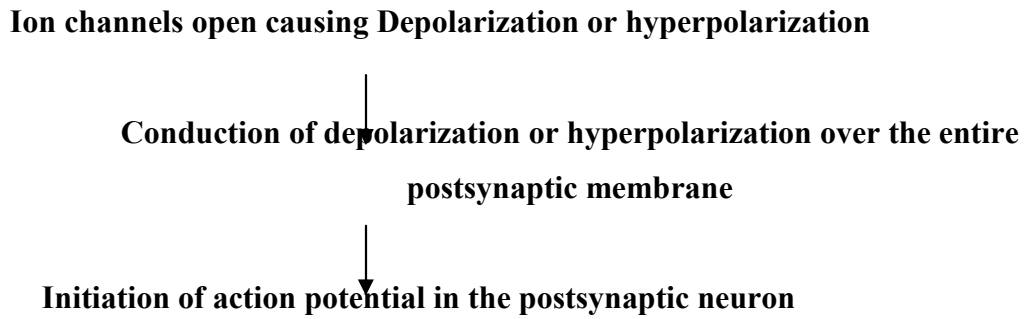


Fig: 5.9 Sequence of events in Synaptic transmission

❖ **Factors affecting synaptic transmission**

1) **Hypoxia: As synaptic transmission requires energy expenditure**, so oxygen deficiency stops it.
2) **Acidosis:** It depresses neuronal activity.A fall in pH ^{from} 7.4-7 may cause comma stage.
3) **Alkalosis:** It increases neuronal activity
4) **Drugs:** Like caffeine, threophylline and theobromine found in coffee, tea and cocoa increases neuronal activity
5) **Hypocalcemia:** or lack of calcium increase synaptic transmission while hypercalcemia retards synaptic transmission.

5.5 TRANSMISSION OF NERVE IMPULSE

In a neuron, nerve impulse is conducted from axon terminal of one neuron to dendrites of next neuron, so it a unidirectional process. The nerve impulse travels along axon in the form of a self-propagative wave of certain fixed electrochemical changes, The conduction of nerve impulse depends upon following facts like: Permeability of axolemma., Osmotic equilibrium between axoplasm and extracellular fluid, Electrical equivalence between axoplasm and extracellular fluid.In thin nerves the impulses travel at less than 1m/sec, but in large nerves they travel much faster at 100m/sec.

1. Resting Membrane Potential (Polarization)

Membrane of nerve cells have electrical potential difference (voltage), known as membrane potential. Resting nerve cell has -70mv (ranging from – 40 to – 90mv) electrical potential on the inner side of membrane. It is called resting membrane potential. This state of nerve cell is, called polarized state. Resting membrane potential is the unequal distribution of ions on both sides of the membrane of neuron determined by the concentration of ions. During polarized state, membrane is negatively charged from inner side and positively charged on outer side. The resting membrane potential is determined primarily by three factors

1) Concentration of ions on the inside and outside of the cell.

2) Permeability of membrane to the ions through specific ion channels

3) By the activity of the electrogenic sodium potassium pump.

Thus polarization is established by maintaining excess sodium ions (Na+) on the outside and an excess of potassium ions K+ on the inside. A certain amount of Na+ and K+ ions is always leaking across the membrane through leakage channels but the sodium –potassium pumps in the membrane actively restore the ions to the appropriate side.

The main factor that determine the resting membrane potential is the difference in permeability of K+and Na+. (fig:5.9).The resting membrane is more permeable to K+ than to Na+ resulting in slightly more net K+ diffusion (from inside of the neuron to the inside) causing a slight difference in polarity along the membrane.

Graded potential

It is a change in the resting potential of the plasma membrane in response to a stimulus .A graded potential occurs when the stimulus causes Na+or K+ gated channels to open .If Na+ channels open Na+ enters inside and the membrane depolarizes (becomes more positive).If K+channels open K+ exit across the membrane and the membrane hyperpolarizes (becomes more negative).A grade potential is a local event that does not travel far from its origin .It occurs in cell bodies and dendrites. Light, heat, mechanical.

Pressure and chemicals may generate potential depending upon the neuron (Fig: 5.10).

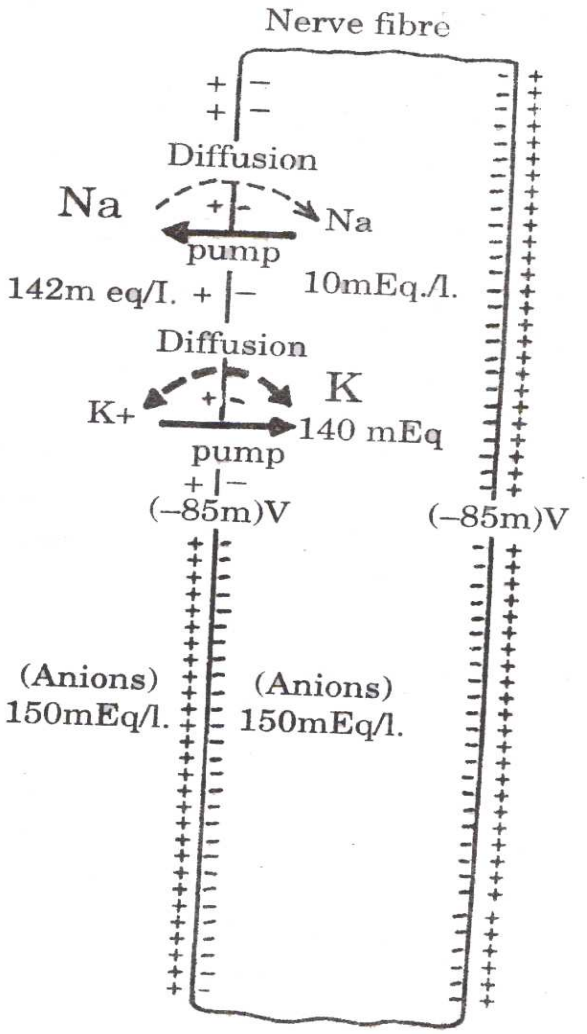


Fig 5.10 :Establishment of a membrane potential of -85mv in the normal resting nerve fibre and development of concentratin differences of sodium and potassium ions

Excitation of Nerve fibre

When a nerve fibre is stimulated it propagates a nerve impulse and the conduction of such an impulse along the axon is associated with an action **potential**. The factors which can elicit an action potential of a nerve fibre are-

a**) Chemical stimulation**: Certain chemicals such as acids, bases, salt solutions of strong concentrations and some hormones stimulate nerve fibres by disturbing resting potential.

b) **Mechanical stimulation**: Crushing, pinching or pricking a nerve fibre can cause a suddensurge of Na+ influx and cause stimulation of the nerves. There are numerous mechanoreceptors found distributed throughout the body which pick up even slightest sensation of pressure, pain, or vibrations.

c) **Electrical stimulation**: Electrical charge can also initiate an action potential because it causes an excess flow of ions across the membrane.

❖ **Action Potential**

Any factor that suddenly increases the permeability of the nerve membrane to sodium ions is likely to elicit a sequence of changes in the membrane potential lasting a fraction of a minute followed immediately by the return of the membrane potential to its resting value.This sequence of potential changes by a factor or stimulus is called action potential.Following sequence of events occur during an action potential (fig: 5.10).

Depolarization: When the stimulus picked up by a nerve is strong enough the sodium channels in the trigger zone open increasing the flow of Na+.The permeability of the nerve membrane to Na+increases and the ions rush to the inside of the membrane.This is known as activation of the membrane at the onset of ac tion potential.As sodium diffuse into the interior the internal negativity becomes less and there is reversal potential.Thus the membrane become depolarized. (Fig: 5.11).

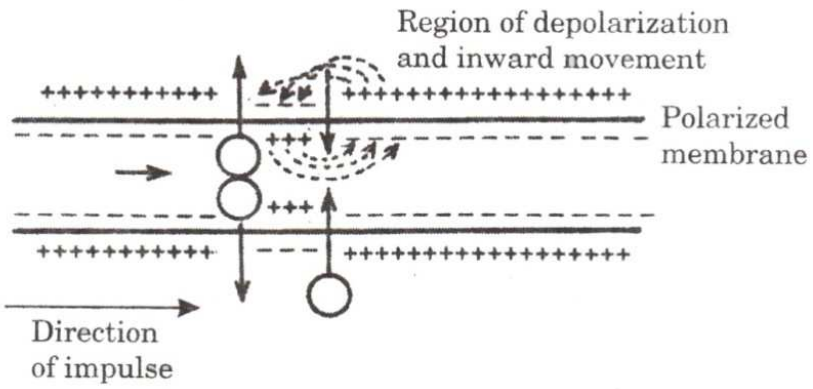


Fig:5.11: Distribution of charges before transmission on right,during it,in the middle,and after on the left.

❖ Repolarisation

Almost immediately after depolarization the sodium channelsclose and the nerve membrane again become impermeable to Na+ .As soon as sodium channels close potassium channels open ,thus allowing K+ from inside to rush out of the cell. This causes repolarizatareion by restoring g the original membrane polarization.Unlike the resting potential,in repolarization the K+are on the outside and Na+ are on the inside.

❖ Hyper polarization

By the time potassium channels close more K+ have moved out of the cell than is actually necessary to establish original polarized potential.Thus,the membrane is said to be hyperpolarized(-80mV).

* Na+and K+diffuse through sodium and potassium channels present in the membrane.(fig 374)The sodium channels are believe d to be oval in shape and having a dia of 3x5ang while potassium channels are rounded,with a dia of 3x3Aº.Each channel is believed to be guarded by a gate which can open and close the channel. Under resting condition both sodium and potassium channels are completely closed .The sodium and potassium gates are positively charged. The positive charge creates a +ve electric field that spread far into the channels and thus blocks ion permeability. The opening and closing of the gate is caused by electrical potential called gating potential.

❖ All or none response

Once an action potential has been set up by a stimulus above the threshold potential at any point on the membrane of a resting nerve fibre, the process of nerve depolarization will travel over the entire membrane. This process of impulse formation and transmission is independent of the strength of stimulus .Had the stimulus been less strong than the threshold value (sub-threshold potential), the impulse would have not generated at all. This is because the conduction follows an all or none response.i.e.the stimulus either fails to set up an impulse or it sets up a full sized impulse.

5.6 TRANSMISSION OF NERVE IMPULSE

Nerve impulse is transmitted from axon of one neuron to dendrites of next neuron, through synapse (fig: 5.12). If dendrites of more than one neuron are in contact of one axon, then nerve impulse will be transmitted to all neurons with same velocity. This transmission of nerve impulse from is a chemical process that is stored in synaptic vesicles.

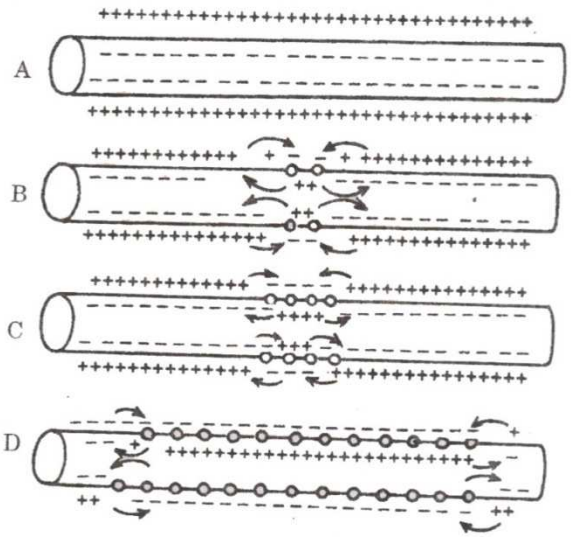


Fig: 5.12: Propagation of action potential in both directions along a conductive nerve fibre ,arrows showing local circuit. Impulse

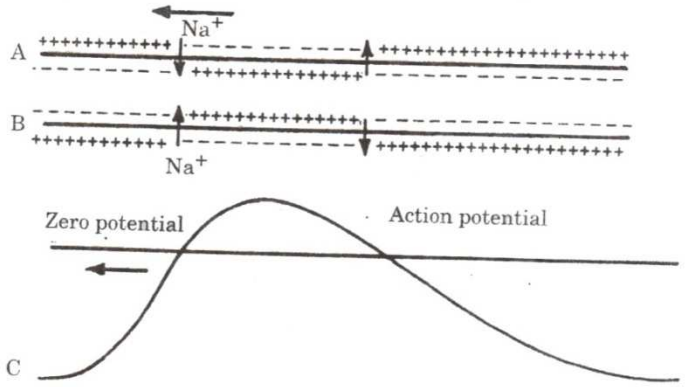


Fig:5.13 Conduction of a nerve impulse along an axon

A) and B) –The distribution of charge and the movement of ions along an axon and across the membrane during conduction of an impulse in the ditection of the arrow.
C) Action potential

At a synapse, telodendria of one axon are not in direct contact of dentrites of next neuron, but are separated by a space called synaptic cleft. Synaptic cleft is from 200 to 400 E wide. Tissue fluid is filled in the synaptic cleft. When nerve impulse reaches telodendria Ca^{++} from tissue fluid diffuse into synaptic vesicles. The concentration of Ca^{++} is 10,000 times more outside the cells than in the axoplasm. After entering inside synaptic vesicles, Ca^{++} stimulates release of neurotransmitters at the synapse.

Many synaptic vesicles fuse with the plasma membrane and release the neurotransmitters in the fluid of synaptic cleft. The molecules of neurotransmitters bind to some surface receptors of dendrites, which change the permeability of postsynaptic membranes and generate nerve impulse in the next neuron. In this manner neurotransmitters transmit nerve impulse to next neuron.

Neurotransmitters are also called neurohumor. Adrenaline, dopamine, serotonin and sympathin are some other excitatory neuron transmitters that are secreted at some nerve endings. Glycine and GABA (gamma amino butyric acid) are impulse inhibitory substances.

Nerve impulse is transmitted from one neuron to next neuron within milliseconds after which neurotransmitters are hydrolyzed. Enzyme acety-cholinesterase (AChE) breaks acetylcholine at synaptic cleft of cholinergic neurons. At synapse of adrenergic neurons, norepinephirine is released. Norepinephrine is disintegrated by atechol-O-methyltransferase enzyme (COMT).

The time required for the impulse to cross at the synapse is, called synaptic delay. Synaptic delay is of about 0.8 milliseconds.

Propagation of the impulse

Once elicited at one spot on an excitable membrane ,the action potential then excites adjacent portions of the membrane resulting inpropagation of the action potential .The fig A shows a reting nerve and a nerve excited in its middle portion has been shown in fig B which has developed a local circuit of current flow between the polarized and the resting membrane.Fig c show that more areas on the membrane become depolarized and the process of depolarization occur in both directions along the nerve fibre. The transmission of this depolarization along a nerve fibre is called a nerve impulse (Fig: 5.12).

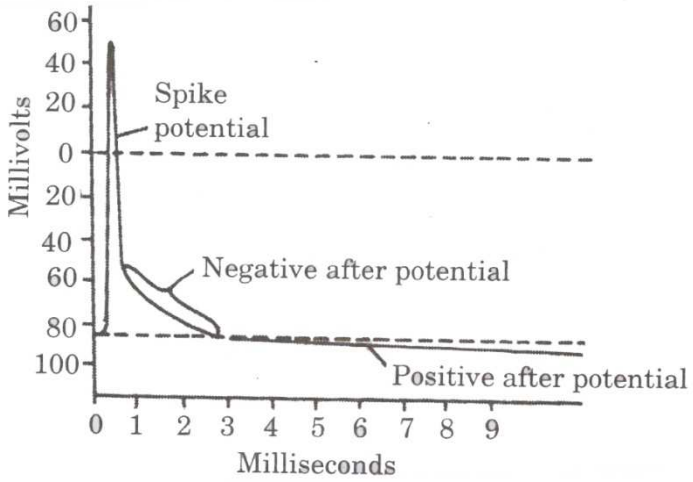


Fig: 5.14 An idealized action potential, showing the initial spike followed by a negative after potential and a positive after potential.

- **The Refractory Period**

After a nerve impulse has passed, there is a short period of time during which the nerve is not able to respond to another stimulus as it is still depolarized from the previous action potential .This brief interval of inexcitability is known as absolute refractory period and it is about 1/1000 second for a large myelinated nerve. Thus, there is limit to the frequency of the impulses the nerve fibre can transmit and it is usually 500-1000 impulses per second. In the later part of the refractory period second stimulus of higher intensity can generate a fresh action potential by it, second stimulus of same intensity cannot. This is known as **relative refractory period.**

- **The velocity of conduction**

The velocity of conduction through nerve fibres varies from as little as 0.5 meter per second in small unmyelinated fibres up to as high as 130metres per second in large myelinated fibres**.**

- **Saltatory conduction**

In 1925, **Lillie** observed that when the velocity of propagation of nerve impulse in an unmyelinated axon is compared with that in a myelinated axon of the same diameter, it is found

that the impulse travels at a much greater rate in myelinated fibre .This suggests that the mechanism of propagation may be different and has led to the hypothesis of saltatory conduction. Saltatory means "Leaping"(saltere-jump) and the term is used to describe a process in which the active process of conduction 'leaps'from one node of Ranvier to another node of Ranvier where the membrane is some 500times more permeable.

Saltatory conduction is of physiological importance for two reasons: First, by causing the depolarization process to jump long intervals along the axon, which greatly increases the velocity of conduction in myelinated fibres. Second, saltatory conduction conserves energy for the axon, for only the nodes depolarize and that metabolic energy is saved which would have been otherwise required to retransport the ions across the membrane.

5.7 SUMMARY

All we know of the world and of our own body, as well as all the conscious and unconscious control that we have over our behavior and physiology, we owe to our nervous system .It is made up of a number of components capable of feeding the main system with moment to moment information about the changes that are taking place in the external and internal environment of the animal. In all metazoans, specialized cells have evolved for the purpose of communication between the receptors the nervous system and the effectors termed as neurons. A neuron is thus the basic structural and functional element of the nervous system which is considered to be an evolutionary product of three lines of cellular specialization: an input or receptor surface, a conducting fibre for transmitting region of cell membrane and an output region usually concerned with the release of a specific transmitter substance (**Hoar, 1976**).

The overall mechanism by which the nervous system performs various regulatory and integrating functions involves the transmission of an electrochemical signal along the nerve fibre called impulse. It passes through the axons and dendrites of the neurons via the dendrites from the skin and then reaches the cell body, axon terminals and the synapse of the neurons. The synapse is the junction between neurons where the impulse moves from one to the other Neurotransmitters present in the synapses are the chemical transmitters that transmits the impulses by releasing acetylcholine and non-adrenaline..These impulses are then continued with the next dendrite in a chain reaction till it reaches the brain that in turn instructs the skeletal muscle to work.

5.8 GLOSSARY

- **Axons** are long nerve processes which carry nerve impulses from the Soma to other neurons; they vary in length but can become almost as long as half of the human body.
- **The soma** (body) of the neuron contains the nucleus which acts as the cell's control centre.
- **Dendrites** are short, thick processes which branch out of the soma in a tree like manor. They conduct nerve impulses to the soma.
- **Afferent (Sensory) Neurons** have the dendrites connected to receptors such as the eyes, ears etc.
- **Efferent (Motor) Neurons** have the dendrites connected to other neurons.
- Effectors are either glands or a muscle cell that is the receiving end of the nerve impulse.
- **Internuncial Neurons** have both the dendrites and the axons are connected to other neurons.
- **Impulse:** Self propagated disturbance induced by excitation.
- **Intereuron:** A nerve cell connecting two or more other neuron.
- A **nerve** is a bundle of fibres (axons and/or dendrites) outside the CNS.
- **Neuroglias are** cells of the nervous system that help protect and support it.
- **Ganglia** are groups of nerve cell bodies lying outside the CNS.
- **Neuron:** Nerve cell.
- **Neurosecretory cells:** Nerve cells that liberate neuro hormones.
- **Neurotransmitter:** A chemical mediator released by a pre-synaptic nerve ending.
- **Node of Ranvier:** Regularly spaced interruptions of the myelin sheath along an axon**.**
- **Post synaptic**: Located distal to the synaptic cleft**.**
- **The soma** (body) of the neuron contains the nucleus which acts as the cell's control centre. these contain many small neuron fibrils which project from the nucleus into the dendrites.
- **Synapse**: A conjunction between two directly interacting nerve cells, where impulses in the pre synaptic cell influence the activity of the post synaptic cell
- **Synaptic cleft**: The space separating the nerve cells at a synapse
- **Spinal Cord:** A spinal tract is a bundle of fibres in the CNS that travel long distances up or down the spinal cord.

5.9 SELF ASSESSMENT QUESTIONS

1) Describe the structure of a neuron in detail.

2) How nerve impulse is conducted through axon of nerve fibre? Explain with the help of suitable diagram?

3) How many different types of cells are found in nerve tissue? Describe the structure of a nerve cell with the help of diagram.

4) Explain the conduction and transmission of a nerve impulse.

5) Give a brief account of the mechanism of synaptic transmission.

6) What is synapse?

7) How many types of synapses are found in neurons? Describe each type?

8) What causes the excitability of neurons?

5.10EFERENCES

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5.12 Terminal Questions/Answer

1) Longest cell in human body may be:

a) Leg muscle cells b) Cells of femur

c) Nerve cells d)Cardiac muscle cell

2) Which of these is the characteristic of nervous tissue?

a) Sensitivity b) Irritability

c) Responsiveness d) All

3) In a neuron, the node of Ranvier is present in:

a)Cyton b)Medullary sheath

c) Telodendria d) Neurilemma

4) A nerve cell divides by:

a) Amitosis b) Mitosis

c) Both d) None

5) A chemical substance which is involved in conduction of impulse across a synapse is

a) Acetylcholine b) Adrenaline

c) Glycine d) All

6) The process of conduction of nerve impulse is

a) Electrical b) Mechanical

c) Electro-chemical d) Physio-chemical

7) Total no. of neurons in the central nervous system of an adult man is about:

A) 100million b) 1000

c)10,000million d)100,000million

8) Which of these is the axon of a neuron?

a) Neurite b) Neuraxis

c) Neuraxon d) All

9. Schwann cells are concerned with:

a)Muscular tissue b)Neuron

c) Olfactory epithelium d) All

10) Nissl's granules are found in:

a) Cyton b) Dendrites

c) Both d) Cyton, dendrite and axon

Answers:

1) C	2) D	3) B	4) D	5) D
6) C	7) C	8) D	9) B	10) C

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UNIT 6- MUSCULAR SYSTEM

CONTENT

6.1 OBJECTIVES

After reading this unit the reader will be able to

- Distinguish between three types of muscles tissue.
- Enlist the components of muscle system
- Classify the muscle according to their shape, direction of fibre and their action.
- Describe the ultra structure of smooth, striated and cardiac muscle
- Compare skeletal, cardiac and smooth muscles
- Explain the mechanism of muscle contraction based on theories
- Write short note on Simple twitch, muscular fatigue and tetanus.
- Explain the importance of Cori's cycle

6.2 INTRODUCTION

Muscular movement is a characteristic of the all animals above the level of coelenterates. Study of muscles is called myology. About 50% of the body bulk is formed of muscles. Muscular system is composed of specialized cells which are always elongated, spindle shaped, fibre-like cells called **muscle fibres** .Their predominant function is contractibility.

It is an organ system composed of skeletal, smooth and cardiac muscles. It permits movement of the body, maintains position and circulates blood throughout the body. The muscular tissue are able to convert chemical energy into mechanical form by translating signals from central nervous system and is initiated by synaptic transmission. Except for iris and ciliary body (of eye which are ectodermal)all muscles of metazoan body are mesodermal in origin and form about 40-50% of our body weight.

Muscular tissue contains all usual cell organelles, but also contains some special structures such as layer of glycoprotein on plasmalemma. Called sarcolemma. While, cytoplasm of muscle cells is also called **sarcoplasmic** reticulum Sarcoplasmic reticulum and sarcolemma remain connected by transverse tubules or T tubules. In sarcoplasm two types of proteins are present, soluble proteins and insoluble proteins. The soluble proteins present in sarcoplasm of muscle are, **myoalbumin, myoglobin** and myogen. While insoluble proteins are **troponin, tropomyosin,**

actin and myosin. In sarcoplasm many minerals such as sodium, calcium, phosphorus and magnesium are also present in traces. While, Potassium is the most abundant mineral found in muscle fibres.

6.3 MUSCULAR SYSTEM

In human body total number of skeletal muscles found is 639. Muscles are of three types according to their structure and function.

1. Smooth or visceral muscles or involuntary muscles /unstriped
2. Striated or skeletal muscles or voluntary muscles /striped
3. Cardiac muscles

➢ UNSTRIATED MUSCLE

These muscles are also called unstriped muscles because of the absence of striations in them. These muscles are also known as visceral muscles because these are present in visceral organs such as stomach, gut, urinary bladder, uterus, the retractor muscles of the extrovert of sipunculid worms, penis muscles of mollusks etc. As these muscles are not under control of will and contract under the influence of autonomic nervous system, these are also called involuntary muscles (fig: 6.1).

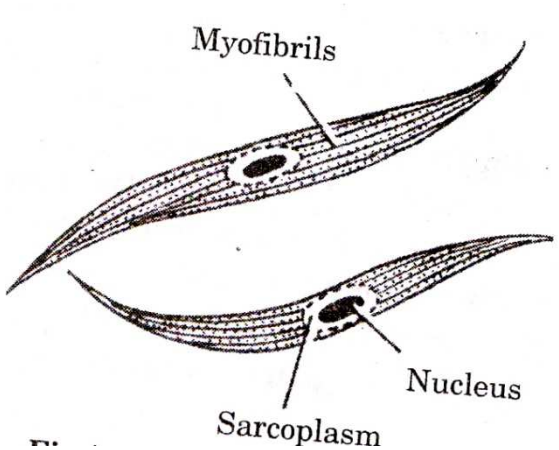

Fig 6.1 Unstriped muscle fibre

6.3.1 ULTRASTRUCTURE OF UNSTRIATED/SMOOTH MUSCLES

Smooth muscle fibres are elongated and spindle shaped.These muscles are widest at middle point arranged in sheets, interspersed with connective tissue. The length of each fibre varies from 20μm to 500 μm in width at the central region. The smooth muscle fibres are longest in pregnant uterus with a length of about 500 μm. These muscle cells have a poorly developed sarcoplasmic reticulum (SR).Sarcoplasm contains longitudinal but somewhat scattered myofibrils formed of many thin actin filaments but only a few thick myosin filaments bearing spurs.

- **Types of smooth muscles**

The smooth muscles of different body organs differ in their physical dimensions, organization into bundles, form of nerve innervations and function. In general smooth muscles have two types of structural organization:

a**) Multi-unit smooth muscles** :In multiunit structure, the smooth muscle fibre operate (fig 6.2 a)independently and are often innervated by a single nerve ending(like skeletal muscles).Ciliary muscles of eyes, Iris of the eyes, nictitating membrane of eyes in lower vertebrates and the erector pili muscles of hair follicle are such multi-unit smooth muscles.

b) Unitary smooth muscles:

In unitary type of smooth muscles whole mass of smooth muscle fibres contract together as a single unit. The muscle fibres of this type of smooth muscles are aggregated into bundles and their cell membranes remain connected at many points enclosing a number of gap junctions for free flow of ions. These muscle fibres contain many nuclei and thus display a synctial structure. Such type of muscles are found in the walls of most visceral organs including gut,bile duct, uterus, ureters and most blood vessels.(fig 6.2b)

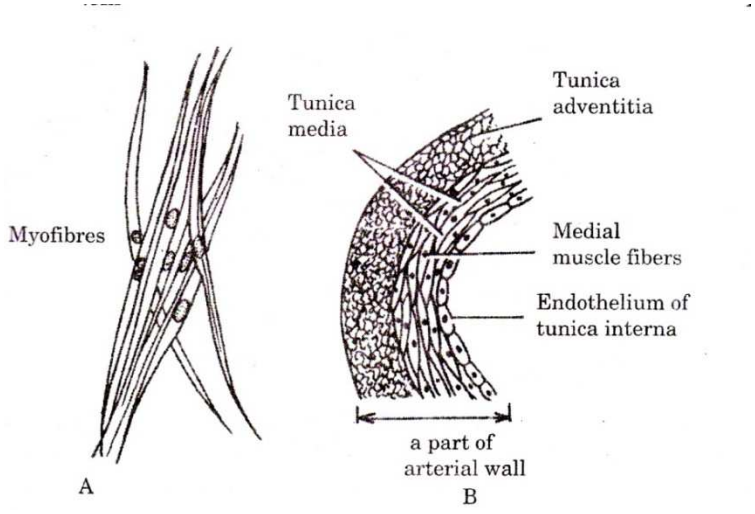

Fig: 6.2 A)Multi-unit smooth muscle; B)Unitary smooth muscle

- **Innervations of smooth muscle fibres**

The nerve fibres innervating the smooth muscles run for long distances among the muscle cells. There are discontinuous and intimate synaptic junctions between the motor terminal and muscle fibre. They have many swelling along their length which contains the chemical transmitter substances. In vertebrate smooth muscles, transmitter is released from many swellings or varicosities, along the length of autonomic axons that travel along the smooth muscle tissue. Transmitter released at a given swelling diffuses over some distance, encountering a number of small, spindles – shaped smooth muscle cells along the way. The postsynaptic receptor molecules in smooth muscles are distributed diffusely over the cell surface. Smooth muscle of vertebrates is entirely under autonomic and hormonal control. It contracts and relaxes more slowly than striated muscle and is capable of more sustained contractions.

- **Mechanism of Contraction of Smooth Muscles**

Smooth muscle fibre contains both actin and myosin filaments and their contraction are usually slow and free from conscious or willful control of brain. They are supplied by the nerve fibres of autonomic nervous system alone and multi-unit fibres contract on nervous stimulation(i.e., neurogenic),while the unitary fibres generate the action potential spontaneously within the muscles themselves due to intrinsic stimuli(like those of hormones, temperature, mechanical stress or other chemical stimuli)causing a myogenic origin. A large number of actin filaments remain attached to the dense bodies which are in turn attached to the cell membrane. The myosin

filament is located midway between the dense bodies and remains attached with the actin filaments on both ends (functioning like the Z-discs of skeletal muscles. During muscle contraction, myosin and actin react to form cross-bridges of actomyosin at the spurs of myosin filament but these cross-bridges are side polar, i.e., the bridges on the side hinge in one direction and those on other side hinge in the opposite direction. Due to pull generated by the cross-bridges the actin filaments slide over the myosin filaments bringing about the contraction of a smooth muscle fibre.

6.3.2 STRIATED MUSCLES/STRIPED MUSCLE

Striated muscles are called so because of the horizontal bandings on these muscles that gives them cross- striations.(fig 6.3)Striated muscles are also called **voluntary** muscles, because these act under conscious control and can be moved according to will. These are also called **skeletal muscles**, because these are attached to skeleton. These muscles are attached at both ends to bone by tendons. But skeletal muscles in the upper portion of oesophagus are not attached to skeleton.

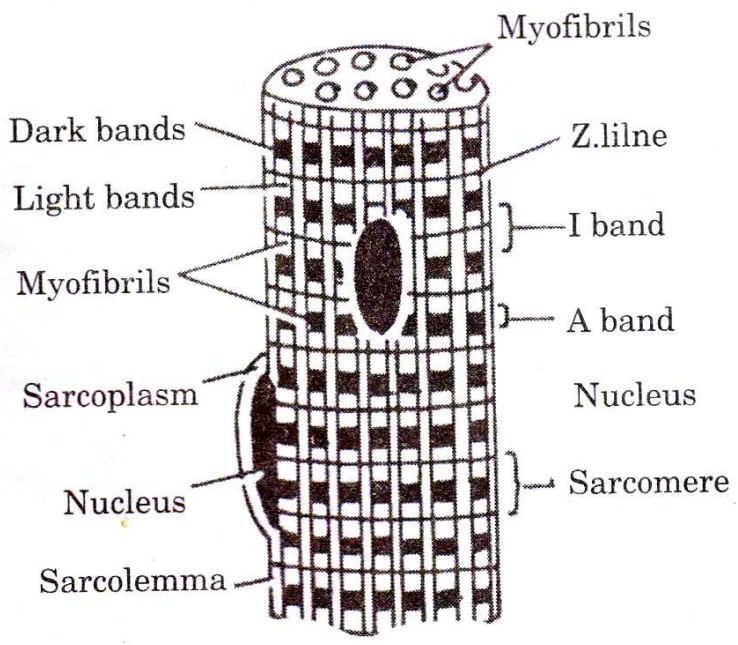

Fig 6.3 :Striated muscle fibre in magnification

- **Structure of Striated Muscles**

Histologically, a single muscle is a complex organ, composed of many cells held together by connective- tissue fibers and encased in a tough connective- tissue sheath. A striated muscle consists of many parallel bundles of muscles fibres. A bundle of muscle fibres is called **Fasciculus.** Muscle fibres are surrounded by connective tissue, called e**ndomycium**. Each fasciculus is bounded by layer of connective tissue called. **perimycium.** The connective tissue in which fasciculi are embedded is also called perimycium. Entire muscle is again bounded by a connective tissue sheath called **epimycium.** Tendons are attached to muscle through epimycium.

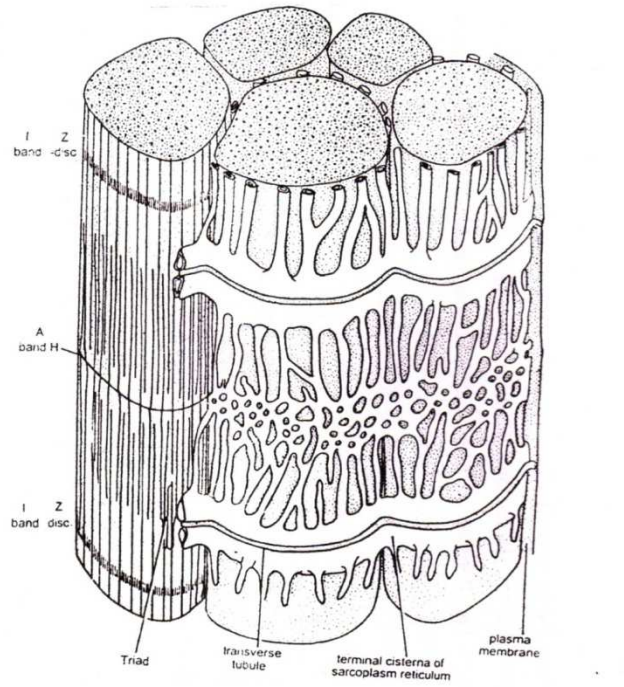

Fig: 6.4 Ultrastructure of a striated muscle

- **Ultra structure of Muscie Fibre/ myofilaments**

A skeletal muscle consists of parallelly arranged striated muscle fibres. Striated muscle fibres are long, unbranched and synctial, with many nuclei located at the periphery. Many embryonic myoblasts combine to form a fibre. Length of striated muscle fibres varies from 1 mm to 30 cm and diameter varies from 10 to 100 5µ (Fig. 6.5 a & b).

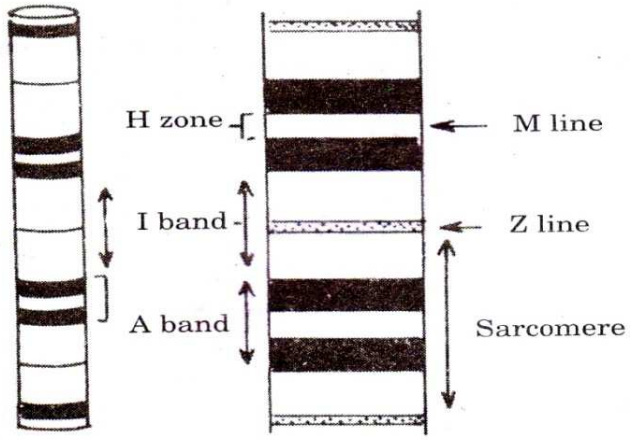

Fig:6.5a &b Structure of a myofibril

Muscle cells or fibres are long and spindle- shaped. The outermost covering of muscle cell is called the sarcolemma, which is visible and permanent structure. The sarcolemma is an electrically excitable membrane. Lying outside the sarcolemma there is a 50 nm wide basal lamina followed by connective tissue containing reticular fibres. Just beneath it, in muscle cell are found numerous parallel thread- like structures, called the myofibrils.These are 1 to 3µm in diameter. Myofibrils are arranged in closely packed groups of 4 to 20 or more. Usually each group is separated from its neighbours' by spaces 0.2 to 0.5µm wide filled with sarcoplasm. The myofibrils are composed of myofilament fibrous elements composed of the muscle's contractile proteins.

In the sarcoplsam are present the sarcosomes (mitochondria), which are larger than those of other cells and more in number. They are distributed between groups of fibrils either in longitudinal rows or in horizontal planes at the level of I bands, A – I junctions or triads of sarcomere. The sarcoplasm reticulum is arranged in network around or between myofilaments.It consists of two types of tubules-

1. Running parallel to the length of muscle fibre is the longitudinal tubule or L-tubule

2. Running towards the centre of the fibre from the surface membrane is the transverse tubule or T-tubule .The T-tubule is situated at the junctions of A and I band. The space enclosed in the T-tubule is extracellular space and it is filled with extracellular fluid. At the junction of T-tubule

and L-tubule there is a swelling called **cisternae**. This is filled with calcium ions which play an important role in contraction of and relaxation of muscle.The T-tubule provide a simple means of conveying an electrical signal derived from the depolarization of the sarcolemma. Amount of sarcoplasmic reticulum is correlated with the contractile activity of the fibre. Those muscles which have slower contractions possess less developed sarcoplasmic reticulum, while fast muscles fibres have an extensive reticulum. The myofibrils are the contractile units of the muscle fibre. They have a striated appearance because conspicuous cross striations divide the myofibrils into alternating light and dark bands called I-band (**Isotropic**) and A-band (**anisotropic band)** respectively. The A-band is made up of about 120A^0thick and 1.8µ long myosin filaments while I-band consists of about 60A^0thick and 1.0 µ long actin filaments.The A band has the lighter region (a region of low density) in the centre known as H-Zone (named after its discoverer **Hensen**) which is visible in relaxed fibrils.In the centre of H-zone tere is a dark M-Line. The I-band is divided by a dark central Z-band or Z-Line (from the German word **Zurischenscheibe** meaning central membrane).The area between two adjacent Z-lines is called **Sarcomere** (Fig: 6.6) which is the unit of muscle contraction. While the uncontracted sarcomere is of a mammalian striated muscle is about 2.1 -2.3 µ long.

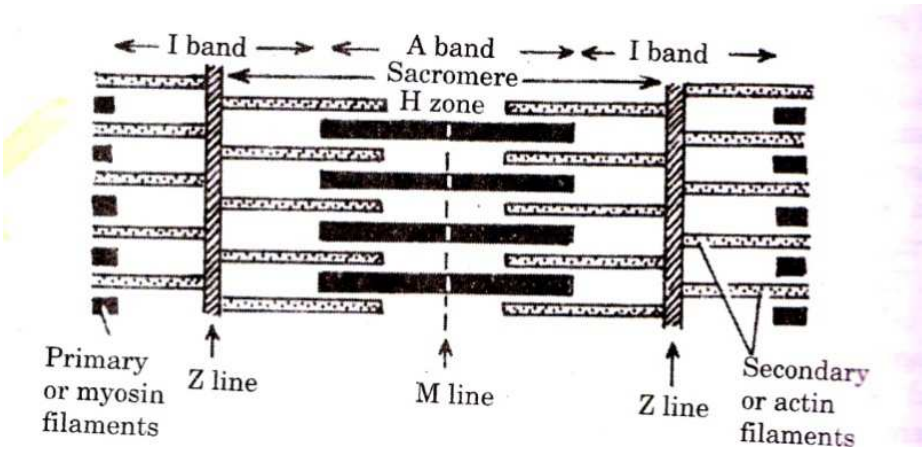

Fig:6.6 (a) A single sarcomere in magnification(At rest)

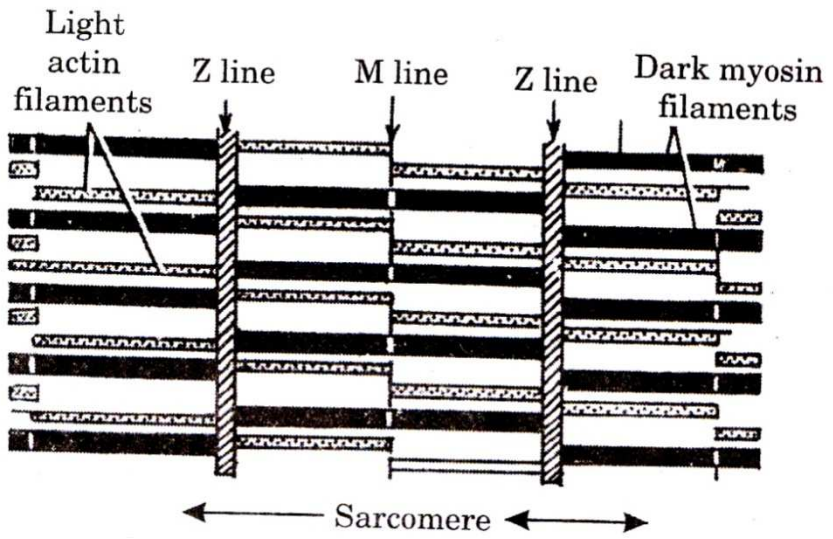

Fig:6.6 (b) A sarcomere contracted

➢ **Sarcomere**

From one Z-band to another Z-band, muscle fibre functions as a **contractile unit,** called **sarcomere.** Each I-band is bisected by narrow dark line, the Z-line (from the German Zwischenscheibe. Central membrane) or **Krause'smembrane.** This is the region where thin filaments meet. The sarcomere is often called the smallest functional unit of muscle.

Length of uncontracted mammalian sarcomere is 2.3µ. (Fig:6.6a&b)

➢ **Chemical composition of a muscle fibre**

The skeletal muscles contain about 75% of water and nearly 25% of solid matter like proteins, lipids, carbohydrates,inorganic salts, extractives, pigments ,enzymes and coenzymes. The muscle proteins are of following types such as:

a) **The stroma proteins**: It constitutes about $1/5^{th}$ of total muscle protein. They are collagen and elastin elements that bind muscle fibre and transmit tension to tendons.

b) **The cellular proteins**: They constitute about $1/5^{th}$ of the total muscle protein. They include various enzymes which catalyze various chemical reactions occurring in muscle cells.

c) **The contractile proteins:** The thick filaments of muscle fibre are made up of myosin protein and the thin filaments are made up of three proteins actin troponin, and tropomyosin.

Actin has a molecular weight of about 70,000.It occurs in two forms a globular or **G-form** and a filamentous (fibrous) or **F-form**.Actin attain a globular form in the presence of salts. In the presence of KCl and ATP it polymerises to form long fibres or F-actin, which is made up of two helical chains twisted around each other.

Myosin (fig: 6.6) has a molecular weight of about 450,000.It is tadpole shaped and compose of two globular heads and a long tail. The heads form cross bridges with actin A myosin molecule can be broken into head and tail fragments by the enzyme trypsin. The head fragment is globular about 150-250ºA long 40ºA in dia and a molecular weight of about 350000 called heavy meromyosin**(HMM)**.It has two active sites :one binds with actin molecules and other is the catalytic site where hydrolysis of ATP occurs. The tail fragment is known as light meromyosin(**LMM)**.It is about 1000ºA long and 15-20ºA in dia having the molecular weight of about 150,000 (fig: 6.7).

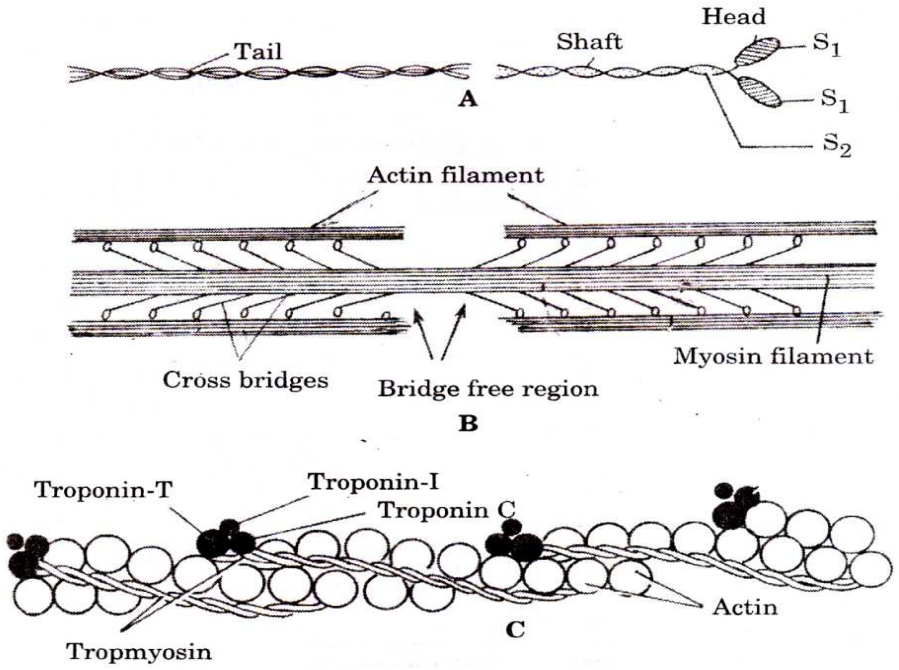

Fig: 6.7 A) Myosin molecule B)Schematic representation of contact of actin and myosin in a skeltal muscle.,C) Relative position of troponin,tropomyosin,and actin in thin filament of muscle.

Tropomyosin: It is another important major protein and constitutes about 2.5% of muscle proteins and has a molecular weight of 65,000. Each thin filament contains 40-60tropomyosin molecule, It is rod shaped about 41mmlong and consists of two unidentical α and β chains coiled around each other. There are two forms of tropomyosin: Tropomyosin A (paramyosin) and Tropmyosin B. Tropmyosin plays an important role in sensitizing the contractile proteins to calcium (Fig: 6.8).

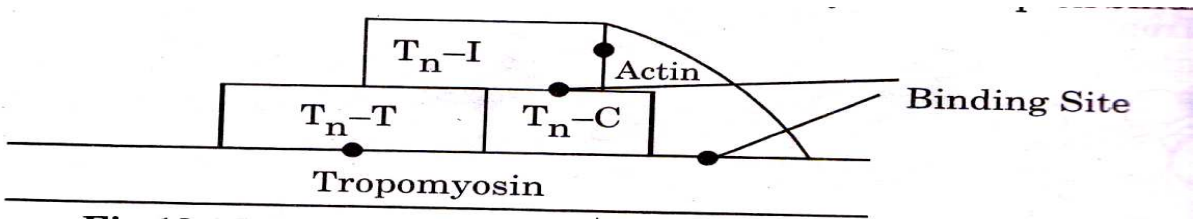

Fig: 6.8 Diagrammatic relationships between actin ,tropomyosin and the 3 subunits of troponin

- **Cross bridges**

The head of myosin is linked with actin filaments by cross bridges which play an important role in muscle activity .Electron microscopic study has revealed that the cross bridges projects outwards from both the ends of a myosin filament at regular interval of about 60-70º A. In the centre of myosin filament there is a region of about 0.15to 0.24µ length where cross bridges are absent .During muscular activity each cross bridge is first attached to an active site on the actin filament at a definite rate and then detached from it .Thus, cross bridges have an important role in muscular activity during muscular contraction .

6.3.3 CARDIAC MUSCLES

Cardiac muscles are found only in the heart and at the end of large veins, at the places of their entry into the heart, these are special muscles with characters of both smooth and striated muscles. Cardiac muscles are not under voluntary control and show fainter cross – striations. Cardiac muscles are striated and involuntary. Special conducting system of heart consists of special cardiac muscle fibres. Three functional synctia form heart are atrial muscle, ventricular muscle and conducting system of heart.

- **Ultra Structure of Cardiac Muscles**

Cardiac muscle has a feature in common with both striated and smooth muscle. Its cells are cross-striated and contain a single central nucleus. Cardiac muscle cells are branched. The length of each cardiac muscle fibre is 85 to 100 μm long. In sarcoplasm one or two nuclei are present. The branches of cardiac muscle fibres form junctions with neighbouring cells end to end by electrical junctions and are therefore electrically continuous. At junctions of two neighbouring cells intercalated disc are present. The presence of intercalated discs is the characteristic feature of cardiac muscles (fig: 6.9).

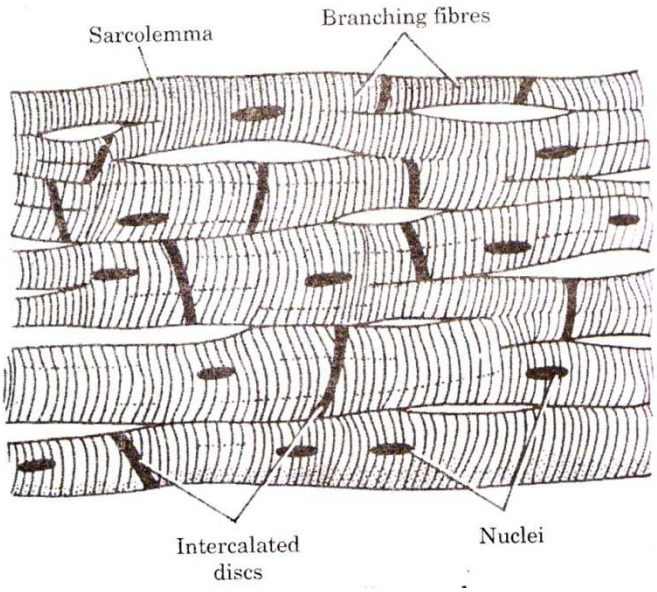

Fig: 6.9 Structure of cardiac muscle

Cardiac muscles fibres have same contractile filaments as in skeletal muscles. In cardiac muscles faint but regular alternate dark and light bands have sarcomeres. Mitochondria and glycogen granules are abundant. In cardiac muscles mitochondria are quite large in size, sometimes up to a full sarcomere in length. Cardiac muscle cells of mammal possess a highly developed sarcoplasmic reticulum (SR) and system of T-tubules. T-tubules are larger in diameter and surround the myofibrils at the level of Z line. T- Tubules are regularly arranged in ventricular muscles but quite rare in atrial muscles. These muscles are immune to fatigue.

- **Contraction of Cardiac Muscles**

Cardiac muscle is activated by the release of Ca^{2+} from SR Ca^{2+}.diffuses into the cells because of the increased calcium conductance of the surface membrane. Cardiac muscle has a relatively rapid contraction. special conductive tissue of heart is self excitatory and has three kinds of fibres-nodal fibres forming the S-A node and A-V node ,transitional fibre which form the inter-nodal and connecting fibre and Purkinje fibre found ramified into the walls of ventricles. The conductive tissue of heart contracts only feebly because it contains only few contractile fibres instead, they provide excitation and rapid conduction of impulses through the heart. The heart muscles thus keep on contracting and relaxing involuntarily throughout the life rhythmically and without fatigue performing a cardiac cycle. Self-excitation of S-A node produces the action potential for contraction and as cardiac muscles is myogenic in nature like most smooth muscles.

6.4 MUSCLE CONTRACTION

A muscle fibre contracts when it is stimulated by nerve impulse, electric or mechanical stimulus.

6.4.1 MECHANISM OF MUSCLES CONTRACTION

The skeletal muscle contracts when it is excited by a stimulus .Excitation (depolarization) of muscle fibre causes development of action potential. This is an electrical phenomenon. The action potential travels along the muscle fibre and through a sequence of events causes' muscular contraction. This is a mechanical phenomenon. The two phenomenon are linked together by release of Ca++ion. Therefore the process by which depolarization of muscle fibre initiates contraction is known as **Excitation-Contraction coupling**.

- **Physiology of muscle contraction**

  In a resting muscle fibre, the sarcolemma is electropositive outside and electronegative inside.Thus, it has a resting potential of -90mv and is said to polarize.The skeletal muscles are supplied by large medullated nerve fibres arising from motor neurons of spinal cord. Each nerve fibre innervating a voluntry muscle divides many times to stimulate some 3-2000 muscle fibres through a neuromuscular junction .Thus,only a nervous stimulus causes the contraction of a skeletal muscle which spreads into the muscle fibres through its neuromuscular contact.

- **Physioanatomy of the Neuromuscular junction**

The following Fig.6.10 depicts the neuromuscular junction between a myelinated nerve ending and a skeletal muscle fibre.The nerve divides to form a complex of nerve endings called end plate which rests over the plasma membrane of the muscle fibre.The junction is covered by one to many Schwann cells insulating the end plate from the surrounding fluids.A synaptic cleft of 200-300ºA width also formed between the azxon terminal and the muscle fibre membrane. Several folds of the muscle membrane also form secondary synaptic clefts increasing the contact surface area of the junction. The axon terminal buttons contain numerous synaptic vesicles having acetylcholine as an excitatory transmitter.Acetylcholine is synthesized in the cytoplasm of the axon terminals but it is absorbed and stored in synaptic vesicles.

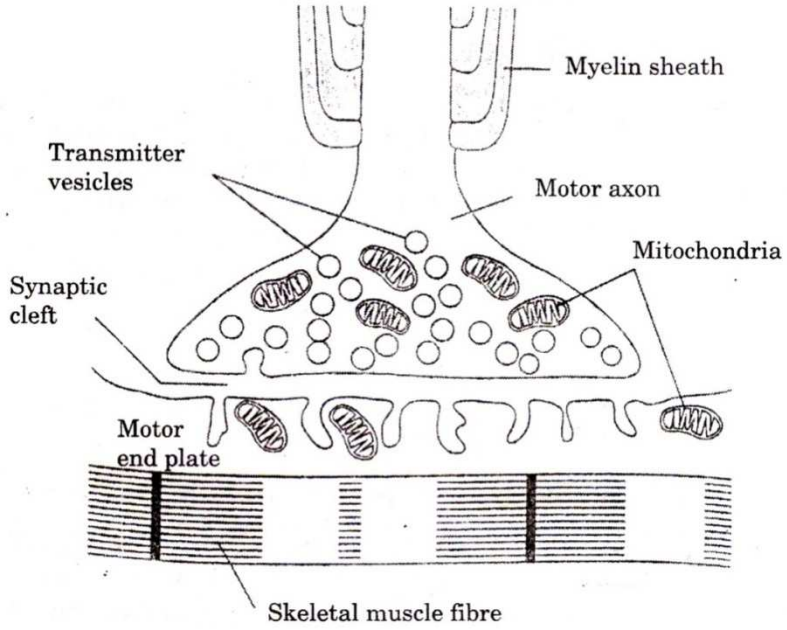

Fig.6.10 Neuromuscular Junction

Theories of muscle contraction

A number of theories have been proposed to explain the mechanism of muscle contraction.

1. **Sliding filament Theory** the sliding filament theory was proposed by A. F. Huxley and H.E Huxley in striated muscle fibres under electron microscope in 1954. The theory explains that the contraction of muscle is brought by sliding movement of an actin filament over myosin filament towards M-line of H-Zone. The sliding movement of actin filaments occurs by forming and

breaking of cross bridges over the myosin filaments.(fig 6.12)In this process there are no changes in actual length of thick and thin filaments, rather there is increased overlap within the muscle cell. It has also been found that during both contraction and relaxation the length of A-band remain static and the length of I-band changes in accordance with the length of the muscle.

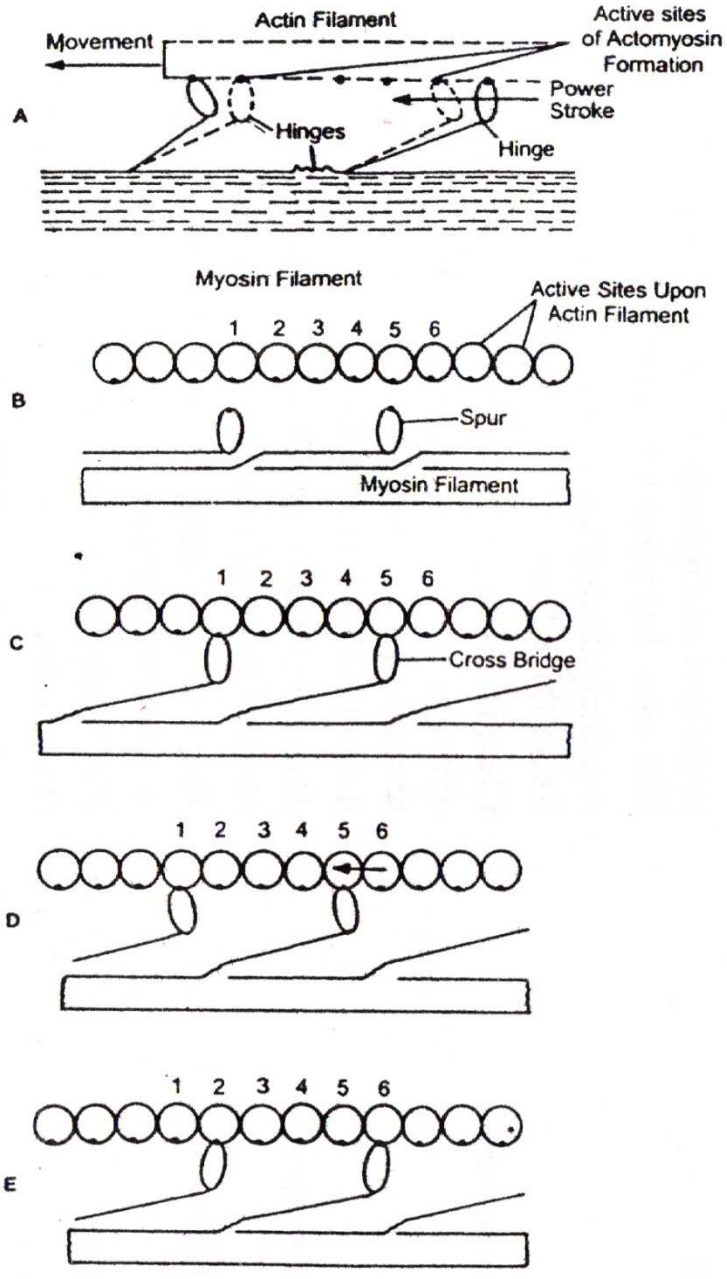

Fig:6.12 Formation of cross bridges between actin and myosin filaments

Under resting condition ,no cross bridges are formed since toponin "I:is lightly bound to actin and tropomyosin covers the actin sites where myosin heads bind to actin ,Therefore the troponin tropomyosin complex constitutes a relaxing protein which inhibit the interaction between actin and myosin. When an action potential travels over the muscle fibre membrane, large quantities of Ca++ are released into the sarcoplasm surrounding the myofibrils. The Ca++activate the attractive forces between the filaments by first binding with troponin.

Neilson (2002) has listed the sequence of events in muscular contraction as follows:

1. **Stimulation**

ii) Stimulation of motor nerve produces as action potential which is propagated to the neuromuscular junction.

iii) Acetylcholine (Ach) is released into the synaptic cleft which binds with Ach receptors present in the muscle membrane generating end plate potential.

iv) Depolarization of the muscle membrane due to increase in permeability to Na+.

v) Generation of action potential in the muscle fibre which spread inwards along the I-system.

vi) Spread of polarization to the terminal cisternae causing the release of Ca++ in the muscle fibre.

vii) Increase in concentration of Ca++in the intracellular fluid by 2000times, Ca++ diffuse into thin filaments.

2. Contraction

i) Ca++ binds with troponin causing tropomyosin to move laterally exposing the binding sites for myosin heads on actin.

ii) Actin activates ATPase activity catalyzing the breakdown of ATP which induces conformational changes in heads of myosin filaments that cause cross bridges to revolve freely causing muscular contraction.

3. Relaxation

A few milliseconds after the action potential is over the Ca++ are actively pumped by Ca++.Mg++ATPase (the Ca++pump) into the sarcoplasmic reticulum for storage in the terminal cisternae. Once Ca++concentration decreases in ICF sufficiently to 10^{-7} moles/L,chemical

interaction between myosin and actin ceases and muscles relaxes.Thus the sliding movement is mediated via cross bridges. If the active transport of Ca++is inhibited relaxation does not occur, even though there is no action potential. This result is sustained contraction of the muscle, called **contracture.**

Summary of events during relaxation

Ca++ pumped back into sarcoplasmic reticulum ⟶ Myosin-ATPase activity depressed ⟶ Cross bridges broken ⟶ Myosin and actin return back to resting stage ⟶ Tension (contraction) disappears

- **Neuromuscular Blocking agents**

These are the substances which block activities at the neuromuscular synapse. They are of two types-

1) Depolarizing Inhibitor: These act as Acetylcholine but are resistant to the action. **of** AcH esterase.They polarize the muscle membrane but since they are not hydrolysed by AcH esterase.eg,succinyl choline

2**) Non-polarizing Inhibitor**: These drugs act by competing with AcH for receptor.They block the receptors but donot have biological activity of AcH.eg gallamine

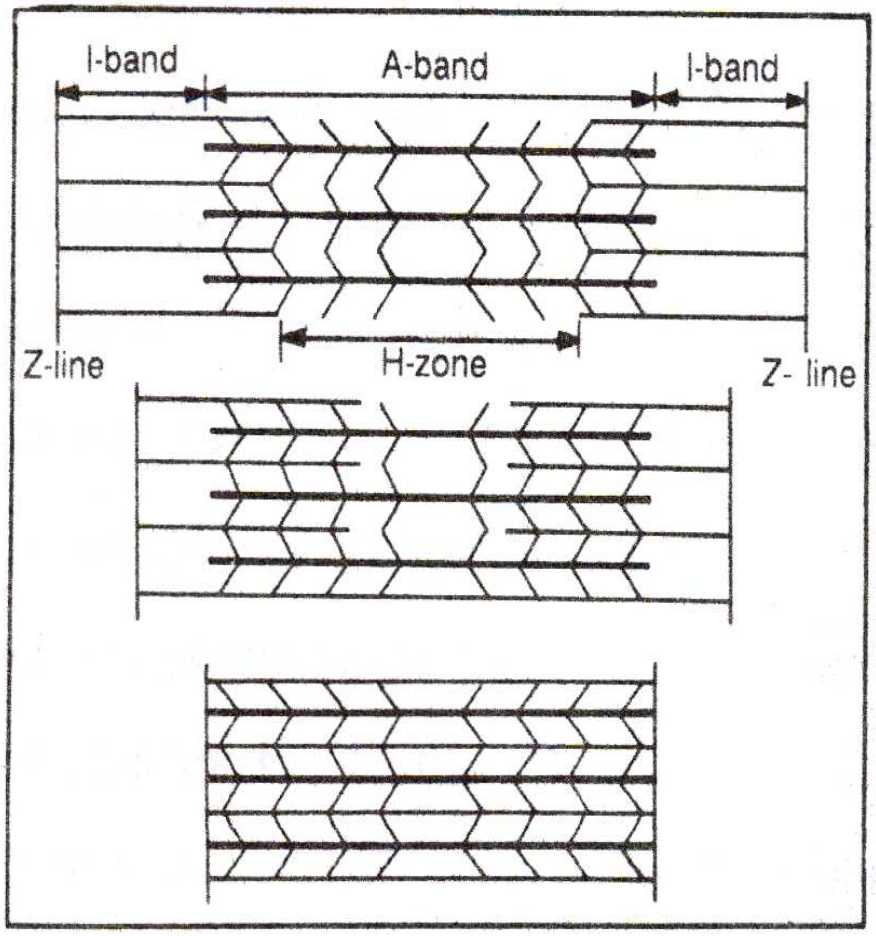

Fig: 6.13 Diagrammatic representation of the nature of contraction according to sliding filament hypothesis.

- **Davies' Theory of Muscle Contraction**

This theory was proposed by **R.E.Davies** in 1963. A fixed negative charge is present in side chain of myosin filament. Near the base of the side chain is a fixed negative charge also. This repulsion pressure keeps the side chain extended in resting molecule. The side chains of myosin are called cross bridges, because they form ionic bond or bridges with neighboring actin filaments. When during activation calcium ions are released, two positive charges of Ca++ between the two sites with minus charges causes coiling of side chains of myosin filaments, causing sliding or actin filaments over myosin filaments. This causes contraction of muscle fibre.(Fig:6.14)

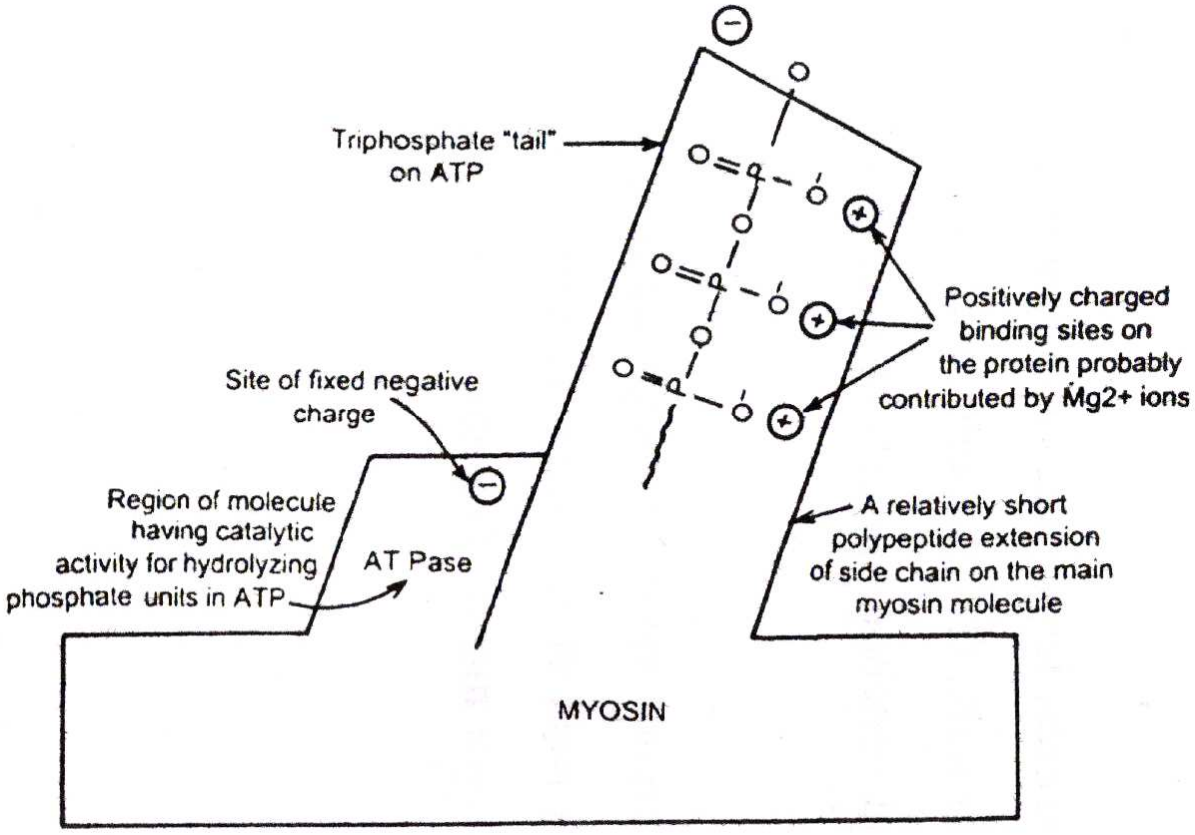

Fig 6.14: Mechanism of muscle contraction according to Davis' Theory

- **Calcium release theory**

This theory was first forwarded **by Alexander Sandow** and later modified by **Ashley (1967), Winegard (1968) and Graham Hoyle (1970)**.This theory state that the muscle contraction is induced by calcium ions released from a binding site in sarcoplasmic reticulum. The end plate potential and the action potential of the muscle fiber is responsible for relaxing the calcium channels gates present in the tubules of sarcoplasmic reticulum. Thus, the calcium ions are responsible for initiating the muscle contraction.

Recent researches in the mechanism of muscle contraction reveal that the role of calcium ions is quite significant in muscle movement and contraction. At rest, when the concentration of free Ca++ is low; the molecules of troponin serve to inhibit the interaction between actin and myosin molecules. However ,at the event of muscle contraction when Ca++ are released from sarcoplasmic reticulum (due to an action potential)and where there is a higher calcium ion concentration it forms a complex with troponin molecules. Troponin –Ca++ combination inactivates troponin –tropomyosin unlocking the actin filaments. The free actin filaments interact with myosin spurs to form cross-bridges of actomyosin causing sarcomere contraction.Thereafter,the calcium ion are forced into sarcoplasmic reticulum using energy from ATP. Actomyosin split at cross-bridges to form actin and myosin,spurs breakaway and come to lie in original position and the muscle relaxes.Thus,the muscle contraction begins with the release of calcium ions and it turned off by their withdrawal.(Fig:6.15)

Released Ca^{2+} → troponin → Tropomyosin → actin

(TN-C)

→ (combines with) Myosin → Actomyosin (Muscle contraction)

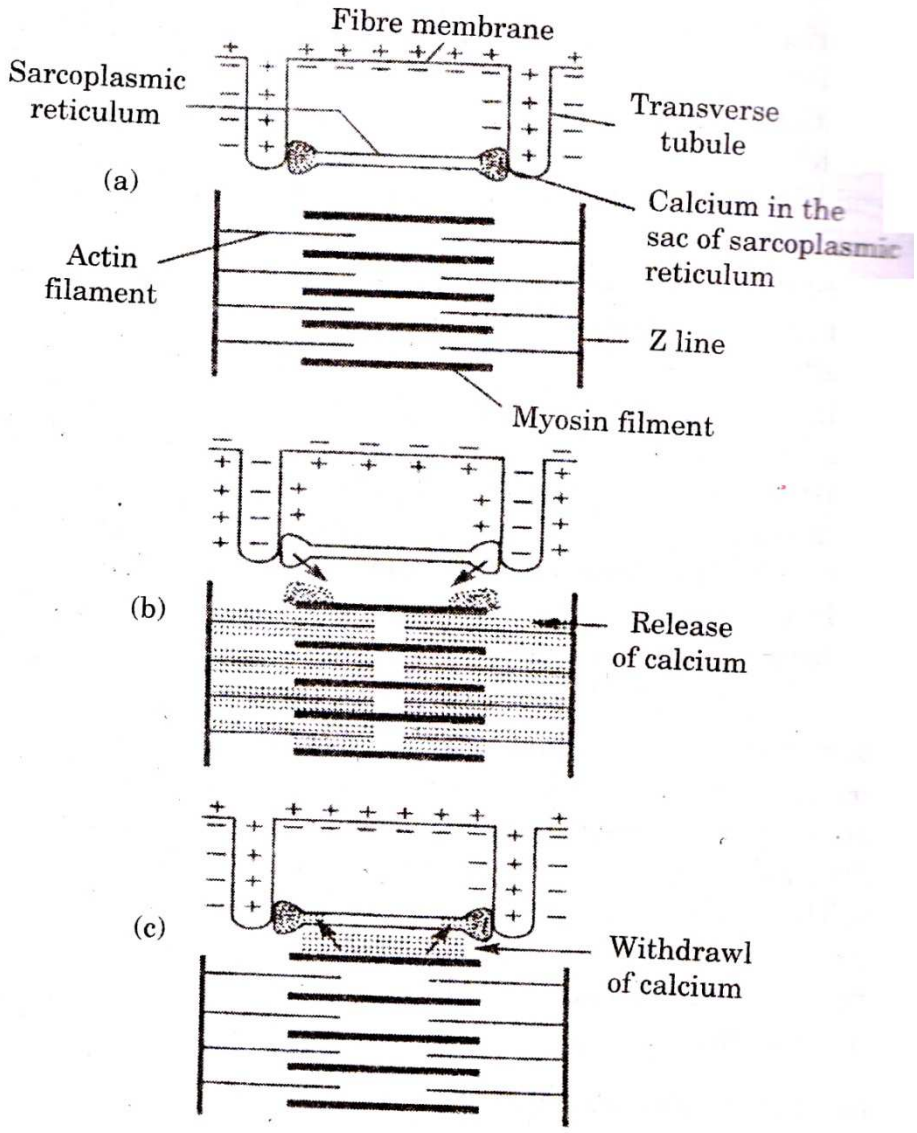

Fig: 6.15 Role of calcium in muscle contraction

➢ **Changes taking place during muscular contraction**

During the contraction of muscle a number of changes take place such as-

- **Mechanical change:** During muscle contraction the muscle fibre shortens in length but its, thickness increases. Volume remains same or slightly increases, in isometric contraction, length remain same but tension increases.

- **Viscosity:** Muscle contraction causes densening of the sarcoplasm .or increase in the viscosity of cytoplasm during muscle contraction

- **Tone:** Isometric contraction of the muscles causes increase in tonicity; however it does not change during isotonic contraction**.**

➢ **Chemical changes:**

Glycolysis and oxidative breakdown

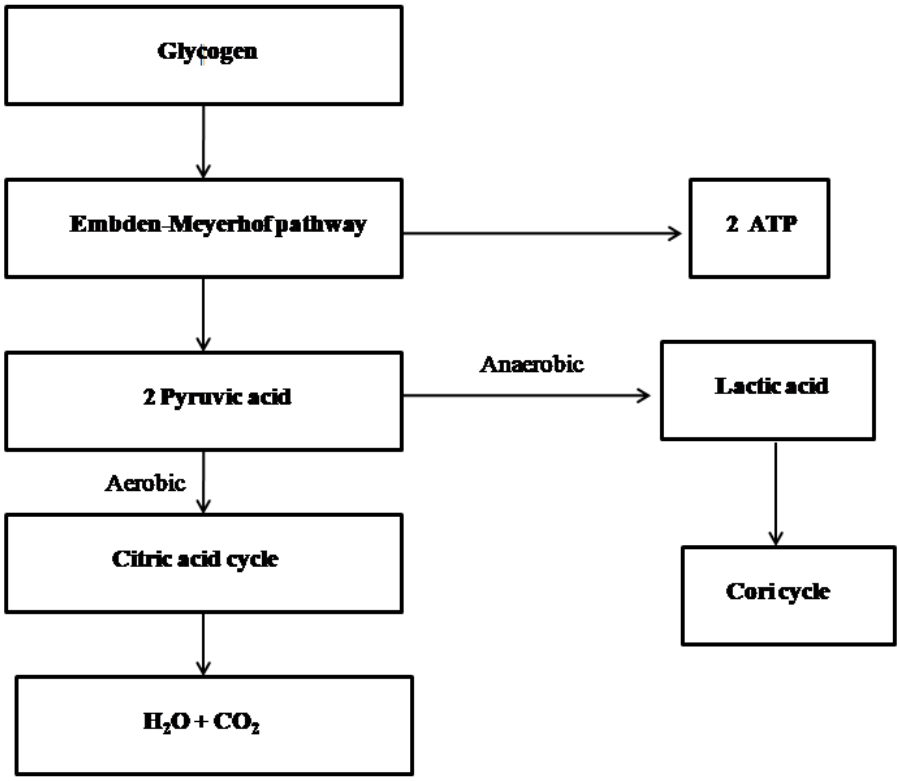

Fig: 6.16: Glycolysis and oxidative breakdown

End products are pyruvate and lactic acid .Out of the total quantity of lactic acid formed under anaerobic conditions $1/5^{th}$ of it is oxidized to Co_2 and H_2O, $4/5^{th}$ is resynthesized into glycogen in the liver.(fig:6.16). Cori cycle involves the cyclic changes that occur in the muscle and liver during heavy exercises for quick removal of acid from the muscle.

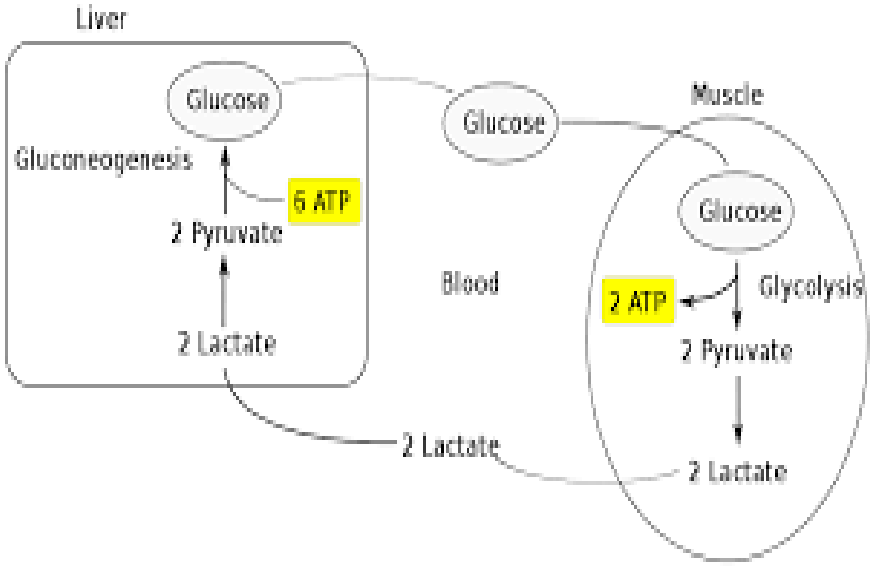

Fig: 6.17 Cori Cycle

In the presence of O_2 pyruvates acid is oxidized through Kreb TCA cycle. There is net production of 38ATP per glucose molecule, under aerobic condition and under anaerobic conditions only 2 ATP molecules are formed. Utilization of other fuels during muscle contraction are-β oxidation of fatty acids for the synthesis of ATP Ketone bodies.

- **Changes in H+concentration or pH**: During molecular activities both acidic and alkaline products are formed .They try to neutralize each other and try to maintain constant pH .However, in the initial stages of muscle contraction the pH becomes more alkaline due to the release of creatinine (which is highly alkaline).In the prolonged activity pH becomes acidic due to the accumulation of lactic acid.
- **Thermal changes:**

During muscular contraction heat is produced in two stages-

i) Initial heat occurs at the onset of contraction

ii) Recovery heat is delayed heat occurs following contraction

Initial heat is rapid outburst of heat, While recovery heat is slow and prolonged production .Considerable amount of this energy is converted into heat energy which is also helpful in

maintaining body heat in homeotherms.That is why we feel hot after exercise and the body temperature shoots up after shivering in malaria.

- **Electrical changes**: This includes generation and spread of action potential or impulses in the muscle. Spread of action potential is followed with contraction. Efficiency of muscle contraction

Total energy output=work done + heat liberated

Efficiency=Work done/Total energy output x100

Following sequence of events electrical events occur during muscle contraction-

1) Resting potential (-70mv) is disturbed

2) The potential difference along the two surfaces of sarcolemma comes to 0.0mv**(depolarization).**

3) The potential difference reaches to +35mv which suggests that the inner surface of sarcolemma is positive by 35mv (reverse polarization).A potential difference of -70mv (resting potential) is set in **repolarization.**

- **Histological changes:** Following histological changes occur during interdigitization of actin and myosin

1) The two adjacent Z-lines come closer

2) I-band disappear

3) There is no change in the A-band except that Hzone disappears.

All these histological changes in a sarcomere are dependent on the strength of its contraction. In a strong contraction the two Z-lines come closest and H-Zone disappears almost completely.Thus Ca++ and the ATP are very essential for muscular contraction.

- **Role of ATP in muscle contraction:**

Muscle contraction require a large amount of energy to work.ATP is the source of energy for muscle contraction, was confirmed by **R.E Davies**.ATP forms actomyosin –ATP complex with actin and myosin during muscular contraction .It is not only the energy source but also a structural component of contraction apparatus because it makes actomyosin elastic and able to contract. When ATP separates from the complex,as in extreme fatigue, the muscle becomes rigid and stiff.When muscle is active, the energy of ATP is used up and if the muscle is to be re-energised new actomyosin-ATP complex must be formed. Muscle requires ATP not only for contraction but also for relaxation when Ca++ is pumped back into the sarcoplasmic reticulum. The main source of energy for muscular activities comes from ATP which is generated by oxidative phosphorylation of glucose in the mitochondria. This is why; the mitochondria are abundantly present in the sarcoplasm of skeletal muscle fibres. Besides, creatine phosphate is also present in the muscles which serves as a reservoir of high energy phosphate bonds ,During muscle contraction myosin ATPase,Ca++ and Mg++ causes this energy release:

$$\text{ATP} \xrightarrow[\text{Ca++, Mg++}]{\text{MyosinATPase}} \text{ADP+Pi+energy}$$

The ADP is phosphorylated to regenerate ATP with the help of creatine-phosphate catalysed by enzyme **creatine kinase.**

The pathway of ATP synthesis involves the conversion of muscle glycogen into lactic acid. In the event of vigorous muscle activity, muscle glycogen is broken down anaerobically to form lactic acid which diffuses out into the blood. Lactic acid is transformed back into glycogen by reverse glycolysis in liver and then released into blood as glucose. This free glucose moves to the muscles and is again converted into glycogen through the **Cori cycle.**

➢ **Types of muscular contraction**

1. Isotonic contraction: In isotonic type of contraction, the muscle shortens in length but the tension of muscle remains same. Thus, it causes movements and locomotion in the form of some mechanical work.(Fig:6.18)

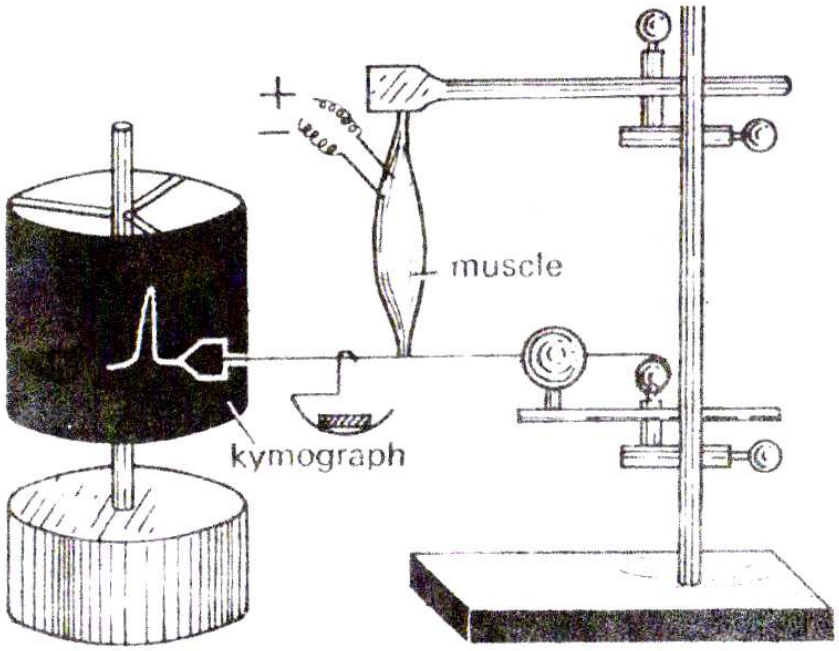

Fig: 6.18 isotonic contraction of muscle

2. Isometric contraction: In isometric contraction**, length** of the muscle remains almost constant but tension increases sharply .Thus, movement of body parts does not occur and the energy expenditure is recorded as heat. (Fig: 6.19)

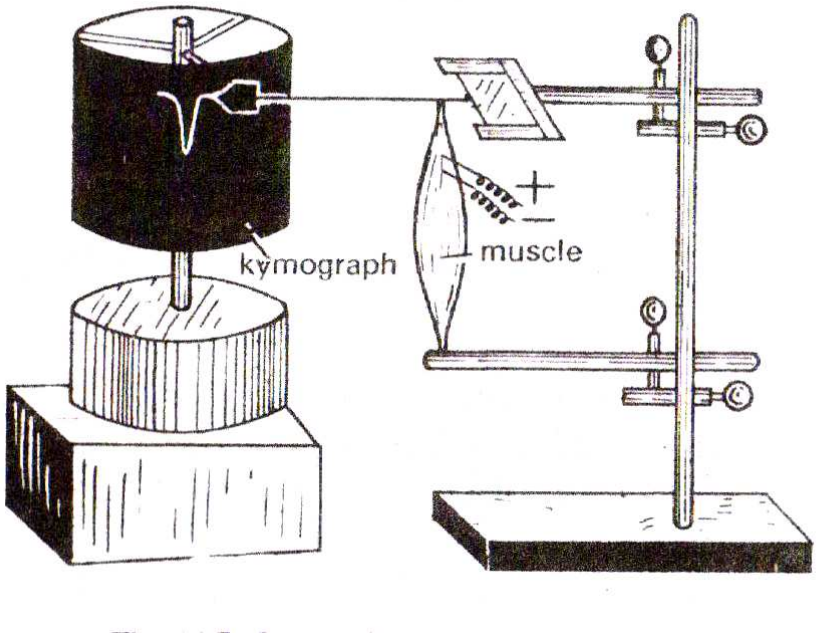

Fig: 6.19 Isometric contraction of muscle

- **3. Rigor mortis:** Several hours after the death of an animal, the muscles start contra cting and become rigid even without action potentials. This state of body when all the muscles go into a state of contracture is termed as **rigor mortis**. Main reason of rigor is supposed to be the loss of all the ATP of body which is needed to separate the cross-bridges from the actin filaments during muscular relaxation. The state of rigor continues until muscle proteins start degenerating due to autolysis caused by lysosomal enzymes. Rigor mortis appears in the muscles of jaws.

6.4.2 MUSCLE TWITCH AND FATIGUE

A muscle twitch can be defined as a simple single muscular contraction caused by a single short stimulus. It can be recorded by kymograph and consists of three successive phases (Fig 6.20)

A) **Latent period**. It is the lag period or time interval between the application of the stimulus and the actual beginning of visible muscle contraction. In the frog, the latent period lasts about 0.01 second and in the figure it is indicated by the distance from A to B.

b) **Contraction phase**: it follows the latent period, as indicated by the portion of the curve from B to C: it is the time interval in which the muscle reaches the peak of contraction and performs work. It has duration of about 0.04 second

c) **Relaxation phase**: it is the final phase of the muscle twitch, or the time interval in which the muscle returns to its original length. This phase lasts about 0.05 second and is represented by the portion of the curve between C and D. Thus, it is the longest of the three phases.

Summation: if a second stimulus is applied to a muscle that is still in the contraction phase, a second contraction is added to the first and results in a greater shortening of the muscle than caused by a single stimulus. This phenomenon is known as **summation. (Fig 6.20)**

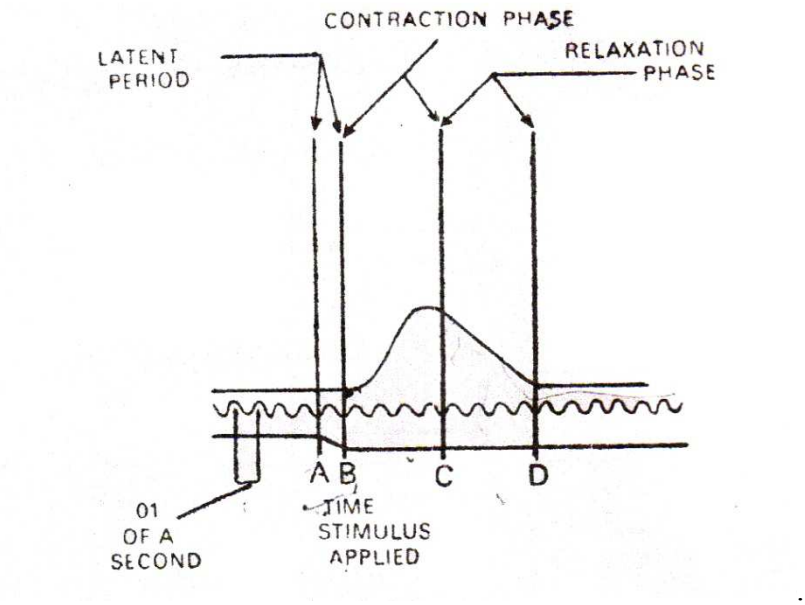

Fig:6.20. A single muscle twitch and its phases

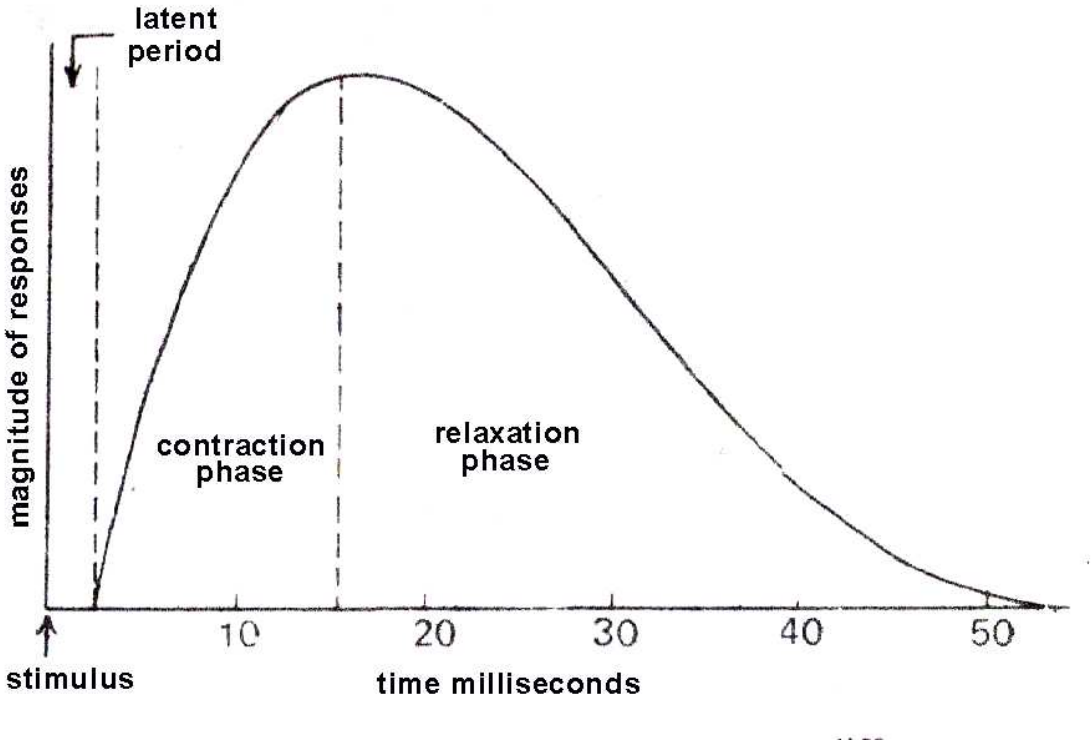

Fig: 6.21. Single contraction of skeletal muscle showing different components

- **Tetanus:**

When a muscle fibre is given threshold stimulus it contracts, it is called single muscle twitch. Single muscle twitch is of 0.1 second in frog and 0.5 second in man. After this there is a recovery or refractory period of 0.002 second, so that the muscle can get ready for next stimulation. If we continue closely spaced stimuli, we get a smooth, sustained contraction, called **tetanus or titanic contraction**. Thus, a complete tetanus may be defined as a sustained contraction of a muscle due to the fusion of many twitches following each other in rapid succession. In case the successive stimuli arrive at the muscle with long time intervals between them, the individual contractions can still be recognised since some degree of relaxation occurs between them to give a curve with a wavy plateau as shown in the Fig. **(6.22)** .This condition is called **incomplete tetanus**.

- **Refractory period:** Skeletal muscle, like nerves, exhibits a refractory period. It is an extremely short time interval lasting about 0.002 to 0.005 seconds, during which a muscle will not respond to a second stimulus when given immediately after the first one. It represent the time necessary for the occurrence of various physical and chemical changes which will allow subsequent contraction.

In skeletal muscles the refractory period is extremely short and for this reason a skeletal muscle will contract to a second stimulus while it is still contracting in response to the first stimulus. the second contraction is reacting superimposed upon the first to give the **summation effect**.

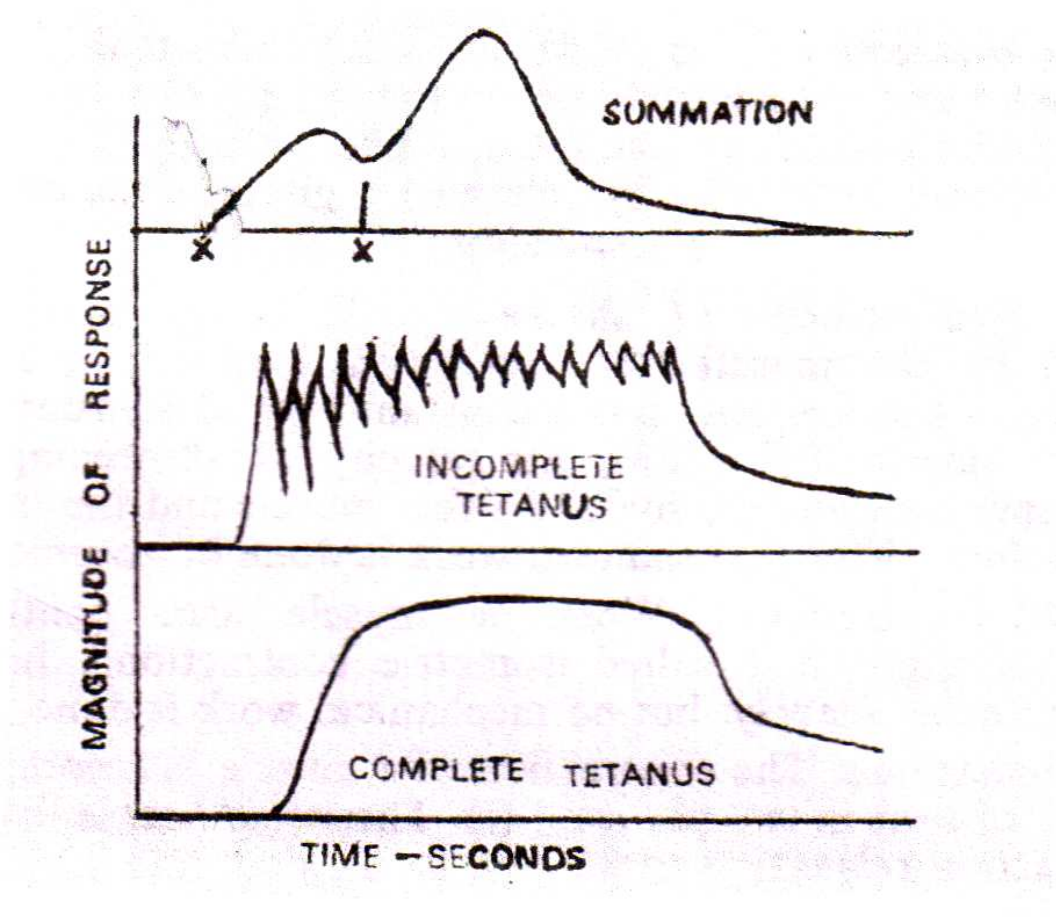

Fig: 6.22 Diagram showing summation, incomplete tetanus and tetanus

- **Muscle fatigue:**

Fatigue is a temporary reduction of the working of muscles resulting from prolonged exertion. When a muscle is repeatedly stimulated, initially the amplitude of contraction gradually increases because of beneficial effect, then it gradually falls to zero and the muscle no longer responds to stimulation. There are two main causes of muscle fatigue: the limitations of a nerve's ability to generate a sustained signal (**neural fatigue**) and the reduced ability of the muscle fiber to contract **metabolic fatigue** (fig**:6.23)**

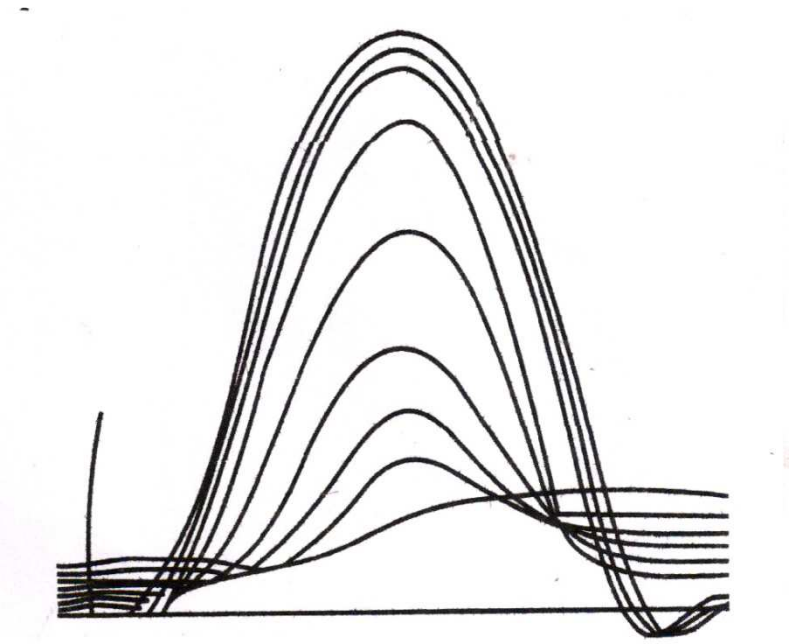

Fig: 6.23 Muscle fatigue

This could be due to -

1) Lack of nutrition

2) Accumulation of waste metabolites

3) Depletion of acetylcholine

4) Lack of oxygen

5) Lack of ATP

Fatigue is a temporary reversible phenomenon and passes off after a rest after the removal of lactic acid (Cori's cycle).

6.5 SUMMARY

Muscles are specialized for contractile ability. There are three types of muscles in the human body: skeletal muscle, smooth muscle and cardiac muscle.Skeltal muscles are attached to the skeletal bones, and provide movement of the body. Smooth muscles are found in our internal organs such as digestive system, respiratory system, blood vessels, and bladder. Cardiac muscle is found only in heart, and is responsible for the heart beating or pumping action. There are about 650 skeltal muscles in the human body. They provide strength, balance .posture, and movements for the body .They also provide heat to keep the body warm.Skeltal muscles are attached to bone by tendons. When muscle contract, they pull on tendons, that in turn pull on the bone. Muscles are controlled by our brain and central nervous system through the peripheral nervous system. This is initiated by the synaptic transmission, however, in certain types of muscle contraction it can be produced by the excitation of the motor nerve action. Muscle cells called myofibrils, are composed of strands of protein molecules the actin and myosin which can be differentiated into many kinds. To maintain proper muscle function one requires proper and regular muscle exercise Contraction of muscle fibre cause contraction of the muscles. They provide strength, balance, posture, and movement for the body. They also provide heat to keep the body warm, without muscles and joints we wouldn't be able to do much,

6.6 GLOSSARY

Actin: A contractile protein of muscle that makes up the thin filaments in the myofibrils

Actomyosin: A complex of the muscle proteins actin and myosin.

Effector: An organ which react to stimulus by producing work or substance.

Myofibril Basic unit of a muscle; contain actin and myosin filaments; has many sarcomeres.

Myosin: A principal contractile proteins found in muscle; forms thick filaments in myofibrils of muscle fibers.

Sarcolemma: The surface membrane of muscle fibre.

Sarcomere: Contractile unit of a myofibre.

Tetanus: State of a muscle undergoing a continuous fused series of contraction due to electrical stimulation.

6.7 SELF ASSESSMENT QUESTIONS

1. Describe the structure of various types of muscles found in the vertebrates.
2. Describe the structure of striated muscle fibres in detail.
3. How muscles contract? Give various theories of muscle contraction.
4. What is sliding filament theory? Describe it in detail giving suitable diagrams.
5. Describe structure and contraction of smooth muscle fibre.
6. Write about proteins of muscle fibre.
7. Describe the chemical changes taking place during contraction of muscle fibre

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6.10 TERMINAL QUESTIONS

1. Contractile proteins are present in:

(a) Muscle cells (b) Fibroblasts (c) Macrophages (d) All of these

2. Smooth muscle fibres are widest at:

(a) Anterior end (b) Posterior end (c) Middle point (d) Not definite

3. Muscle fatigue sets in due to non-availability of:

(a) Calcium (b) Actin binding sites

(c) Mg cofactor (d) ATP

4. The sliding filament theory of muscle contraction was proposed by :

(a) A. Pullman and B. Pullman (b) Striated muscle

(C) A. F. Huxley and H.E. Huxley (d) A.F.Huxley and A.Pullman

5. among the following which is multinucleated:

(a) Non – striated muscle (b) Striated muscle

(c) Erythrocytes of reptiles (d) Nervous tissue

6. The presence of intercalated discs is characteristic feature of

(a) Smooth muscles (b) Striated muscles

(c) Cardiac muscles

(d) Intercalated discs are found in all muscles

7. Which of the following is correct related to contraction of muscles:

(a) Width of the A band remains constant

(b) Width of the I band remains same

(c) Width of H zone remains same

(d) Width between two Z lines remains same

8. Red muscles are rich in:

(a) Myoglobin and cytochrome (b) Lactic acid and acetic acid

(c) Haemoglobin and glucose (d) only myosin

9. Sarcomere is represented between:

(a) Two Z discs (b) Between two H discs

(c) Between two A lines (d) Between two I lines

10. Contractile unit of muscle fiber is:

(a) Z- lines (b) H- lines (c) Sarcomere (d) I- band

Answers

1. (a) 2.(c) 3.(d) 4.(c) 5.(b) 6.(c) 7.(a) 8.(a)

9. (a) 10.(c)

UNIT-7: ENDOCRINE SYSTEM

Contents

7.1 OBJECTIVES

To study the General characters of endocrine system and Basic introduction of Pituitary and general study of Testis and ovary in mammals.

7.2 INTRODUCTION

The various physiological activities of trhe body are controlled by different ductless gland knowns as endocrine gland in mammals & HumansThe endocrine system, along with the nervous system, functions in the regulation of body activities. The nervous system acts through electrical impulses and neurotransmitters to cause muscle contraction and glandular secretion. The effect is of short duration, measured in seconds, and localized. The endocrine system acts through chemical messengers called hormones that influence growth, development, and metabolic activities. The action of the endocrine system is measured in minutes, hours, or weeks and is more generalized than the action of the nervous system.The endocrine glands are located in particular parts of the body & they directly librate secretion in the blood.

7.3- General characteristics of endocrine system

Endocrine Glands

The endocrine glands do not have ducts to carry their product to a surface. They are called ductless glands. The word endocrine is derived from the Greek terms "endo," meaning within, and "krine," meaning to separate or secrete. The secretory products of endocrine glands are called hormones and are secreted directly into the blood and then carried throughout the body where they influence only those cells that have receptor sites for that hormone.

In evolutionary history, Metazoan appeared as the earliest multicellular organisms. In these organisms different cell groups assumed specific functions. The emergence of these animals coincided with development of systems that allowed for communication and functional integration among the specialized groups. Two types of intercellular communication system evolved to assume important physiological roles- one **endocrine** and the other **nervous**. These

both the systems acquired the same basic component- **sender cells** that produce and release chemical messengers and **target cells** that receive and respond to the chemical messengers.

In nervous system, the communication occurs between the neurons or from neurons to peripheral tissues. It is mediated by excitation of sender cell which release neurotransmitter into a synapse. The neurotransmitter diffuses across the synaptic cleft and binds to receptors of target cell, which in turn activate a cascade of signals to the interior of the target cell.

In endocrine system, the sender cell produces and secretes hormones into bloodstream. Once hormones enter the blood, they travel throughout the body and reach and bind to receptors of distantly placed target cell. Once a target site is bound by a particular hormone, a cascade of cellular events follows that culminates in the physiological response to a particular hormone.

Exocrine Glands

Exocrine glands have ducts that carry their secretory product to a surface. These glands include the sweat, sebaceous, and mammary glands and, the glands that secrete digestive enzymes.

7.4- Basic introduction of Pituitary

The pituitary gland is a tiny organ, the size of a pea, found at the base of the brain. As the "master gland" of the body, it produces many hormones that travel throughout the body, directing certain processes or stimulating other glands to produce other hormones. The pituitary gland makes or stores many different hormones. The following hormones are made in the anterior (front part) of the pituitary gland

Consisting of two lobes i.e., the anterior and posterior lobes The two lobes constituting the pituitary are essentially two different endocrine glands, as are the adrenal medulla and adrenal cortex, and the thyroid and parathyroid glands.

(a) Adenohypophysis: The upper or anterior lode, (anterior pituitary) now usually called the *adenohypophysis,* is derived from the epithelial tissue of the mouth. In the course of embryonic development the *neurohypophysis* moves downward, becoming posterior in position to the adenohypophysis which has developed upward to assume an anterior location relative to the neurohypophysis. The adenohypophysis in a mammal consists of the following parts:

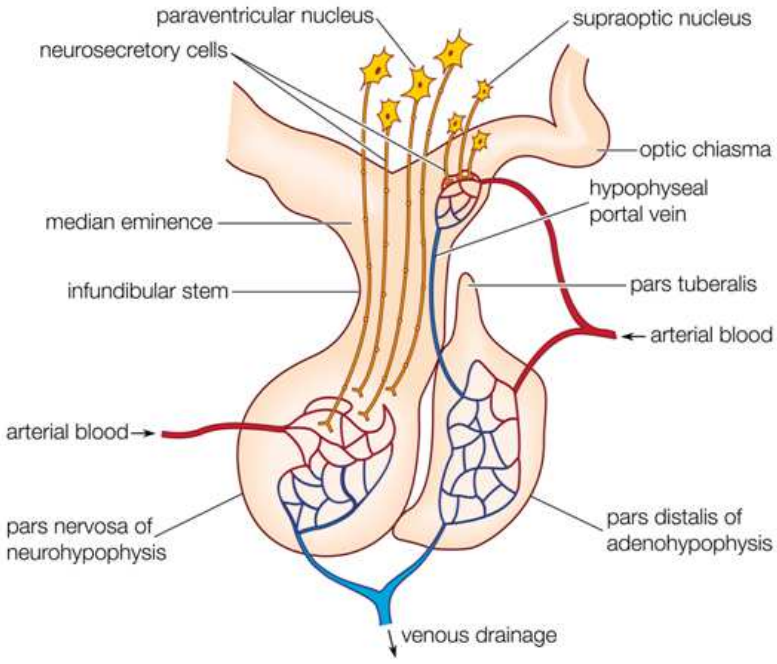


Fig.7.1A section through a human Pituitary & adjacent structure

(i) Pars tuberalis: The pars tuberalis forms a collar of cells 25 to 60 μ thick around the neural stalk. It is a thickest anterior to the stalk and frequently incomplete on the posterior aspect. The cells are arranged in short cords or globular clusters. They are polyhedral, with centrally placed nuclei and the cytoplasm contains fine granules, and is weakly basophilic. Nests of squamous cells are often found around the pars tuberalis. They are important because they may give rise to tumours (craniopharyngiomas).

(ii) Pars intermedia (middle or intermediate lobe): In most species this portion of the adenohypophysis is quite distinct. However, in other (e.g., the whale) and in birds, it is lacking. In man, the pars intermedia are rudimentary and its cells often surround colloid-filled cysts, and merge imperceptibly with those of the pars distalis. They are either *chromophobes*or *basophils*. The cells lining the follicles may be ciliated. A unique feature in humans is the invasion of the pars nervosa by basophil cells of the pars intermedia.

(iii) Pars distalis: It forms about 75% of the hypophysis. The cells in the pars distalis are arranged mainly in cords between which are large-bore capillaries. On the basis of the staining properties, three types of cells are distinguished in it, *acidophils, basophils,* and *chromophobes.*

These are said to constitute about 40, 10 and 50 per cent of the anterior lobe (pars distalis) cells respectively.

***(b) Neurohypophysis*:** The posterior (posterior pituitary) lobe or the *neuro hypophysis* has two subdivisions, *pars nervosa* or *infundibular process,* and *infundibulum* (median eminence and infundibular stem). While developing from the floor of the diencephalon behind the optic chiasma, the *infundibulum* and the *pars nervosa* at first contain the continuation of the cavity of the third ventricle. The pituitary gland can be divided into two different parts: the anterior and posterior lobes.

Anterior lobe

The anterior lobe of your pituitary gland is made up of several different types of cells that produce and release different types of hormones, including:

- **Growth hormone.** Growth hormone regulates growth and physical development. It can stimulate growth in almost all of your tissues. Its primary targets are bones and muscles.
- **Thyroid-stimulating hormone.** This hormone activates your thyroid to release thyroid hormones. Your thyroid gland and the hormones it produces are crucial for metabolism.
- **Adrenocorticotropic hormone.** This hormone stimulates your adrenal glands to produce cortisol and other hormones.
- **Follicle-stimulating hormone.** Follicle-stimulating hormone is involved with estrogen secretion and the growth of egg cells in women. It's also important for sperm cell production in men.
- **Luteinizing hormone.** Luteinizing hormone is involved in the production of estrogen in women and testosterone in men.
- **Prolactin.** Prolactin helps women who are breastfeeding produce milk.
- **Endorphins.** Endorphins have pain-relieving properties and are thought to be connected to the "pleasure centers" of the brain.
- **Enkephalins.** Enkephalins are closely related to endorphins and have similar pain-relieving effects.
- **Beta-melanocyte-stimulating hormone.** This hormone helps to stimulate increased pigmentation of your skin in response to exposure to ultraviolet radiation.

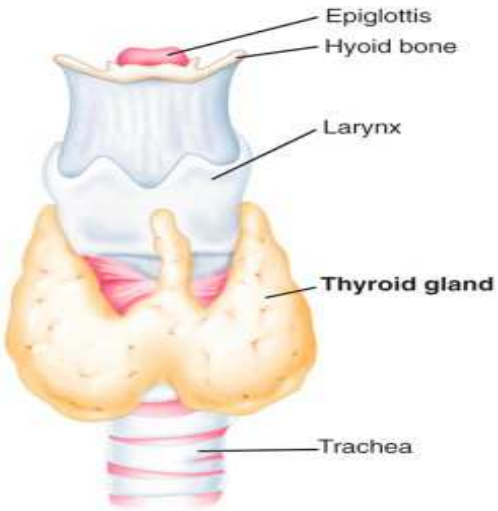
Posterior lobe

The posterior lobe of the pituitary gland also secretes hormones. These hormones are usually produced in your hypothalamus and stored in the posterior lobe until they're released.Hormones stored in the posterior lobe include:

- **Vasopressin.** This is also called antidiuretic hormone. It helps your body conserve water and prevent dehydration.
- **Oxytocin.** This hormone stimulates the release of breast milk. It also stimulates contractions of the uterus during labor.

7.4.1- THYROID

The thyroid gland is the largest of all the endocrine glands, weighing from 20 to 30 grams in adult human being. The two lobes of the gland, and the isthmus of tissue joining them, lie just below the larynx or voice box, just anterior to the upper portion of the trachea or windpipe (Fig. 7.2). The gland is well supplied with blood, has a connective tissue framework, and contains many microscopic cavities or vesicles. The vesicles are lined with a single layer of cuboidal epithelial cells which are the actual secretory cells responsible for the production of the thyroid hormones. The hollow centre of each vesicle is filled with a gelatinous colloidal material made up mostly of a large complex glycoprotein called *thyroglobulin.*



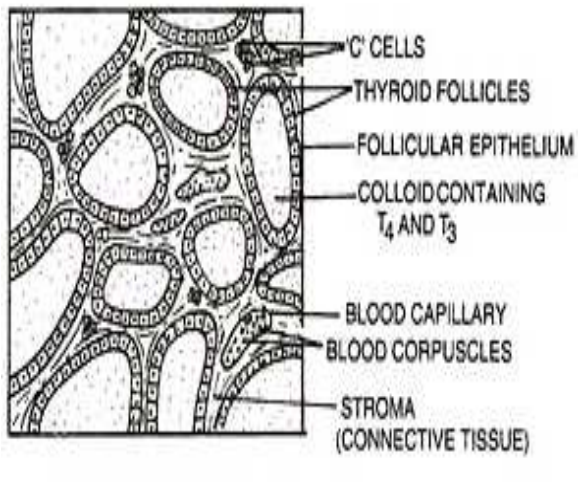


Fig7.2 (A) Thyroid Gland *Fig7.2 (B) T.S of Thyroid Gland*

The hormones remain bound to thyroglobulin until secreted. When they are secreted, colloid is ingested by the thyroid cells, the peptide bonds are hydrolysed, and free T_4 and T_3 are discharged into the capillaries.

7.4.2- PARATHYROID GLAND

In human, the parathyroid glands consist of two pairs of small oval glands attached to the thyroid gland. These glands are absent in fishes, but are present in amphibians, reptiles, birds and mammals in either one or two pairs, depending on the species.

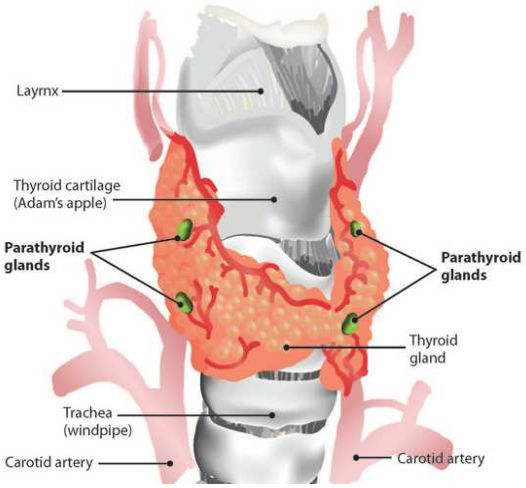


Fig 7.3 Parathyroid Gland

In adult human beings, the parathyroid gland consists of mainly *chief cells* and a small to moderate number of *oxyphil cell* , but oxyphil cells are absent in many animals and in young human beings. The chief cells are believed to secrete most of the parathyroid hormone. However, the function of oxyphil cells is not certain.

7.4.3- ENDOCRINE PANCREAS

In human adults, the pancreas is an elongated gland weighing about three ounces. It lies behind the stomach close to the posterior abdominal wall, and functions both as a digestive gland and an endocrine gland. Besides the bulk of the glandular cells, which secrete a mixture of several digestive enzymes, the pancreas also contains scattered, rounded microscopic clusters or islets of tissue approximately 0.3 mm in diameter and about a million or so in number. These islets, discovered by *Langerhans in 1869,* are not connected with any ducts and perform in important endocrine function.

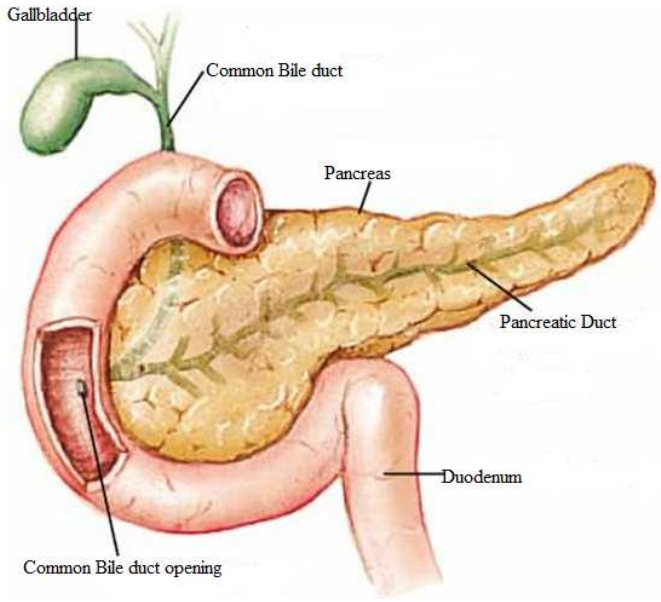


Fig.7.4Common Pancreatic Structure

The *islets of Langerhans* in mammals consist of four known types of cells. The so-called beta-cells constitute majority (about 70-80% in the islets and secrete the hormone *insulin.*

Structure: In ordinary haemotoxylin-eosin preparation the islets of Langerhans look like spheroidal bodies scattered in the pancreatic mass. These are aggressions of endodermal.

There are different types of cells in the islets of Langerhans. They are:-

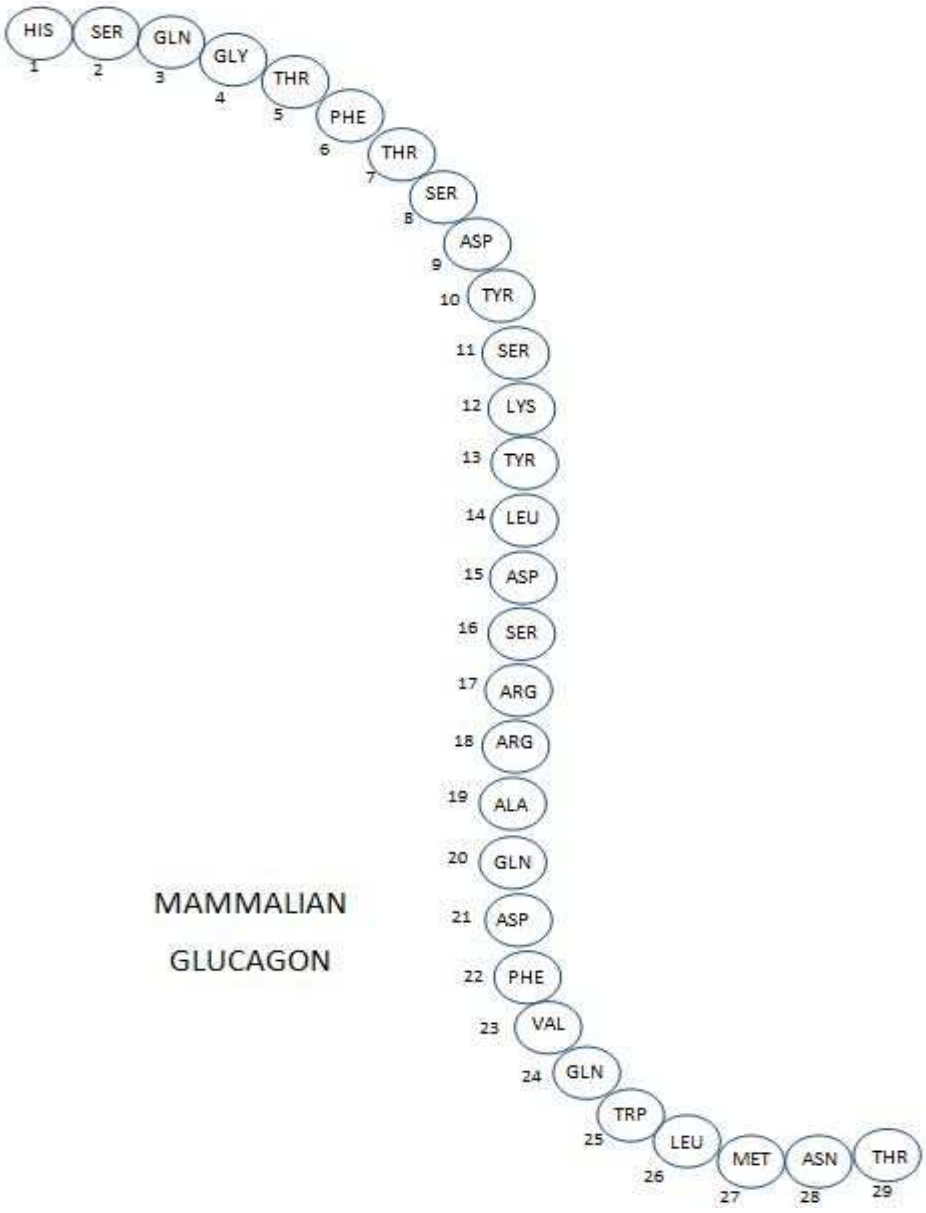
A or α-cells (alpha cells)
B or β-cells (Beta cells) and
D-cells.

Another type of cells has also been described and they are known as C-cells. There is abundance of A and B cells. The number of β-cells is greater than α-cells. These cells have been observed under the microscope when several staining techniques (Masson's trichrome, chrome haemotoxylin- phloxine, aldehyde fuchsin etc.) were employed.in α-cells, numerous red staining; fine granules are present (by chrome haemotoxylin-phloxine method). The cell membranes in these cells are also distinct. The β-cells have comparatively coarse granules and they are stained dark blue by chrome-haematoxylin method. The cells membranes of these cells are not distinct. There are very few D-cells which can be differentiated by Masson's triple stain. C-cells have been described by Bensley in the pancreas of guinea pig. These cells are devoid of granules. These cells are probably the progenitors of α-cells.

Hormones of the Islets of Langerhans

Insulin and Glucagon are the main hormones of the islets of Langerhans.insulin are secreted by the β –cells and glucagon is the product of α –cells. D –cells are said to secrete gastrine which affects the release of insulin in addition to activating gastric glands. Sanger et al. (1945-1955) determined the complete amino acid sequence of insulin in various mammalian species. Kotsoyannis and his colleagues (1963) synthesized sheep insulin. Human insulin was synthesized later on by separate contribution of Dixon, Du, kotsoyannis and others.

The ox insulin consists of two polypeptide chains, one chain is known as A-chain and the chain as B-chain. A-chain consists of 21 amino acids with glycine as the N-terminal residue. B-chain consist of 30 amino acid



Residues with phenylalanine as the N-terminal residue. The two chains (A and B) are linked by two Disulphide Bridge at positions 7 and 20 in the A-chain and 7 and 19 in the B-chain as shown in the figure. When they are joined by disulphide bridges it becomes physiologically active. In addition to these, one intradisulphide bridge is also present in the A-chain which of insulin is 6000(in man).

Glucagon is the product of α-cells. It is a straight chain polypeptide consisting of 29 amino acid residues. There is no disulphide bridge in this case. The glucagon molecule is devoid of cystine. It is resistant to alkali. The molecular weight of glucagon is 3485.

7.4.4- ADRENAL GLAND

Each adrenal or suprarenal gland in man weighs about 6 grams, measures 25 to 50 mm in length, and lies at the top of each kidney (Fig 7.5). The adrenal glands are somewhat pyramidal in shape and have a rich blood supply. They are innervated by the sympathetic nervous system, and consist of two parts that are embryologically, anatomically and functionally different. In the typical mammal, each gland consists of an external reddish brown *cortex* which surrounds an internal grayish *medulla.* The adrenal cortex has arisen from the same origin *(mesodermal)* as that of the sex glands, whereas the medulla has an ectodermal origin similar to that of the postganglionic fibre cells of the sympathetic nervous system.

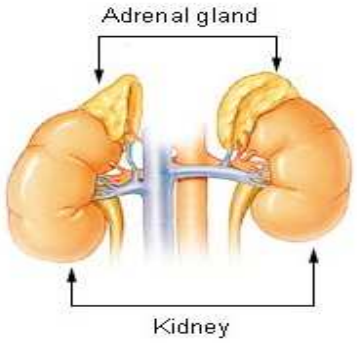


Fig7.5Structure of Adrenal Gland

The cortex is made up of three zones, the cells of which differ in form and function. The outermost *zona glomerulos*is formed by closely packed, low columnar cells and it secretes mineral corticoids, hormones concerned with water and electrolyte mechanism and the most important of which are aldosterone and deoxycorticosterone.

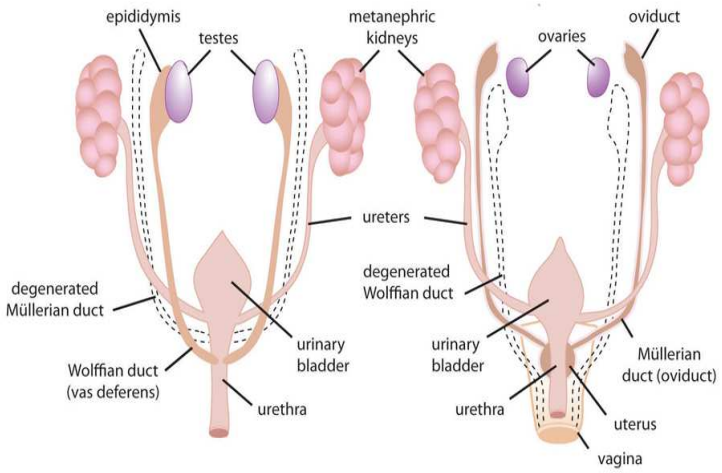
7.5- TESTIS AND OVARY IN MAMMALS

The specialized organs which eventually produce the reproductive cells (Germ cells) are termed the *gonads.* In higher animals, the male gonad produces the sperm cells and is called the *testis.* The female gonad which produces the egg cells or ova is called the *ovary.* The mature gonads of

both sexes are not only the centres for production of the germ cells but they also secrete the *sex hormones* which control the maturation and function of the reproductive system and certain other tissues of the body.

Testes

Each of the two testes in the adult male is an ovoid body about the size of a walnut. Both are located outside the body cavity within a skin covered pouch called the *scrotum.* The wall of each testis consists largely of connective tissue which is continuous with wall-like partitions dividing the interior of the tests into approximately 250 small compartments called *lobules*. Each lobule contains from one to three tiny convoluted somniferous tubules within which the sperms are formed. The special cells located among the *somniferous lobules* are collectively called the *intestinal issue,* which performs the important functions of producing and secreting the male sex hormone. The interstitial cells are not present during childhood but are found after puberty.



Male *Fig 7.6 Gonads* ***Female***

Ovaries

Each of the two ovaries in the adult female is about the size and shape of a large almond and is separately located in the lower or pelvic region of the abdominal cavity. Each ovary consists of a large number of *ovarian follicles*. The ovarian follicles produce the *female sex hormones* while undergoing maturation.

Male Sex Hormones and Their Physiological Effects

The male sex hormones are usually referred to as androgens, including *testosterone, dihydro-testosterone* and *androstenedione.* The term "androgen" means any steroid hormone that has masculinising effects. The male sex hormones have not only been obtained from the testes but from the adrenal cortex, placenta, and the ovaries as well.

The principal male sex hormone is *testosterone.* It is a steroid, and is secreted interstitial cell of the testes of most mammals, including man. Much of the testosterone that becomes fixed to the tissues is converted within the cells to *dihydrotesiosteron.*

7.6- SELF ASSESSMENT QUESTION

Question no.1 Describe in detail about Pituitary Gland?

Question no.:2 Explain in detail the Thyroid and parathyroid Gland?

Question no.:3 give the Physiological effect of Male sex hormones?

Question no.:4 what do you Understand by Endocrine and Exocrine Gland?

Question no.:5 write the Function of Master gland?

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9. Some material taken from https://www.mayoclinic.org.

UNIT-8: HORMONAL DYSFUNCTION AND DISEASES

Contents

8.1 OBJECTIVES:-

To study the Basic characters of Disease and Basic introduction of Hormonal Dysfunctioning.

8.2 INTRODUCTION:-

Hormonal imbalances occur when there is too much or too little of a hormone in the bloodstream, Because of their essential role in the body; even small hormonal imbalances can cause side effects throughout the body. Hormones are chemicals that are produced by glands in the endocrine system. Hormones travel through the bloodstream to the tissues and organs, delivering messages that tell the organs what to do and when to do it. Hormones are important for regulating most major bodily processes, so a hormonal imbalance can affect a wide range of bodily functions. Men and women alike can be affected by imbalances in insulin, steroids, growth hormones, and adrenaline. Women may also experience imbalances in estrogen and progesterone levels, while men are more likely to experience imbalances in testosterone levels.

8.3- DWARFISM

Dwarfism is a condition of short stature. It is defined by the advocacy group Little People of America (LPA) as an adult height of 4 feet 10 inches or under, as a result of a medical or genetic condition. Although other groups may extend the criteria for certain forms of dwarfism to 5 feet, the average height of an adult with dwarfism is 4 feet.

There are two main categories of dwarfism:-

1. Disproportionate 2. Proportionate

1. Disproportionate: Disproportionate dwarfism is characterized by an average-size torso and shorter arms and legs or a shortened trunk with longer limbs.

2. Proportionate: - In proportionate dwarfism, the body parts are in proportion but shortened.

Causes of Dwarfism

Dwarfism can be caused by any of more than 200 conditions. Causes of proportionate dwarfism include metabolic and hormonal disorders such as growth hormone deficiency.

The most common types of dwarfism, known as skeletal dysplasia's, are genetic. Skeletal dysplasias are conditions of abnormal bone growth that cause disproportionate dwarfism.

Achondroplasia

The most common form of dwarfism, achondroplasia occurs in about one out of 26,000 to 40,000 babies and is evident at birth. People with achondroplasia have a relatively long trunk and shortened upper parts of their arms and legs. Other features of achondroplasia include:

- a large head with a prominent forehead
- a flattened bridge of the nose
- protruding jaw
- crowded and misaligned teeth
- forward curvature of the lower spine
- bowed legs
- flat, short, broad feet

Spondyloepiphyseal dysplasia's (SED)

A less common form of dwarfism, SED affects approximately one in 95,000 babies. Spondyloepiphyseal dysplasia refers to a group of conditions characterized by a shortened trunk, which may not become apparent until a child is between ages 5 and 10. Other features can include:

- club feet
- cleft palate
- severe osteoarthritis in the hips
- weak hands and feet
- barrel-chested appearance

Diastrophic dysplasia

A rare form of dwarfism, diastrophic dysplasia occurs in about one in 100,000 births. People who have it tend to have shortened forearms and calves (this is known as mesomelic shortening).Other signs can include:-

- deformed hands and feet
- limited range of motion
- cleft palate
- ears with a cauliflower appearance

8.4- Basic introduction of Gigantism

Gigantism is usually caused by a tumor on the pituitary gland of the brain. It causes growth of the hands, face, and feet. In some cases the condition can be passed on genetically through a mutated gene.

Gigantism is characterized by an excess of growth hormone (GH). This overproduction of growth hormone that brings about gigantism is virtually always caused by pituitary growths (adenomas).These adenomas are on the anterior pituitary gland. They can also cause overproduction of GH's hypothalamic precursor known as growth hormone releasing hormone (GHRH).

As a result of the excessive amounts of growth hormone, children achieve heights that are well above normal ranges. The specific age of onset for gigantism varies between patients and gender, but the common age that excessive growth symptoms start to appear has been found to be around 13 years. Other health complications, such as hypertension, may occur in pediatric patients with hyper-secretion of growth hormone. Characteristics more similar to those seen in acromegaly may occur in patients that are closer in age to adolescence since they are nearing growth plate fusion.

Hormonal Causes Gigantism:-

Growth hormone (GH) and insulin-like growth factor-I (IGF-I) are two substances that have been identified as influencing growth plate formation and bone growth and, therefore, gigantism. Their specific mechanisms are still not well understood.

More broadly, GH and IGF have both been identified to be involved in most stages of growth: embryonic, prenatal, and postnatal. Moreover, the receptor gene for IGF has been shown to be particularly influential throughout various stages of development, especially prenatally. This is the same for GH receptor genes which have been known to drive overall growth throughout various pathways.

Growth hormone is a precursor (upstream) of IGF-I, but each has its independent role in hormonal pathways. Yet both seem to ultimately come together to have a joint effect on growth.

8.4.1- Acromegaly

Acromegaly is a hormonal disorder that develops when your pituitary gland produces too much growth hormone during adulthood. When this happens, your bones increase in size, including those of your hands, feet and face. Acromegaly usually affects middle-aged adults.

Hypersecretion of growth hormone after growth period also causes gigantism but the long bones do not grow in length due to closed hypothesis at their ends. The bones of hand, feet, lower jaw and rib cage become thick. The lips, eyelids, tongue, nose, chin etc also enlarge. The person has ugly face because of growth and thickness of facial bones, long jaws and protruding forehead. The body becomes gorilla like. This is called **disproportionate gigantism** or **acromegaly**. Sometimes a person develops hump due to bending of vertebral column. It is called **kyphosis**.

Acromegaly usually affects middle-aged adults, though it can develop at any age. In children who are still growing, too much growth hormone can cause a condition called gigantism. These children have exaggerated bone growth and an abnormal increase in height.

Because acromegaly is uncommon and physical changes occur gradually, the condition sometimes takes a long time to recognize. If it's not treated promptly, acromegaly can lead to serious illness and may even become life-threatening. But available treatments can reduce your risk of complications and significantly improve characteristics of the condition, including the enlargement of your features.

Symptoms

A common sign of acromegaly is enlarged hands and feet. People with this disorder often notice that they aren't able to put on rings that used to fit and that their shoe size has progressively increased.Acromegaly may also cause gradual changes in the shape of your face, such as a protruding lower jaw and brow, an enlarged nose, thickened lips, and wider spacing between your teeth. Because acromegaly tends to progress slowly, early signs may not be obvious for years. Sometimes, people notice the condition only by comparing old photographs with newer ones.Acromegaly may produce the following signs and symptoms, which can vary from one person to another:

- Enlarged hands and feet
- Coarsened, enlarged facial features
- Coarse, oily, thickened skin
- Excessive sweating and body odor
- Small outgrowths of skin tissue (skin tags)
- Fatigue and muscle weakness
- A deepened, husky voice due to enlarged vocal cords and sinuses
- Severe snoring due to obstruction of the upper airway
- Impaired vision
- Headaches
- Enlarged tongue
- Pain and limited joint mobility
- Menstrual cycle irregularities in women
- Erectile dysfunction in men
- Enlarged organs, such as the heart

- Loss of interest in sex

8.4.2- DIABETES INSIPIDES

Diabetes insipidus is a rare disorder that occurs when a person's kidneys pass an abnormally large volume of urine that is insipid—dilute and odorless. In most people, the kidneys pass about 1 to 2 quarts of urine a day. In people with diabetes insipidus, the kidneys can pass 3 to 20 quarts of urine a day. As a result, a person with diabetes insipidus may feel the need to drink large amounts of liquids.

Diabetes insipidus and diabetes mellitus—which includes both type 1 and type 2 diabetes—are unrelated, although both conditions cause frequent urination and constant thirst. Diabetes mellitus causes high blood glucose, or blood sugar, resulting from the body's inability to use blood glucose for energy. People with diabetes insipidus have normal blood glucose levels; however, their kidneys cannot balance fluid in the body.

Different types of diabetes insipidus include as:

- Central
- Nephrogenic
- Dipsogenic
- Gestational

Each type of diabetes insipidus has a different cause.

8.4.3- DIABETES MELLITUS

Diabetes mellitus, commonly known as diabetes, is a metabolic disease that causes high blood sugar. The hormone insulin moves sugar from the blood into your cells to be stored or used for energy. With diabetes, your body either doesn’t make enough insulin or can’t effectively use the insulin it does make.

Untreated high blood sugar from diabetes can damage your nerves, eyes, kidneys, and other organs.

There are a few different types of diabetes:

- Type 1 diabetes is an autoimmune disease. The immune system attacks and destroys cells in the pancreas, where insulin is made. It's unclear what causes this attack. About 10 percent of people with diabetes have this type.
- Type 2 diabetes occurs when your body becomes resistant to insulin, and sugar builds up in your blood.
- Prediabetes occurs when your blood sugar is higher than normal, but it's not high enough for a diagnosis of type 2 diabetes.
- Gestational diabetes is high blood sugar during pregnancy. Insulin-blocking hormones produced by the placenta cause this type of diabetes.

A rare condition called diabetes insipidus is not related to diabetes mellitus, although it has a similar name. It's a different condition in which your kidneys remove too much fluid from your body.

Each type of diabetes has unique symptoms, causes, and treatments.

The general symptoms of diabetes include:

- increased hunger
- increased thirst
- weight loss
- frequent urination
- blurry vision
- extreme fatigue
- sores that don't heal

Symptoms in Men

In addition to the general symptoms of diabetes, men with diabetes may have a decreased sex drive, erectile dysfunction (ED), and poor muscle strength.

Symptoms in Women

Women with diabetes can also have symptoms such as urinary tract infections, yeast infections, and dry, itchy skin.

Symptoms of type 1 diabetes can include:

- extreme hunger
- increased thirst
- unintentional weight loss
- frequent urination
- blurry vision
- tiredness

Symptoms of type 2 diabetes can include:

- increased hunger
- increased thirst
- increased urination
- blurry vision
- tiredness

8.4.4- Goiter & Cretinism

A goiter (GOI-tur) is an abnormal enlargement of your thyroid gland. Your thyroid is a butterfly-shaped gland located at the base of your neck just below your Adam's apple. Although goiters are usually painless, a large goiter can cause a cough and make it difficult for you to swallow or breathe.

The most common cause of goiters worldwide is a lack of iodine in the diet. In the United States, where the use of iodized salt is common, a goiter is more often due to the over- or underproduction of thyroid hormones or to nodules that develop in the gland itself. Treatment depends on the size of the goiter, your symptoms and the underlying cause. Small goiters that aren't noticeable and don't cause problems usually don't need treatment.

Basically not all goiters cause signs and symptoms but when signs and symptoms do occur they may include:

- A visible swelling at the base of your neck that may be particularly obvious when you shave or put on makeup
- A tight feeling in your throat
- Coughing
- Hoarseness
- Difficulty swallowing
- Difficulty breathing

CRETINISM

Congenital iodine deficiency syndrome, previously known as **cretinism**, is a condition of severely stunted physical and mental growth owing to untreated congenital deficiency of thyroid hormone (congenital hypothyroidism) usually owing to maternal hypothyroidism. It is caused by hypothyroidism during fetal life, infancy and childhood. It leads to failure of growth and mental retardation. In the sufferer, skeletal growth is more inhibited than soft tissue growth. The body becomes deformed with large head, short hand and feet, and enlarged belly. The skin becomes rough, thick and dry. These are called cretins because of poor mental development. Their reproductive organs also remain poorly developed.

Around the world, the most common cause of congenital hypothyroidism is iodine deficiency. It has affected many people worldwide and continues to be a major public health problem in many countries. Iodine is an essential trace element, necessary primarily for the synthesis of thyroid hormones. Iodine deficiency is the most common preventable cause of brain damage worldwide.

Although iodine is found in many foods, it is not universally present in all soils in adequate amounts. Most iodine, in iodide form, is in the oceans, where the iodide ions oxidize to elemental iodine, which then enters the atmosphere and falls to earth as rain, introducing iodine to soils. Earth deficient in iodine is most common inland and in mountainous areas and areas of frequent flooding, but can also occur in coastal regions owing to past glaciations, and leaching by snow, water and heavy rainfall, which removes iodine from the soil. Plants and animals grown in iodine deficient soils are correspondingly deficient. Populations living in those areas without outside food sources are most at risk of iodine deficiency diseases.

8.5- Myxoderma and Addison's disease

MYXODERMA

It is caused by hypothyroidism in adults. Mucus accumulates in the subcutaneous tissues, holding a lot of water which results puffiness to the skin. So body becomes swollen and heavy. It is characterized by low basal metabolic rate, low body temperature and low body pressure. Some other symptoms which may appear in patients are paleness and dryness of skin, loss of eyesight due to vitamin A deficiency, muscular weakness, cramps and stiffness, insomnia and degenerated sex organs and impotency.

ADDISON'S DISEASE

Addison's disease also known as **primary adrenal insufficiency** and **hypocortisolism**, is a long-term endocrine disorder in which the adrenal glands do not produce enough steroid hormones. Symptoms generally come on slowly and may include abdominal pain, weakness, and weight loss. Darkening of the skin in certain areas may also occur. Under certain circumstances, an adrenal crisis may occur with low blood pressure, vomiting, lower back pain, and loss of consciousness. An adrenal crisis can be triggered by stress, such as from an injury, surgery, or infection.

Addison's disease, also called adrenal insufficiency, is an uncommon disorder that occurs when your body doesn't produce enough of certain hormones. In Addison's disease, your adrenal glands, located just above your kidneys, produce too little cortisol and, often, too little

aldosterone. Addison's disease occurs in all age groups and both sexes, and can be life-threatening. Treatment involves taking hormones to replace those that are missing.

8.6-Terminal & Model Questions

1. Describe causes of gigantism and acromegaly. How will you distinguish between two?

2. Briefly describe cretinism?

3. Write a short note on Addison Disease?

4. Describe the Diabetes Insipides and Diabetes mellitus?

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9. Some material taken from https://www.mayoclinic.org.

UNIT 9: AMINO ACIDS AND PEPTIDES

CONTENT

9.1 OBJECTIVES

1. The objective of this chapter is to understand about amino acids and peptides.
2. To understand structures and classification of amino acids.
3. To understand properties of amino acids and peptides.
4. Understanding reactions of amino acids.
5. Biological role of peptides.

9.2 INTRODUCTION

Amino acids are the essential components of all living cell as they are the building blocks of proteins. Although over 300 different amino acids are found in nature, only 20 amino acids are involved in protein and are called as standard amino acids. Amino acids are also metabolically active and supply substrates for many other biochemical reactions. Chemically, amino acids have a carboxyl group and an amino group, bonded to the same carbon atom. The general structural formula of the amino acid: (fig. 1).

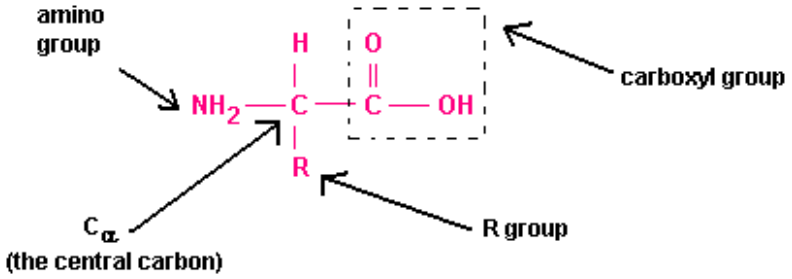


Fig.9.1. General Structure of an Amino Acid

In protein amino acids, it is always the α-carbon atom which is aminated and hence they are called α-amino acids. A α -amino acid consists of a central carbon atom, called the carbon, linked to an amino group, a carboxyl group, a hydrogen atom, and a distinctive R group. The R group is often referred to as the side chain. With four different groups connected to the tetrahedral a-carbon atom, a-amino acids are chiral (with the exception of glycine- for which R is the H atom); the two mirror-image forms are

called the L- isomer and the D-isomer (fig.2). All the amino acids which occur in proteins belong to L-form however D-amino acids have been discovered in antibiotics.

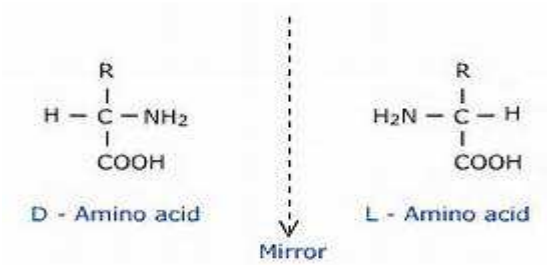


Fig.9.2. Two mirror image forms of amino acid

Peptides are chains of amino acids. Two amino acid molecules are linked together by a covalent bond, called a peptide bond. Peptide bond formation is an example of a condensation reaction, which is formed by removal of the elements of water from the α-carboxyl group of one amino acid and α-amino group of another. The product formed by a peptide bond is a peptide. Two amino acids can be joined by peptide bond to form a dipeptide (fig.3); similarly, amino acids can be linked to form tripeptides, tetrapeptides, pentapeptides and so forth.

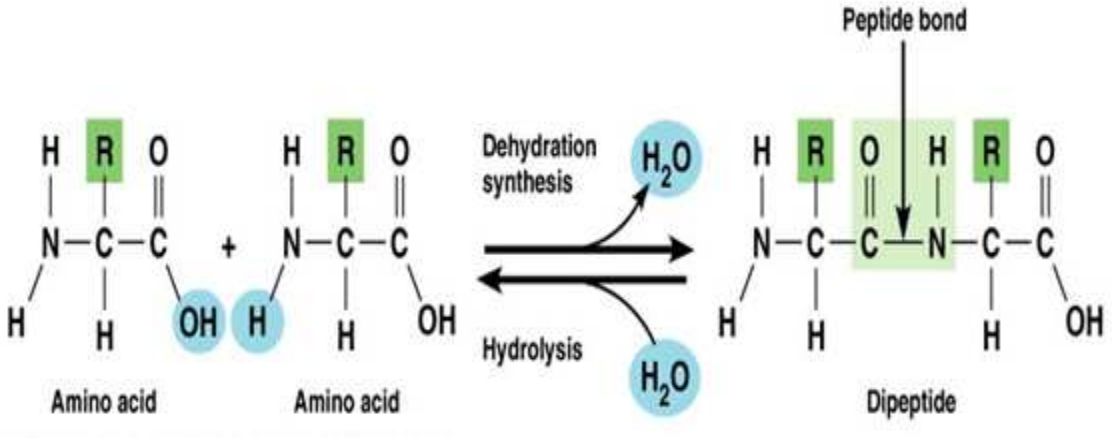


Fig.9.3. Peptide Bond, Formation of a Dipeptide

When a few amino acids can be joined in this manner, the structure is called an oligopeptide. When many amino acids are joined, the product is called a polypeptide. Polypeptides generally have molecular weights below 10,000. The polypeptide chains form proteins. A protein may consist of a single or more polypeptide chains and has higher molecular weight.

An amino acid unit in a peptide is often called a residue. In a peptide, the amino acid residue at the end with a free α-amino group is the amino terminal (N-terminal) residue, the residue at the other end, which has a free carboxyl group, is the carboxyl terminal (C-terminal) residue (fig.4).

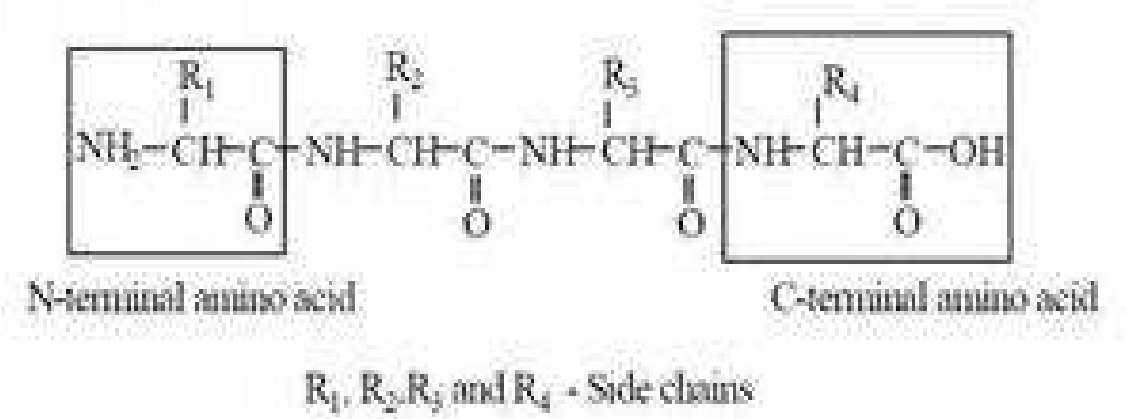


Fig9..4. C-terminal and N-terminal ends in a peptide chain

9.3 CLASSIFICATION OF AMINO ACIDS

Amino acids can be classified on the basis of their R groups (side chain) into the following classes:

1. Amino Acids with Nonpolar, Aliphatic R groups

The R groups in this class of amino acids are nonpolar and hydrophobic. The following amino acids are classified as having nonpolar side chains (fig.5).

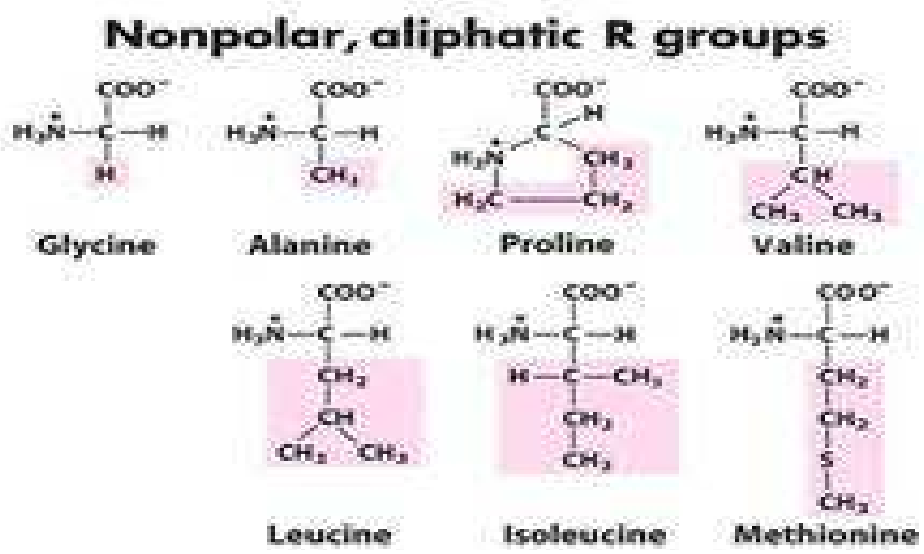


Fig.9.5. Amino Acids with Nonpolar, Aliphatic R groups

2. Amino Acids with Nonpolar, Aromatic R groups

The R groups in this class of amino acids are relatively nonpolar aromatic and hydrophobic in nature. The following amino acids are classified as having non polar aromatic side chains (fig.6).

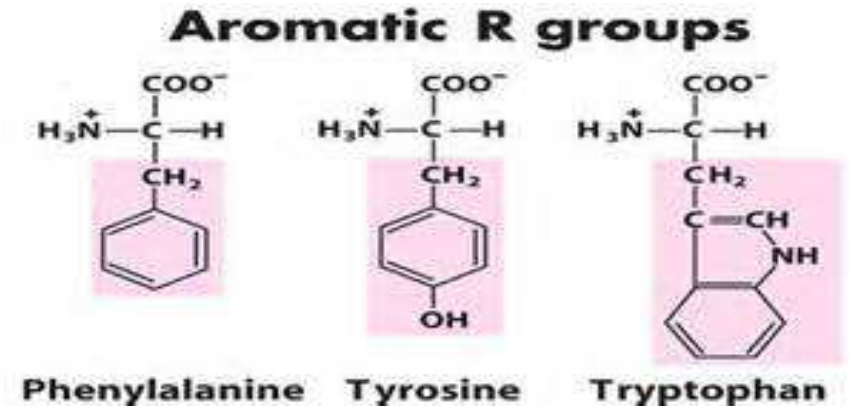


Fig.9.6. Amino Acids with Nonpolar, Aromatic R groups

3. Amino Acids with Polar, Uncharged R groups

The R groups in these amino acids are more soluble in water or more hydrophilic than those of the nonpolar amino acids as they contain functional groups that form hydrogen bonds with water. The following are classified under this class (fig.7).

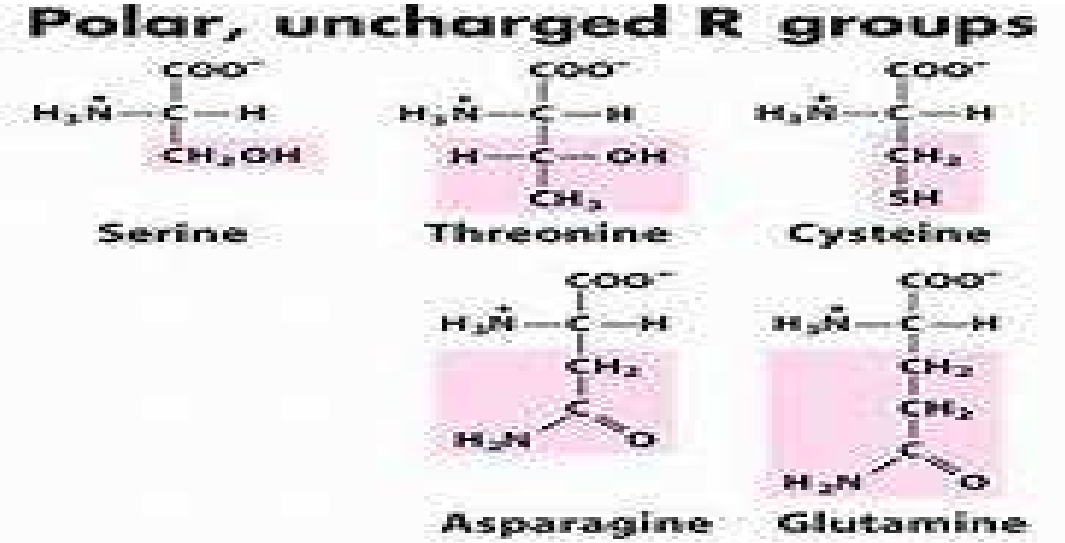


Fig.9.7. Amino Acids with Polar, Uncharged R groups

4. Amino Acids with Positively Charged Polar R groups

The R groups in this class of amino acids are polar and positively charged. These are hydrophilic in nature. The following amino acids are classified as having positively charged R groups (fig.8).

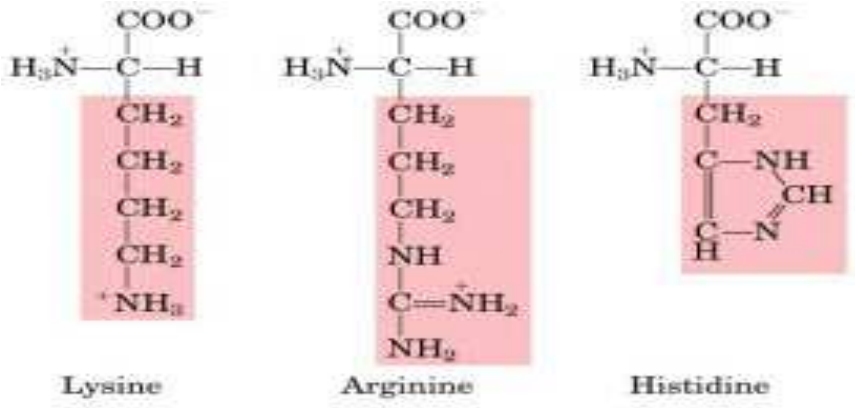


Fig.9.8. Amino Acids with Positively Charged Polar R groups

5. Amino Acids with Negatively Charged Polar R groups

The R groups in this class of amino acids are polar and negatively charged. These are hydrophilic in nature and have a second carboxylic group. The following amino acids are classified under this class (fig.9).

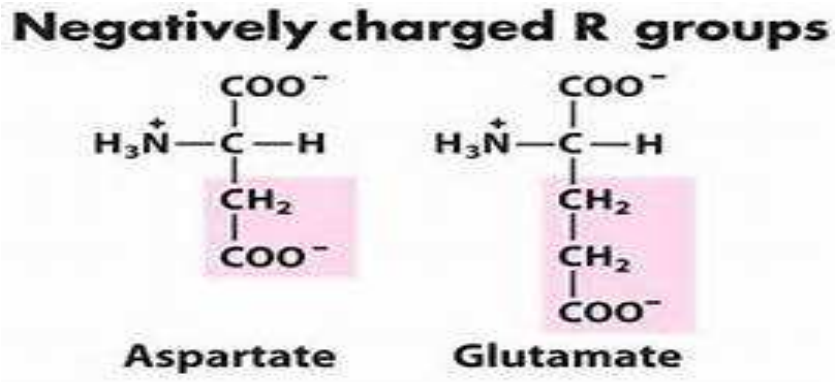


Fig.9.9. Amino Acids with Negatively Charged Polar R groups

9.4 ABBREVIATIONS OF AMINO ACIDS

Amino acids are often designated by either a three letter abbreviation or a one letter symbol (table1). The abbreviations for amino acids are the first three letters of the names except for typtophan (Trp), aspargine (Asp), glutamine (Gln) and isoleucine (Ile).

Table 1. Abbreviations of Amino Acids

Amino acid	Three-letter code	One-letter code
Glycine	Gly	G
Proline	Pro	P
Alanine	Ala	A
Valine	Val	V
Leucine	Leu	L
Isoleucine	Ile	I
Methionine	Met	M
Cysteine	Cys	C
Phenylalanine	Phe	F
Tyrosine	Tyr	Y
Tryptophan	Trp	W
Histidine	His	H
Lysine	Lys	K
Arginine	Arg	R
Glutamine	Gln	Q
Asparagine	Asn	N
Glutamic Acid	Glu	E
Aspartic Acid	Asp	D
Serine	Ser	S
Threonine	Thr	T

9.5 ESSENTIAL AND NONESSENTIAL AMINO ACIDS

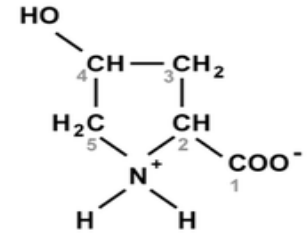
Most prokaryotic cells and some eukaryotic cells (e.g. plants, yeast) are capable of synthesizing all the amino acids present in the proteins but higher animals including man possess this ability for certain amino acids only. Those amino acids that can be synthesized from amphibolic intermediates are known as nutritionally non essential amino acids, while those which cannot be synthesized in the body are known as essential amino acids. The essential amino acids must be obtained from the diet (table 2).

Table 2. Essential and nonessential amino acids

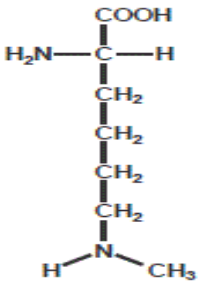
Essential	Non-Essential
Histidine	Alanine
Isoleucine**	Arginine
Leucine**	Asparagine
Lysine	Aspartic acid
Methionine	Cysteine
Phenylalanine	Glutamic acid
Threonine	Glutamine
Tryptophan	Glycine
Valine**	Proline
	Serine
* Branch Chain Amino Acids	Tyrosine

9.6 NON STANDARD AMINO ACIDS

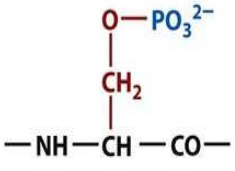
In addition to the 20 standard amino acids there are certain other amino acids which are either the constituents of proteins or biologically active peptides. In addition many amino acids may independently play a variety of biological roles Some of these amino acids result from the specific modification of an amino acid residue after the polypeptide chain has been synthesized. Amino acid modifications include the simple addition of small chemical groups to certain amino acid side chains-hydroxylation (4-hydroxyproline), methylation (6-N-methyllysine), carboxylation (γ-carboxyglutamate), acetylation (ε-N-acetyllysine) and phosphorylation (O-phosphoserine) (fig.10). 4-hydroxyproline is found in collagen, a fibrous protein of connective tissue. 6-N-methyllysine is a constituent of myosin.



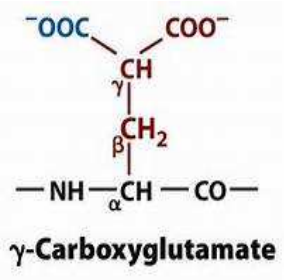
4-Hydroxyproline (Hyp)



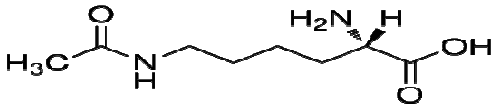
6-N-Methyllysine



***O*-Phosphoserine**



γ-Carboxyglutamate



ε-N-acetyllysine

Fig.9.10. Structures of Some Non-Essential Amino Acids

9.7 PROPERTIES OF AMINO ACIDS

The properties of amino acids are given below:

9.7.1 PHYSICAL PROPERTIES

1. General: Amino acids are usually colourless. Crystalline solids, with high melting points (usually > 200 ^{0}C). These are relatively smaller molecules with average molecular weight about 120 D. They have high boiling points.

2. Solubility: Amino acids are usually soluble in water. However, the solubility varies from different amino acids.

3. Optical Isomerism: All amino acids except glycine have at least one chiral (asymmetric) carbon atom and therefore they are optically active. They exist in D- and L-isomeric form.

4. Absorption of U.V.: Three amino acids tryptophan, tyrosin and phenylalanine (aromatic amino acids) absorb ultraviolet light. Since most biological proteins contain at least a tyrosine residue, the absorption of light by protein in UV (280 nm) range has been used as a conventional method for determining protein content spectrophotometrically.

9.7.2 CHEMICAL PROPERTIES

1. Amphoteric Nature: Amino acids in aqueous solutions are ionized and can act as acids or bases. Substances having this dual nature are amphoteric substances and are called ampholytes, since amino acids contain both amino and carboxyl group, they are capable of both donating and accepting protons (fig.9.11).

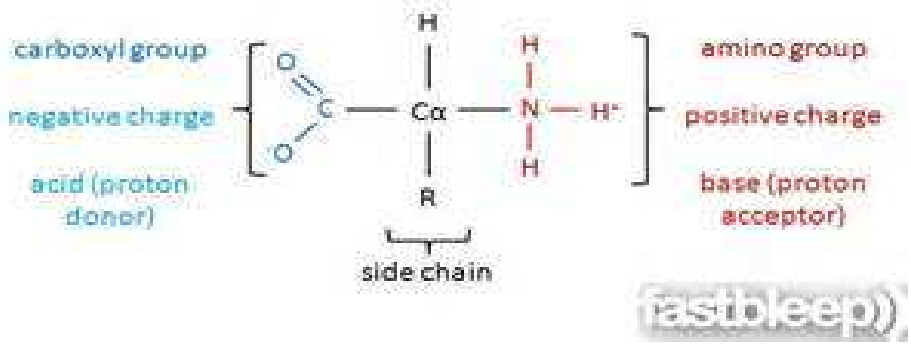


Fig. 9.11. Amphoteric Nature of an Amino Acid

2. Zwitterion: At a certain pH (isoelectric pH, pI), the amino acid molecules in aqueous solution carry both the positive and negative charges in equal amount and exist as zwitterions (dipolar ion). At this

point the net charge in it is zero (fig.12). The zwitterions solutions of amino acids do not migrate to any pole in an electric field. However, if acid is added to the solution they become positively charged ions and move to the cathode. Addition of alkali makes them negatively charged and they move to anode.

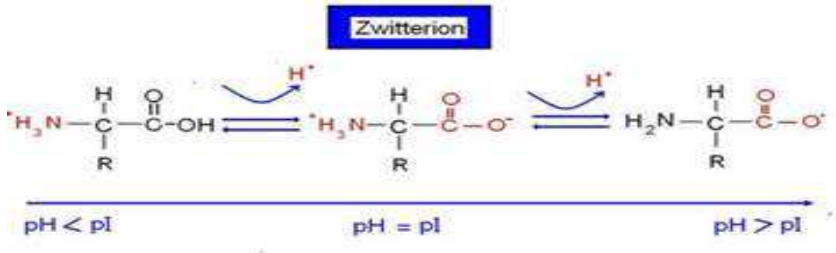


Fig.9.12. Formation of Zwitterion

3. Peptide Bond Formation: Amino acids are joined together in a protein molecule by peptide bonds (-CO-NH-) formed by the condensation of α-COOH of one amino acid with α-NH_2 group of another one with the release of one molecule of water (fig.9.13).

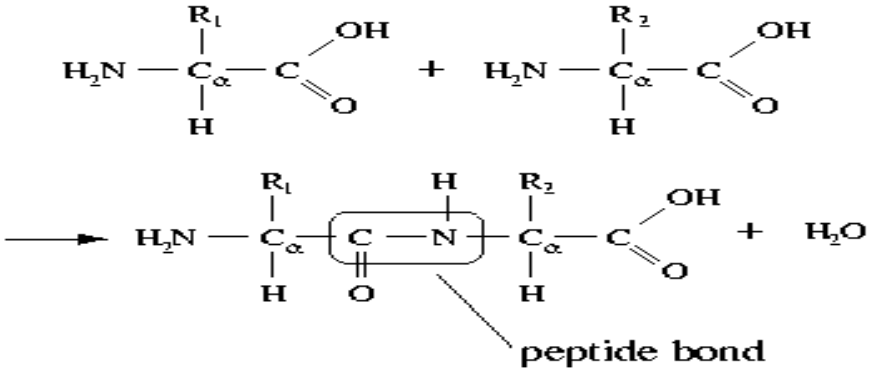


Fig.9.13. Formation of Peptide Bond

4. Ninhydrin Reaction: Amino acids yield coloured products with ninhydrin (triketohydrindene hydrate)(fig.9.14).

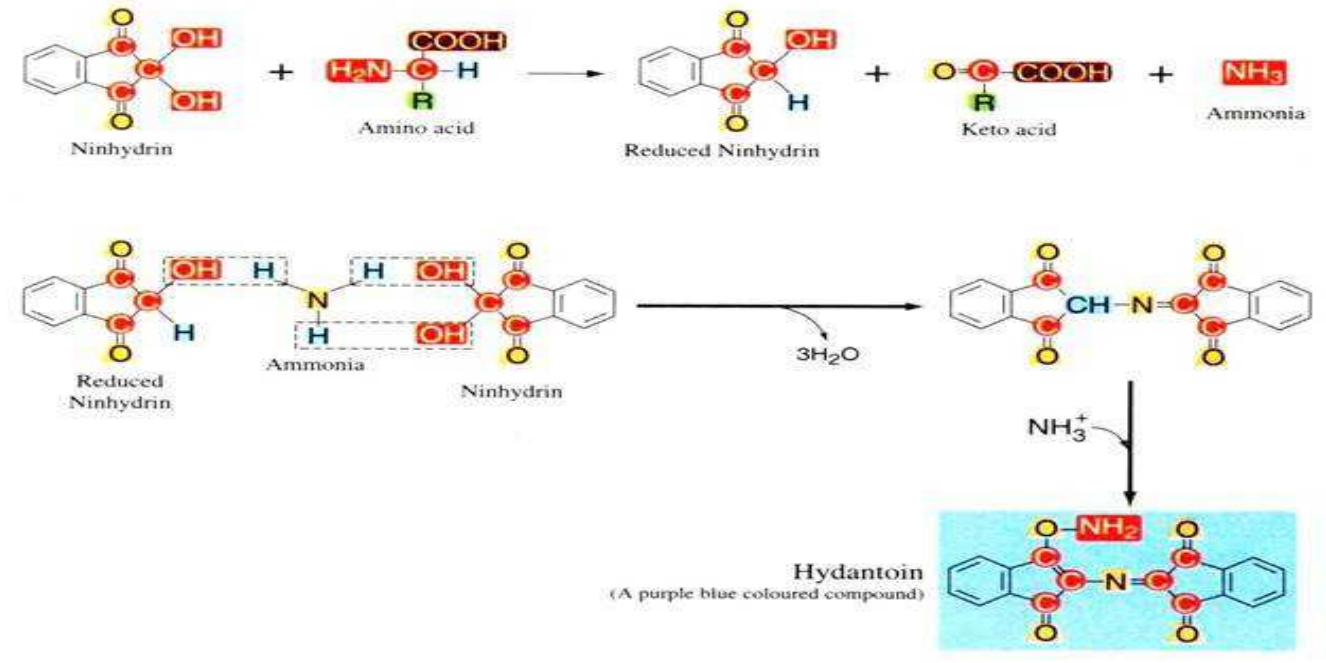


Fig9..14. Ninhydrin Reaction: amino acid yields coloured product with ninhydrin

This reaction is most widely used for quantitative estimation of amino acids. This is an oxidation-reduction reaction in which the amino acid is oxidized to an aldehyde with the release of CO_2 and NH_3. The reduced ninhydrin then reacts with ammonia and some more ninhydrin to form blue-violet compounds. The intensity of the colour is determined colorimetrically, which is proportional to the amount of amino acid present.

9.7.3 GENERAL REACTIONS

1 Formation of Esters: Amino acids can form esters with alcohols. The –COOH group can be esterified with alcohol (fig9.15).

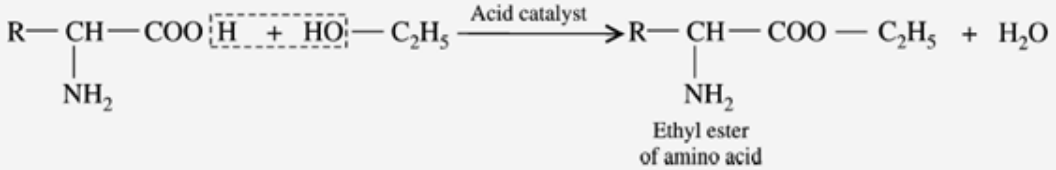


Fig.9.15. Formation of Esters

2. Formation of Amines: Action of specific amino acid decarboxylase, dry distillation or heating with Ba (OH) $_2$ evolves Co_2 from –COOH group and changes the amino acid into its amine (fig.9.16).

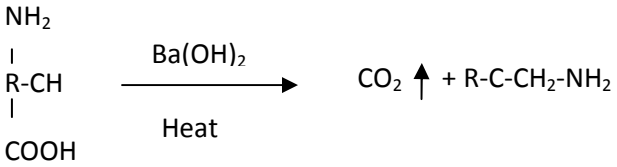


Fig.9.16. Formation of Amines

3. Salt Formation with Acids: The basic amino acids react with mineral acids to form salts like hydrochlorides (fig.9.17).

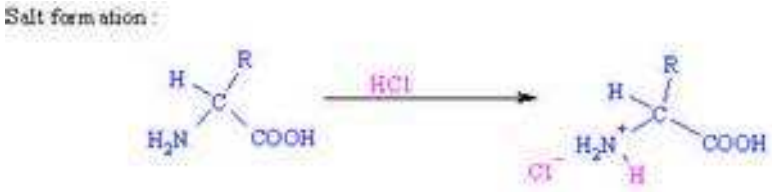


Fig.9.17. Formation of salt with acid

4. **Reaction with HNO_2:** The amino acids except proline and hydroxyline react with HNO_2 (nitrous acid) and librate N_2 from NH_2 group (fig.9.18).

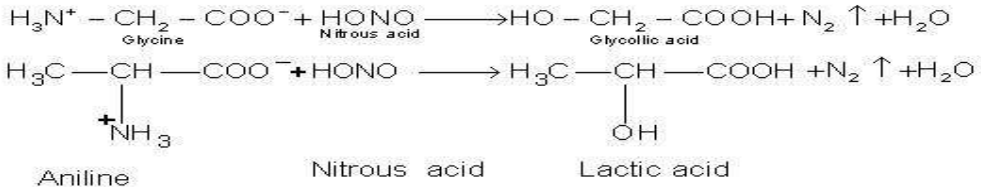


Fig.9.18. Reactions of amino acids with nitrous acids

This forms the basis for Van Slyke method for determining amino nitrogen in amino acids and proteins.

9.8 BIOLOGICAL ROLE OF AMINO ACIDS

Amino acids participate in a number of biological activities:

1. They are building blocks of proteins. Proteins form structural as well as functional components of the cell.
2. Some amino acids give rise to other compounds, such as:
    i. Tyrosin is part of hormone thyroxine and adrenaline
    ii. Glycine participates in the synthesis of heme
    iii. Tryptophan produces vitamin nictotinamide and plant hormone indole-3-acetic acid (IAA)
    iv. B-alanine is needed in the synthesis of coenzyme-A and vitamin pantothenic acid.
3. Nonprotein amino acids are components of antibiotics.
4. Because of amphoteric nature they act as buffers in the body fluids.
5. They also synthesize glucose by gluconeogenesis.
6. Histamine is an important vasodialator, derived from amino acid histidine.

9.9 PROPERTIES OF PEPTIDES

Peptides can be distinguished by their ionization behavior as they contain only one free α-amino group and one free α-carboxyl group, at opposite ends of the chain. These groups ionize as they

do in free amino acids. However, the R groups of some amino acids can ionize and in a peptide these contribute to the overall acid-base properties of the molecule. Thus the acid-base behavior of a peptide can be predicted from its free α-amino and free α-carboxyl group as well as the nature and number of its ionizable R groups. Like free amino acids, peptides have characteristic titration curves and a characteristic isoelectric pH (pI), at which they do not move in an electric field. These properties are exploited in some of the techniques used to separate peptides.

9.10 NAMING OF PEPTIDE CHAIN

In naming a polypeptide the convention is that the N-terminal residue is written first and the C-terminal residue in the end. The names of the intermediary amino acid residues are written in the same sequence as they are placed. The name of all the amino acid residues except the last one are wrillen by adding the suffix –yl because all these are the acyl groups. The name of the last amino acid , however is written as such. For example, a tripeptide containing glycine, alanine and leucine is named as glycyl-Lalanyl-L leucine and abbreviated as Gly-Ala-Leu (fig.9.19).

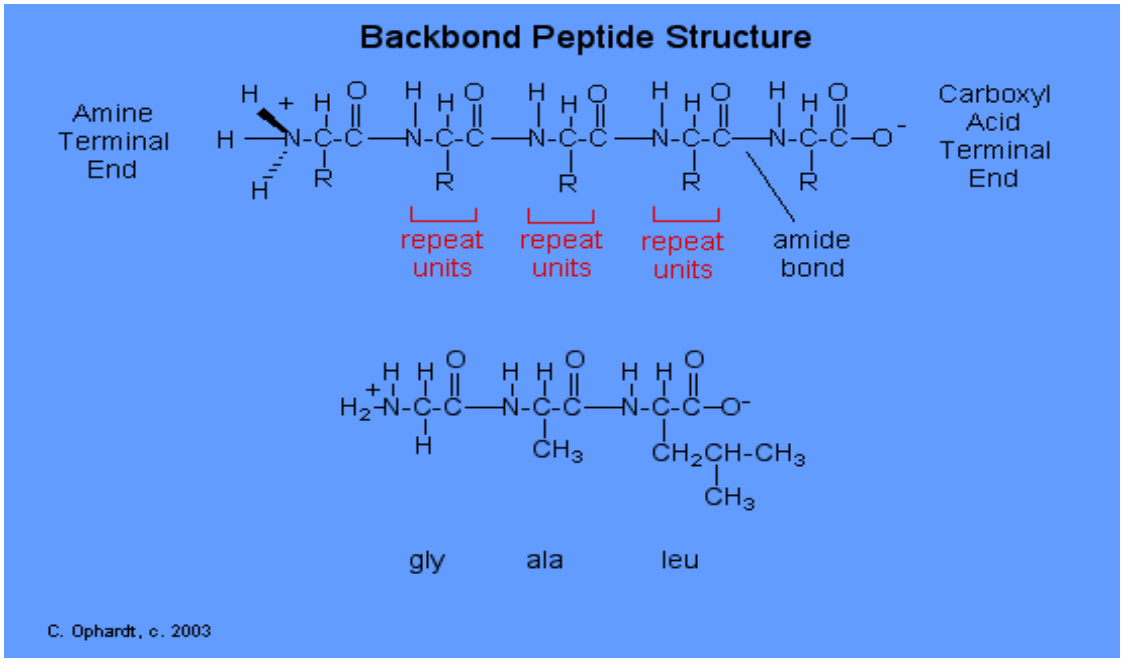


Fig.9.19. Naming of Peptide Chain

9.11 BIOLOGICAL ROLE OF PEPTIDES

Peptides participate in a number of biological activities:

1. They serve as intermediates in the formation of proteins.
2. They appear as constituents in a group of compounds called alkaloids. Majority of these have been isolated from fungi, although they are also found in higher plants. Ergotamine is a peptide alkaloid from rye ergot and has pronounced pharmacological properties.
3. Many of them possess antibacterial activities and are usually present in fungi and bacteria. Penicillin G is a common antibiotic.
4. Certain other peptides serve as growth factors. Folic acid, a water soluble vitamin is an example of it. Another group of peptides serving as growth factor for a variety of microorganisms is streptogenins.
5. Higher animals do synthesize certain peptides serving as hormones e.g. oxytocin, bradykinin etc.
6. Certain peptides like glutathione participate in controlling the oxidation-reduction potential of the cell. This may also serve as a key intermediate in electron transfer systems.
7. Enkephalins (pentapeptides) formed in certain part of brain. These are associated with the perception of pain and pleasure.
8. Some peptides are components of peptidoglycans which form bacterial cell wall.

9.12 SUMMARY

- Amino acids are the building blocks of proteins. Chemically they have a carboxyl group and an amino group bonded to same carbon atom.
- Amino acids are colourless, crystalline solids with high melting points.
- Amino acids which can be synthesized by the body are called non essential amino acids and those which cannot be synthesized in the body of the animals and human are called essential amino acids.
- The R-group present in the amino acids may be either polar or non polar.

- In aqueous solutions, amino acids are ionized and act as ampholytes.
- Amino acids give all the typical reactions associated with compounds containing carboxyl and amino groups.
- Peptides are chains of amino acids which are linked together by covalent bonds called peptide bonds.
- Peptides can be named on the basis of number of amino acid residues are present as dipeptide, tripeptide etc.
- When few amino acids are joined, the product is called as oligopeptide while many amino acids are present then the product is termed as polypeptide.
- A peptide has a carboxyl terminal residue and an amino terminal residue also termed as C-terminal and N-terminal residues respectively.
- Peptides can be distinguished by their ionization behavior.
- Peptides have characteristic titration curves and a characteristic isoelectric pH (pI), at which they do not move in an electric field.

9.13 GLOSSARY

Amino acids: Building blocks of proteins. An amino acid consists of a carbon atom to which are attach a primary amino group, a carboxyl group, a side chain group and an H atom.

Essential amino acids: Amino acids which are not synthesized by the body. These must be supplied in diet.

Non-essential amino acids: Amino acids which can be synthesized in the body and hence need not to be obtained from the diet.

Peptide: A short linier chain of amino acids linked by peptide bonds.

Peptide bond: Linkage between the α-amino group of one of the amino acid and α-carboxylic group of another amino acid.

Polypeptide: A polymer consisting of amino acids linked together by peptide bonds.

Zwitter ion: A molecule which bears oppositely charged groups.

9.14 LONG ANSWER TYPE QUESTIONS

1. What are amino acids? Describe briefly the physical and chemical properties of amino acids.
2. Classify various amino acids on the basis of their side chains and give their structure.
3. Describe the general reactions of amino acids and their biological significance.
4. What are peptides? Describe the structure and biological importance of peptides.

9.15 SHORT NOTES

1. Peptide Bond
2. Amphoteric Property of amino acids
3. Essential and Nonessential Amino Acids
4. Non standard amino acids
5. Zwitterion

9.16 FILL IN THE BLANKS

1. Compounds having both acidic and alkaline groups are called ----------.
2. ----------and ----------are two polar negatively charged amino acids.
3 ----------and---------- groups are involved in the formation of peptide bond.
4.----------amino acid forms part of vitamin nicotinamide.
5. A molecule which bears oppositely charged groups is called ---------- .

9.17 ANSWER

Fill in the blanks:

1. Amphoteric compounds
2. Aspartate and Glutamate
3. Amino ($-NH_2$) group and Carboxyl (-COOH) group
4. Tryptophan
5. Zwitterion

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UNIT 10: CARBOHYDRATES AND LIPIDS

Content:

10.1 OBJECTIVES

1. The objective of this chapter is to understand about carbohydrates and lipids.
2. To understand structures and classification of carbohydrates and lipids.
3. To understand the properties of carbohydrates and lipids.
4. Understanding the metabolism of carbohydrates.
5. Major sources of carbohydrates and lipids.
6. Biological significance and deficiency diseases of carbohydrates and lipids.

10.2 INTRODUCTION TO CARBOHYDRATES

Carbohydrates are the most abundant class of biomolecules in nature. They are widely distributed in plants and animals. Carbohydrates are the compounds of carbon, hydrogen and oxygen with empirical formula $C_n(H_2O)_n$, where n represents number of carbon atoms. However, not all the carbohydrates have this empirical formula; some also contain nitrogen, phosphorus and sulpher. They are also called hydrates of carbon because in them hydrogen and oxygen occur in the radio of 2:1, similar to that found in water. Carbohydrates are broadly defined as polyhydroxy aldehydes or ketones and their derivatives or substances that yield one of these compounds on hydrolysis or in other words they are defined as the compounds having either an aldehyde (-CHO) or a ketone (=CO) group or a modified aldehyde or ketone group and other carbon atoms with alcoholic (-OH) groups.

10.3 CLASSIFICATION OF CARBOHYDRATES

Carbohydrates are classified into three main classes:

1. Monosaccharides: These are the simplest carbohydrates, cannot be hydrolyzed into small molecules.
2. Oligosaccharides: This class includes, the carbohydrates comprise of short chains of monosaccharide units, most common are disaccharides comprise of two monosaccharide units.
3. Polysaccharides: This class includes the carbohydrates consists of long chains of monosaccharide units generally hundreds or thousands.

10.3.1MONOSACCHARIDES

Monosaccharides are simple carbohydrates having single or one saccharide (Sugar) molecule. These cannot be hydrolyzed further into simpler molecules. They are colourless crystalline, sweet tasting substances soluble in water, sparingly soluble in alcohol and insoluble in ether. They are reducing agent as they reduce the oxidizing agent and get oxidised at the carboxyl group. They are called monomers and form structural units of oligo and polysaccharides.

The number of carbon atoms in monosaccharides varies from 3 to 7. The carbon atoms form an unbranched straight chain, joined together by single covalent bonds. On the basis of number of carbon atoms monosaccharides are classified into 5 classes-

Trioses: with three carbon atoms ($C_3H_6O_3$)
Tetroses: with four carbon atoms ($C_4H_8O_4$)
Pentoses: with five carbon atoms ($C_5H_{10}O_5$)
Hexoses: with six carbon atoms ($C_6H_{12}O_6$)
Heptoses: with seven carbon atoms ($C_7H_{14}O_7$)

A monosaccharide molecule has two functional groups: A carbonyl (-C=O) group in which oxygen is attached to carbon atom by a double bond. Hydroxyl (-OH) groups which are attached to all other carbon atoms of the chain. On the basis of the position of the carbonyl (-C=O) group at the chain, monosaccharides are divided into two families: A. Aldoses B. Ketoses

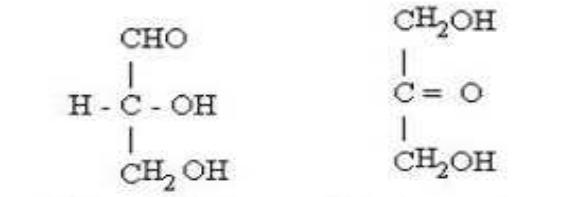
A. Aldoses: If the carbonyl group is present at the end of the carbon chain, aldehyde (-CH=O) group is formed and the sugar is called as an aldose sugar.

B. Ketoses: If the carbonyl group is present at any other position, ketone (-C=O) group is formed and the sugar is called as ketose sugar.

Table1. Some Monosaccharides

Carbohydrates	No. of C atoms	Aldoses	Ketoses
Trioses ($C_3H_6O_3$)	3	Glyceraldehyde or Glyserose	Dihydroxyaceton
Tetroses ($C_4H_8O_4$)	4	Erythrose	Erythrulose
Pentoses ($C_5H_{10}O_5$)	5	Ribose	Ribulose
Hexoses ($C_6H_{12}O_6$)	6	Glucose	Fructose
Heptoses ($C_7H_{14}O_7$)	7	Mannoheptulose	Sedoheptulose

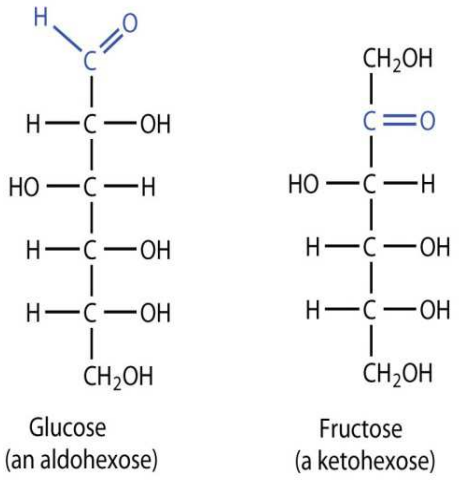
Glyceraldehyde and dihydroxyacetone are the simplest monosaccharides with three carbon atoms. Here glyceraldehyde is an aldose as it contain an aldehyde group while, dihydroxyacetone is a ketose because it contains a keto group.



Glyceraldehyde (An aldotriose of sugar)

Dihydroxyacetone (A ketotriose sugar)

Glucose is a hexose sugar with six carbon atoms ($C_6H_{12}O_6$). It is an aldose as it contains an aldehyde group, while fructose is a keto hexose sugar because it contains a keto group.



10.3.2 OLIGOSACCHARIDES (GK. OLIGOS = FEW)

Oligosaccharides are composed of few monosaccharide units (2-20). During union of monosaccharide units, water molecule is eliminated and the units are linked through an oxygen bridge. This chemical bond that joins the two monosaccharide units is called a glycosidic bond. Depending upon the steric configuration at C1, which is involved in the formation of glycosidic linkage of monosaccharide unit, the bond is called α- and β- bond. The natural source of oligosaccharides is green plants. They are crystalline substances and readily soluble in water, most of them are obtained as colourless solids. Oligosaccharides are classified according to the number of their constituent monosaccharide units. In the modern system of nomenclature these are named according to the name of their constituent monosaccharide units and the position of glycosidic bond such as: 1. Disaccharides (2 sugar units), 2.Trisaccharides (3 sugar units), 3.Tetrasaccharides (4 sugar units) etc. Out of these most common are disaccharides.

1. Disaccharides: Disaccharides consists of two monosaccharide units covalently bound to each other with a glycosidic bond. Their empirical formula is $C_{12}H_{22}O_{11.}$ These can be hydrolyzed to yield their free monosaccharide compounds by boiling with dilute acid. Following are the examples of disaccharides-

a. Maltose: It is the simplest disaccharide, composed of two units of D-glucose joined together through their 1 and 4 carbon atoms. It does not occur in nature and also called as malt sugar. It is the product of

hydrolysis of starch by the enzyme amylase. Maltose is hydrolyzed to two molecules of glucose by the intestinal enzyme maltase.

b. Sucrose: It is the most abundant disaccharide, made up of glucose and fructose also known as cane sugar. It is obtained commercially from cane or beet. It is very sweet, crystalline and freely soluble in water. It can be cleaved into its components by the enzyme sucrase.

c. Lactose: It occurs naturally only in milk thus also known as milk sugar. It is made up of glucose and galactose units joined together through carbon 1 of galactose and carbon 4 of glucose. It is not much sweet to taste and hydrolyzed to its monosaccharides by enzyme lactase in human beings.

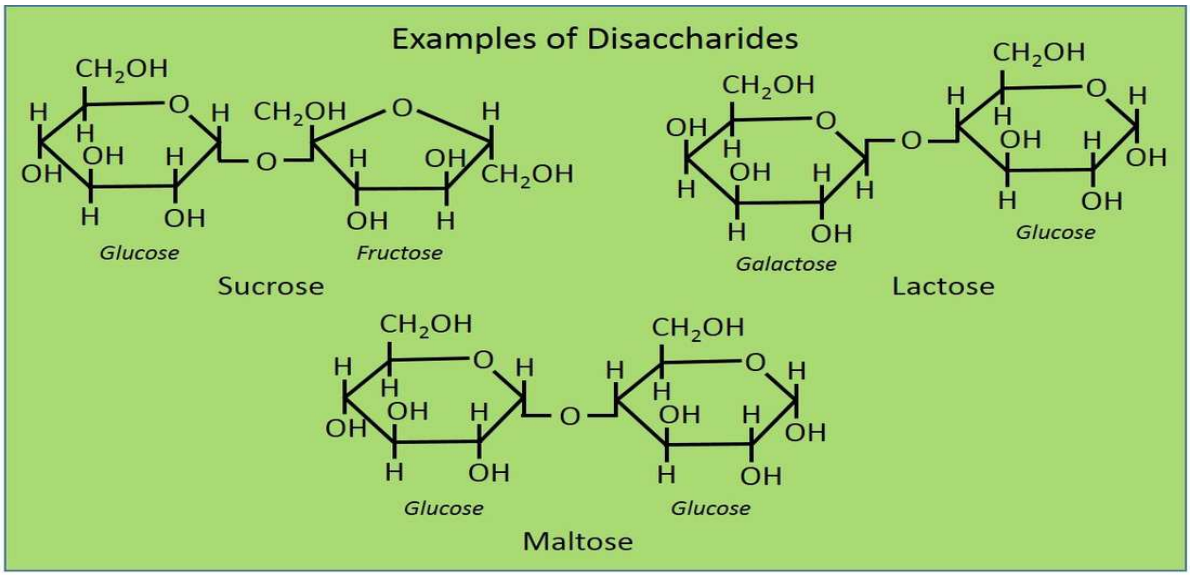


Fig.10.1. Examples of Disaccharides

2. Trisaccharides: These are composed of three molecules of monosaccharide units. Examples of trisaccharides are-

a. Raffinose: It is made up of glucose, galactose and fructose units. It is found in sugar beet and cotton seed meal and in some fungi.

b.Gentianose: It is composed of one unit of fructose and two units of glucose. It is found in rhizomes of several gentian species.

3. Tetrasaccharides: These are composed of four molecules of monosaccharide units. Example of tetrasaccharide is-

a. Stachyose: It is composed of two units of galactose, one unit of glucose and one unit of fructose. It is found in the hair cot beans.

10.3.3 POLYSACCHARIDES

Polysaccharides are composed of several monosaccharide units linked together by glycosidic bond. Polysaccharides are tasteless and colourless amorphous powder which are little soluble in water, although some may form colloidal solutions. The most common monosaccharide units in polysaccharides are glucose, although fructose, galactose and other hexoses also occur. If all the monosaccharide units are the same in a polysaccharide, it is called a homopolysaccharide. For example starch, cellulose and glycogen, they all are polymers of glucose. If the monomeric units are different in a polysaccharide, it is called a heteropolysaccharide. For example, Hyaluronic acid, made up of alternating residues of uronic acid and N-acetyl glucosamine. On the basis of their function, polysaccharides are classified into two classes: 1. Storage polysaccharides 2.Structural polysaccharides.

1. Storage Polysaccharides: Polysaccharides are stored as reserve products in many tissues. They occur as starch in plant cells and as glycogen in animal cells.

a. Starch: Starch is a storage polysaccharide as it is the reserve food material in the plants. It is a homopolysaccharide made up of glucose units. It is especially abundant in tubers such as potatoes, in seeds especially corn, but most plant cells are able to produce starch. It contains two types of glucose polymers- α-amylose and amylopectin. A-amylose consists of long chain of D-glucose units linked by α (1→4) linkage while amylopectin is a branched chain of D-glucose units. The glycosidic linkages in linear chain are α (1→4) but at the branch points these are α (1→6) occurring every 24 to 30 residues.

b. Glycogen: Glycogen is a storage polysaccharide as it is the major reserve food material in animals, the counterpart of starch in plant cells. It is a homopolysaccharide made up of glucose units. It is especially abundant in the liver, where it may constitute as much as 7% of the wet weight; it is also present in the skeletal muscle. Glycogen is a branched polymer of glucose in which glucose residues are linked by α(1→4) and α(1→6) glycosidic linkages just like in amylopectin but glycogen is more extensively branched (on average every 8 to 12 residues) and more compact than starch.

2. Structural Polysaccharides: Polysaccharides are involved in the structural organization of many tissues in microorganisms, higher plants and animals. They occur as cellulose in plant cells and as chitin in animal cells.

a. Cellulose: Cellulose is a major structural polysaccharide in plant cells and also present in some microorganisms. It is a fibrous, tough and water insoluble substance. Cotton is almost pure cellulose while wood is largely made up of cellulose. Cellulose is a linear, unbranched, homopolysaccharide of 10,000 or more D-glucose residues, connected by β (1→4) glycosidic linkage. However, cellulose resembles with amylase and the main chain of amylopectin of glycogen but in those, the linkage is α (1→4).

b. Chitin: Chitin is the structural component of exoskeleton of invertebrates such as insects and crustaceans. It is also present in the cell-walls of fungi and many algae. Chitin is a homopolysaccharide made up of N-acetyl-D-glucosamine linked by β (1→4) glycosidic linkage.

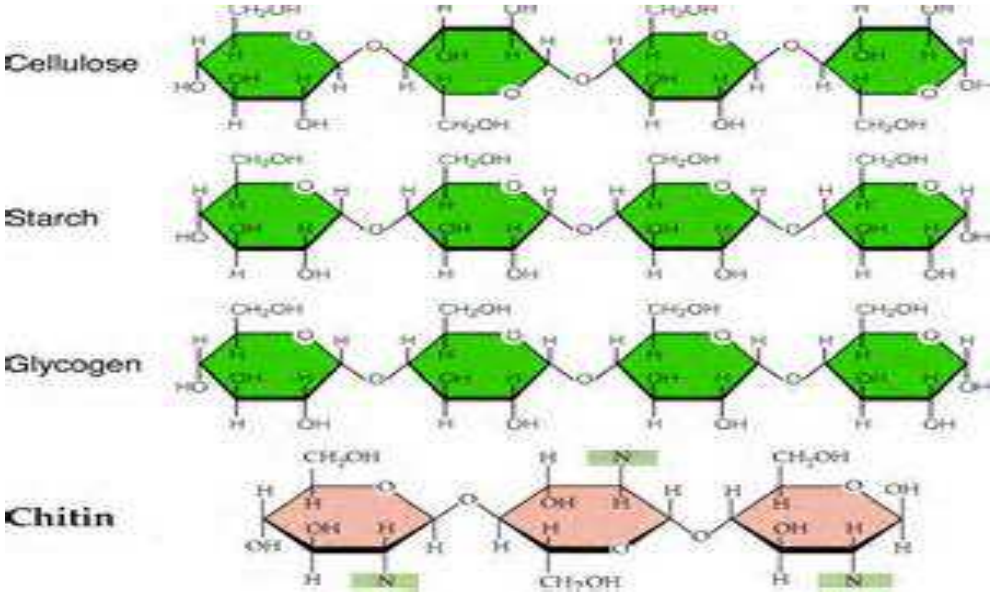


Fig.10.2. Examples of Polysaccharides

10.4 METABOLISM OF CARBOHYDRATES

Carbohydrates are the major source of energy in the organisms. Dietary carbohydrates mainly consist of polysaccharides (starch) and disaccharides (sucrose, lactose and maltose). These are hydrolyzed into monosaccharides and are absorbed in the blood. Glucose is an important monosaccharide which served as the major metabolic fuel of the cell and tissues. Non-glucose monosaccharides are also converted into glucose. Glucose is metabolized in different ways to produce energy in the body. The main pathways of carbohydrates metabolism are –

I. Glycolysis
II. Citric acid cycle
III. Glycogenesis
IV. Glycogenolysis
V. Gluconeogenesis
VI. Pentose Phosphate Pathway

I. Glycolysis

Glycolysis is a process in which one molecule of glucose is converted into two molecules of pyruvate. The pathway is also known as Embden-Meyerhof Parnas (EMP) Pathway after two pioneer investigators Gustav. Embden and Otto Meyerhof. Glycolysis occurs in the cytoplasm of the cell and virtually in all tissues. The process is completed in 10 steps and each step is catalyzed by a specific enzyme. Glycolysis is divided into two phases. In the first phase, one molecule of glucose is converted into two molecules of glyceraldehydes-3-phosphate. The process requires 2 molecules of ATP. In the second phase the molecules of glyceraldehydes-3-phosphate are converted into pyruvate with the release of 4 ATP and 2 NADH molecules. Steps of glycolysis are as follows.

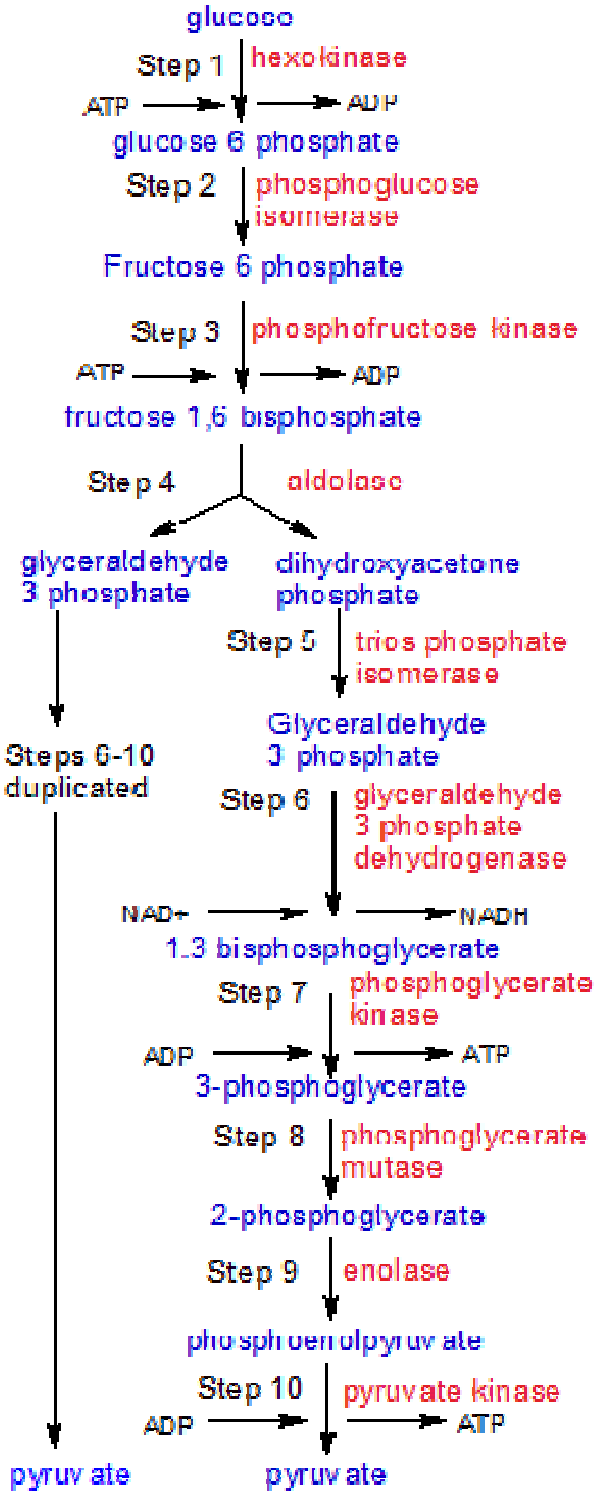


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Fig.10.3. the ten steps of glycolysis. Note that steps 6-10 are completed twice because two molecules of G3P were created in steps 4 and 5.

End products of glycolysis

1. From one molecule of glucose two molecules of pyruvate are formed.
2. 2 ATP molecules are used while 4 ATP molecules are formed. Therefore, there is net gain of 2ATP molecules.
3. $2H^+$ atoms are formed. These are accepted by NAD, which changes into $NADH+H^+$. Hydrogen ions are transferred to electron transport system.

Fate of pyruvate

The pyruvate formed, as a result of glycolysis, can be further metabolized via any of the three catabolic pathways-

- **I.** Under aerobic conditions, pyruvate is oxidised completely to yield CO_2 and H_2O by citric acid cycle.
- **II.** Under anaerobic conditions, in some microorganisms and under hypoxia in skeletal muscles, pyruvate is reduced to lactate via lactic acid fermentation.
- **III.** In some plant tissues invertebrates and microorganisms under hypoxic or anaerobic conditions pyruvate is converted into ethanol and CO_2 via ethanol (alcohol) fermentation.

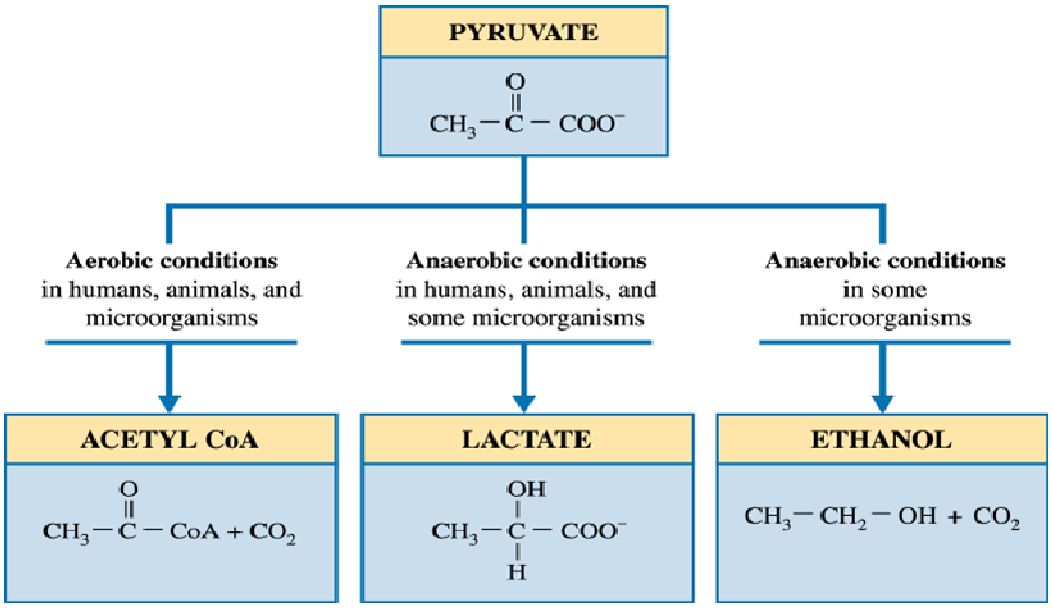
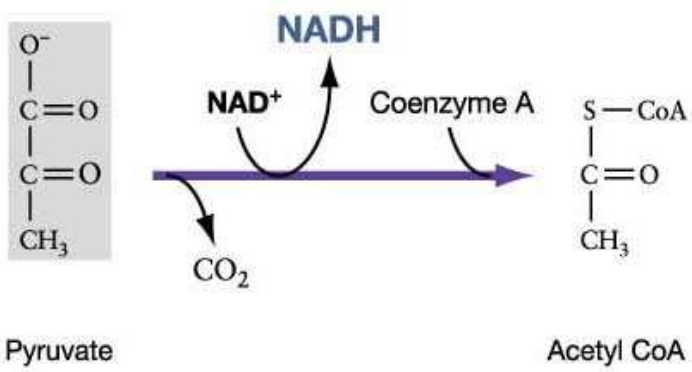


Fig.10.4 Fate of Pyruvate

II. Citric Acid Cycle

Under aerobic conditions pyruvate is completely oxidised to yield CO_2 and H_2O. Pyruvate is first converted to acetyl Co-A which is then oxidised to produce CO_2 and H_2O. The whole process occurs in a cyclic manner and is called citric acid cycle. The cycle is also known as TCA cycle (Tricarboxylic acid cycle) or Krebs cycle after its discoverer Hans Krebs. The enzymes for TCA cycle located in the mitochondrial matrix.



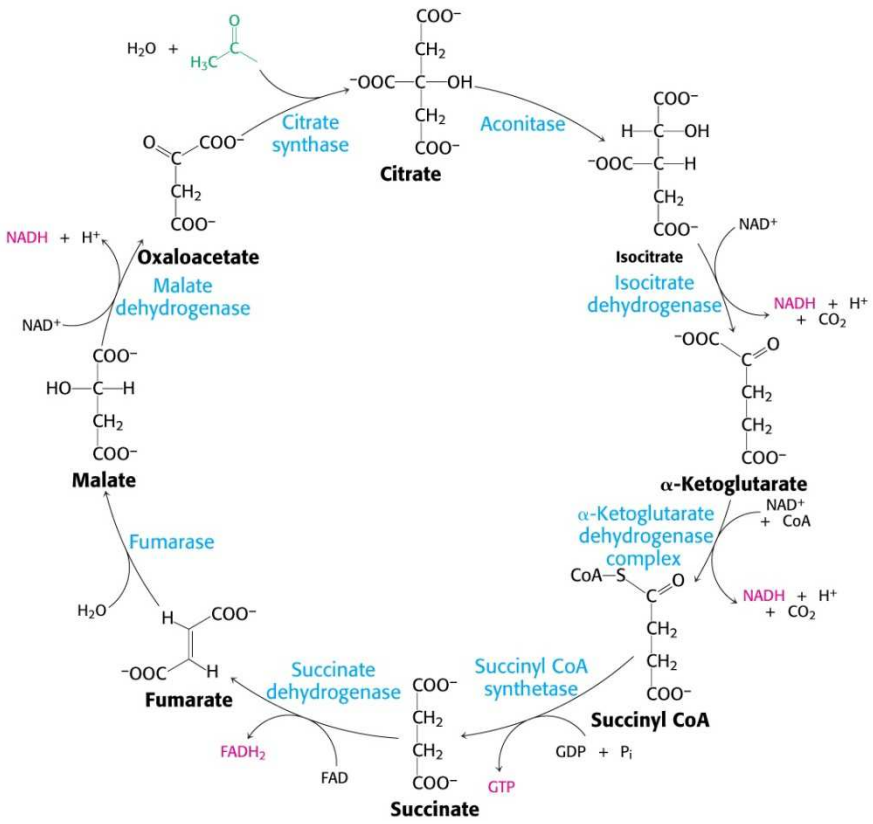


Fig.10.5. Citric acid cycle

During glycolysis each glucose molecule yields 2 molecules of pyruvate and 8 ATP are gained. Hence when these two molecules of pyruvate undergo complete oxidation by TCA cycle 24 ATP are formed. In addition, 2NADH are formed during the formation of acetyl Co-A by pyruvate through dehydrogenase complex which yields 6 ATP molecules. Thus complete oxidation of one molecule of glucose will yield 38ATP molecules (glycolysis 8, Pyruvate dehydrogenase complex 6, TCA cycle 24).

III. Glycogenesis

Glycogenesis is the process of formation of glycogen from glucose. The major sites of glycogenesis are liver and muscles but it can occur in every tissue in the body to some extent. Glycogenesis is a very important process as excess of glucose is converted to glycogen and is stored in this form for utilization at the time of requirement.

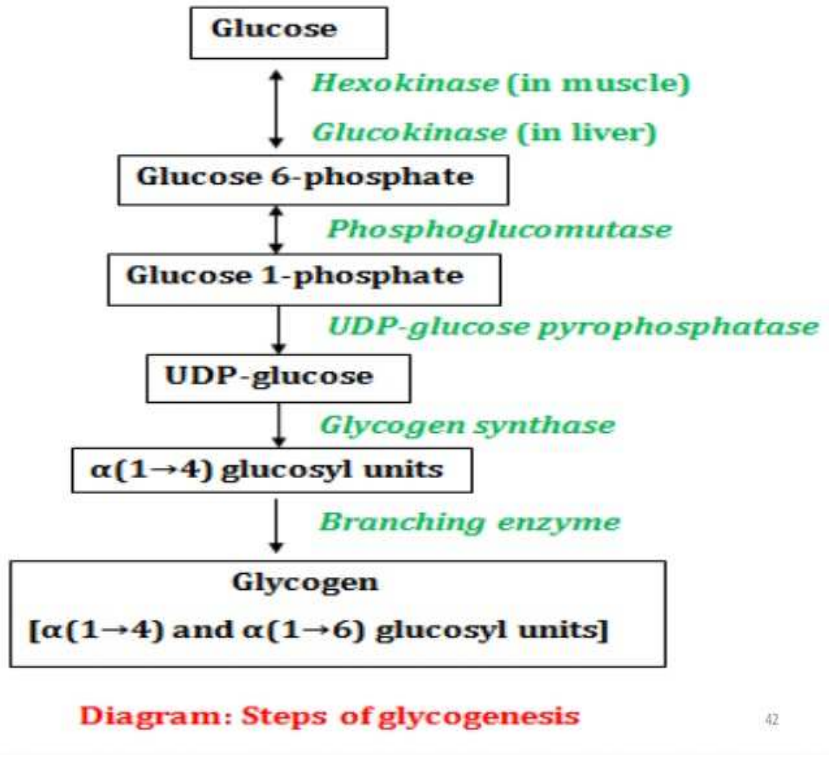


Fig.10.6. Glycogenesis

For synthesis of glycogen, a pre-existing glycogen chain (glycogen primer) is required. UDP-G transfers the glucose molecule to a pre-existing glycogen primer. C-1 of the glucose of UDP-G forms a glycosidic bond with the C-4 of a terminal residue of glycogen primer in the presence of enzyme glycogen synthase. UPD is liberated in this process.

In this way an existing glycogen chain is repeatedly extended by one glucose unit at a time by the successive α 1-4 linkage. Glycogen synthase is a principle enzyme which regulates glycogen formation. Glycogen synthase can add glycosyl residue only if polysaccharide chain already contains more than four residues. When the chain has become minimum of 11 glucose residues, another enzyme, branching enzyme transfers a part of α 1-4 chain (at least 6 glucose residues) to a neighboring chain to form α 1-6 linkage, thus forming a branching point in the molecule. The branches now grow further by further addition of α 1-4 glycosyl units and further branching. The glycogen formed is stored in liver and muscles.

IV. Glycogenolysis

The breakdown of glycogen to glucose is called glycogenolysis. When the blood glucose level falls, glycogen is broken down to glucose to maintain the normal level of blood glucose.

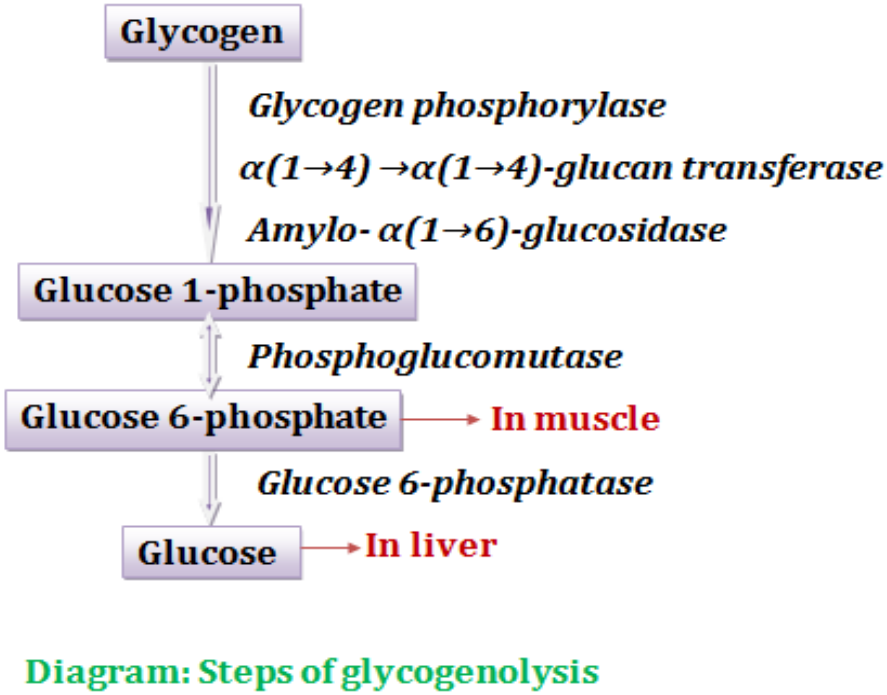


Fig.10.7. Glycogenolysis

The first step in the breakdown of glycogen is catalyzed by the enzyme glycogen phosphorylase which catalyzed the cleavage of terminal α 1-4 linkage of glycogen to remove one glucose residue as glucose-1-phosphate. The removal of glucose residues continues until about four to five glucose residues remain on either side of the α 1-6 branch. Further degradation by glycogen phosphorylase can occur only after the debranching enzyme (a bifunctional enzyme), catalyzes two successive reactions:-

1. The transferase activity of the enzyme shifts a block of three glucose residues from the branch to a nearby non reducing end to which they reattached in α 1-4 linkage.
2. Then the 1-6 glucosidase activity releases a single glucose residue remaining at the branched point in α-1-6 linkage.

After this glycogen phosphorylase activity can continue. Glucose1-phosphate formed on the phosphorylatic cleavage of glycogen is converted into glucose 6-phosphate by phosphoglucomutase. The glucose 6-phosphate formed from glycogen in muscles enters into glycolysis and serve as an energy source to support the muscle contraction. In liver, enzyme glucose 6-phosphatase cleaves the phosphoryl group from glucose 6-phosphate to form free glucose which release into the blood when the blood glucose level drops.

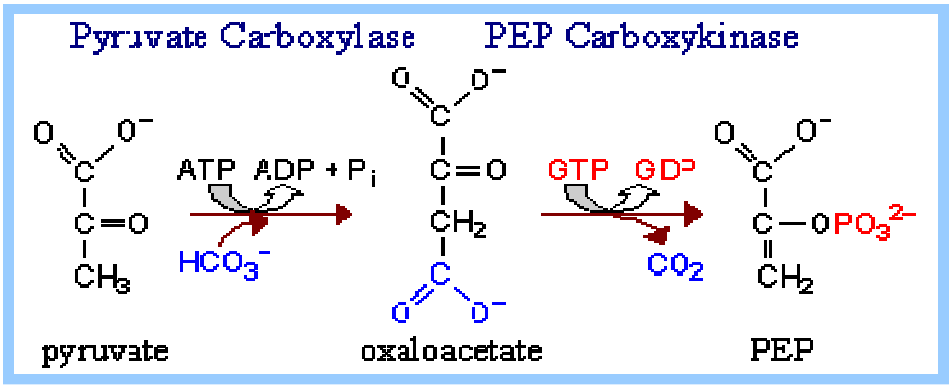
V. Gluconeogenesis

It is the formation of glucose from non-carbohydrate sources, such as lactate, pyruvate, citric acid cycle intermediates and most of the amino acids. Gluconeogenesis mainly occurs in liver and to a lesser extent in kidney. It takes place only when carbohydrates are not available in sufficient amount from the diet.

Though most of the reactions of gluconeogenesis are the reversal of glycolysis, there are certain reactions which are specific to gluconeogenesis. These are:

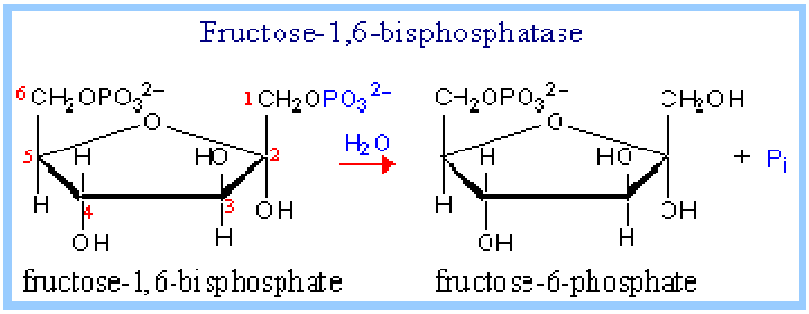
1. Conversion of pyruvate to phosphoenol pyruvate,
2. Conversion of fructose 1, 6, bisphosphate to fructose 6-phosphate,
3. Formation of glucose from glucose 6- phosphate

1. Conversion of pyruvate to phosphoenol pyruvate: In this step pyruvate is first converted to oxaloacetate, which is then converted to phosphoenol pyruvate.

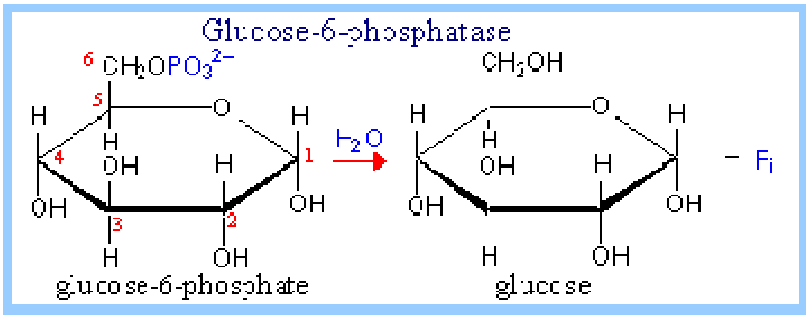


As oxaloacetate is formed inside the mitochondria it must come out to the cytosol for conversion to phosphoenol pyruvate because the enzyme phosphoenol pyruvate kinase is present in cytosol. As oxaloacetate is not permeable to mitochondria it is converted to malate and cross the mitochondria. In the cytosol malate is again converted to oxaloacetate. Oxaloacetate can also combine to acetyl co-A in the mitochondria to form citrate which is permeable to mitochondria. In the cytosol, citrate is again converted to oxaloacetate. Once phosphoenol pyruvate is formed it goes into reverse glycolytic pathway to form fructose 1, 6, bisphosphate.

2. Conversion of fructose 1, 6, bisphosphate to fructose six phosphates:



3. Formation of glucose from glucose 6 phosphate:



Glucose produced by gluconeogenesis in liver or kidney is released into blood stream to be carried to the tissues.

VI. Pentose phosphate pathway

Pentose phosphate pathway (PPP) is also known as phospho gluconate pathway (PGP) and the hexose monophosphate shunt (HMP), operates in the cytosol o the cell. The pathway serves following purpose:

1. It provides cytosolic NADPH for use in biosynthesis of fatty acids and steroids,
2. It synthesizes ribose phosphate, a precursor of the nucleotide biosynthesis.
3. The pathway ultimately yields glyceraldehydes 3-phosphate which can be then oxidised to produce ATP.
4. In dark reactions of photosynthesis, a variation of pentose phosphate pathway leads to the synthesis of glucose.

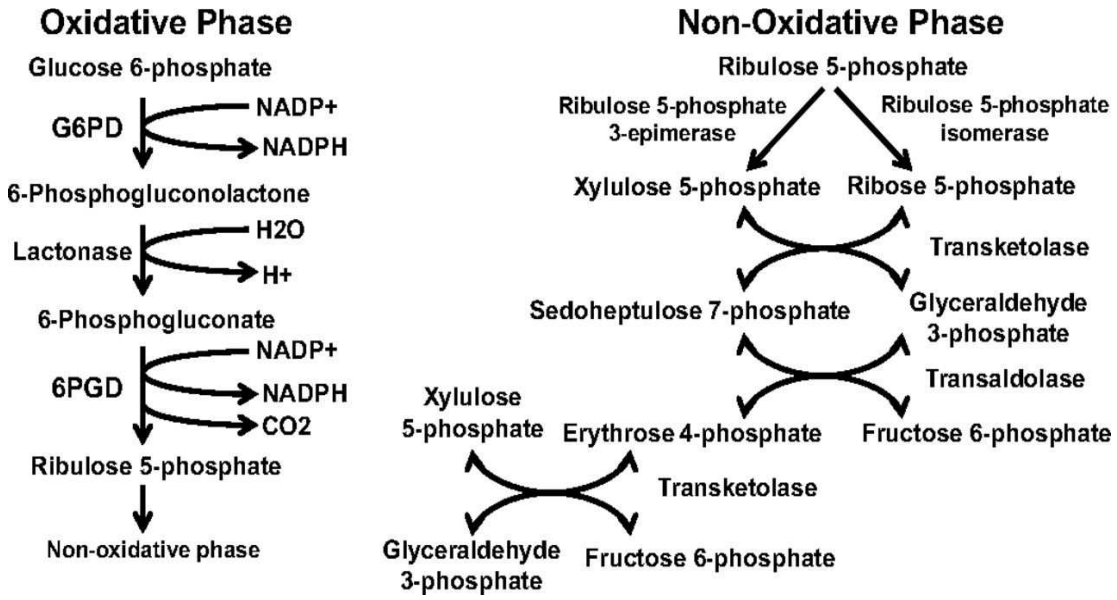


Fig.10.8. Pentose Phosphate Pathway

Pentose phosphate pathway comprises of some oxidative and some non-oxidative reactions. In oxidative phase, glucose 6-phosphate is converted to a pentose sugar, ribulose 5-phosphate with the

elimination of NADPH and CO_2. In non-oxidative phase, ribulose 5-phosphate molecules undergo a series of conversions and ultimately produce fructose 6-phosphate and glyceraldehyde 3-phosphate.

10.5 ELECTRON TRANSPORT SYSTEM OR RESPIRATORY CHAIN

During the process of respiration, both oxidation and reduction go on simultaneously. When a substance releases hydrogen, it is called hydrogen donor and said to be oxidized. The substance accepting hydrogen is called hydrogen acceptor and is said to be reduced.

At various stages in glycolysis, in oxidation of pyruvic acid and Kreb's citric acid cycle, hydrogen atoms are released.

1. In glycolysis: 2 x 2H,
2. During decarboxylation of pyruvic acid and formation of acetyl coenzymes A: 2 x 2H,
3. During kreb's cycle: 2(4x2H.)

Each hydrogen atom consists of one proton and electron $2H \rightarrow 2H^+ + 2e$. Protons are soluble in water but not the electrons. The protons are released into aqueous solution of cell, the electrons are accepted by the electron acceptors of electron transport system. ETS or electron transport system is a system of enzyme and coenzymes present in the inner mitochondrial membrane. These enzymes and coenzymes act as hydrogen and electron accepters. In ETS several hydrogen and electron acceptors are present alternately. Hydrogen and electrons are passed from one acceptor to another. Finally hydrogen is accepted by molecular oxygen and water is formed.

There are three major classes of enzymes involved in electron transport in mitochondria,
(i) Pyridine linked dehydrogenase catalyses reversible transfer of electron from intermediates of TCA cycle to NAD^+ or $NADP^+$ and produce NADH or NADPH respectively,
(ii) The flavin linked dehydrogenase catalyses transfer of electrons from either succinate or NADH to FMN or FAD and
(iii) The cytochromes acting in series and transferring electrons from flavoproteins to molecular oxygen.

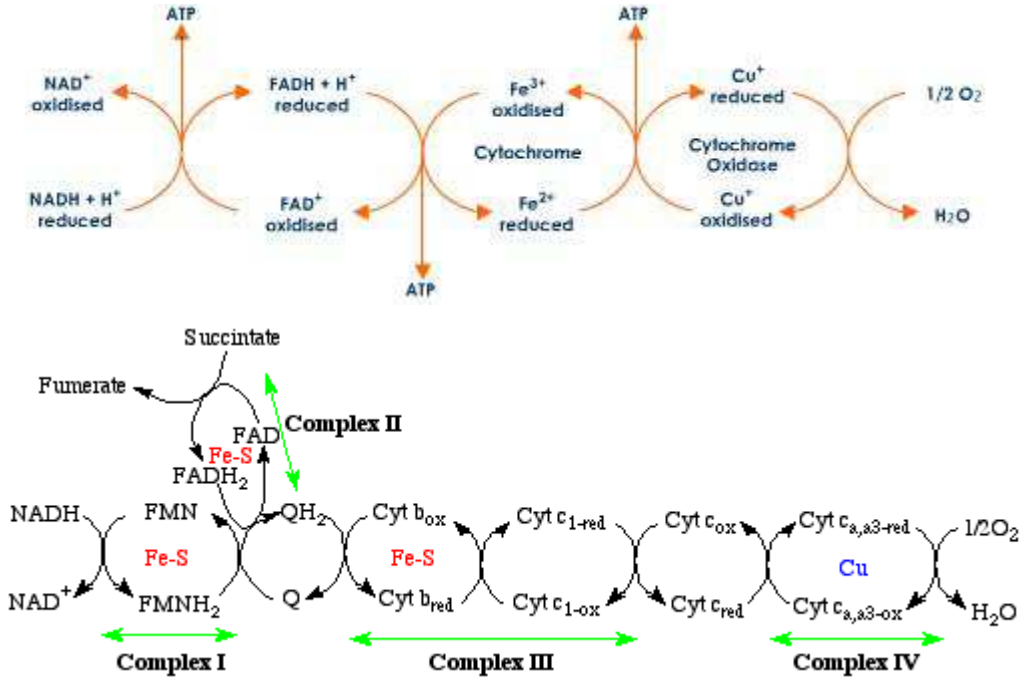


Fig.10.9. Electron Transport System

Oxidative Phosphorylation in Electron Transport System

Electrons released during transport of H^+ along ETS are accepted by electron accepters of ETS and are passed on to F_o-F_1 complex or ATPase complex where ADP is phosphorylated into energy rich ATP molecules. This process of ATP synthesis from ADP and inorganic phosphate (pi) during oxidation of acetyl coenzyme-A is called oxidative phosphorylation. During this oxidation energy is released in graded sequence. Some of this energy is utilized by F_1 particles containing there coupling factors and ATPase enzyme for the synthesis of ATP molecules from ADP.

Thus for the reduction of one molecule of oxygen, six protons are translocated from matrix to perimitochondrial space. This creates an increase of proton concentration outside the inner membrane and sets up a proton gradient or electrical potential difference. The resulting potential difference forces the proton to return towards the mitochondrial matrix. They pass through the coupling factors of complex V or F_o-F_1 complex or ATPase complex. This provides energy for synthesis of ATP for every pair of proton driven inwards one ATP molecule is synthesized.

10.6 SOURCES OF CARBOHYDRATE

Carbohydrates are the most abundant biological molecules. They are widely distributed molecules in plants and animals both tissues. Plants are considerably richer in carbohydrate in comparison of animal. Plants synthesize glucose during photosynthesis from carbon dioxide and water. It is stored in the form of starch. Cellulose, pectin and lignin are the common structural polysaccharides present in plants, which forms the plants framework, which is generally present in seeds, tubers and rhizomes of the plants. In animal cells, glycogen is present as a storage polysaccharide. Glucose is present in the human blood in a concentration of 1g/L. Chitin, hyaluronic acid and chondritin sulphates serve as structural polysaccharides in animals, present in the shells of lobsters, crabs, insects and in the cartilage, adult bones heart valves and cornea. Lactose occurs naturally in milk of mammals also detected in the flowers of some plants.

10.7 BIOLOGICAL SIGNIFICANCE OF CARBOHYDRATES

1. The most important information role of carbohydrates is the production of energy in the form of ATP both in plants and animals, the energy being derived as a result of their oxidation.
2. They are indispensable for living organisms, serving as skeletal structures in plants (e.g. cellulose, hemicellulose, lignin and pectin) and animals (e.g. Chitin, hyaluronic acid chondritin sulphates)
3. They also occur as food reserves in the storage organs of plants (e.g. seeds, tubers and rhizomes) in the form of starch and inulin. Sucrose (sugar cane, beet), glucose (grapes) and fructose (fruits) are also stored. In animals, storage form of carbohydrate is glycogen. Glucose is the most important carbohydrate. The bulk dietary carbohydrates are absorbed in the form of glucose, which is present in blood as a most common carbohydrate in a concentration of 1g/L.
4. Deoxyribose and ribose sugar form part of the structural framework of DNA and RNA.
5. Carbohydrates also play role as mediators of cellular interactions.
6. They play a key role in the synthesis of acids, lipids, fatty acids and steroids.
7. They also serve to lubricate skeletal joints to provide adhesion between cells and to confer biological specificity on the surface of animal cells.

10.8 DEFICIENCY DISEASES OF CARBOHYDRATES

1. Acidosis: In carbohydrate starvation, there is a shift from glycolysis (breakdown of glucose) to lipolysis (breakdown of lipids) and ketogenesis for energy needs. The resulting product, keto acids increases acidity in the blood and other body tissues. These changes in the pH of arterial blood outside 7.35 pH -7.45 pH result in irreversible cell damage.
2. Ketosis: Carbohydrate deficiency causes the production of ketone bodies in liver formed by the breakdown of fatty acids and by the deamination of amino acids, leading to a state of ketosis. Ketosis results in chronic dehydration and reduced body secretions.
3. Hypoglycemia: The non availability of glucose due to severe lack of carbohydrate causes drop in the blood sugar levels. It occurs when blood glucose level drop under 70 mg/L with typical symptoms like giddiness, fatigue and distress.
4. Constipation: Dietary fiber is an essential component of carbohydrate food, which is known to prevent recto colon cancer and help digestion. The absence of dietary fiber can cause constipation.

10.9 INTRODUCTION TO LIPIDS

Lipids are a very important heterogonous group of organic substances which are widely distributed throughout the plant and animal kingdom. The plant they are present in seeds, nuts and fruits, while in animals they are stored in adipose tissues, bone marrow and nervous tissues. Chemically they are various types of esters of fatty acids and alcohol. The addition to fatty acids and alcohols, some of the lipids may contain phosphoric acid, nitrogenous group and carbohydrate. Lipids are relatively insoluble in water and readily insoluble in water and readily soluble in organic solvents such either, chloroform, carbon disulphide benzene, hot alcohol etc. Bloor [1947] defined lipids as “naturally occurring compound which are insoluble in water, and soluble in one or more organic solvent such as benzene, chloroform, either acetone the so called fat solvent on hydrolysis field fatty acids which are utilized by the living organisms’’

10.10 CLASSIFICATION OF LIPIDS

Lipids are generally classified into three main classes

1. Simple Lipids
2. Compound Lipids
3. Derived Lipids

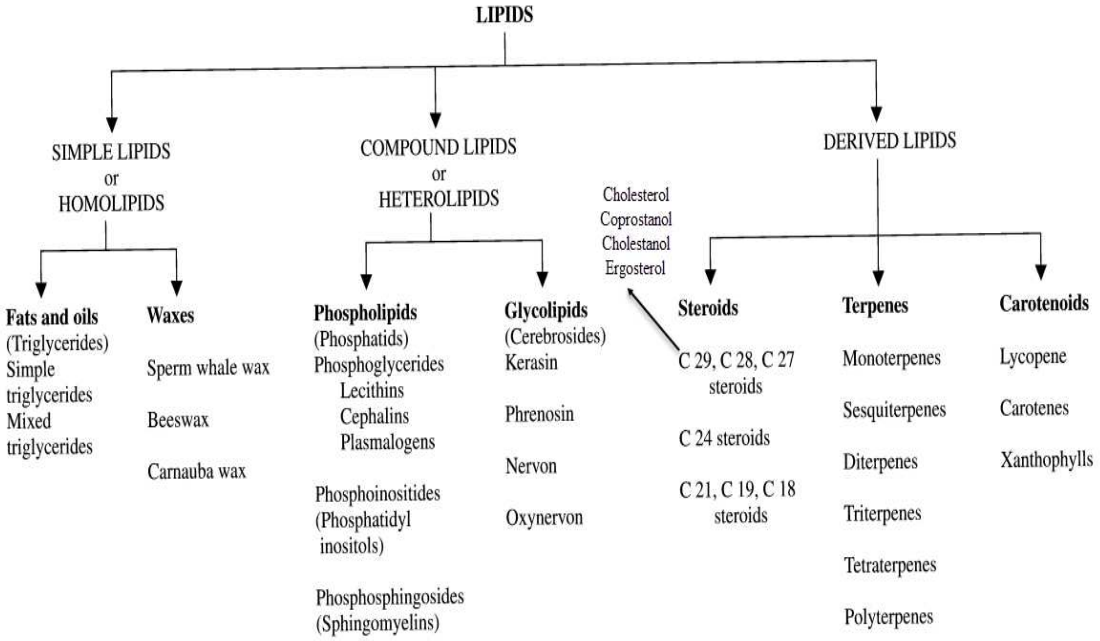


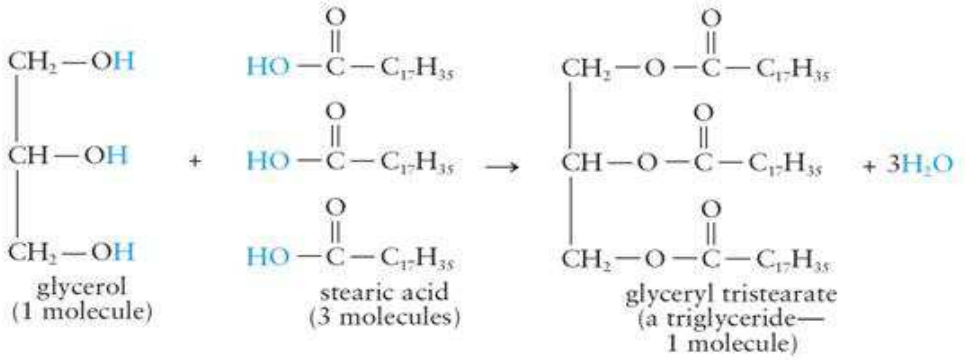
Fig.10.10. Classification of Lipids

10.10.1 SIMPLE LIPIDS

Simple lipids are the esters of fatty acids with various alcohols. These can be further categorized into fats and waxes. Fats are triesters of glycerol and fatty acids. A fat in the liquid state is called oil. Waxes are the esters of fatty acids with long chain (higher mol.wt.) monohydric alcohols.

Fats

Fats are solids at room temperature. Chemically, fats are triglycerides since, three molecules of fatty acids condense with one molecule of glycerol, e.g. three molecules of stearic acid are linked to glycerol to yield glyceryl tristearate, a fat.



If all the three molecules of fatty acids are similar, the product is a simple glyceride. If fatty acids molecules are different, it is called a mixed glyceride. Natural fats are largely composed of mixed glycerides. Since these glycerides have no acid or basic groups, they are often called natural fats. The melting point of fats depends upon the chain length and degree of saturation of fatty acids. The melting points of fats are always higher than the solidification point, e.g. tristearin melts at 72^0C but solidifies on cooling at 52^0C.

Fats develop unpleasant odor on aging, this is due to auto oxidation of fat. This is called rancidification.

The chemical changes that occur during rancidification are called rancidity. Fats which are liquid at room temperature are called oils. Oils are also esters of fatty acids and glycerol, but the fatty acids found in oils are unsaturated fatty acids. The unsaturated fatty acids have one or more double bonds. They have low melting point and are insoluble in water. Hydrolysis of fats with alkali or enzymes lipase yields fatty acids and glycerol. When the fats are hydrolyzed with alkali, the few fatty acids react with alkali to form salts. These salts are soaps and this process is called saponification.

Waxes

Waxes are another class of simple lipids. These are the esters of fatty acids with high molecular weight alcohol. Waxes contain one molecule of fatty acids and one molecule of alcohol. The bees wax, the fatty acid constituent is a smaller chain acid, palmitic acid and [16^0C] and alcohol is myristic palmitate. Ambretolide found in the seeds of abelmoschus esculentus is a hay hydro nil acid and is responsible for the characteristic smell of the seed. Being highly insoluble in water and having no double bonds in their hydrocarbon chains, waxes are chemically insert. And very resistant to atmospheric condition also not digested by the fat splitting enzymes. They can be split slowly with hot alcoholic KOH, however. They also have higher melting point. They serve as protective coating on

fruits and leaves. They play on important role in provide water barrier for insects, birds and animals. They are used in furniture polishing.

Fatty Acids

Fatty acids are obtained from the hydrolysis of fats. Since all the fats contain glycerol, their properties differ according to the nature of fatty acids present in them. A fatty acid can be defined as an organic acid that occurs in a natural triglyceride and is a monocarboxylic acid ranging in chain length from C_4 to about 24 carbon atoms. A few have branched chain or contain hydromel group or have a cyclic chain at the end. Fatty acids that occur in natural fats usually contain an even number of carbon atoms, one carboxylic group and are straight chain derivatives. On the basis of presence or absence of double bonds, fatty acids may be classified into two main classes-

i. Saturated fatty acids (saturated with hydrogen)
ii. Unsaturated fatty acids

1. Saturated fatty acids

The fatty acids which do not contain any double bond are called saturated fatty acids. The general formula is $C_n H_{2n+1}$ COOH e.g. Butyric acid $C_3 (CH_2)_2$ COOH. The most abundant saturated fatty acids in nature are palmitic acid (C_{18}) and stearic acid (C_{16}). The saturated fatty acids are straight chain acids. In addition to these straight chain acids, there are some branched chain acids, with odd or even number of carbon atoms.eg. Isopalmitic acid, anti-isopalmitic acid and tuberculostearic acid.

2. Unsaturated fatty acids

The fatty acids which contain one or more double bonds are called unsaturated fatty acids. On the basis of number of double bonds, the unsaturated fatty acids may be divided into two groups-
Monosaturated fatty acids: - having one double bond e.g. crotonic acid, oleic acid, palmitoleic acid, nervonic acid etc.
Polyunsaturated fatty acids:-having more than one double bonds e.g. linoliec acid, eleostearic acid etc.
In most of the monosaturated fatty acids there is a single bond lying between carbon atoms 9 and 10. This is designated as Δ^9. The symbol Δ with the superscript number nine (9) indicates the positions of the double bond. When there are more than one double bond (polyunsaturated fatty acids), the additional bonds occur between the Δ^9 double bond and the methyl terminal end of the chain. The

symbol 18:3 signifies that there are three double bonds and symbol $\Delta^{9,12,15}$ signifies that the position of double bonds are between carbon atom 9 and 10, 12 and 13 and 15 and 16. Presences of double bonds in the fatty acids lower their melting point considerably. Most plant fats contain unsaturated fatty acids as oleic acid and linoleic acid and hence they are liquid at room temperature. Contrary to this animal fats have more of saturated fatty acids and hence solid at room temperature.

FATTY ACIDS

Common Saturated Fatty Acids

Number of Carbon Atoms	Formula	Common Name	Source
4	$CH_3(CH_2)_2COOH$	Butyric acid	Butter
6	$CH_3(CH_2)_4COOH$	Caproic acid	Butter
8	$CH_3(CH_2)_6COOH$	Caprylic acid	Coconut oil
10	$CH_3(CH_2)_8COOH$	Capric acid	Coconut oil
12	$CH_3(CH_2)_{10}COOH$	Lauric acid	Palm kernel oil
14	$CH_3(CH_2)_{12}COOH$	Myristic acid	Oil of nutmeg
16	$CH_3(CH_2)_{14}COOH$	Palmitic acid	Palm oil
18	$CH_3(CH_2)_{16}COOH$	Stearic acid	Beef tallow
18	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	Oleic acid	Olive oil
18	$CH_3(CH_2)_4CH{=}CHCH_2CH(CH_2)_7COOH$	Linoleic acid	Soybean oil
18	$CH_3CH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	Linolenic acid	Fish oils
20	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COOH$	Arachidonic acid	Liver
22	$CH_3(CH_2)_{20}COOH$	Behenic acid	Sesame oil

Common Unsaturated Fatty Acids

Number of Carbon Atoms	Formula	Common Name	Source
16	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	Palmitoleic acid	Whale oil
18	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	Oleic acid	Olive oil
18	$CH_3(CH_2)_4CH{=}CHCH_2CH(CH_2)_7COOH$	Linoleic acid	Soybean oil, safflower oil
18	$CH_3CH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	Linolenic acid	Fish oils, linseed oil
20	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COOH$	Arachidonic acid	Liver

Essential and Non Essential Fatty Acids

Essential fatty acids: The fatty acids which cannot be synthesized by human body but are essential for the normal maintenance of the body are called essential fatty acids .These fatty acids must be included in our diet. Three polyunsaturated fatty acids, linoleic acid, linolenic acid and arachidonic acid are the essential fatty acids.

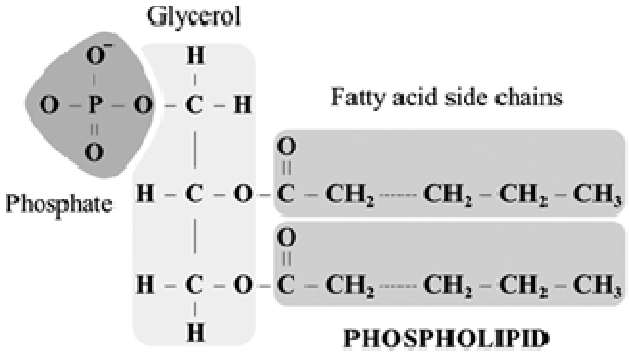
Non essential fatty acid: These are the fatty acids which can be synthesized by our body. Thus they need not be included in our diet. They are unsaturated fatty acids and are synthesized from their corresponding saturated fatty acids by introducing a single bond .e.g. palmitoleic acid and oleic acid.

10.10.2 COMPOUND LIPIDS

Compound lipids contain some additional groups or elements besides fatty acids are alcohol. The addition group may contain phosphorus, nitrogen, sulpher or it may be a protein. Compound lipids can be categorized into the following: Phospholipids, Glycolipids, Other compound lipids.

Phospholipids

Phospholipids are those compound lipids which contain a phosphorus atom. Phospholipids are wide spread in bacteria, animal and plant tissues and their general structures are quiet similar. These have been termed as amphipathic and compound since they process both polar and non polar function.

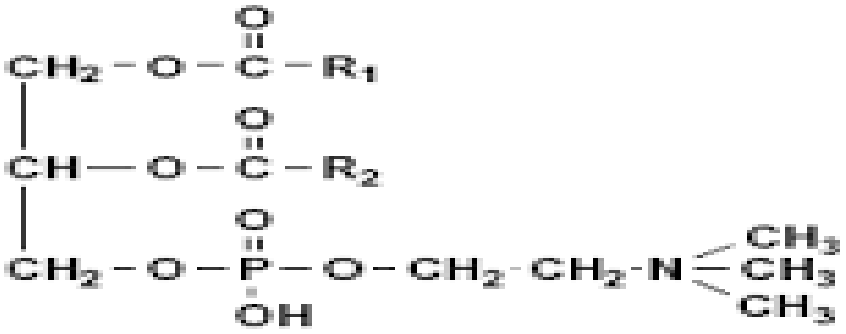


In addition to phosphorus, phospholipids may also contain nitrogen as a key component. There are various types of phospholipids including- lecithin, cephalins, plasmalogens, phosphoinositides and phosphosphingosides.

Lecithin

Lecithin is widely distributed in nature. In animals it is found in liver, brain, nerve tissues, sperm and egg yolk. In plant it is abundant in seeds and sprouts. On hydrolysis, lecithin yields glycerol, fatty acids, phosphoric acid and nitrogenous base - choline. It is also called phosphatidyl choline. The fatty acids commonly found in lecithin are palmitic, stearic, oleic, linolenic and arachidonic acids. Lecithin

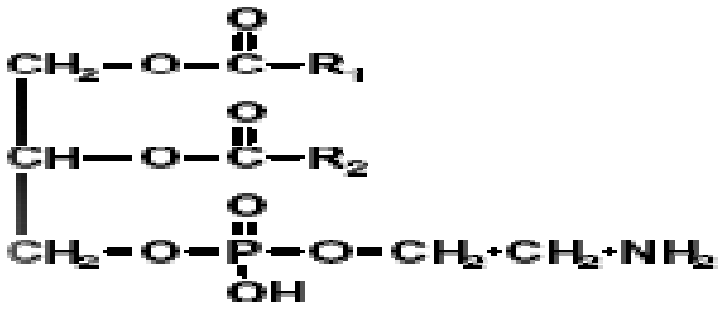
is yellowish grey solid which is soluble in with soluble in ether and alcohol but insoluble in acetone. On exposure to air they rapidly darken colour and absorb water, forming dark grassy mass. Lecithins are broken down by the enzyme lecithinase to lysolesithin which is present in venoms of bee and cobra. When injected into the blood, lysolecithins cause rapid haemolysis of the red blood cells.



Lecithin (phosphatidyl choline)

Cephalins

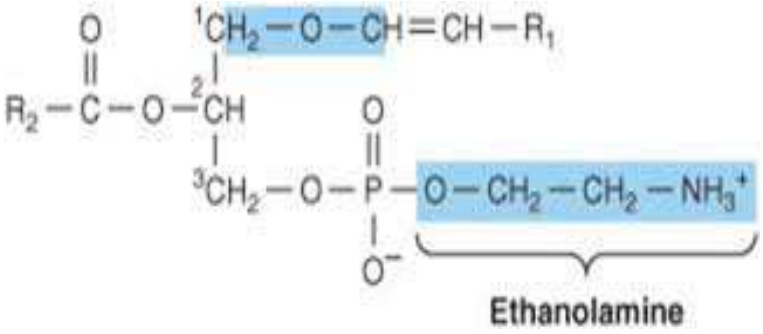
Cephalins are found in animal tissues in close association with lecithin. They are also found in soya bean oil. The basic difference between cephalins and lecithin is the nature of nitrogenous base. Cephalins contain ethanolamine in place of choline. The fatty acid components of cephalins are stearic, oleic, linoleic and arachidonic acid. They are less soluble in alcohol then lecithin.



Cephalin
(Phosphatidyl ethanolamine)

PLASMALOGENS

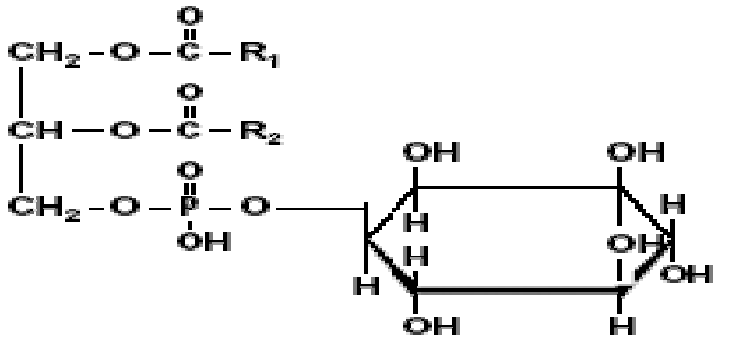
Plasmalogens are abundant in brain and muscles they are also found in the seeds of higher plants. Structurally, they resemble lecithin and cephalins except in having an aldehyde group attached to ex – carbon atoms of glycerol. They are soluble in all lipid solvents.



Plasmalogen

Phosphoinositides

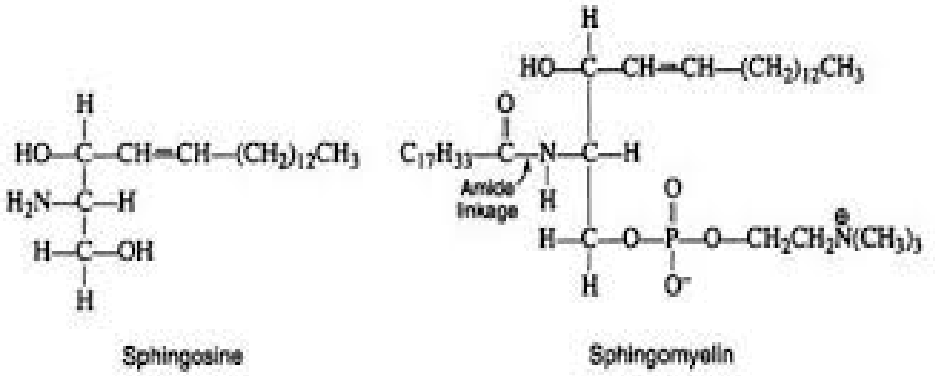
They are present in brain tissues and nervous tissues. They can be either mono or diphosphoinositides. Monophosphoinositides contain hexahydric alcohol inositol. The name lipoinosital was also proposed for them.



Phosphatidyl inositol

PHOSPHOSPHINGOSIDES

These lipids are abundant in lacking in plant and microorganisms. In these lipids glycerol is replaced by an 18 carbon unsaturated amino alcohol called ‘sphingosine’. On hydrolysis they yield fatty acids, phosphoric acid, choline and sphingosine.

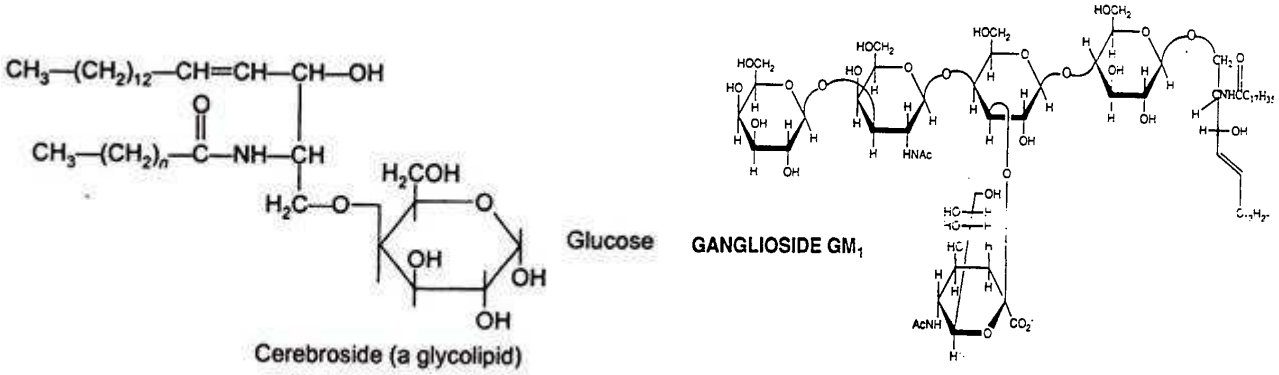


Glycolipids

Glycolipids are compounds containing carbohydrates and high molecular weight fatty acids like sphingosine but no phosphoric acid. These are of two types: Cerebrosides and Gangliosides.

Cerebrosides: They occur in large amount in brain and myelin sheath of nerves. The structure of cerebrosides is somewhat similar to sphingomyelin. Here the fatty acid ceramide is linked either to galactose or glucose.

Gangliosides: These are found in ganglion cells of nervous tissues and also in parenchymatous tissues like spleen and RBCS, They are the most complex glycosphingolipids. They are ceramides with attached oligosaccharides that include at least one sialic acid residue.



Other compound lipids

Sulfolipids: Lipids containing sulfer, widely distributed in plant (localized in chloroplast) and bacteria.

Lipoprotein: They are the component of membranes found in the membranes of mitochondria, ERL

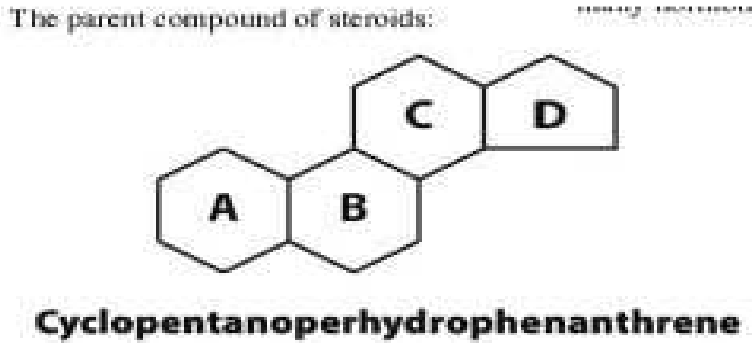
The lipid component consists of triacylglycerol, phospholipids and cholesterol. The protein components of lipoprotein have a relatively high portion of non polar acid residues.

10.10.3 DERIVED LIPIDS

Derived lipids are the product of hydrolysis of simple lipids and compound lipids and in addition other lipid like compounds such as steroids, terpenes, fatty acids, alcohols, carotenoids, essential oils etc.

Steroids

Steroids are the derivatives of cyclopentanoperhydrophenanthrene, a compound consisting of four fused non planer rings. They are named as A, B, C and D. The rings A, B and C are hexagons and are called cyclohexane rings while D is a pentagon and is called cyclopentane.



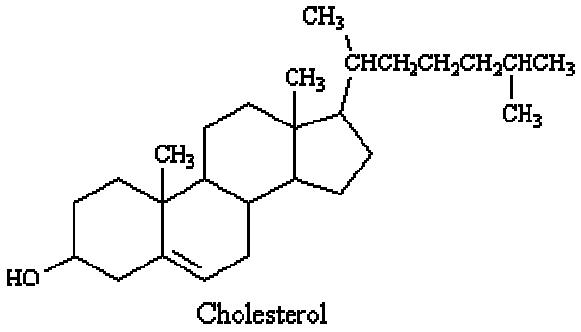
Sterols

The steroids may have one or more alcoholic groups. The steroids having alcoholic groups are called sterols. They are crystalline compounds and differ from alcohols in being solids that is the reason they are called so. Steroids are widely distributed in plant, animals and microorganism. They are found in cell membranes and other cellular component containing lipids. Unlike other lipids, sterols cannot be saponified and by this process they can be separated from other lipids. Ergosterol is present in food in small amount. It has been isolated from parasitic fungus Claviceps pupurea (Ergon) growing on rye plants. Other plant sterols are spinasterol obtained from spinach and cabbage, stigmasterol from coconut and soyabeans and sitosterol from cereal seeds.

Cholesterol

The best known animal sterol is cholesterol, which is a major component of animal plasma membrane. It is classified as sterol because of its C30H group It is present in relatively high concentration in

nervous tissues and in bile. Cholesterol is a crystalline solid with rhombic crystals and its solution is levorotatory. It has a high melting point.



Cholesterol

In mammals, cholesterol is the metabolic precursor of steroid hormones – adrenocorticoids and sex-steroids.

Adrenocorticoids

They are secreted by the adrenal cortex hormone contains – glucocorticoids, aldosterone, cortisterone, desoxycortisterone adrenosterone and other mineral corticoids influence a wide variety of vital functions.

Sex steroids

The testes secrete testosterone, andosterone and adrenosterone, while estrogens and progesterone are secreted by the ovary. They affect sexual development and functions.

10.11 SOURCES OF LIPIDS

Lipids are widely distributed in plants and animals. Common sources of fatty acids are butter, coconut oil, animal fats and some bacteria. Lecithin, a type of phospholipids is found in liver, brain, nerve tissue, sperm and yolk sac in animals while in plants it is abundant in seeds and sprouts. Similarly other phospholipids and glycolipids are also found in animal tissues like brain, muscles and nervous tissues. Terpenes and carotinoids are types of derived lipids which are exclusively of plant origin.

10.12 BIOLOGICAL SIGNIFICANCE OF LIPIDS

1. Rich source of energy: fats provide food of high calorific value (1g fat produces about 9.3 kilo calories of heat).
2. As food reserve: Fats are stored in body as reserve food material, because these could be readily stored in the body on account of insoluble character sticks. Triglycerides stored in adipocytes (fat cells) of adipose tissue are the principal fat reserve.
3. As heat insulators: Fats deposited in the subcutaneous tissues act as insulators conserving body heat.
4. Solvent: Lipids act as a solvent for fat soluble vitamins like vitamin A, D and E.
5. Structural constituents: Phospholipids, glycolipids and sterols are structural components of all the membrane system of cell (i.e. cell membrane, nuclear membrane, membranes of the endoplasmic reticulum etc.)
6. Fat transport: Phospholipids play an important role in the absorption and transportation of fatty acids.
7. Hormone synthesis: Adrenocorticoids, sex hormones, vitamin D and cholic acids are synthesized from cholesterol.
8. As shock absorber: The fat deposited around the visceral organs and underneath the skin acts as cushion and absorbs mechanical shocks.
9. As electric insulators: Myelin sheath around medullated nerve fibres forms an electric insulation.
10. Prostaglandins: They control local activities in the body.
11. Protective layers: Lipids form a protective waxy covering on the aerial parts of plants to check loss of water by evaporation.
12. Thrombokinase: helps blood clotting.
13. Leukotrienes: a group of eicosanoid helps in respiration.
14. Some isoprenoids form insect hormones.
15. Some insoprenoids form volatile oil and pigments. Natural rubber is also an isoprenoid.
16. Glycolipids help in cell recognition.
17. Complex lipids form phospholipid bilayer of plasma membrane.
18. Steroid act as hormones and neurotransmitters in mammals.

10.13 DEFICIENCY DISEASES OF LIPIDS

The deficiency of lipids in human body causes dry, scaly skin, hair loss, loss of mensuration, cold intolerance, power resistance to infection and bruising, poor growth, poor wound healing and low body weight.

10.14 SUMMARY

- Carbohydrates are the compounds of carbon, hydrogen and oxygen are the major source of energy in body.
- Carbohydrates are classified into three major groups- monosaccharides, oligosaccharides and polysaccharides.
- Monosaccharides are the simplest carbohydrates and are either aldoses or ketoses.
- Oligosaccharides consist of few molecules of monosaccharide, most common are disaccharides consist of two molecules of monosaccharides.
- In polysaccharides a number of monosaccharides are linked by glycosidic bonds.
- Polysaccharides maybe either homopolysaccharide or heteropolysaccharide.
- Monosaccharides are colourless and crystalline compounds which are optically active.
- Lipids are a heterogeneous group of molecules with are soluble in organic solvents.
- Chemically lipids are the esters of fatty acids with alcohols.
- Lipids are good source of energy and important constituent of biological membranes.
- Lipids are generally classified into simple, compound and derived lipids. Fats and wax are simple lipids.
- Fatty acids are obtained from the hydrolysis of fats and can be saturated or unsaturated.
- Phospholipids are the compounds lipids which contains a phosphorus group.
- Glycolipids contain carbohydrate and high molecular weight fatty acids.
- Derived lipids are the products of hydrolysis of simple and compound lipids and include steroids, terpenes, carotenoids etc.

10.15 GLOSSARY

Aldose: A sugar in which the carboxyl group is an aldehyde.

Carbohydrates: Carbohydrates, the major source of energy, are the polyhydroxy aldehydes or ketones.

Citric Acid Cycle: A series of reactions which result in the oxidation of pyruvic acid into CO_2 and H_2O. It is also known as Krebs cycle or tricarboxylic acid cycle (TCA cycle).

Disaccharide: A carbohydrate composed of two monosaccharides covalently joined by a glycosidic bond.

Fat: Lipid which is solid at room temperature.

Fatty acid: A carboxylic acid with a long chain hydrocarbon side group.

Gluconeogenesis: The synthesis of glucose from non-carbohydrate precursors.

Glycolipid: A lipid containing carbohydrate.

Glycolysis: The process of conversion of glucose into pyruvic acid.

Lipids: Esters of alcohols and fatty acids.

Monosaccharide: A carbohydrate which cannot be further hydrolyzed to simple sugars.

Oil: Lipid which is liquid at room temperature.

Oligosaccharide: Carbohydrate containing a few monosaccharide residues.

Pentose: A sugar containing five carbon atoms.

Polysaccharide: A carbohydrate containing multiple units of monosaccharides.

Saponification: The process of formation of soap. Fats form soap with alkali.

Saturated fatty acid: A fatty acid which does not contain any double bond.

Unsaturated fatty acid: a fatty acid which contains one or more double bonds.

10.16 EXERCISE

Long answer type questions

1. What are carbohydrates? Discuss the chemical composition and molecular structure of carbohydrates.

2. Classify carbohydrates on the basis of their composition. Write the structure of one typical example representing each class.

3. Give an account of the structure, properties and uses of monosaccharides.

4. Describe structure, classification and functions of polysaccharides.

5. Describe the pathway of glycolysis. How many molecules of ATP are produced from glycolytic oxidation of one molecule of glucose?

6. Give an account on citric acid cycle.

7. What are lipids? Give structure and classification of lipids.

8. What are the sources and biological significance of lipids?

Short Notes

1. Oligosaccharides
2. Gluconeogenesis
3. Pentose phosphate pathway
4. Oxidative phosphorylation
5. Electron transport system
6. Biological significance of carbohydrates.
7. Lipid deficiency diseases
8. Phospholipids
9. Glycolipids
10. Derived lipids

Fill in the blanks

1. …….. is the process of hydrolysis of glycogen into glucose molecules.
2. ……. and ……. are two examples of storage polysaccharides.
3. Glycolysis takes place in the …….. .
4. Krebs cycle occurs in the mitochondrial …… .
5. When the fats are hydrolyzed with alkali, the free fatty acids react with alkali to form salt, this process is called …….. .
6. Saturated fatty acids have …….. double bonds.

Answers:

Fill in the blanks

1. Glycogenolysis 2. Starch and Glycogen 3. Cytoplasm 4. Matrix 5.Saponification 6. No

10.17 REFERENCES

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Note: All the images are taken from internet sources.

UNIT 11: VITAMINS

CONTENT

11.1 OBJECTIVES

1. The objective of this chapter is to understand vitamins.
2. To understand the basis of their classification
3. To understand the structure, occurrence and functions of vitamins.
4. Detailed study of the source, significance and deficiency related diseases of vitamins.

11.2 INTRODUCTION

The term vitamin was introduced by Funk in 1912. The deficiency of vitamins causes some diseases. The term vitamin refers to vital amines.

Vitamins are the disparate group of organic vital nutrients required in limited quantities for a number of metabolic functions. They cannot be synthesized by the body and must therefore be supplied in the diet.

Although not all vitamins are amines, they are organic compounds required by humans in small amounts from the diet .Vitamins are organic compounds which are needed in small quantities in the diet and essential for the functioning of an organism.

Thirteen vitamins are universally documented at present, required to maintain our normal heath.

11.3 CLASSIFICATION

Vitamins are classified by their structure but on the basis of biological and chemical activity.

The main classification for vitamins is based on the solubility, as some are soluble in water while others are soluble in fat.

FAT SOLUBLE

Vitamin A, D, E and K are fat soluble. These are stored in liver and adipose tissues and hence are called fat soluble vitamins. Fat-soluble vitamins except for vitamin K, are stored for long periods at a time and then excreted. For this reason overdosing on fat-soluble vitamins is highly feasible. If ingested at high, toxic levels it could possibly result in hypervitaminosis.

WATER SOLUBLE

Vitamins in B-group and vitamin C are water soluble and cannot be stored in our body as they excreted out of the body via urine. These vitamins must be supplied to our body with regular diets.. As a result of this type of vitamin that can be dissolved in water, individuals cannot overdose on them because all excess will simply be excreted.

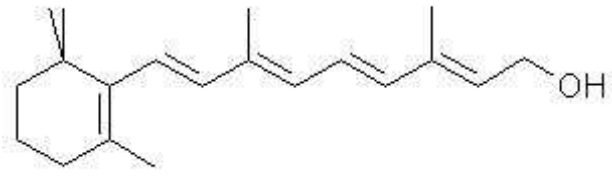
11.4 STRUCTURE OCCURRENCE AND FUNCTIONS OF FAT SOLUBLE VITAMINS

- **VITAMIN A** (Axerophthol)

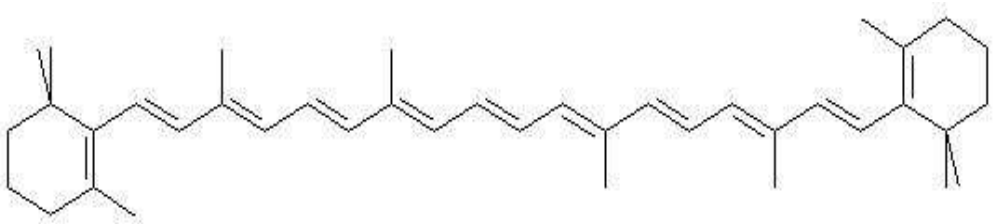
Vitamin A is a group of unsaturated nutritional organic compounds that includes retinol, retinal (found only in food of animal origin), and carotenoids (carotenes and related compounds, (beta-carotene found in plants known as provitamin A.

STRUCTURE

Retinol is the immediate precursor to two important active metabolites: **retinal**, which plays a critical role in vision, and **retinoic acid**, which serves as an intracellular messenger that affects transcription of a number of genes. Vitamin A does not occur in plants, but many plants contain **carotenoids such as beta-carotene** that can be converted to vitamin A within the intestine and other tissues. It has following structure; ring shown is called β-ionone ring. There are several isomers of vitamin A; the most important of these are 13-cis retinol found in many fish liver oils and 11-cis retinol which occurs in the retina. Vitamin A is quite heat stable but it is destroyed at high temperature in the presence of O_2 or air.



11.1Structure of Retinol



11.2Structure of Beta Carotene

OCCURRENCE & SOURCES

Vitamin A occurs in nature exclusively in the animal kingdom. It occurs in highest concentration in the liver oils of certain species of fish, e.g. halibut (richest source), shark and cod; it also occurs in the livers of other animals, egg yolk, whole milk and dairy products. In plants it occurs in carrots, yellow corn, sweet potato, peaches and green leafy vegetable such as spinach. The carotenes do not have any vitamin A activity but these are converted to vitamin A in the liver. Carotenes, however, poor sources of vitamin A because they are not absorbed completely and their conversion to the active vitamin A is not 100% complete.

Good sources of vitamin A include:

1. Cheese
2. Eggs
3. Oily fish
4. Fortified low-fat spreads
5. Milk and yoghurt

Liver is a particularly rich source of vitamin A.

The main food sources of beta-carotene are:

1. Yellow, red and green (leafy) vegetables, such as spinach, carrots, sweet potatoes and red peppers

2. Yellow fruit such as mango, papaya and apricots

FUNCTIONS AND SIGNIFICANCE

Vitamin A is required for several vital functions in the body. Some of the most important functions of Vitamin A are described below.

Vision

Vitamin A is required for the maintenance of normal vision. A deficiency in vitamin A can lead to visual disturbances. In the eyes, a form of vitamin A called retinal is combined with a protein called opsin to give rhodopsin, an essential light absorbing molecule needed for color vision and seeing in dim light.

Immune system

Vitamin A is essential for maintaining healthy immune function and deficiency can lead to an impaired response to infection.

Cell growth

Retinoic acid is, a crucial hormone-like growth factor for epithelial cells and other cell types in the body. It plays an important role in reproduction, cell division, and cell differentiation.

Gene transcription and protein formation

Vitamin A in the form of retinoic acid is essential for gene transcription.

Skin health

Vitamin A, and more specifically, retinoic acid, appears to maintain normal skin health by activating genes and differentiating keratinocytes (immature skin cells) into mature epidermal cells.

Antioxidant

Beta-carotene is an antioxidant. Antioxidants protect cells from damage caused by substances called free radicals. Antioxidants prevent naging process risk of cancer etc.

Bone growth

Vitamin A plays an important role in bone growth. However, *too much* vitamin A has been linked to bone loss and an increase in the risk of hip fracture.

VITAMIN A RELATED DISEASES

1. One of the earliest and specific signs of vitamin A deficiency is impaired vision, particularly in reduced light – night blindness. Vitamin A deficiency is the leading cause of xerophthalmia in childrens.
2. It also increases the risk of death from common childhood conditions such as diarrhea.
3. Mild deficiency of Vitamin A leads to increased susceptibility to infectious diseases.
4. Due to limited capacity to metabolize vitamin A, and excessive intakes lead to tissue damage. Excessive vitamin A consumption can lead to nausea, irritability, anorexia (reduced appetite), vomiting, blurry vision, headaches, hair loss, muscle and abdominal pain and weakness, drowsiness, and altered mental status.
5. In chronic cases, hair loss, dry skin, drying of the mucous membranes, fever, insomnia, fatigue, weight loss, bone fractures, anemia, and diarrhea can all be evident on top of the symptoms associated with less serious toxicity.

- **VITAMIN D**

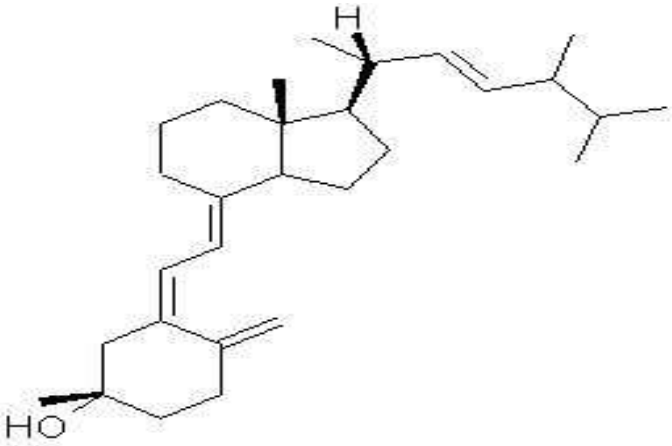
Vitamin D comprises a group of fat-soluble secosteroids/secosterols that are not strictly a vitamin since they can be synthesized in the skin. In humans, the most important compounds in this group are vitamin D_3 (also known as cholecalciferol) and vitamin D_2 (ergocalciferol). Cholecalciferol and ergocalciferol can be ingested from the diet and from supplements. Very few foods naturally contain vitamin D. Synthesis of vitamin D (specifically cholecalciferol) in the skin is the major natural source of the vitamin that depends on the sun exposure (specifically UVB radiation). Vitamin D2 is produced by UV irradiation of plant sterol ergosterol, which occurs in molds, yeast, and higher-order plants. Under conditions of regular sun exposure, dietary vitamin D intake is of minor importance. However, latitude, season, aging, sunscreen use, and skin pigmentation influence the production of vitamin D3 by the skin .

STRUCTURE

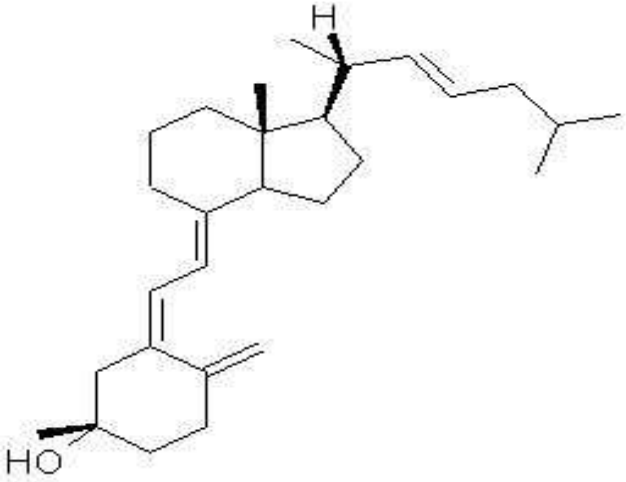
Several vitamers of vitamin D exist. The two major forms are vitamin D_2 or ergocalciferol, and vitamin D_3 or cholecalciferol. These are known collectively as calciferol.

7-Dehydrocholesterol (an intermediate in the synthesis of cholesterol), undergoes a nonenzymic

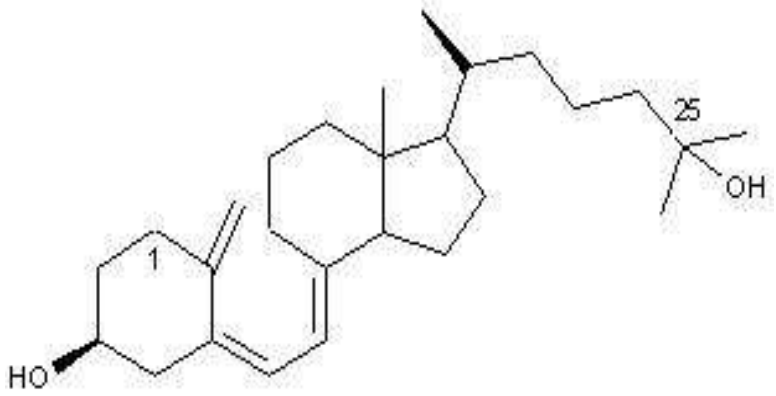
reaction on exposure to ultraviolet light, yielding previtamin D (Cholecalciferol Vitamin D3)



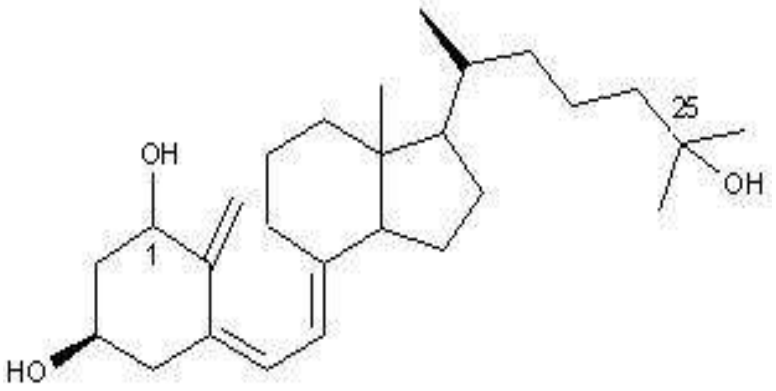
11.3Structure of Vitamin D2 (Ergocalciferol)



11.4Structure of Vitamin D3 (Cholecalciferol)



11.4Structure of Calcidiol



11.5Structure of Calcitriol

Vitamin D, as either D_3 or D_2, does not have significant biological activity. Rather, it must be metabolized within the body to the hormonally-active form known as 1, 25-dihydroxycholecalciferol. This transformation occurs in two steps.

1. **in the liver,** Cholecalciferol (vitamin D_3) is hydroxylated to *25-hydroxycholecalciferol* (calcidiol) by the enzyme 25-hydroxylase. Whereas Ergocalciferol (vitamin D_2) is converted in the liver to 25-hydroxyergocalciferol

2. **In the kidney,** part of calcidiol is converted by the kidneys to calcitriol, the biologically active form of vitamin D by 1-hydoxylation or inactive metabolite 24- hydroxycalcidiol by 24-

hydroxylation. Ergocalciferol from fortified foods undergoes similar hydroxylations to yield ercalcitriol.

All the forms of vitamin D is hydrophobic, and is transported in blood bound to carrier proteins.

OCCURRENCE AND SOURCES

There are two sources of vitamin D: dietary consumption and endogenous production. Vitamin D is found in few dietary sources..Vitamin D may be consumed in the diet as either ergocalciferol (D_2) from plant sources or cholecalciferol (D_3) from animal sources. Sunlight exposure is the primary source of vitamin D for the majority of people, other than supplements. Beside this mushroom, Alfa Alfa, lichen, Fish liver oils, such as cod liver oil, Fatty fish species, such as Salmon, Tuna.

FUNCTIONS AND SIGNIFICANCE

1. Vitamin D regulates and is regulated by Calcium homeostasis; it behaves like a **steroid hormone,** binding to a nuclear receptor protein. Vitamin D is essential for strong bones, because it helps the body use calcium from the diet.
2. In addition, calcitriol is involved in insulin secretion, synthesis and secretion of parathyroid and thyroid hormones.
3. Vitamin D functions to activate the innate and dampen the adaptive immune systems. Deficiency has been linked to increased risk of viral infections.

VITAMIN D RELATED DISEASES

1. In the vitamin D deficiency, due to poor calcium absorption**,** the bones of children are undermineralized results in condition known as **rickets**. Similar problems occur in adolescents who are deficient during their growth spurt.
2. **Osteomalacia**in adults results from demineralization of bone in women who have little exposure to sunlight.
3. Vitamin D toxicity is rare, however too much vitamin D can make the intestines absorb too much calcium resulting inHypercalcemia, noted with an increase in urination and thirst. If hypercalcemia is not treated, it results in excess deposits of calcium in soft tissues and organs such as the kidneys, liver, and heart, resulting in pain and organ

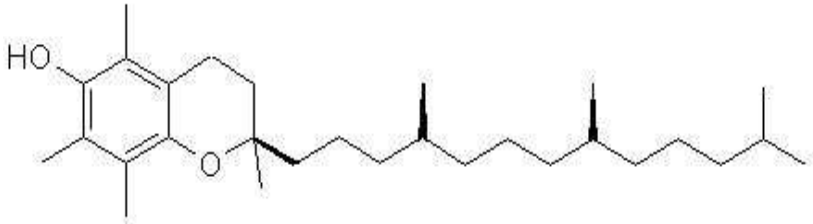
damage, Confusion and disorientation, Nausea, vomiting, constipation, poor appetite, weakness, and weight loss.

- **VITAMIN E (Tocopherol)**

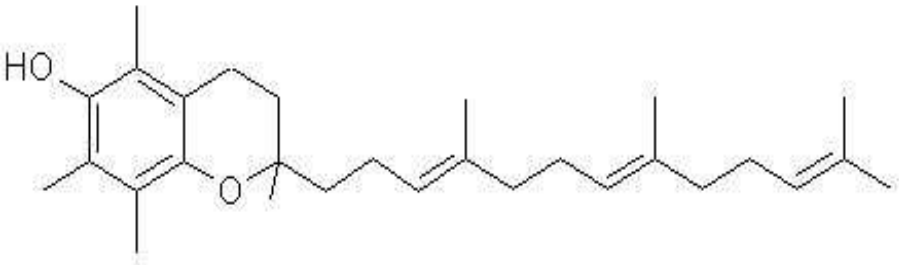
Vitamin E alphatocopherol is the most important and most active. It acts as a lipid-soluble an- tioxidant in cell membranes.

STRUCTURE

The nutritional content of vitamin E is defined by α-tocopherol activity. Vitamin E comprises 8 forms split into two families of compounds, the **tocopherols**and the **tocotrienols**, identified by the prefixes alpha- (α-), beta- (β-), gamma- (γ-), and delta- (δ-). The different vitamers (compounds having similar vitamin activity) have different biologic potencies; the most active is D-α-tocopherol, and it is usual to express vitamin E intake in milligrams of D-α-tocopherol equivalents.



α-tocopherol



11.5Structure of alpha-Tocotrienol

OCCURENCE AND SOURCES

Γ-Tocopherol can be found in corn oil, soybean oil, margarine, and dressings. α-tocopherol, the most biologically active form of vitamin E, is the second-most common form of vitamin E in the diet. Good dietary sources of vitamin E include nuts such as almonds, peanuts and hazelnuts, and vegetable oils such as sunflower, wheat germ, safflower, corn and soybean oils. Sunflower seeds and green leafy vegetables such as spinach and broccoli also contain vitamin E.

FUNCTIONS AND SIGNIFICANCE

1. Vitamin E does not have precisely defined metabolic function. The main function of vitamin E is as a free radical trapping antioxidant in cell membranes and plasma lipoproteins. Due to the potent antioxidant properties of tocopherols, the influence of α-tocopherol is believed to be associated with oxidative stress. It protects body tissue from damage caused by substances called free radicals. Free radicals can harm cells, tissues, and organs. They are believed to play a role in certain conditions related to aging.
2. The body also needs vitamin E to help keep the immune system strong against viruses and bacteria.
3. As an enzymatic activity regulator, for instance α-tocopherolinhibitsprotein kinase C
4. Vitamin E also has an effect on gene expression
5. Vitamin E also plays a role in neurological functions,and inhibition of platelet coagulation.
6. Vitamin E also protects lipids and prevents the oxidation of polyunsaturated fatty acids.
7. Vitamin E thickens endometrium.
8. Vitamin E protects red blood cells and helps prevent destruction of vitamin A and C.
9. The lack of vitamin E causes sterility and death of the embryos in some animals.

VITAMIN E RELATED DISEASES

Vitamin E deficiency is rare and is almost never caused by a poor diet.Instead; there are three specific situations when a vitamin E deficiency is likely to occur

1. Premature, very low birth weight infants

2. Rare disorders of fat metabolism
3. Fat malabsorption

Signs of vitamin E deficiency include the following:

1. Neuromuscular problems
2. Neurological problems
3. Anemia-due to oxidative damage to red blood cells.
4. Retinopathy
5. Impairment of the immune response.
6. In rats deficiency produces muscular dystrophy with progressive paralysis of hindlegs.

- **VITAMIN K**

Vitamin K is known as the “blood-clotting vitamin” for its important role in healing wounds. The "K" is derived from the German word koagulation. Vitamin K was discovered as a result of investigations into the cause of a bleeding disorder. It is a group of structurally similar, fat-soluble vitamins the human body requires for complete synthesis of certain proteins that are prerequisites for blood coagulation.

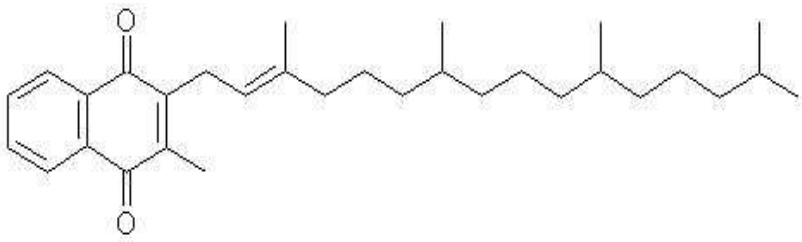
STRUCTURE

Chemically, the vitamin K family comprises 2-methyl-1,4-naphthoquinone (3-) derivatives. Vitamin K includes two natural vitamers: vitamin K_1 and vitamin K_2.[1] Vitamin K_2, in turn, consists of a number of related chemical subtypes, with differing lengths of carbon side chains made of isoprenoid groups of atoms.

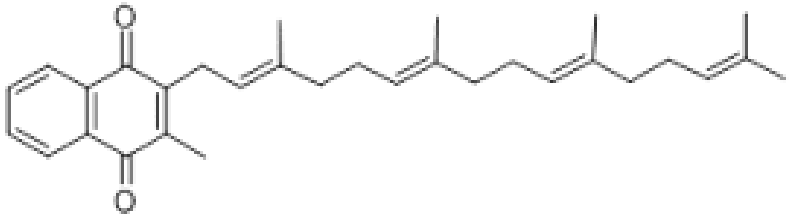
Vitamin K_1, also known as **phylloquinone** or **phytomenadione** is synthesized by plants. It may be thought of as the "plant" form of vitamin K. It is active as a vitamin in animals and performs the classic functions of vitamin K, including its activity in the production of blood-clotting proteins. Animals may also convert it to vitamin K_2.

Bacteria in the gut flora can also convert K_1 into vitamin K_2 **(menaquinone),** with differing lengths of side-chain. Moreover, **menadione, menadiol,** and **menadioldiacetate, are** synthetic

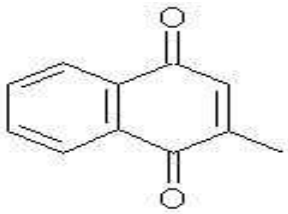
compounds that can be metabolized to phylloquinone.



11.6Structure of vitamin K1 (Phylloquinone)



11.7Structure of Vitamin K2 (menaquinone)



11.8Structure of Vitamin K3 (Menadione)

OCCURRENCE AND SOURCES

Vitamin K_1, functions as an electron acceptor in photosystem I during photosynthesis. For this reason, vitamin K_1 is found in large quantities in the photosynthetic tissues of plants (green leaves, and dark green leafy vegetables such as Brussels sprouts, parsley, bokchoy, asparagus, cabbage, turnip, broccoli,lettuce and spinach), but it occurs in far smaller quantities in other plant

tissues (roots, fruits, etc.).Besides this it is also found in avocados, plums, raspberry, soyabeans, dairy products, eggs.

FUNCTIONS AND SIGNIFICANCE

1. Vitamin K is best recognized for its role in the blood clotting process, it acts as a cofactor for some of the blood clotting factor's activity.
2. Vitamin K plays an important role in bone formation. It is also involved in the prevention of bone loss. Vitamin K modifies the protein osteocalcin and makes it able to bind to calcium. Calcium can then aid to form the bone matrix.
3. Recent research has shown that vitamin K also works with vitamin D to facilitate the function of osteoblasts, the bone building cells. On the other hand, it works to inhibit the production of osteoclasts
4. Gas6 a protein important for regulating cell growth, proliferation and preventing cell death is vitamin K dependent.

VITAMIN K RELATED DISEASES

1. **Haemorrhagic disease of the newborn**, also known as **vitamin K deficiency bleeding** (**VKDB**), is a coagulation disturbance in newborn infants due to vitamin K deficiency.
2. Vitamin K_1 deficiency can result in coagulopathy, a bleeding disorder.Symptoms of K_1 deficiency include anemia, bruising, and bleeding of the gums or nose in both sexes, and heavy menstrual bleeding in women.
3. Vitamin K_2 deficiency is associated with the inhibition of calcification and arterial stiffening of arteries.
4. Osteoporosisand coronary heart diseaseare strongly associated with lower levels of K_2 (menaquinone)

11.5 STRUCTURE OCCURRENCE AND FUNCTIONS OF WATER SOLUBLE VITAMINS

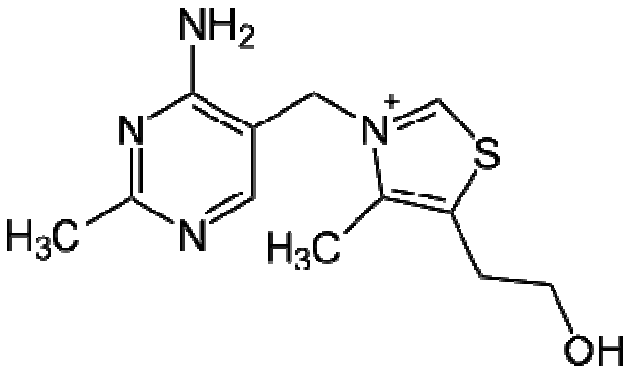
- **VITAMIN B_1 (THIAMIN)**

All B vitamins help the body convert food (carbohydrates) into fuel (glucose), which the body uses to produce energy. These B vitamins, often referred to as B-complex vitamins, also help the body metabolize fats and protein. All B vitamins are water soluble, meaning that the body does not store them.

Thiamine, **thiamin**, or **vitamin B_1**, named as the "thio vitamine" ("sulfur-containing vitamin") is one of the 8 vitamin of the B complex. Its phosphate derivatives are involved in many cellular processes.

STRUCTURE

Thiamine is a colorless organosulfur compound with a chemical formula $C_{12}H_{17}N_4OS$. Its structure consists of an aminopyrimidine and a thiazole ring linked by a methylene bridge. Thiamine is soluble in water, methanol, and glycerol and insoluble in less polar organic solvents.



11.9Structure of Vitamin B1 (thiamine)

OCCURRENCE AND SOURCES

Thiamine occurs widely in foods, but generally in small amounts. In general, cereal grains are the

most important dietary sources of thiamine. The most highly concentrated sources of thiamine are Yeast, yeast extract, and pork. Some other foods naturally rich in thiamine are oatmeal, flax, and sunflower seeds, brown rice, whole grain rye, asparagus, kale, cauliflower, potatoes, oranges, liver (beef, pork, and chicken), and eggs.

FUNCTIONS AND SIGNIFICANCE

1. The main functions of vitamin B_1 (thiamin pyrophosphate) are linked to its role as a coenzyme. **Thiamin** has a crucial role in energy-yielding metabolism, and especially of carbohydrate in which **Thiamin diphosphate** acts as the coenzyme.
2. Thiamin triphosphate helps in conduction of nerve impulses.
3. It is involved in RNA and DNA production

VITAMIN B1 RELATED DISEASES

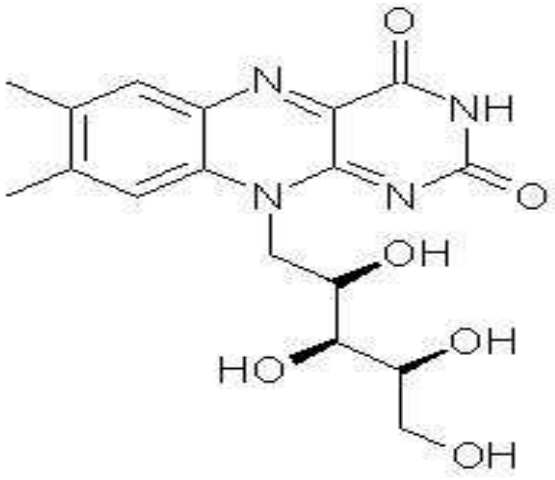
1. Thiamine deficiency would seem to adversely affect all of the organ systems; however, the nervous system is particularly sensitive to thiamine deficiency, because of its dependence on oxidative metabolism. Well-known syndromes caused by thiamine deficiency include beriberi, Wernicke-Korsakoff syndrome, and optic neuropathy.
2. Vitamin B1 (thiamin) deficiency is rare, but can occur in people who get most of their calories from sugar or alcohol. People with thiamin deficiency have difficulty digesting carbohydrates, causing a loss of mental alertness, difficulty breathing, and heart damage.

- **VITAMIN B_2 (RIBOFLAVIN)**

Riboflavin (**vitamin B_2**) formerly knowm as vitamin G is part of the vitamin B group. It is the central component of the cofactorsFAD and FMN and as such required for a variety of flavoprotein enzyme reactions including activation of other vitamins.

STRUCTURE

Riboflavin's Chemical Formula is C17H20N4O6. It is a polar and therefore water-soluble compound. The aspects of this compound which would prove it to be polar include its two polar bonds between carbon and oxygen, its polar bond between hydrogen and nitrogen and, most significantly, Riboflavin's chemical structure includes 4 'H-Bonds' between hydrogen and oxygen. The structure of Vitamin B2 features 3 benzene rings, an abundance of 6 tetrahedron bond shapes.



11.10The Chemical Structure of Riboflavin (Vitamin B2)

OCCURRENCE AND SOURCES.

The best sources of riboflavin that provide riboflavin without fortification are milk, cheese, eggs, leaf vegetables, liver, kidneys, legumes, mushrooms, almonds, brewer's yeast, organ meats, mushrooms, soybeans, yogurt, eggs, broccoli, Brussels sprouts, and spinach. Flours and cereals are often fortified with riboflavin.

Riboflavin is destroyed by light, so food should be stored away from light to protect its riboflavin content. While riboflavin is not destroyed by heat, it can be lost in water when foods are boiled or soaked. Riboflavin is also best absorbed when taken between meals.

Synergistic Nutrients

Nutrients that can help with absorption of vitamin B2 are vitamins A, B1, B3, B5, B6, and B12, as well as biotin, chromium, copper, cysteine, folate, glutathione, iron, magnesium, phosphate, potassium, and zinc.

FUNCTIONS AND SIGNIFICANCE

The active forms of riboflavin flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) plays its vital role in metabolism as the coenzymes in variety of reactions:

1. The primary coenzyme form of vitamin B_6 (pyridoxal phosphate) is FMN dependent
2. Oxidation of pyruvate, α-ketoglutarate, and branched-chain amino acids requires FAD
3. Fatty acyl CoA dehydrogenase requires FAD in fatty acid oxidation
4. FAD is required to convert retinol (vitamin A) to retinoic acid
5. Synthesis of an active form of folate (5-methyltetrahydrofolate) is $FADH_2$ dependent
6. FAD is required to convert tryptophan to niacin (vitamin B_3)
7. Reduction of the oxidized form of glutathione (GSSG) to its reduced form (GSH) by Glutathione reductase is FAD dependent

Riboflavin is also an antioxidant working to rid the body of free radicals. Other important functions include body growth and red blood cell production.

- **VITAMIN B_2 RELATED DISEASES**
  1. Riboflavin deficiency (also known as ariboflavinosis) results in stomatitis including painful red tongue with fissured lips (cheilosis) and sore throatinvovinginflammation of the corners of the mouth (angular stomatitis).
  2. Oily scaly skin rashes appearson the scrotum, vulva, lip, or the nasolabial folds. The eyes can become itchy, watery and sensitive to light.
  3. Mild to moderate riboflavin deficiency results in an anemia with normal cell size and normal hemoglobin content (i.e. normochromic normocytic anemia), due to interference with iron absorption.
  4. Deficiency of riboflavin during pregnancy can result in birth defects including limb deformities and congenital heart defects

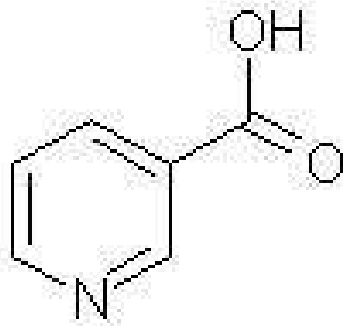
5. In other animals, riboflavin deficiency results in lack of growth weakness, ataxia, and inability to stand.

- **VITAMIN B_3 (NIACIN)**

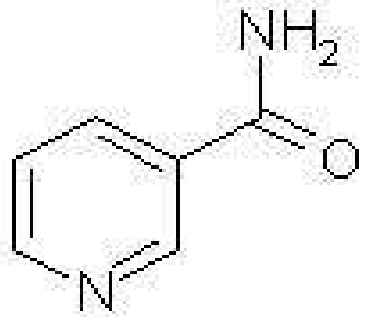
Niacin also known as **vitamin B_3** and **nicotinic acid** was discovered as a nutrient and is not strictly a vitamin since it can be synthesized in the body from the essential amino acid tryptophan. The vitamin is obtained from the diet in the form of nicotinic acid, nicotinamide and tryptophan, which are transformed to nicotinamide adenine dinucleotides, NAD and NADP (serves as coenzymes). NAD converts to NADP by phosphorylation in the presence of the enzyme NAD+ kinase.

STRUCTURE

This colorless, water-soluble solid is a derivative of pyridine, with a carboxyl group (COOH) at the 3-position. Other forms of vitamin B_3 include the corresponding amide and nicotinamide ("niacinamide"), where the carboxyl group has been replaced by a carboxamide group (CONH2), as wel l as more complex amides and a variety of esters.



11.11STRUCTURE OF NICOTINIC ACID



11.12STRUCTURE OF NICOTINAMIDE

OCCURRENCE AND SOURCES

Both forms of niacin are widely occurring in nature, in variety of foods, including liver, chicken, beef, fish, cereal, peanuts, and legumes, and is also synthesized from tryptophan, an essential amino acid found in most forms of protein.

Animal products: liver, chicken, chicken, breast, beef, fish (tuna, salmon), eggs

Fruits and vegetables: avocados, dates, tomatoes,leaf vegetables, broccoli, carrots, sweet potatoes, asparagus

Seeds: nuts, whole grain products, legumes

Fungi: mushrooms, brewer's yeast

Other: beer ,Peanut butter , Tofu

FUNCTIONS AND SIGNIFICANCE

1. Niacin and nicotinamide are precursors of the coenzyme snicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP).
2. NAD is the source of ADP-ribose for the **ADP-ribosylation** of proteins and poly ADP-ribosylation of nucleoproteins involved in the **DNA repair mechanism.**
3. It also produces macromolecules, including fatty acids and cholesterol and Normal psychological functions
4. Facilitate DNA repair and stress responses.
5. Involves in normal energy-yielding metabolism
6. Plays role in the normal function of the nervous system and the maintenance of normal skin and mucous membranes
7. The reduction of tiredness and fatigue.

VITAMIN B3 RELATED DISEASES

1. Symptoms of mild niacin deficiency include: indigestion due to slow metabolsim, fatigue, canker sores, vomiting, depression, intolerance to cold
2. Severe deficiency, called pellagra characterized by a photosensitive dermatitis, hyperpigmentation, thickening of the skin, inflammation of the mouth and tongue, digestive disturbances. As the condition progresses, there is dementia, possibly diarrhea, and, if untreated, death
3. Hartnup disease is a hereditary nutritional disorder resulting in niacin deficiency.
4. It is due to a deficit in the intestines and kidneys, making it difficult for the body to break

down and absorb dietary tryptophan. The resulting condition is similar to pellagra, including symptoms of red, scaly rash, and sensitivity to sunlight.

5. Niacin synthesis is also deficient in carcinoid syndrome, because of metabolic diversion of its precursor tryptophan to form serotonin.

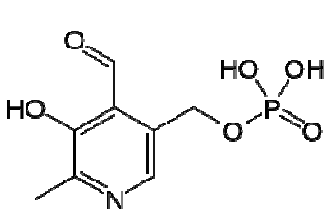
- **VITAMIN B_6 (Pyridoxine)**

Vitamin B_6 is a water-soluble vitamin refers to six common forms, namely pyridoxal, pyridoxine (pyridoxol), pyridoxamine, and their phosphorylated forms. The phosphate ester derivative pyridoxal 5'-phosphate (PLP) is the bioactive coenzyme form involved in over 4% of all enzymatic reactions mostly involved in amino acid metabolism.

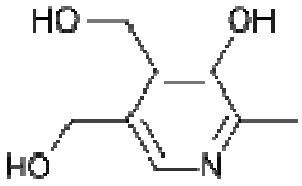
STRUCTURE

It has the structure, of 3-hydroxy-4, 5-bis (hydroxymethyl)-2-methylpyridine. The trivial name "pyridoxine", proposed for this compound by P. György, came into general use as a synonym for "vitamin B_6". Two other natural compounds possessing vitamin B_6 activity was recognized as the aldehyde, or 4-formyl analogue of pyridoxine, and the corresponding amine, or 4-aminomethyl analogue were designated "pyridoxal" and "pyridoxamine" respectively.

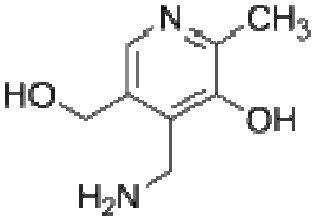
It was demonstrated that a phosphoric derivative of pyridoxal, later identified as pyridoxal 5'-phosphate, is the coenzyme of a large group of specific enzymes catalysing reactions of amino-group transfer, decarboxylation and other metabolic transformations.



Pyridoxal phosphate



Pyridoxine



Pyridoxamine

OCCURRENCE AND SOURCES

Vitamin B_6 is widely distributed in foods. Plant foods contain mostly pyridoxine. Vitamin B_6 is found in the germ and aleurone layer of grains, and milling results in the reduction of this vitamin in white flour.

Foods that contain large amounts of vitamin B_6 include pork, turkey, beef, bananas, chickpeas, potatoes, pistachios, fortified ready-to-eat cereal, chicken, tuna, salmon, shrimp, beef liver ,milk, cheese, lentils, beans, spinach, carrots, brown rice, bran, sunflower seeds ,whole-grain flour ,yeast, egg yolk and germ seeds etc.

FUNCTIONS AND SIGNIFICANCE

1. Pyridoxal phosphate is a coenzyme for many enzymes involved in amino acid metabolism
2. PLP, the metabolically active form of vitamin B_6, is involved in many aspects of macronutrient metabolism, neurotransmitter synthesis, histamine synthesis, hemoglobin synthesis and function, and gene expression
3. PLP is a required coenzyme of glycogen phosphorylase, the enzyme necessary for glycogenolysis to occur.
4. PLP is an essential component of enzymes that facilitate the biosynthesis of sphingolipids
5. PLP aids in the synthesis of hemoglobin, by serving as a coenzyme

VITAMIN B_6 RELATED DISEASES

Although vitamin B_6 deficiency is not very usual, studies have linked a vitamin B_6 deficiency with an increased risk for a range of different disorders and symptoms.

1. Several observations have suggested that low dietary intake of vitamin B_6 is associated with higher risk of having heart disease.

2. An adequate vitamin B_6 intake is especially important in the elderly, as this group often suffers from impaired immune function.

3. In Excess, Vitamin B_6 Causes Sensory Neuropathy.

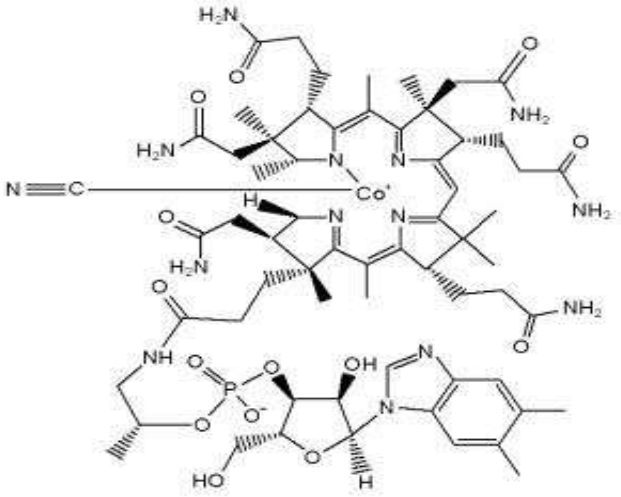
4. Vitamin B_6 deficiency is linked most commonly with neuropsychiatric disorders, including seizures, migraines, chronic pain and mood disorders like depression and **confusion** with inflammation of the tongue, sores or ulcers of the mouth, and ulcers of the skin at the corners of the mouth.
5. Its deficiency can worsen the symptoms of premenstrual syndrome and anemia.
6. Essential for growth of microorganisms, chick, dog, mice and infants.

- **VITAMIN B_{12}**

The term "vitamin B12" (**cobalamins), corrinoid** (cobalt containing compounds possessing the corrin ring) having the biologic activity of the vitamin. It is the most chemically complex of all the vitamins and the only known essential biomolecule with a stable metal-carbon bond, that is, it is an organometallic compound. Vitamin B12 is involved in cellular metabolism in two active coenzyme forms—methylcobalamin and 5-deoxyadenosylcobalamin.

STRUCTURE

Cyanocobalamin's structure is based on a corrin ring, which, although similar to the porphyrin ring found in heme, chlorophyll, and cytochrome, has two of the pyrrole rings directly bonded. The central metal ion is Co (cobalt).



11.13Structure of Vitamin B12

OCCURRENCE AND SOURCES

The vitamer is produced by bacteria as hydroxocobalamin, but conversion between different forms of the vitamin occurs in the body after consumption. A common synthetic form of the vitamin is cyanocobalamin, produced by chemically modifying bacterial hydroxocobalamin. Because of superior stability and low cost this form is used in many pharmaceuticals and supplements as well as for fortification of foods. In the body, it is converted into the human physiological forms methylcobalamin and 5'-deoxyadenosylcobalamin. Animals must obtain vitamin B_{12} directly or indirectly from bacteria thus, herbivorous animals must either obtain B_{12} from bacteria.

Vitamin B_{12} is found in most animal-derived foods, including fish and shellfish, meat (especially liver), poultry, eggs, milk, and milk products. Besides this, Foods fortified with B_{12} are also dietary sources of the vitamin. Foods for which B_{12}-fortified versions are widely available include breakfast cereals, soy products, energy bars, and nutritional yeast.

FUNCTIONS AND SIGNIFICANCE

1. B_{12} is required for some essential biochemical reactions in most cells of the body. The products of these reactions are needed to make DNA, and many proteins, hormones, and fats.
2. B_{12} works together with folate, another important B vitamin, in many biochemical pathways. B_{12} helps convert a substance called homocysteine into methionine, a major route for lowering homocysteine levels. This reaction also transforms folate into the active form needed to make DNA.
3. Vitamin B_{12} is an essential vitamin that's crucial for addressing adrenal fatigue, multiple metabolic functions — including enzyme production, DNA synthesis and hormonal balance — and maintaining healthy nervous and cardiovascular systems.
4. Vitamin B_{12} benefits the central nervous system by maintaining the health of nerve cells including those needed for neurotransmitter signaling — and helps form the protective covering of nerves, called the cell's myelin sheath.
5. Vitamin B_{12}, sometimes also called cyanocobalamin, also helps with digestion and heart health, so a deficiency can lead to both digestive disorders and an increased risk for heart

disease.

VITAMIN B_{12} RELATED DISEASES

1. Vitamin B_{12} deficiency can cause irreversible damage, specifically to the brain and nervous system. At levels only marginally lower than normal, symptoms such as fatigue, depression, and poor memory may result.
2. Vitamin B_{12} deficiency can also cause symptoms of mania and psychosis.
3. Pernicious anemia appears when vitamin B_{12} deficiency blocks the metabolism of folic acid, leading to functional folate deficiency. This impairs erythropoiesis, causing immature precursors of erythrocytes to be released into the circulation (megaloblastic anemia).
4. Other symptoms as a result of its deficiency are:
   Yellowing of the skin, Sore, red tongue, Mouth ulcers, Changes or loss of some sense of touch, Feeling less pain, Walking problems, Vision problems, Mood changes, Symptoms of dementia

- **VITAMIN B_9 (FOLIC ACID)**

Folic acid, also known as folate, is one of the B vitamins that stimulate the hematopoietic system. It is used as a supplement during pregnancy to prevent neural tube defects (NTDs). The active form of folic acid (pteroyl glutamate) is tetrahydrofolate. The folates in foods may have up to seven additional glutamate residues linked by γ-peptide bonds. It is the most important medications needed in a basic health system.

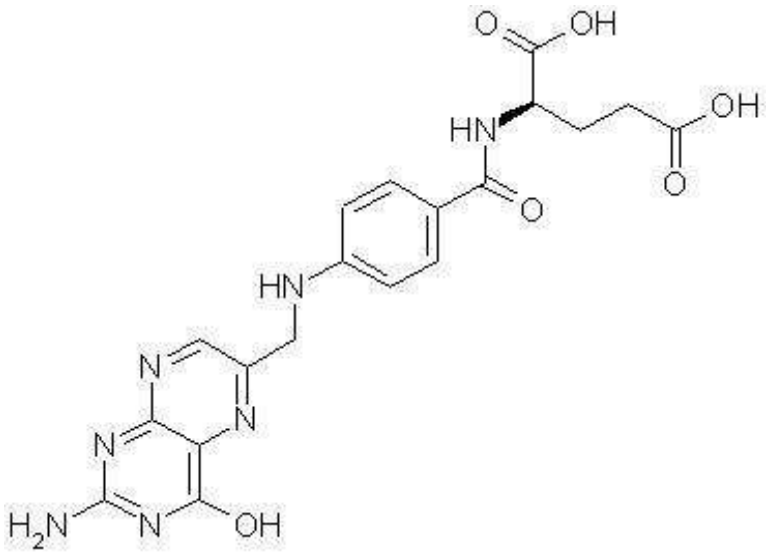
STRUCTURE

The folates are a group of heterocyclic compounds based on the 4- [(pteridin-6-ylmethyl) amino] benzoic acid skeleton conjugated with one or more L-glutamate units. Folic Acid is a collective term for pteroylglutamic acids and their oligoglutamic acid conjugates. Folate and folic acid are the preferred synonyms for pteroylglutamate and pteroylglutamic acid, respectively.

OCCURRENCE AND SOURCES

Folic acid is pteroylmonoglutamic acid. Naturally occurring folates exist in many chemical forms; folates are found in food, as well as in metabolically active forms in the human body. Folic acid is the major synthetic form found in fortified foods and vitamin supplements. Reduced polyglutamates are found in animal and plant foods.

There are many food sources containing folic acid, the most important being:
Green leafy vegetables, Beans, Liver, mushrooms, spinach and grasses (POACEAE), Yeast Extract, Whole grains, Egg yolk, Milk and milk products, Oranges, Beets, Wholemeal bread, Beer.



11.14Chemical Structure of Folic Acid

FUNCTIONS AND SIGNIFICANCE

1. Tetrahydrofolic acid, or tetrahydrofolate, is a folic acid derivative. The only function of folate coenzymes in the body appears to be in mediating the transfer of one-carbon units. Folate coenzymes act as acceptors and donors of one-carbon units in a variety of reactions critical to the metabolism of nucleic acids and amino acids.
2. Folate coenzymes play a vital role in DNA metabolism. The synthesis of DNA from its

precursors (thymidine and purines) is dependent on folate coenzymes.

3. Folate coenzymes are required for the metabolism of several important amino acids, namely methionine, cysteine, serine, glycine, and histidine.
4. Folic acid intake during pregnancy has been linked to a lessened risk of neural tube defects and some other specific kinds of birth defects.
5. Folate is important for cells and tissues that rapidly divide.

VITAMIN B_9 RELATED DISEASES

1. Folate deficiency leads to megaloblastic anemia, which is reversible with folic acid treatment. Symptoms include headache, fatigue, weight loss, anemia, nausea, anorexia, diarrhea, insomnia, irritability; Signs are macrocytic red cells and megaloblasts in the bone marrow.
2. Deficiency may result in neural tube defects. The importance of adequate folate intake at conception and for the first 3 weeks when the neural tube closes is obvious to few mothers.
3. Defieciency may result in elevated homocysteine (HCS) which is associated with increased risk for coronary disease.
4. Folate deficiencies are seen under conditions of poor nutrition, heavy alcohol.
5. Folate deficiency may lead to glossitis, diarrhea, depression, confusion, fatigue, gray hair, mouth sores, poor growth and swollen tongue.

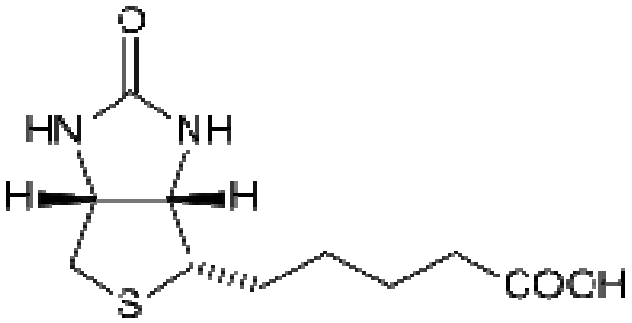
- **VITAMIN B_7 BIOTIN**

Biotin is a water-soluble B-vitamin (vitamin B_7), formerly known as vitamin H or coenzyme R.Biotin is an important component of enzymes in the body that break down certain substances like fats, carbohydrates, and others.It is also used orally for hair loss, brittle nails, skin rash in infants (seborrheic dermatitis), diabetes, and mild depression.

STRUCTURE

Biotin has the chemical formula $C_{10}H_{16}N_2O_3S$. Biotin is a heterocyclic, S-containing monocarboxylic acid. Biotin is composed of an ureido (tetrahydroimidizalone) ring fused with a tetrahydrothiophene ring, which is an organic compound consisting of a five-membered ring

containing four carbon atoms and a sulfur atom. A valeric acid substituent—straight chain alkyl carboxylic acid with the chemical formula CH_3 $(CH_2)3COOH$)—is attached to one of the carbon atoms of the tetrahydrothiophene ring.



11.15Structure of biotin

OCCURRENCE AND SOURCES

Biotin is consumed from a wide range of food sources in the diet, but few are particularly rich sources. Biotin is found in many foods, either as the free form that is directly taken up by enterocytes or as biotin bound to dietary proteins. The richest sources of vitamin B7 (biotin) are yeast, liver, egg yolk, soybeans, nuts, cereals, peanuts, swiss chard, leafy green vegetables and egg yolk.

Besides these almonds, sunflower seeds, walnuts, sweet potatoes, strawberries, mushrooms, broccoli, avocado, spinach and carrots are other good sources.
Interestingly, normal intestinal bacteria synthesize biotin, but it is not known if it is absorbed.

FUNCTIONS AND SIGNIFICANCE

1. In humans, vitamin B_7 (biotin) is the coenzyme attached at the active site of several ‘carboxylases’ enzymes. The attachment of biotin to another molecule, such as a protein (e.g. histone), is known as ‘biotinylation’.

2. Carboxylases catalyze an essential metabolic reaction required for the synthesis of fatty acids, gluconeogenesis, the catabolism of leucine (an essential amino acid), and the metabolism of certain amino acids, cholesterol, and certain fatty acids.

3. Biotinylation of histones plays a role in regulating DNAreplication and gene expression as well as cell division and other cellular responses.

4. Biotin serves as a key element in maintaining healthy hair and skin.

VITAMIN B_7 RELATED DISEASES

Biotin deficiency is quite rare. Bacteria in the intestines make biotin, or vitamin B_7, and the human body actually recycles unused biotin through the intestines too, making a biotin deficiency unlikely. Certain medications and dietary practices (consumption of raw eggs), however, can predispose individuals to a deficiency.

1. Biotin deficiency can arise due to various inborn genetic errors that affect the activity of biotin-related enzymes. Since endogenous biotin production occurs in the gut, dysbiosis could also upset the metabolic processes that allow the body to generate biotin on its own.
2. Inherited metabolic disorders characterized by deficient activities of biotin-dependent carboxylases are termed multiple carboxylase deficiency results in inhibition of the use biotin from the body's cells. Biochemical and clinical manifestations include: ketolactic acidosis, organic aciduria, hyperammonemia, skin rash, feeding problems, hypotonia, seizures, developmental delay, alopecia, and coma.

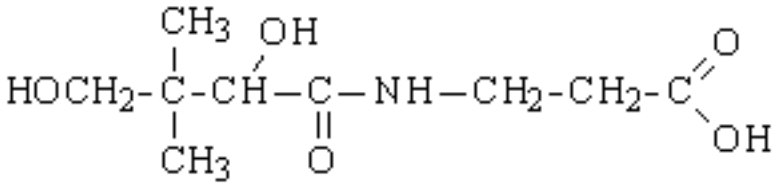
Besides this other deficiency symptoms include:

- Brittle and thin fingernails
- Conjunctivitis
- Dermatitis in the form of a scaly, red rash around the eyes, nose, mouth, and genital area.
- Neurological symptoms in adults, such as depression, lethargy, hallucination, and numbness and tingling of the extremities
- **VITAMIN B_5**

Vitamin B_5, otherwise known as pantothenic acid or pantothenate is also commonly called the anti-stress nutrient because it plays a role in the production of stress hormones by the adrenal glands. Pantothenic acid is an essential nutrient.

STRUCTURE

The molecular formula is butyryl-beta-alanine ($C_9H_{17}NO_5$) and it is the **amide** between D-pantoate and beta-alanine; its **IUPAC** name is 3-[(2, 4-dihydroxy-3, 3-dimethylbutanoyl) amino] propanoic acid.



11.16 Vitamin B5, pantothenic acid

OCCURRENCE AND SOURCES

Vitamin B_5 is found in a variety of food sources. Small quantities of pantothenic acid are found in most foods. The major food source of pantothenic acid is meat. The concentration found in human muscle is about double that in other animals' muscle. Whole grains are another good source of the vitamin, but milling removes much of the pantothenic acid, as it is found in the outer layers of whole grains. Vegetables, such as avocadoskale, and other vegetables in the cabbage family and broccoli, also have abundance. In animal feeds, the most important sources are alfalfa, cereal, fishmeal, peanut meal, molasses, milk, mushrooms, rice, wheat bran, and yeasts.

FUNCTIONS AND SIGNIFICANCE

1. Pantothenic acid is used in the synthesis of coenzyme A (CoA). Coenzyme A may act as an acyl group carrier to form acetyl-CoA and other related compounds; this is a way to transport carbon atoms within the cell.
2. CoA is incidentally also required in the formation of ACP, which is also required for fatty acid synthesis in addition to CoA.

3. Vitamin B5 plays a pivotal role in the breakdown of fats, carbohydrates and proteins for providing energy to the cells.
4. It is also required for the production of red blood cells, steroids, neurotransmitters and stress related hormones.
5. It helps in maintaining a healthy digestive tract and also assists the body in making an optimal use of vitamin B2.
6. To synthesize cholesterol, the body makes use of pantothenic acid and thus this vitamin finds its use even in the production of cholesterol.
7. CoA is important in energy metabolism and in the biosynthesis of many important compounds such as fatty acids, cholesterol, and acetylcholine
8. Pantothenic acid in the form of CoA is also required for acylation and acetylation, involved in signal transduction and enzyme activation and deactivation, respectively.

VITAMIN B_5 RELATED DISEASES

1. Pantothenic acid deficiency is exceptionally rare The symptoms are similar to other B vitamin deficiencies including:
2. Impaired energy production, due to low CoA levels, causing symptoms of irritability, fatigue, and apathy
3. Acetylcholine synthesis is impaired resulting in neurological symptoms, like numbness, paresthesia, and muscle cramps.
4. Deficiency in pantothenic acid can also cause hypoglycemia, or an increased sensitivity to insulin.
5. Additional symptoms could include restlessness, malaise, sleep disturbances, nausea, vomiting, and abdominal cramps.

- **VITAMIN C or ASCORBIC ACID**

Ascorbic acid is a naturally occurring organic six carbon compound related to glucose with antioxidant properties. It is an essential nutrient in human diets. A name, ascorbic acid, is derived from *a-* (meaning "no") and *scorbutus* (scurvy), the disease caused by a deficiency of vitamin C. It is derived from glucose, hence many animals are able to produce it, but humans

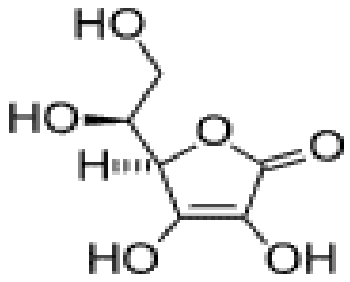
require it as part of their nutrition. Other vertebrates which lack the ability to produce ascorbic acid include some primates, guinea pigs, bats, some fishes and birds, all of which require it as a dietary micronutrient. Vitamin C comprises several vitamers that have vitamin C activity in animals, including ascorbic acid and its salts, and some oxidized forms of the molecule like dehydroascorbic acid.

STRUCTURE

Vitamin C, Ascorbic acid or ascorbate is a weak organic acid that appears as a white, crystalline compound. Structurally, it is related to the six-carbon sugarglucose, from which most animals are able to derive the molecule in a four-step process.

Ascorbate occurs in two forms, both of which are mirror images of the same molecular structure *(enantiomers).* Vitamin C is specifically the L-enantiomer of ascorbate; the D-enantiomer has no physiological significance. L-ascorbate naturally occurs either attached to a hydrogen ion, forming *ascorbic acid,* or joined to a metal ion, forming a mineral ascorbate.

When L-ascorbate carries out its reducing function, it is converted to its oxidized form, L-dehydroascorbate, which can then be converted back to the active form in the body by specialized enzymes and the peptide glutathione.



11.17Ascorbic acid (Vitamin C)

OCCURRENCE AND SOURCES

The richest natural sources are fruits and vegetables. Interestingly richest sources are not citrus juices, but broccoli, brussel sprouts and peppers. The other sources high in ascorbate are citrus products, potatoes, and tomatoes. It is also present in some cuts of meat, especially liver. Vitamin C is the most widely taken nutritional supplement and is available in a variety of forms, including tablets, drink mixes, crystals in capsules or naked crystals. Cereal products, grains, and meats contain very little amount of vitamin C.

The majority of species of animals (but *not* humans, guinea pigs or fruit bats) and plants synthesize their own vitamin C. Therefore, some animal products can be used as sources of dietary vitamin C.

Vitamin C is most present in the liver and least present in the muscle. It is present in human breast milk and only in limited quantity in raw cow's milk. All excess vitamin C is disposed of through the urinary system.

FUNCTIONS AND SIGNIFICANCE

1. The most prominent role of vitamin C is its immune-stimulating effect, e.g., important for defence against infections such as common colds.
2. It also acts as an inhibitor of histamine, a compound that is released during allergic reactions.
3. As a powerful antioxidant it can neutralize harmful free radicals and it aids in neutralizing pollutants and toxins.
4. It acts as a prooxidant, reduces metals to their prooxidant form.
5. Vitamin C is also able to regenerate other antioxidants such as vitamin E.
6. Vitamin C is required for the synthesis of collagen, the intercellular ‘cement’ substance which gives structure to muscles, vascular tissues, bones, tendons and ligaments.
7. Vitamin C especially in combination with zinc – is also important for the healing of wounds.
8. Vitamin C contributes to the health of teeth and gums, preventing haemorrhaging and bleeding.

9. It also improves the absorption of iron from the diet.
10. Vitamin C is also needed for the metabolism of bile acids which may have implications for blood cholesterol levels and gallstones.
11. Vitamin C plays an important role in the synthesis of several important peptide hormones, neurotransmitters and carnitine.
12. Vitamin C is also a crucial factor in the eye's ability to deal with oxidative stress, and can delay the progression of advanced age-related macular degeneration (AMD) and vision-loss.

VITAMIN C RELATED DISEASES

Vitamin C deficiency results in scurvy that includes skin changes, fragility of blood capillaries, gum decay, tooth loss, and bone fracture, many of which can be attrib- uted to deficient collagen synthesis.

The first symptoms of vitamin C deficiency tend to be:

Tiredness and weakness, Muscle and joint pains, easy bruising, Spots that look like tiny red-blue bruises on skin.

Other symptoms can include:

Dry skin, Splitting hair, Swelling, discoloration, Sudden and unexpected bleeding of gums, Nosebleeds, Poor healing of wounds, Problems with fighting infections, Bleeding and severe joint pains, Tooth and Weight loss.

11.6 SUMMARY

- A **vitamin** is an organic compound and a vital nutrient that an organism requires in limited amounts. An organic chemical compound (or related set of compounds) is called a vitamin when the organism cannot synthesize the compound in sufficient quantities, and it must be obtained through the diet.
- Vitamin A, D, E and K are fat-soluble. These are stored in liver and adipose tissues and hence are called fat-soluble vitamins.

- Vitamin A is found in the body in three forms: retinol, retinal, and retinoic acid. Together, they are essential to vision, healthy epithelial tissues, and growth.
- Vitamin D is a steroid prohormone yielding the active derivative calcitriol, which regulates calcium and phosphate metabolism. Vitamin D deficiency leads to rickets. □
- Vitamin E acts as an antioxidant, defending lipids and other components of the cells against oxidative damage and also required for the synthesis of blood clotting proteins.
- Vitamin K functions as cofactor to a carboxylase that acts on glutamate residues of clotting factor precursor proteins to enable them to chelate calcium. □
- Vitamins in B-group and vitamin C are water-soluble and cannot be stored in our body as they excreted out of the body via urine and acts as enzyme cofactors.
- Thiamin has a crucial role in energy-yielding metabolism, and especially of carbohydrate in which Thiamin diphosphate acts as the coenzyme.
- Riboflavin (vitamin B2) works with the other B vitamins. It is important for body growth and the production of red blood cells.
- Niacin is a B vitamin that helps maintain healthy skin and nerves. It is also has cholesterol-lowering effects. Niacin and nicotinamide are precursors of the coenzy mesnicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP).
- Pyridoxal phosphate is a coenzyme for many enzymes involved in amino acid metabolism
- Vitamin B12 is required for some essential biochemical reactions in most cells of the body. The products of these reactions are needed to make DNA, and many proteins, hormones, and fats.
- Folate coenzymes act as acceptors and donors of one-carbon units in a variety of reactions critical to the metabolism of nucleic acids and amino acids and plays a vital role in DNA metabolism

- vitamin B7 (biotin) is the coenzyme attached at the active site of several 'carboxylases' enzymes and catalyzes an essential metabolic reaction required for the synthesis of fatty acids, gluconeogenesisand the metabolism of certain amino acids.

- Pantothenic acid is used in the synthesis of coenzyme A (CoA). Coenzyme A may act as an acyl group carrier to form acetyl-CoA and other related compounds; this is a way to transport carbon atoms within the cell

- Vitamin C is a water-soluble antioxidant that maintains vitamin E and many metal cofactors in the reduced state.

11.7 GLOSSARY

Vitamer: one of multiple related chemical compounds possessing a given vitamin activity.

Retinol: **Retinol** (Vitamin A1) is one of the animal forms of vitamin A.

Carotenes: an organic, strongly colored red-orange pigment abundant in plants and fruits

Antioxidant: An **antioxidant** is a molecule that inhibits the oxidation of other molecules.

Prooxidant: A species that causes or promotes oxidation.

Free radicals: an uncharged molecule (typically highly reactive and short-lived) has an unpaired valency electron.

Steroid: An organic compound with four rings arranged in a specific configuration

Enzyme: **Enzymes** are biological molecules (proteins) that act as catalysts

Cofactor: A **cofactor** is a non-protein chemical compound or metallic ion that is required for a protein's biological activity.

Coenzyme: **coenzymes** are organic molecules that are required by certain enzymes to carry out catalysis

Cholesterol: a sterollipid molecule, biosynthesized by all animal cells

11.8 LONG ANSWER TYPE QUESTIONS

Question 1. Give a detailed account of fat soluble vitamins.

Question 2. Give a detailed account of water soluble vitamins.

Question 3. Describe in detail the deficiency related diseases by vitamin B complex.

11.9 SHORT ANSWER TYPE QUESTIONS

Question 1. What are fat soluble vitamins?

Answer. Vitamin A, D, E and K are fat soluble. These are stored in liver and adipose tissues and hence are called fat soluble vitamins

Question 2. What are water soluble vitamins?

Answer. Vitamins in B-group and vitamin C are water soluble and cannot be stored in our body as they excreted out of the body via urine. These vitamins must be supplied to our body with regular diets.

Question 3. What are vitmaers? Give an example.

Answer. A vitamer of a particular vitamin is any of a number of chemical compounds, generally having a similar molecular structure, each of which shows vitamin-activity in a vitamin-deficient biological system. Eg. Vitamin K includes two natural vitamers: vitamin K_1 and vitamin K_2.

11.10 FILL IN THE BLANKS/ MULTIPLE CHOICE QUESTIONS

1. Vitamin _____are fat soluble.
2. Vitamin _____are water soluble.
3. Scurvy is caused by the deficiency of _____.
4. _____are vitamers of Vitamin D.
5. Vitamin B5 is also known as_____.
6. _____ is known as the "blood-clotting vitamin" for its important role in healing wounds

Answer:

1. A, D, E, K**,**
2. B & C
3. Vitamin C
4. Cholecalciferol and ergocalciferol
5. Pantothenic acid
6. Vitamin K

MULTIPLE CHOICE QUESTIONS

1. The symptoms of retinol excess are
   A. Bone fragility B**.** Nausea
   C**.** Weakness D. All of these

2. Ascorbic acid acts as an

   A**.** reducing agent B**.** oxidizing agent
   C. oxidizing and reducing agent both D**.** none of the above

3. A deficiency of thiamin produces the disease known as

   A**.** Beriberi B. Scurvy

   C**.** Catarac**t** D. Anemia

4. Vitamin B_{12} is useful in the prevention and treatment of

A. Pernicious Anemia B. Scurvy

C. Cataract D. Beri Beri

5. Vitamin B3 is called as

A. Pantothenic acid B. Niacin

C. Retinol D. Folic acid

6. Which of the following acids is a vitamin?

A. Aspartic acid B. Ascorbic Acid

C. saccharic acid D. Adipiic acid

7. Which one of the following is correctly matched?

A. Vitamin E – Tocopherol B. Vitamin D - Riboflavin
C. Vitamin B – Calciferol D. Vitamin A – Thiamine

8. Which one of the following pairs is not correctly matched?

A. Vitamin C- Scurvy B. Vitamin B_4- Pellagra
C. Vitamin B_2- Pernicious Anaemia D. Vitamin B_6- Beriberi

9. Which one of the following is a fat soluble vitamin and its related deficiency disease?

A. Retinol- Xerophthalmia B. Cobalamine-Beriberi
C. Calciferol- Pellagra D. Ascorbic acid- Scurvy

10. The Haemorrhagic disease of new born is caused to the deficiency of

A. Vitamin K B. Vitamin B12
C.Vitamin A D. Vitamin B6

11. Which of the following families do folic acid and panthothenic acid belong?

A. Vitamin C B. Vitamin K
C. Vitamin A D. Vitamin B complex

Answers: 1.D, 2.A, 3.A, 4. A, 5.B, 6.B, 7.A, 8.D, 9.A, 10. A, 11.D

11.11 REFERENCE

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UNIT 12: PROTEINS

Content

12.1 OBJECTIVES

1. The objective of this chapter is to understand about proteins.
2. To understand structures and classification of proteins.
3. To understand the properties of proteins.
4. Understanding the metabolism of proteins.
5. Major sources of proteins.
6. Biological significance and deficiency diseases of proteins.

12.2 INTRODUCTION TO PROTEINS

Proteins are the highly complex chemical compounds present in all living organism. These are the high molecular weight polypeptides, composed of carbon, hydrogen, oxygen, nitrogen, sulpher and phosphorus. Proteins are the most abundant and essential constituents of living cells. All the basic functions of life depend upon specific proteins. The term protein was first suggested by Swedish chemist Berzalius in 1938 and derived from the Greek word "proteios" meaning 'first'. Geradus Mulder for the first time used the term in 1840 and referred it to the complex organic nitrogenous substance found in the cells of living organisms. Proteins are the most significant compound in living beings depending upon their chemical and physical structures they are involved in wide variety of functions i.e. catalysis, conduction, contraction, structure, nutrition, binding and defense.

Chemical Structure of Proteins

Proteins are the linear polymers of amino acids. Proteins can be very long polypeptide chains of hundred to several thousand amino acids. When a large number of amino acids join together they form polypeptides chains. The amino acids molecules in a polypeptide chains are linked by polypeptide bonds.

$$[\text{Amino Acid}]_n \rightarrow \text{Peptide} \rightarrow \text{Polypeptide} \rightarrow \text{Protein}$$

Peptide Bond: The amino acids of a protein are joined to one another by their respective amino and carboxyl groups i.e. the carboxyl group of one amino acid is joined to the amino group of the

next amino acid to form a peptide bond or peptide linkage with the release of one molecule of water.

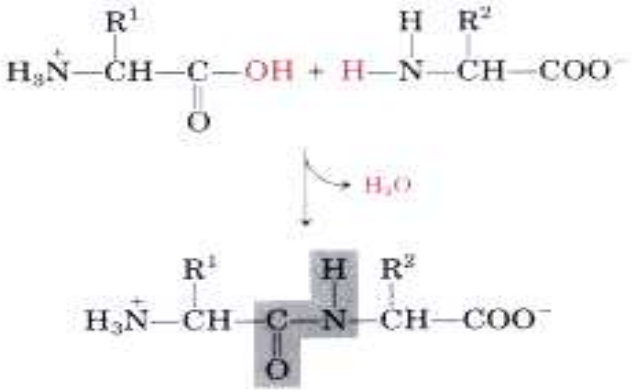


Fig.12.1. Formation of a peptide bond

12.3 CLASSIFICATION OF PROTEINS

Proteins can be classified according to their functions, shape, structures and complexity. On the basis of their conformation the proteins can be classified into two major classes:

1. Fibrous proteins
2. Globular Proteins

1. Fibrous proteins

In fibrous proteins polypeptide chains are arranged in a parallel manner along a single axis producing long fibers or sheet like structures. Fibrous proteins Fibrous proteins are insoluble in water or dilute salt solution. These are the basic structural elements in animal tissues. Keratin of hair and skin, elastin of ligament and collagen of tendons and bone matrix all are examples of fibrous proteins.

2. Globular Proteins

Polypeptide chains are tightly folded into compact spherical or globular shapes. Most of the globular proteins are soluble in water example of globular proteins are all the enzymes, certain hormones, and antibodies etc.

On the basis of their structure and complexity proteins are classified into three major classes:

1. Simple proteins
2. Conjugated or Complex proteins
3. Derived proteins

1. Simple proteins: Proteins which consist solely of amino acid are called simple proteins. These are further classified into the following subclasses

Albumins: They are soluble in water and coagulate on heating. They are precipitated by dilute acids and alkalis. Albumins are widely distributed in nature stored as food reserved e.g. egg albumin, serum albumin, legumelin (legumes), lactalbumin (milk), lecucosin (cereals), gliadin (wheat).

Protamins: They are basic proteins highly soluble in water, dilute acids and ammonium hydroxide. Protamins are not coagulated by heat. These are simplest of all the naturally occurring proteins, isolated from mature sperms e.g. Sturine and salmine.

Histones: These are soluble in water and dilute acids but insoluble in ammonia. They are not coagulated by heat. Histones occur as part of nucleoproteins.

Scleroproteins: These are also known as albuminoids. Scleroproteins are soluble in water and solutions of neutral salts. They are found exclusively in animals e.g. keratin, collagen, elastin and fibroin.

Globulins: These are insoluble in water but are soluble in dilute neutral salt solution such as NaCl. On heating globulin get coagulated. They are precipitated by half saturated with ammonium sulphate examples of globulins are fibrinogen (blood plasma), egg globulin, myogen (muscles), legumin (peas), tuberin (potato) etc.

Glutelins: They are insoluble in water but are soluble in dilute acids and alkalies, Glutelin get coagulate on heating. These are found exclusively in seeds of cereal grains e.g. glutenin (wheat) and oxyzenin (rice).

Prolamins: These are insoluble in water but are soluble in dilute alkalies and 50-80% of alcohol. They are not coagulated by heat and found in plants only e.g. hordein (barley), gliadin (wheat) and zein (maize).

2. Conjugated Proteins: These proteins are composed of not only amino acids but also some non-protein components. This non-protein substance linked to proteins is called "prosthetic group". Conjugated proteins are further classified into the following subclasses on the basis of their prosthetic group.

Glycoproteins: In glycoproteins simple proteins are covalently linked with carbohydrate group. The percent of carbohydrate group in different glycoproteins may vary from less than1% in egg albumin to as high as 80% in mucoproteins. Glycoproteins which have very high carbohydrate content are called proteoglycans. Examples of glycoproteins are mucin (saliva), heparin (bile juice), and immunoglobulins (plasma).

Nucleoproteins: In nucleoproteins protein molecules are combined with nucleic acid. The chromatin material of the nuclei of cells is composed of nucleoproteins e.g. nucleohistones.

Lipoproteins: Lipoproteins are the proteins in combination with lipids. These are present in the brain, egg, milk and plasma.

Phosphoproteins: Phosphoproteins are formed by the combination of simple proteins with phosphoric acid. Examples of phosphoproteins are vitelline (egg) and casein (milk).

Metalloproteins: These are proteins linked to some metallic prosthetic group, which also gives colour to the proteins. They are also known as chromoproteins e.g. haemoglobin, hemocynin, cytochromes and flavoproteins.

3. Derived Proteins: These proteins are derived from some previously existing proteins either by its hydrolysis or by its coagulation. Derived proteins can be divided into two major subclasses-

Primary derived proteins:- These are denaturated or coagulated proteins. The denaturation is caused by heat, acid or alkali treatment. Their molecular weight is same as the native protein but they differ in solubility, precipitation and crystallization. Examples are proteans, metaproteins and coagulated proteins.

Secondary derived proteins:- These are formed by the hydrolysis of complex protein of their peptide linkage. The hydrolysis is caused by the action of digestive enzymes, acids or alkalis. Their molecular weight is different from the native proteins. Examples are proteoses, peptones and peptides. On the basis of their biological functions they are classified into seven major classes:

i. Structural Proteins: Their function is strengthening or protecting biological structures e.g. keratin, fibroin, collagen, elastin etc.
ii. Storage and Nutrient Proteins: Their function is to provide nourishment to growing e.g. ovalbumin, giadin, ferritin etc.
iii. Enzymatic Proteins: Their function is to transport molecules in body e.g. haemoglobin, serum albumin, myoglobin etc.
iv. Transport Proteins: Their function is to transport molecules or ions in body e.g. haemoglobin, myoglobin and serum albumin.
v. Regulatory Proteins: Their function is to regulate cellular or metabolic activities e.g. hormones, repressors etc.
vi. Contractile Proteins: Their function is in contractile system e.g. actin, myosin, tubulin etc.
vii. Defense Proteins: Their function is to provide defense against another organisms e.g. antibodies, ricin etc.

12.4 Structural Organization of Proteins

Proteins are long polypeptide chains formed by the linkages of several thousand molecules of amino acid with a peptide bond. Proteins can be very long polypeptide chains of 100 to several thousand amino acid residues. There are four different structural level of organization are present in proteins, they are:

1. Primary structure
2. Secondary structure
3. Tertiary structure
4. Quartiary structure

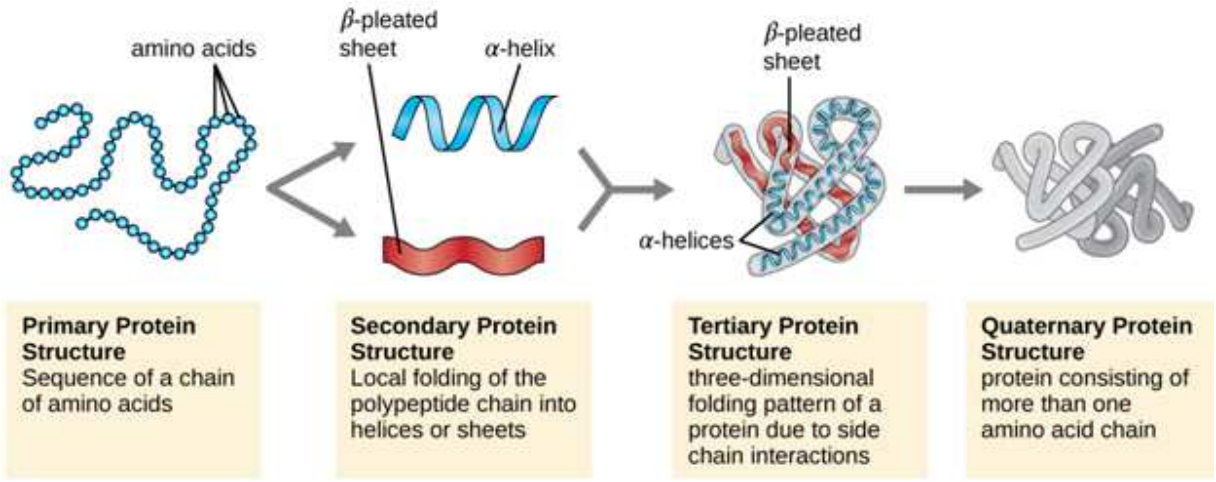


Fig.12.2. Structural organization of proteins

12.4.1PRIMARY STRUCTURE

The sequence of amino acids in a protein and a description of all covalent bonds joining amino acid residues in the protein are called its primary structure. The linear sequence of amino acids in a protein is its characteristics feature. It determines the three dimensional structures of protein and also essential to elucidating mechanism of action of that protein. Fredrick Sanger (1953) for the first time presented the primary structure of protein insulin. Each polypeptide chain has at one end N-terminal amino acid containing a free amino group and at the other ends a C-terminal amino acid containing free carboxyl group. Any single alteration in amino acid sequence can produce a defective protein e.g. sickle cell anemia disease can result from a change in a single amino acid of haemoglobin polypeptide chain.

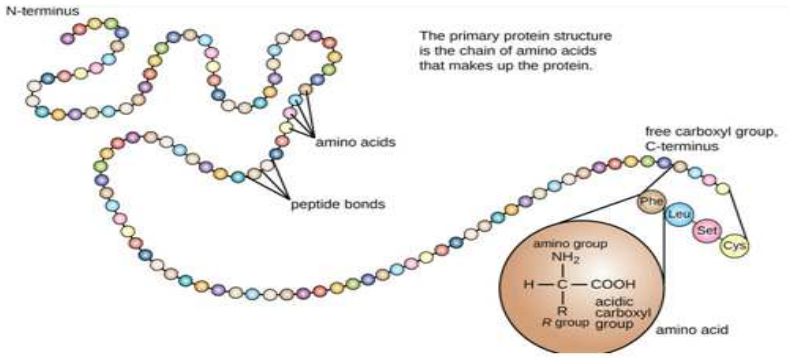


Fig.12.3. Primary structure: Amino acid sequence of proteins

12.4.2 SECONDARY STRUCTURE

Secondary structure refers to regular folding patterns of amino acid residues in a segment of a polypeptide chain. Folding of the polypeptide chain is the result of formation of hydrogen bond interaction between amino acid residues which are close to one another. The most prominent secondary structure which occurs widely in proteins is α-helix and β pleated sheets. In 1951 Robert Corey and Linus Pauling determine these confirmations of protein molecules.

α-helix: The most simple and common type of secondary structure is α-helix. It is a rod like structure in which the polypeptide backbone is tightly wound around an imaginary axis. Longitudinally and the side chains extend outwards a helical backbone. The helical structure of protein is formed by the hydrogen bond between peptide groups within the same polypeptide chains. In the α-helix each amino acid residue is away from the other at a distance of 1.5 Å and there are 3.6 amino acid residues per turn of helix. Almost all naturally occurring proteins have right handed α-helix. Examples of α-helices are α-keratin in hair and nails, fibrin in blood clots, myosin and tropomyosin in muscles etc.

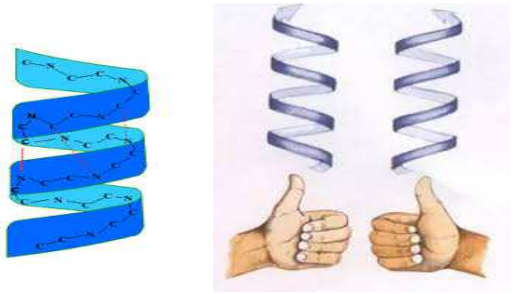


Fig.12.4. α-helix structure of protein (Left and Right handed helix)

β-Pleated Sheets: In this type of confirmation the backbone of polypeptide chain is extended into a zigzag rather than helical structure. The zigzag polypeptides are arranged side by side to form pleats like structure, thus called β-pleated sheets. In this type of structure hydrogen bonds are formed between two adjacent polypeptide chains rather than within the same polypeptide chain as in α-helix. There are two types of β-pleated sheets parallel and anti parallel.

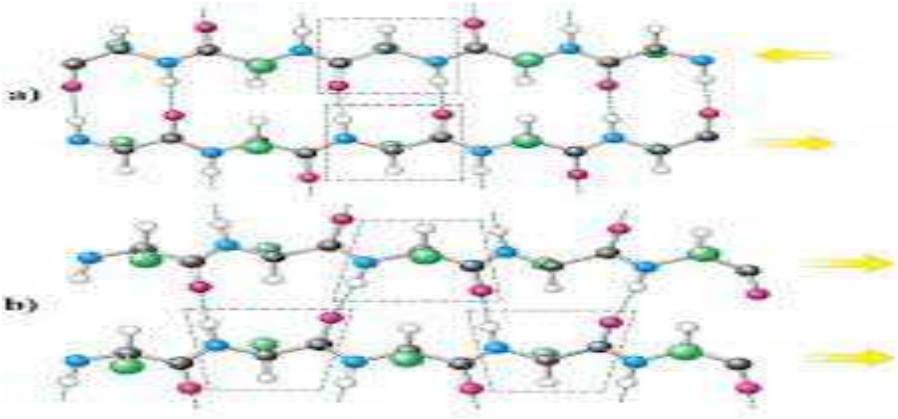


Fig.12.5. β-pleated structure of protein (a) Anti parallel β-pleated sheet (b) Parallel β-pleated sheet

In parallel β-pleated sheets, the adjacent hydrogen bonded polypeptide chains run in the same direction i.e. the N-terminal end of the polypeptide chains point in the same direction while in the antiparallel β-pleated sheets the adjacent hydrogen bonded polypeptide chains run in the opposite directions. Example of parallel β-pleated sheet is β-keratin and antiparallel β-pleated sheet is silk fibroin.

12.4.3 TERTIARY STRUCTURE

Tertiary structure refers to the final 3-dimentional structure that protein molecule assumes under the normal conditions by coiling and folding of the long polypeptide chain with or without a helix. This type of structure is more stable and complex than the secondary structure and is found in globular proteins. Tertiary structure is stabilized by several non covalent interactions such as hydrogen bonds, ionic bonds and hydrophobic interactions. The only covalent linkage involved in tertiary structure is disulphide bond formed between two cystein residues. Myoglobin, cytochrome C and ribonuclease exist in tertiary structure.

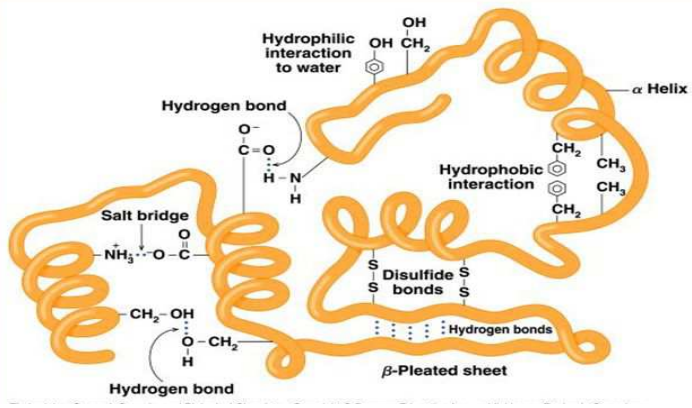


Fig.12.6. Tertiary structure of protein

Myoglobin is a primary oxygen carrying pigment of muscle tissues. In 1957, Jhon Kendrew and associates successfully determined the structure of myoglobin by high resolution X-ray crystallography. Myoglobin is a single polypeptide chain of 153 amino acid residues containing a heme group in the center. The molecular weight of myoglobin is 16700, Daltons. It is an extremely compact molecule with overall dimensions with 45×35×25 Å. A myoglobin polypeptide is made up of eight separate right handed α-helices interrupted by short non helical regions. Each myoglobin molecule contains one heme prosthetic group inserted into a hydrophobic cervice or pocket in the protein. Each heme residue contains one central coordinately bound iron atom that is normally in the ferrous (Fe^{2+}) form. When exposed to oxygen the Fe^{2+} atom of the isolated heme is irreversibly oxidized to the ferric (Fe^{3+}) form. Myoglobin is the oxygen carrier in the muscles and the oxygen carrying (binding) capacity depends on the presence of heme. The protein portion of myoglobin prevents this oxidation and makes it possible for O_2 to bind reversibly to the heme group.

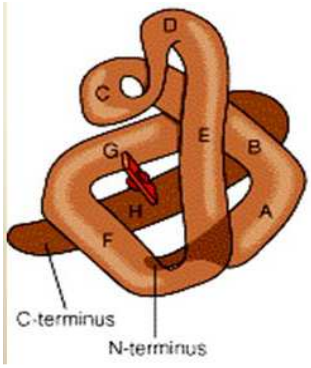


Fig.12.7. Structure of myoglobin

12.4.4 QUATERNARY STRUCTURE

Quaternary structure of proteins concerns the non covalent association of two or more polypeptide chains to form an ordered biologically active protein. The individual polypeptides are called subunits or protomers and ordered total proteins is called oligomer. The forces involved in binding the subunits are same as those in tertiary structure e.g. hydrophobic interaction, hydrogen bonding and ionic bonding are quite common in oligomeric proteins. If the subunits in an oligomer are identical the protein is said to be homogenous e.g. enzyme phosphorylase a, contains two subunits which are identical to each other. However, if the subunits are different, the protein is heterogeneous e.g. human haemoglobin consists of four subunits two subunits of one type α and two subunits of another type β.

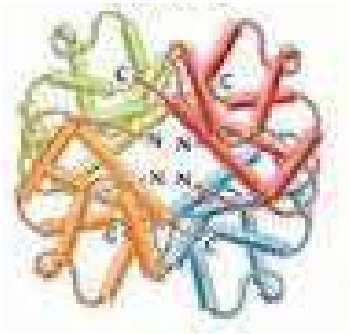


Fig.12.8. Quarternary structure of protein

Hemoglobin is a respiratory pigment present in the red blood corpuscles (RBCs in the blood) of most of the animals. The structure of hemoglobin was determined by Max Perutz and associates by X-ray analysis. They revealed that the hemoglobin molecule is roughly spherical with a diameter of about 5.5 nm. Mammalian hemoglobin is a tetrameric protein consists of two α and two β chains which held together by non-covalent factors. The molecular weight of the molecule is 68000 Daltons and has overall dimensions 64×55×50 Å. Haemoglobin contain four iron containing heme prosthetic groups, each with one subunit. In haemoglobin iron remain in ferrous (Fe^{2+}) form. The protein part of hemoglobin helps heme to keep the iron in Fe^{2+} form and to combine loosely and reversibly with oxygen.

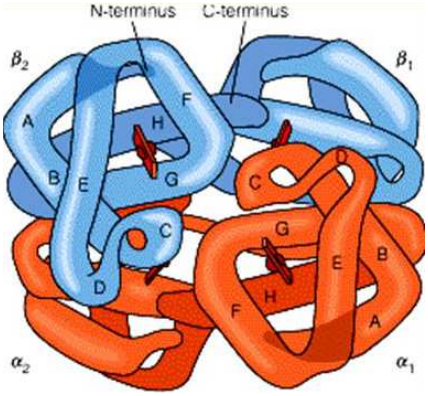


Fig12..9. Structure of haemoglobin

12.5 GENERAL PROPERTIES OF PROTEINS

12.5.1 SOLUBILITY

The solubility of protein varies to the native because proteins are colloids of large-sized molecules these form turbine solution in water. These are also soluble in acid and salt solution while insoluble in alcohol.

12.5.2 AMPHOTERIC NATURE

Like a-acid proteins are amphoteric in nature. They behave as acid alkaline solution and alkaline to acidic solution due to presence of several free- NH_2 and COOH groups.

12.5.3 ZWITTERION FORMATION

The protein is either positively or negatively charged molecule and in an electric field migrate either towards cathode or towards anode. They are electrically neutral and do not move towards any pole.

12.5.4 HYDROLYSIS

The protein undergoes hydrolysis by acid, alkali or hydrolytic enzymes which lead the protein to amino acids. Complete hydrolysis with HCl or H_2SO_4 and yields free a-acid thin breakdown products.

12.5.5 DENATURATION

Under certain conditions there is a disruption of secondary tertiary and quaternary structures of functional protein molecule resulting in the changes of its physical, chemical and biological characteristics. These changes occurring in proteins are collectively called denaturation. During denaturation only primary structure of protein is retained. Various physical and chemical elements such as heat, UV-rays, X-rays, ultrasonic waves, high pressure, acids, alkalis, detergents or certain organic solvents can cause denaturation of proteins. Physical and chemical properties of denatured proteins are different then the native proteins and they lose most of their

biological activities, in denatured proteins the solubility is decreased or lost. During denaturation the soluble globular proteins are changed into insoluble fibrous proteins. The process of denaturation also destroys enzyme and hormonal activity and the proteins become biologically inactive. The process of denaturation in some proteins is returned to its native stable confirmation and regains their native structure and biological activity, this process is called renaturation.

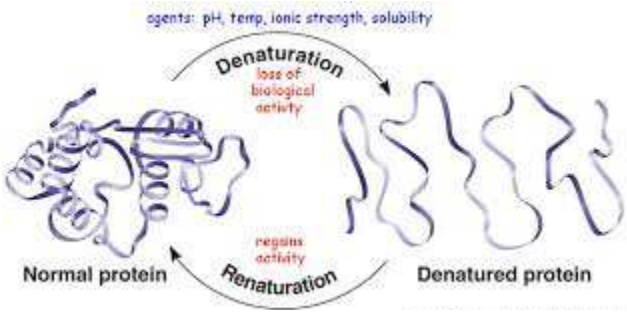


Fig.12.10. Denaturation of protein

12.6 METABOLISM OF PROTEINS

Amino acids are the building blocks of proteins and proteins are the building material in the body. Metabolism of proteins involves both biosynthesis of amino acids as well as breakdown of amino acids.

12.6.1 BIOSYNTHESIS OF AMINO ACID

There are 20 standard amino acids known and these can be classified into the group on the basis of their synthesis in human and animals are called the non essential amino acid whereas the amino acid which cannot be synthesized by the organisms and they must be obtained through diet are called the essential amino acids. The pathways for the synthesis of these two types of amino acids are quite different. Non essential amino acids can be synthesized by quite simple reaction while synthesis of essential amino acids is quite complex.

Biosynthesis of non-essential amino acids:-

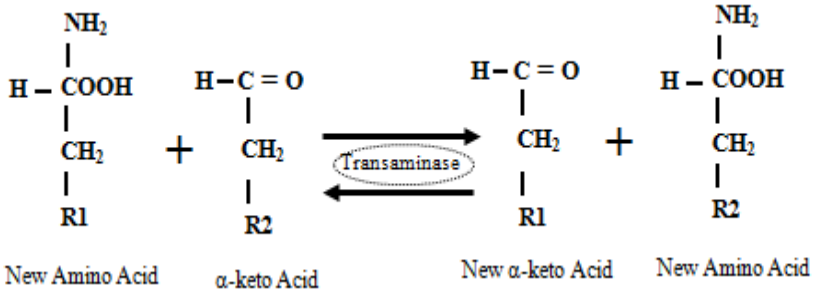
Biosynthesis of essential amino acids:-

12.6.2 CATABOLISM OF AMINO ACIDS

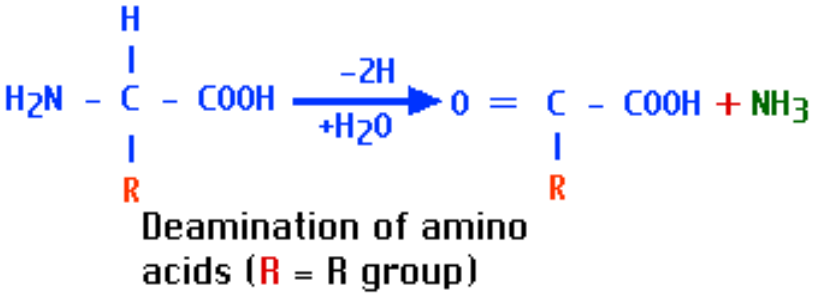
Amino acids are not only served as the building blocks of proteins but also serve as source of carbon and nitrogen, when required. The very first step in their catabolism is removal of $–NH^2$ group and formation of corresponding keto-acid. The ammonia which is librated, quickly converted to urea and it is incorporated in some other a-acid. Catabolism of a-acid involves the following process:

1. Transamination
2. Deamination
3. Urea formation
4. Decarboxylation

Transamination: Russian workers Braunstein and Bychkou had shown the importance of transamination for the first time in 1939. It is the most important process of conversion of amino acid into keto acid. In this process amino group of one amino acid (donor) is transferred to an α-keto acid (recipient) resulting in the formation of a new amino acid and a new keto acid.The donor amino acid is converted into a new keto acid and the recipient keto acid is converted into a new amino acid. Transamination is a reversible process and is catalyzed by the enzyme transaminase or amino transferase. Co-enzyme for the reaction is pyridoxal-5'-phosphate, a derivative of vitamin B_6 (Pyridoxine). Transamination takes place principally in liver, kidney, heart and brain.



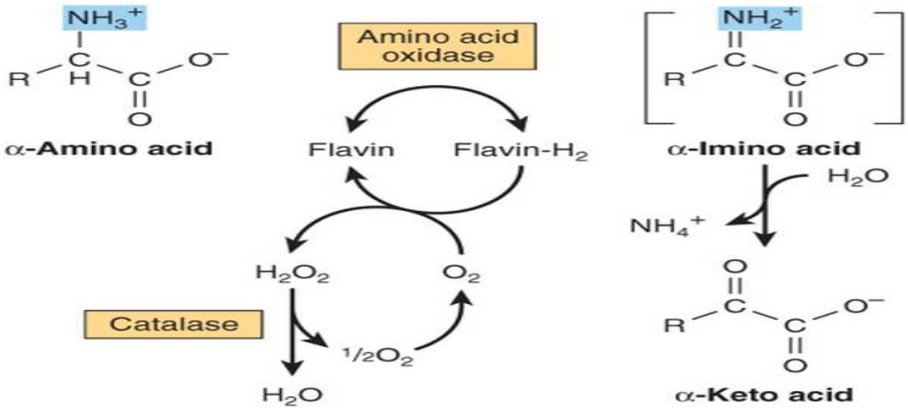
Deamination: Deamination is a process in which amino group ($-NH_2$) is removed from the amino acid, which then changes to a α-keto acid. In this process amino group is removed as ammonia.



Deamination usually takes place in liver and kidney cells to catabolize excess of amino acids. There are two types of deamination:

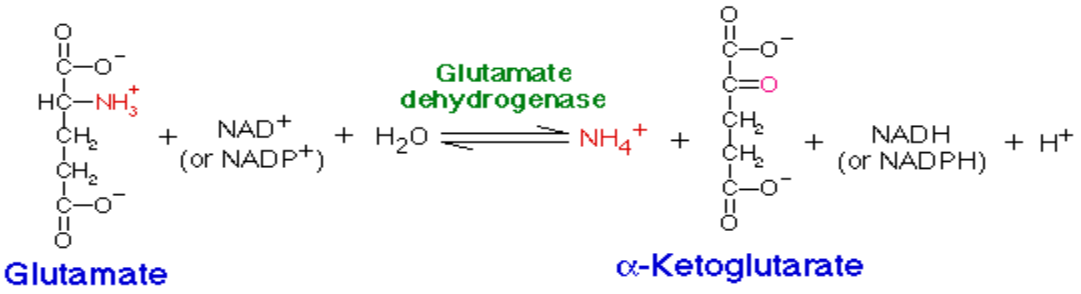
i. Oxidative deamination
ii. Non oxidative deamination

Oxidative Deamination: When deamination process is accompanied by an oxidative reaction, it is known as oxidative deamination. This process is catalyzed by a group of flavin containing enzymes known as amino acid oxidases. This is the two step reaction, in the first step the amino acid is dehydrogenated by the flavoprotein of the enzyme L-amino oxidase to form α-imino acid. In the next step with addition of H_2O molecule, α-imino nitrogen is released as NH_3 and α- keto acid is formed.



The enzyme amino acid oxidase is auto oxidizable flavoprotein. Reduced flavoprotein is oxidized to form hydrogen peroxide, which then broken up into H_2O and O_2 by the enzyme catalase.

Non oxidative Deamination: There are certain amino acids which can be deaminated non oxidatively. Non oxidative deamination is catalyzed by specific enzymes and NH_3 is liberated in this process. One example of non oxidative deamination is deamination of glutamate by the enzyme glutamate dehydrogenase.



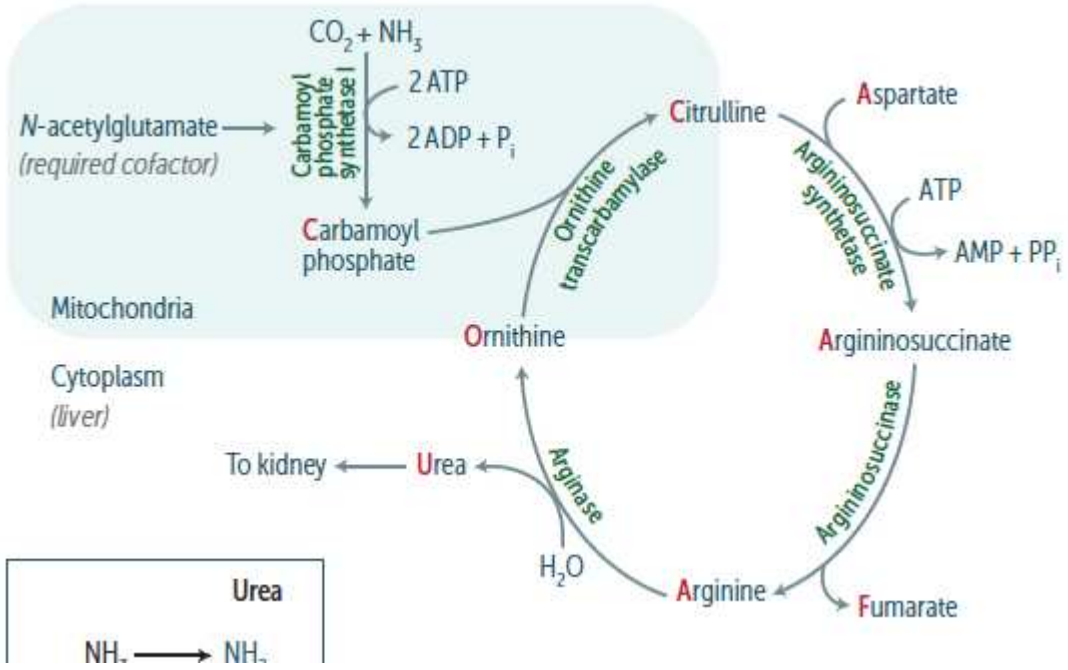
This reaction is reversible in which NAD act as coenzyme.

Fate of Deaminated Amino Acids: Acids produced from transamination and deaminations of amino acids are channeled to several metabolic routes. Some amino acids are deaminated to produce keto acids which are eventually oxidyzed to CO_2 and H_2O through acetoacetate and acety-co-A. ***Acetoacetone*** is one of the chemical constituent of 'ketone bodies' formed in the pathological conditions of urine. Thus the amino acid which leads to the formation of acetoacetate during their metabolism are called ketogenic e.g. leucine & lycine. Some amino acids are deaminated to produce keto acids which are broken down to pyruvate, α-keto glutarate, succinyle co-A, fumerate or oxaloacetate which can be utilized for the synthesis of glucose or glycogen. This amino acid is called glycogenic or antiketogenic amino acid example of glucogenic amino acids are called glucogenic or antiketogenic amino acids. Examples of glucogenic amino acids are alanine, glycine, serine, aspertate, glutamate, valine, histidine argine, proline, metheonine, cystine and arginine. Some amino acids are precursors of both glucose and ketone bodies are known as glucogenic and ketogenic amino acids. Examples are phenylalanine, tyrosine, isoleucine, threonine and tryptophan.

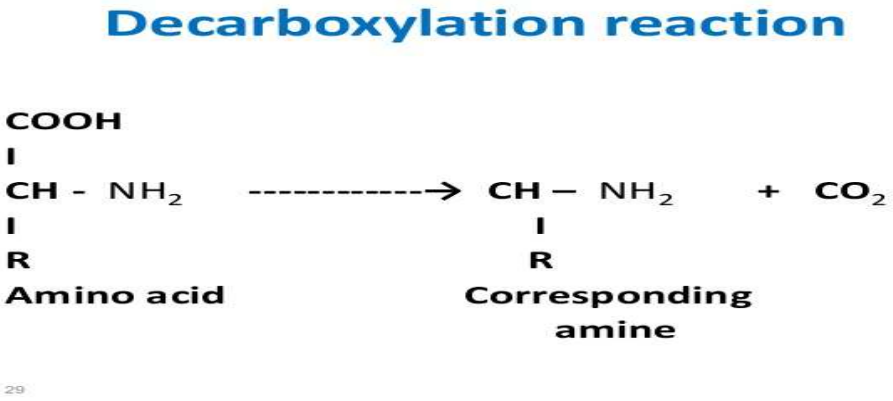
Fate of Ammonia: Ammonia released during deamination is either converted into ammonium salt or into urea.

Formation of ammonium salts: - Ammonium ions (NH_{4+}) are formed from some of the ammonia released by deamination of amino acids. These ammonium ions are excreted out from the body in the form of ammonium salts.

Formation of Urea (Urea cycle): When production of ammonia exceeds beyond a certain level it become toxic. Excess of ammonia produced during the deamination of amino acids is converted to less toxic substance, urea, before been excreted in the urine. Formation of urea is a cyclic process and the cycle is known as urea cycle. This cycle was first outlined by Hans Krebs and Kurt Henseleit in 1932; hence it is also known as Kreb's Hanseleit cycle. The chief site for urine formation is liver and after its formation urea passes into the blood stream and from blood to kidneys and finally excreted into the urine. Urea synthesis takes place in five steps. Each step is catalyzed by a specific enzyme. Out of these five enzymatic reactions, two take place in the mitochondria and three take place in the cytoplasm. This cycle is also known as ornithin cycle as it involves conversion of amino acid ornithin to citrulline though glutamic acid which is derived from aspartic acid by transamination and/or form α-ketoglutaric acid and ammonia.



Decarboxylation: Decorboxylation is the process in which CO_2 is removed from the carboxyl group of an amino acid resulting the formation of an amine. Enzyme aminoacid decarboxylases catalyse these reactions which require pyridoxal phosphate as coenzyme.



For example histdine is decarboxylated to histamine by histidine decarboxylase and 3,4-dihydroxyphenylalanine is decarboxylated to dopamine. These types of amines are called biogenic amines. Many of these amines have strong pharmacological effects and others are important as precursors of hormones or as co-enzymes.

12.7 SOURCES OF PROTEINS

Proteins are widely distributed in plants and animals. Common sources of proteins are milk, yogurt, cheese, fishes, beans, nuts, green peas, meat, eggs, lentils, soy products, quinoa and sea foods.

12.8 BIOLOGICAL SIGNIFICANCE OF PROTEINS

Proteins are the most significant macromolecules in living beings because of the following physiological roles performed by them in all biological processes:

1. Proteins which are involved in the formation and maintenance of various cellular structures are called structural proteins. These proteins form an important part of all membranes and membrane bound organelles of the cell. The cell wall and primary fibrous of the cell have structural proteins e.g. Collagen is the most abundant fibrous protein found in animals forming a major part of the skin, cartilage, ligament, tendons

and bones. Keratin is another animal protein involved in the formation of scales, hair, feather, horns, hoofs, fur wool, nails and claws.

2. Capacity of motion and flexibility in the organisms is due to the presence of certain proteins called contractile proteins e.g. Muscle proteins- actin and myosin.
3. Proteins acts as enzymes or biocatalysts and catalyzes a variety of chemical reactions in the living organisms. Almost all the enzymes are protein in nature.
4. Some proteins bind and transport specific types of molecules via blood e.g. haemoglobin is important protein, transports oxygen from lungs to cell tissues. Myoglobion binds and transports oxygen in the muscles. Certain membrane proteins transport ions and small molecules across the cell membrane.
5. Some proteins are stored as reserve food such as albumin in egg and gluttelin in rice. In the liver ferritin stores iron.
6. A few proteins functions as hormone e.g. insulin.
7. Proteins also play important role in the immune system of vertebrates. Antibodies are immunoglobulins which combine and neutralize the antigen entering the body.
8. Proteins also take part in blood coagulation e.g. thrombin and fibrinogen.

12.9 DEFICIENCY DISEASES OF PROTEINS

Protein deficiency can lead to weak muscle tone, thin and brittle hair, edema or swelling, skin lesions, fatigue, stunted growth and cognitive development as well as mental health in children. Following diseases can occur due to protein deficiency:

Marasmus: It affects infants and very young children, often resulting in weight loss and dehydration. People with marasmus appear bony with little muscle tissue.

Kwashiorkor: It usually affects older children. People with Kwashiorkor appear swollen stomach due to fluid retention.

Cachexia: It is a condition that involves protein deficiency, depletion of skeletal muscle and an increased rate of protein degradation. It causes weight loss and mortality and is associated with cancer, AIDS, heart disease and chronic kidney failure.

12.10 SUMMARY

- Proteins are polymers of amino acids in which amino acids are joined together by peptide bonds.
- Proteins are the most abundant and significant compounds in the living body.
- Proteins are involved in wide variety of functions in living beings. They function as catalyst; take part in structural organization and transportation; control growth and differentiation; provide support and immune protection.
- Proteins can be classified on the basis of their functions, shape, structure and complexity.
- Protein structure can be described by its four levels of organization. Primary structure refers to the amino acid sequence. Secondary structure describes the regular polypeptide folding pattern such as α-helices and β-sheets. Tertiary structure refers to the folding secondary structural elements of the protein. Proteins with more than one polypeptide chains exhibit quaternary structure which describes the spatial rearrangement and noncovalent association of the subunits in a protein.
- Proteins are soluble in acids and salt solutions, amphoteric in nature and denatured by heat, acids, detergents and certain radiations.
- Amino acids are the building blocks of protein thus metabolism of protein refers to the synthesis of amino acids (anabolism) and breakdown of amino acids (catabolism).
- Biosynthetic pathways for essential and non essential amino acids are quite different.
- Non essential amino acids are synthesized in simple pathways from pyruvate, oxaloacetate, α-ketoglutarate or 3-phosphoglycerate whereas the pathways for the synthesis of essential amino acids are much more complicated as compared to the synthesis pathways of non essential amino acids.
- Degradation of amino acids involves transamination, deamination and decarboxylation.
- Urea synthesis takes place in five different steps, each step is catalyzed by a specific enzyme.

12.11 GLOSSARY

Anabolism: A process where complex molecules are synthesized from simple molecules.

Catabolism: The metabolic process in which complex molecules are degraded into simple molecules usually accompanied by the release of energy.

Catalyst: A substance which increases the rate of a chemical reaction without undergoing a permanent change.

Collagen: A group of fibrous proteins which consists of extensively cross linked molecules of tropo-collagen.

Conformation: The three dimensional structure of a molecule.

Deamination: Hydrolytic removal of an amino group of an amino group from an amino acid.

Denaturation: The unfolding of the native conformation of a protein by its exposure to heat or chemicals.

Disulfide bond: A covalent link formed by a cystine residue.

Enzyme: An enzyme is a biocatalyst which increases the rate of a reaction.

Essential amino acids: Amino acids which are not synthesized by the body. These must be supplied in diet.

Globular proteins: Compact and spherical molecules of polypeptide chains which are soluble in water.

Glocogenic amino acids: Amino acids which can be degraded to glucose or glucogenic precursors.

Glycoprotein: A protein containing carbohydrate.

Haemoglobin: A respiratory pigment of animals consisting of protein-globin and an iron containing prosthetic group known as heme.

Heme: An iron containing porphyrin, heme is the prosthetic group in the oxygen transport proteins haemoglobin and myoglobin.

Hormone: A substance released by an endocrine gland which has variety of functions.

Isoelectric point: The pH at which a molecule has no net charge.

Myoglobin: Heme containing oxygen carrying protein of muscles.

Peptide: A short linier chain of amino acids linked by peptide bonds.

Peptide bond: Linkage between the α-amino group of one of the amino acid and α-carboxylic group of another amino acid.

Polypeptide: A polymer consisting of amino acids linked together by peptide bonds.

Primary structure: The amino acid sequence of a protein.

Protein: A macromolecule which consists of one or more polypeptide chain.

Quaternary structure: Spatial arrangement of different polypeptide chains of oligomeric proteins.

Secondary structure: Folding of contiguous segment of polypeptide chains into geometrically ordered units.

Tertiary structure: The three dimensional structure of a protein.

Urea cycle: A metabolic process which results in the formation of urea.

Zwitter ion: A molecule which bears oppositely charged groups.

EXERCISE

Long answer type questions

1. What are proteins? Classify proteins on the basis of their structure and complexity.
2. Describe the α-helix and β-pleated sheet structures of proteins.
3. Discuss the biological significance of proteins in detail.
4. Discuss general properties of proteins.
5. Describe the biosynthesis of non-essential amino acids.
6. What is deamination? Explain the two types of deamination.
7. Describe urea cycle in detail.

Short Notes

1. Denaturation of proteins
2. Conjugated protein
3. Fibrous and globular proteins
4. Haemoglobin and Myoglobin
5. Glycoproteins
6. Biosynthesis of tyrosin

7. Transamination
8. Decarboxylation

Fill in the blanks

1. A disulphide bond is formed between those amino acids which have…….group.
2. ……stores oxygen in the muscles.
3. …… and …….proteins helps in contraction of muscles.
4. ……is the common precursor in the synthesis of lysine, metheonine and threonine.
5. Oxidative deamination is catalyzed by a group of flavin enzymes, known as ……
6. Amino acids which are precursors of glucose are called as……..... amino acids.

Answers:

Fill in the blanks

1) Suphydryl (-SH) 2) Myoglobin 3) Actin and Myosin 4) Aspartate 5) Amino acid oxidases 6) Glucogenic

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Note: All the images are taken from internet sources.

UNIT 13: ENZYMES

CONTENTS

13.1 OBJECTIVES

- To understand what are enzymes.
- To understand basic concepts, classification and their properties.
- Details of their mechanism of action and factors affecting enzyme activity
- To know about their sources, significance and deficiencies of enzymes.

13.2 INTRODUCTION

The life depends on a sequence of chemical reactions. The greatest majority of these biochemical reactions do not take place spontaneously. Most of the chemical reactions proceed too slowly on their own to sustain life. Hence catalysts are required to greatly accelerate the rates of these chemical reactions. Catalysis is defined as the "acceleration of a chemical reaction by some substance which itself undergoes no permanent chemical change". The phenomenon of catalysis makes possible biochemical reactions necessary for all life processes. The catalysts of biochemical reactions are enzymes and are responsible for bringing about almost all of the chemical reactions in living organisms.

The existence of enzymes has been known for well over a century. French chemist Anselme Payen was the first to discover an enzyme, diastase, in 1833. A few decades later Louis Pasteur recognized in 1860 that enzymes were essential to fermentation but assumed that their catalytic action was intricately linked with the structure and life of the yeast cell. In 1877, German physiologist Wilhelm Kuhne (1837–1900) first used the term *enzyme*, which comes from Greek "leavened" (Leavening makes bread rise), to describe this process. Not until 1897 was it shown by German chemist Edward Buchner that cell-free extracts of yeast could ferment sugars to alcohol and carbon dioxide; Buchner denoted his preparation *zymase.* This important achievement was the first indication that enzymes could function independently of the cell.

The first enzyme molecule to be isolated in pure crystalline form was urease, prepared from the jack bean in 1926 by American biochemist J. B. Sumner, who suggested, contrary to prevailing opinion, that the molecule was a protein. In the period from 1930 to 1936, pepsin, chymotrypsin, and trypsin were successfully crystallized; it was confirmed that the crystals were protein, and the protein nature of enzymes was thereby firmly established.

13.3 DEFINITION

Enzymes are macromolecular biological organic catalysts responsible for supporting almost all of the chemical reactions that maintain animal homeostasis proceeds without itself being altered in the process.

13.4 BASIC CONCEPTS/GENERAL PROPERTIES

1. Enzymes differ from ordinary chemical catalysts by:
   - Higher reaction rates, 10^6-10^{12}
   - Milder reaction conditions (temp, pH)
   - Greater reaction specificity (no side products) - Capacity for regulation
2. Enzyme reactions are always reversible: they accelerate, or catalyze, chemical reactions.
3. Enzymes speed up the rates at which the equilibrium positions of reversible reactions are attained.
4. In terms of thermodynamics, enzymes reduce the activation energies of reactions, enabling them to occur much more readily
5. The reactants at the beginning of the process upon which enzymes may act are called substrates and the enzyme converts these into different molecules, called products.
6. Almost all metabolic processes in the cell need enzymes in order to occur at rates fast enough to sustain life.
7. The set of enzymes made in a cell determines which metabolic pathways occur in that cell.
8. The study of enzymes is called *enzymology*.
9. Enzymes are known to catalyze more than 5,000 biochemical reaction types.
10. Most enzymes are proteins, although a few are catalytic RNA molecules.
11. Enzymes' specificity comes from their unique three-dimensional structures, each enzyme catalyzes the reaction of a single type of molecules or a group of closely related molecules.

CHEMICAL NATURE AND STRUCTURE

All enzymes were once thought to be proteins, but since the 1980s the catalytic ability of certain nucleic acids, called ribozymes (or catalytic RNAs), has been demonstrated, contesting this saying.

A large protein enzyme molecule is composed of one or more amino acid chains linked together by peptide bonds. The amino acid sequence determines the characteristic folding patterns of the protein's structure, which is essential to enzyme specificity. If the enzyme is subjected to changes, such as variations in temperature or pH, the protein structure may lose its integrity (denature) and its enzymatic ability. Denaturation is sometimes, but not always, reversible. The key to enzyme activity is a structure called active site. Interactions between residues of polypeptide chain amino acids cause them to create a structure of defined size, shape and sequence. The active site is the region of an enzyme where substrate molecules bind and undergo a chemical reaction. The active site consists of residues that form temporary bonds with the substrate (binding site) and residues that catalyse a reaction of that substrate (catalytic site). The active site is usually a groove or pocket of the enzyme which can be located in a deep tunnel within the enzyme, or between the interfaces of multimeric enzymes.

Moreover, unrelated to its active site, there is an allosteric site on an enzyme, which can bind an effector molecule. This interaction is another mechanism of enzyme regulation. Allosteric modification usually happens in proteins with more than one subunit. Allosteric interactions are often present in metabolic pathways and are beneficial in that they allow one step of a reaction to regulate another step. They allow an enzyme to have a range of molecular interactions, other than the highly specific active site.

Bound to some enzymes is an additional chemical non-protein component called a cofactor, which is a direct participant in the catalytic event and thus is required for enzymatic activity. A cofactor may be either a coenzyme—an organic molecule (which is dialyzable, thermostable and loosely attached to the protein part), such as a vitamin—or an inorganic metal ion; some enzymes require both. A cofactor may be either tightly or loosely bound to the enzyme. If tightly connected, the cofactor is referred to as a prosthetic group (- an organic substance which is dialyzable and thermostable which is firmly attached to the protein or

apoenzyme portion.

This entire active complex is referred to as the holoenzyme; i.e., apoenzyme (protein portion) plus the cofactor (coenzyme, prosthetic group or metal-ion- activator) is called the holoenzyme.

Apoenzyme + Cofactor = Holoenzyme

ZYMOGEN: A zymogen is an enzymatically inactive precursor of an enzyme, often but not always a proteolytic enzyme (or proteinase). Some zymogens are named by adding the suffix -ogen to the name of the enzyme itself, as in trypsinogen or pepsinogen, whereas others are indicated by the prefix pro-, as in pro-collagenase or pro-carboxypeptidase.

13.5 NAMING AND ENZYME CLASSIFICATION

Except for some of the originally studied enzymes such as pepsin, rennin, and trypsin, most enzyme names end in "ase" to the name of their substrate describing their activity. Sometimes the same enzyme has two or more names, or two different enzymes have the same name. Because of such ambiguities, and the ever- increasing number of newly discovered enzymes, biochemists, by international agreement, have adopted a system for naming and classifying enzymes. This system divides enzymes into six classes, each with sub- classes, based on the type of reaction catalyzed. Each enzyme is assigned a four-part classification number and a systematic name, which identifies the reaction it catalyzes. The first Enzyme Commission, in its report in 1961, devised a system for classification of enzymes that also serves as a basis for assigning code numbers to them. These code numbers, proceed by EC (enzyme commission).

(i) The first number shows to which of the six main divisions (classes) the enzyme belongs,

(ii) The second figure indicates the subclass,

(iii) The third figure gives the sub-subclass,

(iv) The fourth figure is the serial number of the enzyme in its sub-subclass.

The main divisions are:

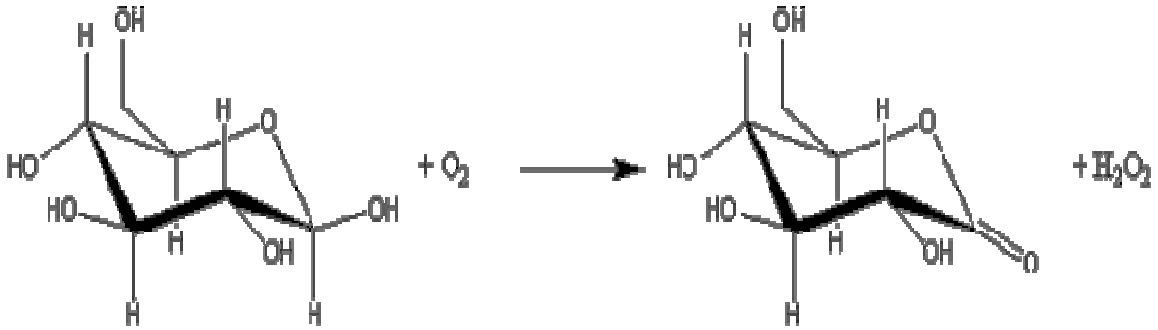
1. OXIDOREDUCTASES

Oxidoreductases are a class of enzymes that catalyze oxidoreduction reactions. Oxidoreductases catalyze the transfer of electrons from one molecule (the oxidant) to another molecule (the reductant). Oxidoreductases catalyze reactions similar to the following, $A^- + B \rightarrow A + B^-$ where A is the oxidant and B is the reductant. Trivial names of Oxidorecuctases include oxidases and dehydrogenases. Oxidases are enzymes involved when molecular oxygen acts as an acceptor of hydrogen or electrons. Whereas, dehydrogenases are enzymes that oxidize a substrate by transferring hydrogen to an acceptor that is either $NAD^+/NADP^+$ or a flavin enzyme. Other oxidoreductases include peroxidases, hydroxylases, oxygenases, and reductases.

An example, would be:

$$A^- + B \rightarrow A + B^-$$

where A=reductant (electron donor) and B=oxidant (electron acceptor).



b-D-glucose + oxygen ⟶ D-glucono-1,5-lactone + hydrogen peroxide

Hydroxylases add hydroxyl groups to its substrates. Oxygenases incorporate oxygen from molecular oxygen into organic substrates. Reductases catalyze reductions, in most cases reductases can act like an oxidases.

Peroxidases are localized in peroxisomes, and catalyzes the reduction of hydrogen peroxide.

Oxidoreductase enzymes play an important role in both aerobic and anaerobic metabolism. They can be found in glycolysis, TCA cycle, oxidative phosphorylation, and in amino acid metabolism.

2. TRANSFERASES

Transferases are the class of the enzymes that catalyze the enact transfer of specific functional groups (e.g. a methyl or glycosyl group) from one molecule (called the donor) to another (called the acceptor). Transaminases, for example, catalyze the transfer of an amino group (−NH2) from an amino acid to an a-keto acid.[2] They are involved in hundreds of different biochemical pathways throughout biology, and are integral to some of life's most important processes.

Common names include acetyltransferase, methylase, protein kinase and polymerase. The first three subclasses play major roles in the regulation of cellular processes. The polymerase is essential for the synthesis of DNA and RNA.

Transferases are involved in innumerable reactions in the cell. For example, the activity of coenzyme A (CoA) transferase, which transfers thiol esters, the action of N-acetyltransferase is part of the pathway that metabolizes tryptophan, and also includes the regulation of pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl CoA. Transferases are also utilized during translation. In this case, an amino acid chain is the functional group transferred by a peptidyltransferase. The transfer involves the removal of the growing amino acid chain from the tRNA molecule in the A-site of the ribosome and its subsequent addition to the amino acid attached to the tRNA in the P-site.

Based on the type of biochemical group transferred, transferases can be divided into ten categories (based on the EC Number classification). In the EC numbering system, transferases have been given a classification of EC2.

Systematically, a reaction would be:

$$X\,group + Y \xrightarrow{transferase} X + Y\,group$$

where, X= donor, and Y = acceptor. "Group" would be the functional group transferred as a result of transferase activity. The donor is often a coenzyme.

Example: Carbamyl phosphate + L-aspartate→ L-carbamyl aspartate + phosphate

3. HYDROLASES

Hydrolases are hydrolytic enzymes that catalyze the hydrolysis of a chemical bond, usually dividing a large molecule into two smaller molecules. Examples of common hydrolases include esterases, proteases, glycosidases, nucleosidases, and lipases.

Hydrolases carry out important degradativereactions in the body. During digestion, lipases hydrolyze lipids and proteases convert protein to amino acids. Hydrolases cleave large molecules into fragments used for synthesis, the excretion of waste materials, or as sources of carbon for the production of energy. In these reactions, many biopolymers are converted to monomers. Some hydrolases release energy as they act.

Systematically, a reaction would be:

$A–B + H_2O \rightarrow A–OH + B–H$

Example: Nucleases splits nucleic acids (DNA and RNA). Based on the substrate type, they are divided into RNase and DNase. RNase catalyzes the hydrolysis of RNA and DNase acts on DNA. They may also be divided into exonuclease and endonuclease. The exonuclease progressively splits off single nucleotides from one end of DNA or RNA. The endonuclease splits DNA or RNA at internal sites.

4. LYASES

Lyase is an enzyme that catalyzes the breaking (an "elimination" reaction) of various chemical bonds (C-C, C-O, C-N) by means other than hydrolysis (a "substitution" reaction) and oxidation. These bonds are cleaved by the process of elimination and the resulting product is the formation of a double bond or a new ring. The reverse reaction is also possible (called a "Michael addition"). For example, an enzyme that catalyzed this reaction would be a lyase:

$ATP \rightarrow cAMP + PP_i$

Lyases differ from other enzymes in that they require only one substrate for the reaction in one direction, but two substrates for the reverse reaction.

These bonds are cleaved by the process of elimination and the resulting product is the formation of a double bond or a new ring. This class of enzymes differs from other enzymes in that two substrates are involved in one reaction direction, but only one substrate is involved in the other direction. To generate either a double bond or a new ring, the enzyme is acted upon the single substrate and a molecule is eliminated.Lyases are classified as EC 4 in the EC number classification of enzymes

5. ISOMERASES

Isomerases are a class of enzymes which convert a molecule from one isomer to another, meaning that the end product has the same molecular formula but a different physical structure. Isomers themselves exist in many varieties but can generally be classified as structural isomers or stereoisomers. They can either facilitate it by intramolecular rearrangements in which bonds are broken and formed or they can catalyze conformational changes. The general form of such a reaction is as follows:

A–B → B–A

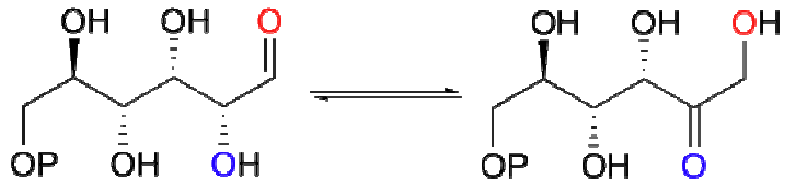
Where A and B = Isomers.

There is only one substrate yielding one product. This product has the same molecular formula as the substrate but differs in bond connectivity or spatial arrangements. Isomerases catalyze reactions across many biochemical pathways, such as in glycolysis and carbohydrate metabolism. All isomerases have Enzyme Commission numbers beginning in EC 5. A variety of isomerizations can be carried out, including racemization, cis-trans isomerization, enolization, and many others. Examples of isomerases include triose phosphate isomerase, and bisphosphoglycerate mutase.

Isomerases can help prepare a molecule for subsequent reactions such as oxidation-reduction reactions. Additionally, isomerases can catalyze phosphorylation reaction pathways throughout the Kreb Cycle by preparing the molecule for oxidation states. The change in

position is facilitated through Isomerases without affecting the overall chemical composition of the substrate or product.

Example:In the phosphoglucoseisomerase reaction, glucose-6-phosphate (an aldehyde sugar) and fructose-6-phosphate (a ketone sugar) are interconverted



glucose-6-phosphate fructose-6-phosphate

6. LIGASES

Ligases also called synthetases are the enzymes that catalyze reactions which make bonds to join together (ligate) smaller molecules to make larger ones. Ligase enzymes tend to raise the energy of a system, but the hydrolysis of ATP is often coupled with these reactions to make the reaction spontaneous. All enzymes tend to have the same basic catalytic effect in that they lower the overall activation energy often by moving the two substituents into close proximity. In general, a ligase catalyzes the following reaction:

$Ab + C \rightarrow A–C + b$

or sometimes

$Ab + cD \rightarrow A–D + b + c + d + e + f$

Where the lowercase letters denote the small, dependent groups. Ligase can join two complementary fragments of nucleic acid and repair single stranded breaks that arise in double stranded DNA during replication.

Example: a tyrosine-tRNA ligase is an enzyme that catalyzes the chemical reaction the 3 substrates of this enzyme are ATP, L-tyrosine, and RNA, whereas its 3 products are AMP, diphosphate, and L-tyrosyl-tRNA (Tyr).

This enzyme belongs to the family of ligases, to be specific those forming carbon-oxygen bonds in aminoacyl-tRNA and related compounds.

Tyrosine t-RNA Synthetase

ATP+ L-Tyrosine+ t-RNA ⟶ L-tyrosyl-tRNA + AMP + PPi

ENZYME UNITS

Enzyme activity is measured as the amount of the substrate lost per unit time.The enzyme commission of the International Union of Biochemistry (IUB) defined enzyme unit (U), later known as (IU), as the amount of enzyme that catalyzes the reaction of 1 μmol of substrate per minute under specified conditions.

Later the term "Katal" was introduced by the Commission on Biochemical Nomenclature as the SI (Systeme International) unit of enzyme activity as follow:The amount of enzyme causing loss of 1 mol substrate per second under specified conditions.

However, there is a direct relationship between the number of units of activity and the amount of sample assayed. Therefore to minimize these problems, units of enzyme activity may be related to the total protein content of the sample assayed, termed as "Specific activity", expressed as

International Units per mg protein or Katals per kg protein.

Turn-Over Number (k_{cat}): The number of substrate molecules converted into product in an enzyme-catalyzed reaction under saturating conditions in a given unit of time on a single enzyme molecule when the enzyme is saturated with substrate.

$k_{cat} = V_{max}/[E_{total}]$.

Where, $[E_{total}]$ = enzyme concentration and V max =maximum reaction rate

13.6 SPECIFICITY OF ENZYME ACTION

A characteristic feature of enzymes that makes them so important as diagnostic and research tools is the specificity they exhibit relation to the reactions they catalyze.some enzymes are specific for a particular type of chemical bond or functional group whereas few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. In general, there are four distinct types of specificity:

1. **Absolute, High or substrate specificity** – Such enzymes catalyze only one particular reaction.

Example:

a) Uricase, which acts only on uric acid.
b) Arginase, which acts only on arginine.
c) Carbonic anhydrase, which acts only on carbonic acid.
d) Lactase, which acts on lactose.

2. **Structural or Group specificity** - these enzymes act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.

Example:

a) Trypsin is an endopeptidase that hydrolyzes central peptide bonds in which the amino group belongs to basic amino acids e.g. arginine, lysine and histidine.
b) Chymotrypsin is an endopeptidase that hydrolyzes central peptide bonds in which the carboxyl group belongs to aromatic amino acids.
c) Aminopeptidaseis an exopeptidase that hydrolyzes peripheral peptide bond at the amino terminal (end) of polypeptide chain.

3. Linkage specificity–These enzyme acts on a particular type of chemical bond regardless of the rest of the molecular structure.

Example:

a) Amylase, which acts on α 1-4 glycosidic, bonds in starch, dextrin and glycogen.

b) Lipase that hydrolyzes ester bonds in different triglycerides

4. Stereo chemical specificity - these enzymes act on a particular steric or optical isomer.

Example:

a) L amino acid oxidase acts only on L amino acids. □

b) D amino acid oxidase acts only on D amino acids. □

c) α- glycosidase acts only on α- glycosidic bonds, which are present in starch, dextrin and glycogen. □

ENZYME CATALYSIS

Enzyme catalysis is the increase in the rate of a chemical reaction by the active site of a protein. The mechanism of enzyme catalysis is similar in principle to other types of chemical catalysis.

An enzyme provides a specific environment within which a given reaction can occur more rapidly. An enzyme-catalyzed reaction takes place within the restricted pocket on the enzyme called the active site. The molecule that is bound in the active site and acted upon by the enzyme is called the substrate. The surface of the active site is lined with amino acid residues with substituent groups that bind the substrate and catalyze its chemical transformation. The enzyme-substrate complex, whose existence was first proposed by Charles-AdolpheWurtz in 1880, is fundamental to the action of enzymes.

MECHANISM OF CATALYSIS

A simple enzymatic reaction is as follows

E+ S ⇔ES ⇔EP ⇔E P

Where E=enzyme, S= substrate and P = product ES and EP are transient complexes of the enzyme with the substrate and with the product.

The function of a catalyst is to increase the rate of a reaction. Catalysts do not affect reaction equilibria. And so are the enzymes, the bidirectional arrows put in the equation on page make the point clear: any enzyme that catalyzes the reaction, S → P also catalyzes the reverse reaction, P → S. Its only role is to accelerate the interconversion of S and P. The enzyme is not consumed in the process, and the equilibrium point remains unaffected. However, the reaction reaches equilibrium much faster when the appropriate enzyme is present because the rate of the reaction is increased.

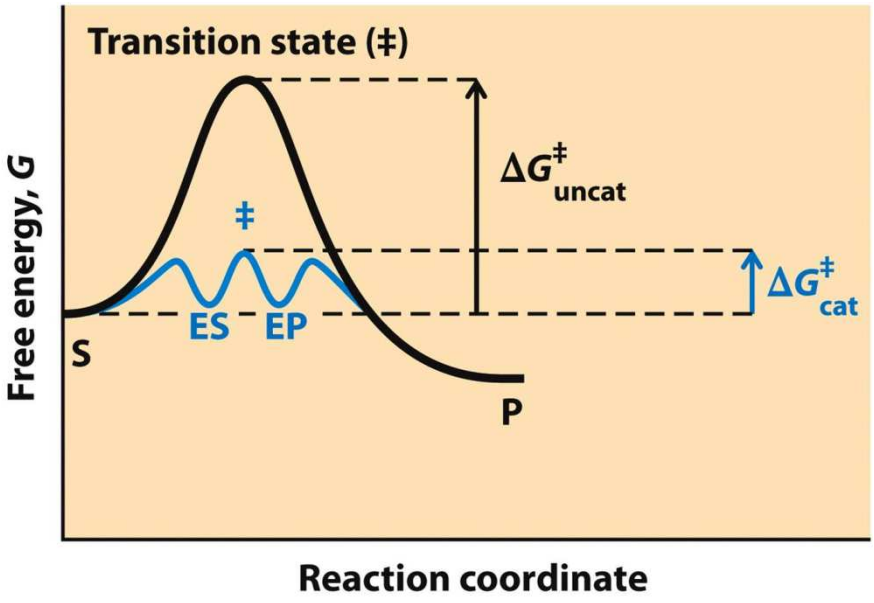
Energy in biological systems is described in terms of free energy, G. In the Fig no. 1-A, the free energy of the system is charted against the progress of the reaction (the reaction coordinate). The starting point for both the reaction (forward or reverse) is called the ground state, the contribution to the free energy of the system by an average molecule (S or P) under a given set of conditions. the free energy change for this reacting system under standard set of conditions (temperature, 298 K; partial pressure of gases, each 1 atm or 101.3 kPa ; pH = 0; concentration of solutes, each 1M) and call this as standard free-energy change, $\Delta G°$. Because biochemical systems commonly involve H+ concentrations far from 1M, biochemists define a constant $\Delta G'°$, the standard free-energy change at pH 7.0.

The equilibrium between S and P reflects the difference in the free energies of their ground states. the free energy of the ground state of P is lower than that of S, so G for the reaction is negative and the equilibrium favors P. The position and direction of equilibrium are not affected by any catalyst.

A favorable equilibrium does not mean that the S →P conversion will occur at a noticeable rate. There is an energy barrier between S and P: the energy required for arrangement of reacting groups, formation of transient unstable charges, bond rearrangements, and other alterations required for the reaction to proceed in both direction. To undergo reaction, the molecules must

overcome this "energetic hill" or barrier and therefore must be raised to a higher energy level. At the top of the energy hill is a point at which deterioration to the S or P state is uniformly possible This is called the transition state not be confused with a reaction intermediate (such as ES or EP). It is basically a transient molecular moment in which events such as bond breakage, bond formation, and charge development have proceeded to the precise point at which decay to either substrate or product is equally possible. The difference between the energy levels of the ground state and the transition state is the activation energy, G. The rate of a reaction reflects this activation energy: higher activation energy corresponds to a slower reaction. Reaction rates can be increased by raising the temperature, thereby increasing the number of molecules with sufficient energy to overcome the energy barrier. Alternatively, the activation energy can be lowered by adding a catalyst (Fig.no.1-B).Catalysts enhance reaction rates by lowering activation energies.

(A)



(B)

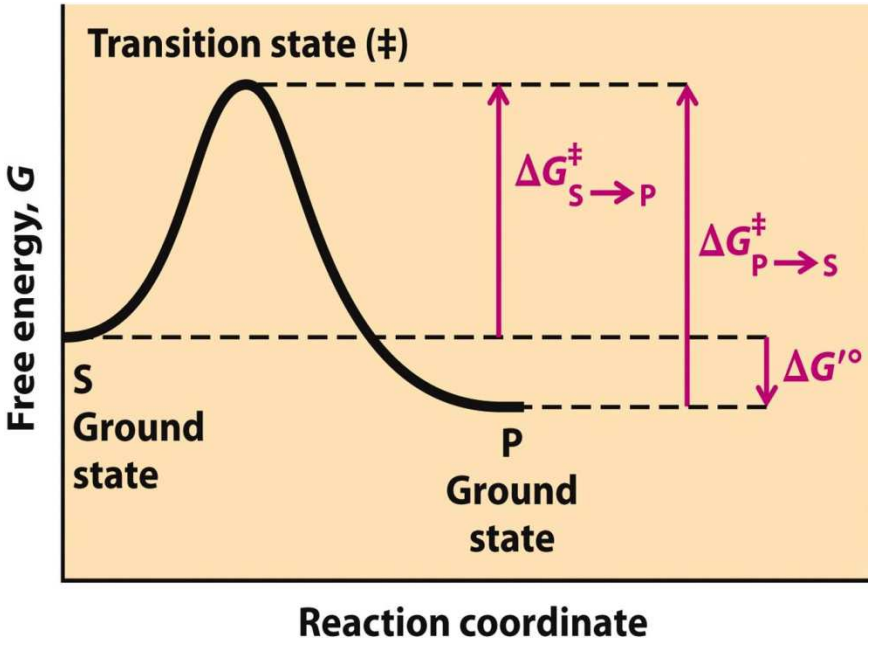


Figure No. 13.1 (A) Reaction coordinate diagram for a chemical reaction. (B)Reaction coordinate diagram comparing enzyme- catalyzed and uncatalyzed reactions

LOCK AND KEY MODEL

In order to explain why enzymes have such a high level of specificity, Emil Fischer in 1894 suggested that both a substrate and an enzyme have specific geometric shapes that fit exactly into each other **(Fig.No.2).** This enzyme-substrate complex is highly unstable and almost immediately this complex decomposes to produce the end products of the reaction and regenerates the free enzyme. The enzyme-substrate union results in the release of energy. It is this energy, which in fact, raises the energy level of the substrate molecule, thus inducing the *activated state*, in which certain bonds of the substrate molecule become more susceptible to cleavage.

This idea of both substrates and enzymes having a natural geometric fit has been called the lock and key hypothesis.

The problem with this hypothesis is that it doesn't explain the stabilization of the enzyme. When an enzyme has a substrate enter into its active site, the enzyme will change its shape slightly to

match the substrate. If the enzymes were to be specifically designed to fit a substrate, then there would be no need for it to have to adjust its shape.

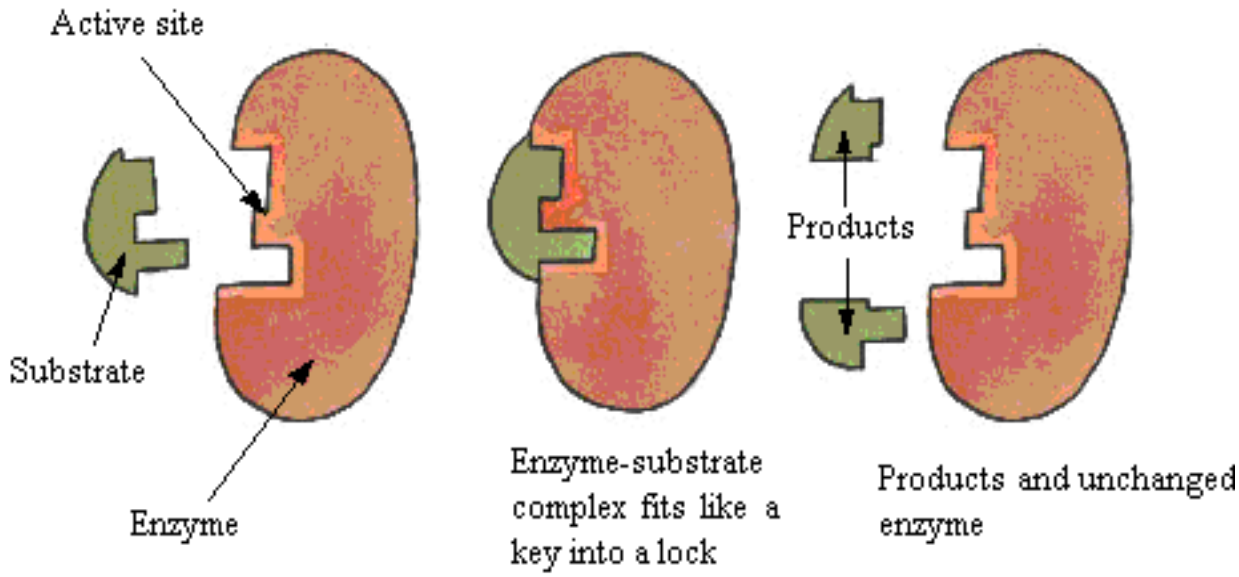


Fig. No. 13.2. Lock and Key model

INDUCED FIT MODEL

In 1958, another scientist named Daniel Koshland suggested a slight modification to the lock and key hypothesisto explain the enzyme properties more efficiently. Koshland's suggestion was that since enzymes were so flexible, the active site is constantly being reshaped by its interaction with the substrate. Koshland presumed that the enzyme molecule does not retain its original shape and structure. But the contact of the substrate *induces* some configurational or geometrical changes in the active site of the enzyme molecule (Fig No. 3). Consequently, the enzyme molecule is made to *fit* completely the configuration and active centres of the substrate.

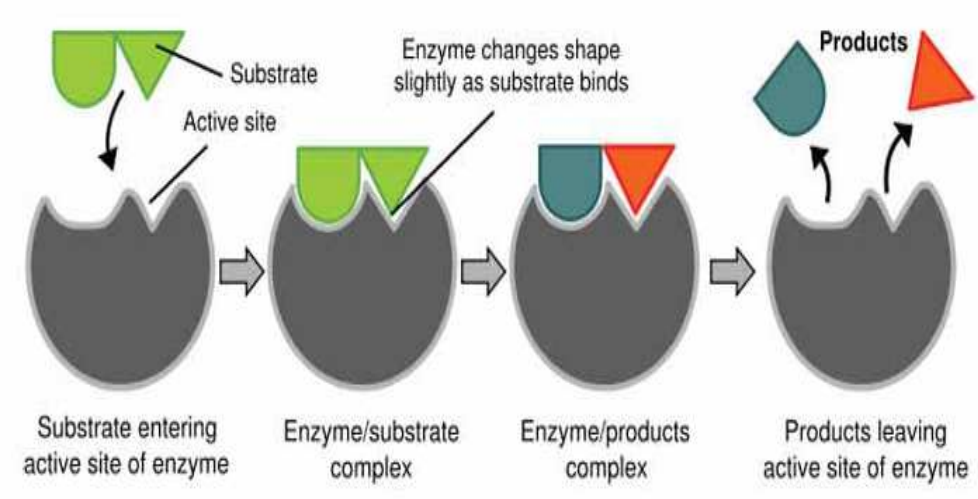


Fig.no. 13.3 Induced-fit Model

Moreover, that substrate doesn't bind to an active site as if it were specifically the right shape, but that the amino acid side-chains that are a part of the active site are molded into a specific position. This position allows the enzyme to start the catalyzing process. Koshland's modified suggestion has been called the induced fit theory.

Types of Catalytic Mechanisms

Enzyme catalyzed reactions are typically 10^7 to 10^{14} times faster than the uncatalyzed reaction.After binding takes place, one or more mechanisms of catalysis lowers the energy of the reaction's transition state, by providing an alternative chemical pathway for the reaction. There are seven possible mechanisms of "over the barrier" catalysis as well as a "through the barrier" mechanism:

1. Acid-base catalysis
2. Covalent catalysis
3. Metal ion catalysis
4. Proximity and orientation effects
5. Electrostatic catalysis
6. Bond strain
7. Quantum tunneling

These mechanisms are not mutually exclusive, and a given enzyme might incorporate several types in its overall mechanism of action. For most enzymes, it is challenging to compute the role of any one catalytic mechanism to the rate or specificity of a particular enzyme-catalyzed reaction.

1. Acid-Base Catalysis

The mechanism of acid- and base-catalyzed reactions is explained in terms of the Bronsted Lowry concept of acids and bases as one in which there is an initial transfer of protons from an acidic catalyst to the reactant or from the reactant to a basic catalyst. In terms of the Lewis theory of acids and bases, the reaction involves sharing of an electron pair donated by a base catalyst or accepted by an acid catalyst.

There are two types of acid-base catalysis:

- General acid-base catalysis
- Specific acid-base catalysis

The term "general" refers to the fact that any acid or base we add to the solution will affect the rate of the reaction, and hence the catalysis is quite general. The term" specific" refers to the fact that just one acid or base, that from the solvent, affects the rate. The catalysis is therefore very specific.

2. Covalent Catalysis

It involves a temporary covalent bond is formation between the enzyme (residues in the enzyme's active site or with a cofactor usually a nucleophile) and the substrate. This adds an additional covalent intermediate to the reaction, more reactive than the substrate itself originally was and helps to reduce the energy of later transition states of the reaction. The covalent bond must, at a later stage in the reaction, be broken to regenerate the enzyme. This mechanism is utilised by the enzymes such as proteases like chymotrypsin and trypsin.

Assume the hydrolysis of a bond between groups A and B:

A-----B $\xrightarrow{H_2O}$ A + B

covalent catalyst (an enzyme with a nucleophilic group Z:) the reaction becomes

A-----B+ Z: ⟶ A-----Z: + B $\xrightarrow{H_20}$ A + Z: + B

This alters the pathway of the reaction, and it results in catalysis only when the new pathway has a lower activation energy than the uncatalyzed pathway. Both of the new steps must be faster than the uncatalyzed reaction. The covalent bond formed between the enzyme and the substrate can activate a substrate for further reaction in a manner that is usually specific to the particular group or coenzyme.

3. Metal Ion Catalysis

Enzymes that bind metal ions tightly are referred to as metalloenzymes. Enzymes that bind metal ions more weakly, perhaps only during the catalytic cycle, are said to be metal activated. One role for metals in metal-activated enzymes and metalloenzymes is to act as electrophilic catalysts stabilizing the increased electron density that can develop during reactions.

The metal ion acts as a bridge between the substrate and the enzyme increasing the binding energy. Alternatively, the metal ion may bridge the substrate to a nucleophilic group. The metal ion may stabilize negative charges on a leaving group to make it a better leaving group, or shield negative charges on the molecule to allow for nucleophilic attack which otherwise may have been repelled. It may also participate in oxidation-reduction reactions by changing their oxidation state. An example is liver alcohol dehydrogenase where a zinc ion stabilizes negative charge development on the oxygen atom of acetaldehyde. Another potential function of metal ions is to provide a powerful nucleophile at neutral pH.

4. Proximity and orientation effects

Enzyme catalytic efficiency arises from the specific physical conditions at enzyme catalytic sites. Enzymes bring reacting species close together. It can accelerate a reaction between two species simply by holding the two reactants close together in an appropriate orientation. Enzymes, which have specific binding sites for particular reacting molecules, essentially take the reactants out of dilute solution and hold them close to each other. This proximity of reactants is said to raise the *effective* concentration over that of substrates in solution, and leads to an increased reaction rate.

Enzymes not only bring substrates and catalytic groups together, they orient (specific geometric alignment) them in a manner suitable for catalysis as well. Clearly, proximity and orientation play a role in enzyme catalysis, but there is a problem with each for comparisons since we cannot separate true proximity and orientation effects from the effects of entropy loss when molecules are brought together.

By simply binding their substrates, enzymes facilitate their catalyzed reactions in three ways (+ electrostatic catalysis):

1. Enzymes bring substrates into contact with their catalytic groups and, in reactions with more than one substrate, with each other.
2. Enzymes bind their substrates in the proper orientations for reaction. Molecules are not equally reactive in all directions. Rather, they react most readily if they have the proper relative orientation.
3. Enzymes freeze out the relative translational and rotational motions of their substrates and catalytic groups.

In short this mechanism increases the rate of the reaction as enzyme- substrate interactions by aligning reactive chemical groups and holding them close together. This reduces the entropy of the reactants and thus makes reactions such as ligations or addition reactions more favorable, there is a reduction in the overall loss of entropy when two reactants become a single product.

5. Electrostatic catalysis

An electrostatic effect gives the largest contribution to catalysis. The enzyme provides an environment that is more polar than water, and the ionic transition states are stabilized by fixed dipoles. This is very different from transition state stabilization in water, where the water molecules must pay with "reorganization energy", in order to stabilize ionic and charged states. Thus, the catalysis is associated with the fact that the enzyme polar groups are preorganized

The magnitude of the electrostatic field exerted by an enzyme's active site is highly correlated with the enzyme's catalytic rate enhancement

Binding of substrate usually excludes water from the active site, thereby lowering the local dielectric constant to that of an organic solvent where electrostatic interactions are much stronger

than they are in aqueous solutions. This strengthens the electrostatic interactions between the charged/polar substrates and the active sites. Furthermore, the charge distributions about the active sites are arranged so as to stabilize the transition states of the catalyzed reactions. In several enzymes, these charge distributions serve to guide polar substrates toward their binding sites so that the rates of these enzymatic reactions are greater than their apparent diffusion-controlled limits.

6 .Bond strain

This is the principal effect of induced fit binding, where the affinity of the enzyme to the transition state is greater than to the substrate itself. This induces structural rearrangements which strain substrate bonds into a position closer to the conformation of the transition state, so lowering the energy difference between the substrate and transition state and helping catalyze the reaction.

However, the strain effect is, in fact, a ground state destabilization effect, rather than transition state stabilization effect. Furthermore, enzymes are very flexible and they cannot apply large strain effect.In addition to bond strain in the substrate, bond strain may also be induced within the enzyme itself to activate residues in the active site.

5. Quantum tunneling

The "over the barrier" mechanisms above have been challenged in some cases by models and observations of "through the barrier" mechanisms (quantum tunneling). Some enzymes operate with kinetics which are faster than what would be predicted by the classical ΔG‡. In "through the barrier" models, a proton or an electron can tunnel through activation barriers. Quantum tunneling for protons has been observed in tryptamine oxidation by aromatic amine dehydrogenase. Interestingly, quantum tunneling does not appear to provide a major catalytic advantage, since the tunneling contributions are similar in the catalyzed and the uncatalyzed reactions in solution. However, the tunneling contribution (typically enhancing rate constants by a factor of ~1000 compared to the rate of reaction for the classical 'over the barrier' route) is likely crucial to the viability of biological organisms. This emphasizes the general importance of tunneling reactions in biology.

In 1971-1972 the first quantum-mechanical model of enzyme catalysis was formulated

13.7 FACTORS AFFECTING ENZYME ACTIVITY

The activity of an enzyme is affected by its environmental conditions. Changing these alter the rate of reaction caused by the enzyme. In nature, organisms adjust the conditions of their enzymes to produce an optimum rate of reaction, where necessary, or they may have enzymes, which are adapted to function well in extreme conditions where they live.Several factors affect the rate at which enzymatic reactions - temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators.

1. Temperature

Increasing temperature increases the kinetic energy that molecules possess, leading to more random collisions between molecules per unit time. Since enzymes catalyze reactions by randomly colliding with substrate molecules, increasing temperature increases the rate of reaction, forming more product.

As temperature increases more bond, especially the weaker ionic bonds will break as a result of this strain. As temperature increases, initially the rate of reaction will increase. However, the effect of bond breaking will become greater and greater, and the rate of reaction will begin to decrease.

The rate of enzyme activity increases with as temperature increases until the optimum temperature because of increased kinetic energy, then falls to zero as the enzyme is denatured. The temperature at which the maximum rate of reaction occurs is called the enzyme's optimum temperature. This is different for different enzymes. *Most enzymes in the human body have an Optimum Temperature of around 37.0 °C*

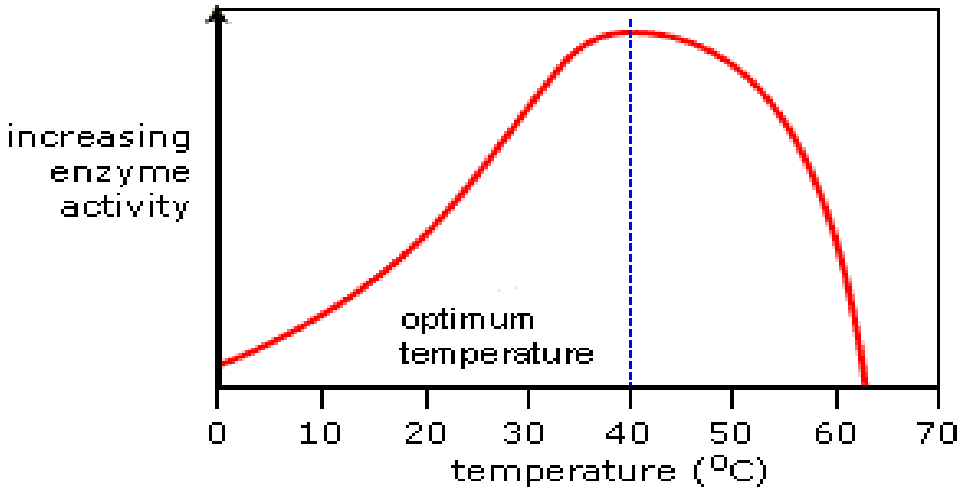


Fig. No. 13.4 Effect of Temperature on enzyme catalyzed reaction

2. pH - Acidity and Basicity

pH measures the acidity and basicity of a solution. It is a measure of the hydrogen ion (H^+) concentration, and therefore a good indicator of the Hydroxide Ion (OH^-) concentration. It ranges from pH1 to pH14. Lower pH values mean higher H^+ concentrations and lower OH^- concentrations. Acid solutions have pH values below 7, and Basic solutions (alkalis are bases) have pH values above 7. Deionised water is pH7, which is termed 'neutral'.

Each enzyme has its own range of pH in which it will work. H^+ and OH^- Ions are charged and therefore interfere with hydrogen and ionic bonds that hold together an enzyme, since they will be attracted or repelled by the charges created by the bonds. This interference causes a change in shape of the enzyme, and importantly, it's Active Site.

Different enzymes have different optimum pH values. This is the pH value at which the bonds within them are influenced by H^+ and OH^- Ions in such a way that the shape of their Active Site is the most Complementary to the shape of their Substrate. At the optimum pH, the rate of reaction is at an optimum. If pH increases or decreases much beyond this optimum, the ionisation of groups at the active site and on the substrate may change, effectively slowing or preventing the formation of the enzyme substrate complex. At extreme pH, the bonds which maintain the tertiary structure – hence the active site – are disrupted and the enzyme is irreversibly denatured. Any change in pH above or below the optimum will quickly cause a

decrease in the rate of reaction, since more of the enzyme molecules will have active sites whose shapes are not (or at least are less) complementary to the shape of their substrate.

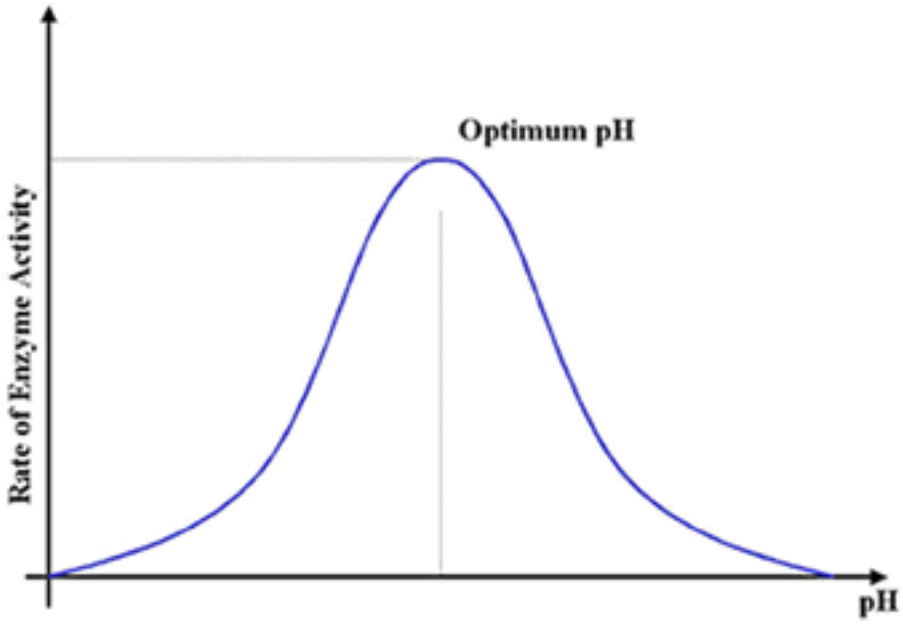


Fig. No. 13.5 Effect of pH on enzyme catalyzed reaction

Small changes in pH above or below the optimum do not cause a permanent change to the enzyme, since the bonds can be reformed. However, extreme changes in pH can cause enzymes to denature and permanently lose their function.

Enzymes in different locations have different optimum pH values since their environmental conditions may be different. *For example, the enzyme Pepsin functions best at around pH=2 and is found in the stomach, which contains Hydrochloric Acid (pH=2).*Most enzymes have an optimum pH that falls within the physiological range of 7.0-7.5.

3. Concentration

Changing the enzyme and substrate concentrations affect the rate of reaction of an enzyme-catalysed reaction. Controlling these factors in a cell is one way that an organism regulates its enzyme activity and so its metabolism.

A. Substrate Concentration

If we keep the concentration of the enzyme constant and increase the concentration of the substrate, it leads to increase in the rate of reaction. This is because more substrate molecules will be colliding with enzyme molecules, so more product will be formed.

However, after a certain concentration, any increase will have no effect on the rate of reaction, since substrate concentration will no longer be the limiting factor. The enzymes will effectively become saturated, and will be working at their maximum possible rate.

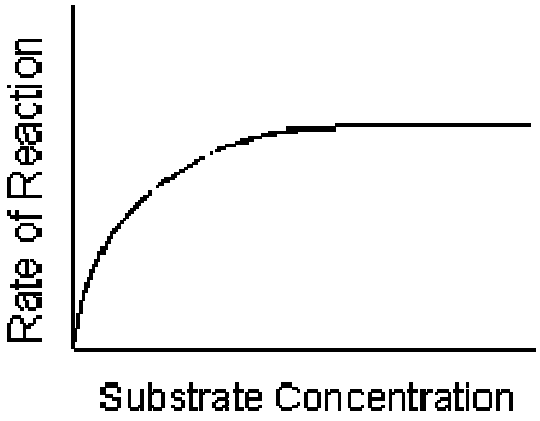


Fig. No. 13.6 Effect of varying substrate concentration on enzyme catalyzed reaction

B. Enzyme Concentration

If we keep the concentration of the substrate constant and increase the concentration of the enzyme, the rate of reaction increases linearly as more enzymes will be colliding with substrate molecules. Moreover, this is because rationally in all enzyme reactions the molar concentration of the enzyme is almost always lower than that of the substrate.

However, this too will only have an effect up to a certain concentration, where the enzyme concentration is no longer the limiting factor.

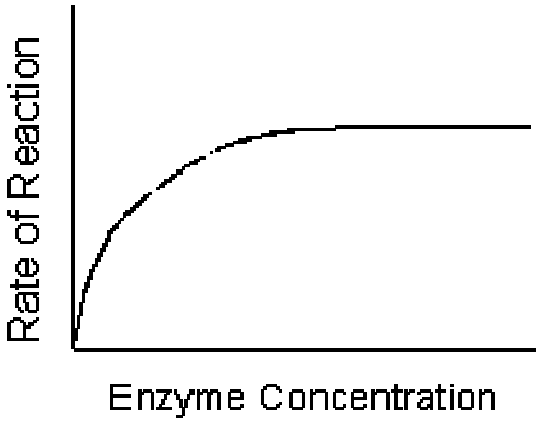


Fig. No. 13.7 Effect of varying enzyme concentration on enzyme catalyzed reaction

4. Cofactors

Many enzymes require cofactors to function properly. There are three main types of cofactor; co-enzymes, inorganic ions and prosthetic groups.

1. Coenzymes are organic molecules, which often contain a vitamin molecule as part of their structure. Coenzymes become loosely bound to the enzyme and move away from the enzyme once the reaction is completed. One coenzyme, e.g. NAD+ may react with many different enzymes in many different types of reaction. NAD+ transfers hydrogen in reactions involving dehydrogenase enzymes.

2. Inorganic metal ions are also known as enzyme activators. They change the charge in the active site, enabling the enzyme substrate complex to form. Some become intimately bound to the enzyme, e.g. Fe_2+ in catalase. Most others accelerate the binding between the enzyme and the substrate, e.g. Mg_2+ in phosphotransferases.

3. Prosthetic groups are coenzymes that bind permanently to the enzyme molecule and remain there even after the reactions are complete, e.g. FAD (flavin adenine dinucleotide). Like NAD+ it carries hydrogen atoms, this time with oxidase enzymes.

5. Inhibitors

Inhibitors slow down the rate of reaction. As such, they are an essential form of cellular control, allowing enzyme reaction rate to be slowed when necessary. Some enzymes are inhibited by the end product of the reaction they catalyse.

(a) Reversible inhibitors

There are two types of reversible inhibitor:

- Competitive reversible inhibitor
- Non-competitive reversible inhibitor

Competitive reversible inhibitors are structurally similar to the normal substrate and compete with the normal substrate for the active sites

However, if the concentration of the normal substrate is increased, reversible inhibitors are displaced from the active site and the normal enzyme substrate complex can form.

Non-competitive reversible inhibitors react with the enzyme but not at the active site. They change the shape of the whole enzyme, including the shape of the active site, hence the reaction cannot proceed and no products are formed on those enzymes

(b) Irreversible inhibitors

Irreversible inhibitors bind covalently and permanently to the enzyme, preventing normal enzyme function. For example, Aspirin is an irreversible inhibitor of cycloxygenase, an enzyme involved in the synthesis of prostaglandins. Substances such as mercury, iron and arsenic bind irreversibly to the SH (sulphydryl) group on enzymes.

13.8 SOURCES OF ENZYMES

Biologically active enzymes may be extracted from any living organism. A variety sources are used for commercial enzyme production. Of the hundred or so enzymes being used industrially, most of them are from fungi, yeast and bacteria with the rest divided between animal and plant sources. Microbes are preferred to plants and animals as sources of enzymes because of low production cost, enzyme contents are more predictable and controllable, easily arranged reliable supplies of raw material of constant composition, andplant and animal tissues comprise more potentially harmful materials than microbes, including phenolic compounds, endogenous enzyme inhibitors and proteases.Attempts are being made to overcome some of these difficulties by the use of animal and plant cell culture.

Enzymes from microbial sources:

Microorganisms are the most significant and convenient sources of commercial enzymes. They can be made to produce abundant quantities of enzymes under suitable growth conditions. Microorganisms can be cultivated by using inexpensive media and production can take place in a short period.

In addition, it is easy to manipulate microorganisms in genetic engineering techniques to increase the production of desired enzymes. Recovery, isolation and purification processes are easy with microbial enzymes than that with animal or plant sources. The great majority of microbial enzymes come from a very limited number of genera, of which *Aspergillus* species, *Bacillus* species and *Kluyveromyces* (also called *Saccharomyces*) species predominate. Most of the strains used have either been employed by the food industry for many years or have been derived from such strains by mutation and selection. For e.g. production of high fructose syrup using glucose isomerase and the use of pullulanase in starch hydrolysis.

Industrial production of enzymes aims at economy, effectiveness and safety and high yield as well. Among the microorganisms, *Aspergillus Niger* (a fungus) occupies a special position for the manufacture of a large number of enzymes in good quantities. There are well over 40 commercial enzymes that are conveniently produced by A. Niger. These include a-amylase, cellulase, protease, lipase, pectinase, phytase, catalase and insulinase.

A common trend in the industry today is that the gene coding for the enzyme with desired characteristics is transferred into one of the selected microbial production strains which have all the required features of safety and high expression levels and for which the growth medium has been optimised, hence avoiding the need for optimization of individual enzyme producing strains.

Enzymes from animal and plant sources:

Animal organs and tissues are very good sources for enzymes such as lipases, esterases and proteases. The enzyme lysozyme is mostly obtained from hen eggs. Some plants are excellent sources for certain enzymes-papain (papaya), bromelain (pineapple). Rennet has been among the most industrially significant enzymes obtained from animal tissue. The other enzymes obtained

from animal sources e.g. proteases like trypsin, chymotrypsin and urokinase, lactate dehydrogenase have diverse applications in industry, analysis,

In recent years, protein production in transgenic animals and –plants has attracted attention. Focus on transgenic animals (e.g. sheep, cattle) has been for the production of therapeutic proteins. The expression of the foreign gene is targeted to the mammary gland so that the protein is secreted directly into the milk.

Although both pharmaceutical and industrial proteins have been expressed in transgenic plants, they are suggested to be ideal bioreactors for production of the latter category of proteins. Production of bulk enzymes like α-amylase, xylanase, phytase, etc. combines the advantages of low production costs of plant biomass with the minimal purification requirements for such products.

Enzymes from mammalian cell cultures:

There exists a possibility of producing commercial enzymes directly by mammalian cell cultures. But the main constraint is the cost factor, which is extremely high. However, certain therapeutic enzymes such as tissue plasminogen activator are produced by cell cultures.

13.9 ENZYME DEFICIENCY

Causes of enzyme depletion

Unfortunately, enzymes are being depleted at every stage from seed to plate. This has caused enzyme deficiencies in the human body that leads to all kinds of health conditions. The main causes of enzyme depletion include:

• Pesticides and chemicals

• Hybridization and genetic engineering

• Bovine growth hormone

• Pasteurization

• Irradiated food

• Excess intake of unsaturated and hydrogenated fats

• Cooking at high temperatures

• Microwaving

• Radiation and electromagnetic fields

• Geopathic stress zones

• Fluoridated water

• Heavy metals

• Mercury amalgam dental fillings

Health disorders caused by enzyme deficiencies

Due to their critical role in a variety of functions in the body, enzyme deficiencies can cause many health related symptoms. The following are some of those health disorders associated with each of the four basic enzymes:

Protease (digests proteins): anxiety, low blood sugar, kidney problems, water retention, depressed immunity, bacterial and viral infections, cancer, appendicitis, bone problems (such as osteoporosis, arthritis, and bone spurs).

Amylase (digests non-fiber carbohydrates): skin problems such as rashes, hives, fungal infections, herpes, and canker sores; lung problems such as asthma, bronchitis, and emphysema; liver or gall bladder disease.

Lipase (digests fats): high cholesterol, obesity, diabetes, hardening of the arteries and other cardiovascular problems, chronic fatigue, spastic colon, dizziness.

Cellulase (digests fibers): gas and bloating, acute food allergies, facial pain or paralysis, candidiasis (bowel and vaginal yeast infections).

Beside these four types of deficiency, others are

Sucrase, Lactase & Maltase Deficiency

People who have malabsorption syndrome and cellulose deficiency also have a tendency towards sugar (sucrose, lactose, & maltose) and/or gluten intolerance. Sucrose, lactose and maltose are

three common sugars which some people cannot tolerate. They are broken down and absorbed into the system by three enzymes; sucrase, lactase and maltase.

Sucrase deficient people cannot split the sucrose disaccharide into glucose and fructose. Glucose is a primary brain food so expect mental and emotional problems in people who are sucrase deficient. Symptoms include depression, moodiness, panic attacks, manic and schizophrenic behavior and severe mood swings.

People who are intolerant of lactose also have classic symptoms, which include abdominal cramps and diarrhea. Other allergic symptoms have been recorded, not the least of which was asthma, from the ingestion of lactose-containing products.

Maltase deficient people are generally sensitive to environmental conditions. An intolerance to sucrose, lactose or maltose may be worsened by a deficiency in sucrase, lactase or maltase.

Combination Deficiency

Combination deficiency is when an individual has more than one of the above deficiencies. The person will most often have the most severe digestive issues. Crohn's disease, Colitis, and Irritable Bowel Syndrome are quite common.

Gluten grains can be a real problem for example. These grains include wheat, oats, rye and barley. Not everyone has to avoid all four grains; however, sometimes it is a must. Gluten intolerance is associated with Celiac Disease and Malabsorption Syndrome. It is also associated with Crohn's Disease. Gluten is actually a protein that exists in these high carbohydrate grains. The best way to address this is usually a high potency protease and amylase enzyme combination.

The insidious thing about gluten intolerance is that it creates a sugar intolerance because when gluten intolerant people eat gluten containing foods, the brush border cells of the jejunum are injured and thus unable to secrete the disaccharidases (sucrase, lactase and maltase) leading to sugar intolerance. The problems discussed here are just the tip of the iceberg. More discoveries continue to emerge as research with food enzymes continues.

SIGNIFICANCE OF ENZYMES

Enzymes are needed for every chemical reaction that takes place in our body and are connected to every organ of the body. They are catalysts and are required by our body to digest food and ensure the delivery of vitamins and minerals. They work within the cells to regulate detoxification and produce energy. Enzymes can prevent partially digested proteins from putrefying, carbohydrates from fermenting, and fats from turning rancid within the body. They have been described as the 'live energy' of all organisms. Cooking, chemicals, and food processing destroy the natural enzymes found in the foods we eat and therefore, enzymes are by far the most important supplement to be taken. Enzymes from a plant-based source become active as soon as they enter the body whereas animal sources are only active within the small intestine in an alkaline setting.

The enzymes ensure the assimilation of vitamins, minerals, proteins, fats and carbohydrates and can help the body by supporting gall bladder function reducing inflammation, decreasing lactose intolerance, and aiding general indigestion.

THERAPEUTIC USES OF ENZYMES

Assay of plasma enzymes

Assay of plasma enzymes have been carried out routinely in clinical biochemistry whereas few of them have a clearly defined role in that particular location, the majority do not. For each of the plasma enzyme there is a normal concentration range or normal range of activity, which can be determined. Since many enzymes or isoenzymes are characteristically associated with the cells of certain tissues, their plasma assay can help to identify the location of damaged cells. This in turns correlate with the symptoms and case history of the patient and with other biochemical parameters. Examples are: Lactate Dehydrogenase (LDH), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), and Creatine Kinase (CK)

Inborn errors of metabolism

It forms a large class of genetic diseases involving congenital disorders of metabolism. The majority is due to defects of single genes that code for enzymes as a result of genetic mutation, that facilitate conversion of various substances (substrates) into others (products). In most of the disorders, problems arise due to accumulation of substances, which are toxic or interfere with normal function, or to the effects of reduced ability to synthesize essential compounds.

The primary diagnosis is usually made by observing a build-up in plasma or urine of the metabolic intermediate, which is the substrate for the defective enzyme. There are many different types of inborn errors of metabolism.

A few of them are: Fructose intolerance, Galactosemia, Maple Sugar Urine Disease (MSUD), Phenylketonuria (PKU

ENZYMES AS REAGENTS IN CLINICAL BIOCHEMISTRY

Enzymes may be employed in a solution medium, immobilized on a surface of the reaction vessel the enzyme, as a catalyst, can be used as a label in various immunoassay techniques. Thus or in a reagent strip. The requirements imposed on the reagent enzyme may be different in all of these situations. For eg. D-glucose in blood and other physiological fluids is commonly analysed by involving glucose oxidase, this can be of use in diagnosis of diabetes mellitus. Blood lactate and pyruvate are usually determined by means of LDH-catalysed methods and blood urea is analysed by procedures involving urease. Beside this blood cholesterol may be analyse by using cholesterol oxidase. Interestingly, luciferase can be utilized that uses luciferin as a substrate to analyse the product oxyluciferin (exhibiting green chemiluminisence) on a spectrofluorimeter or luminometer

APPLICATION IN FORENSIC SCIENCE

A test for seminal acid phosphatase activity is used as a presumptive identification of semen. Detection of the presence of saliva in forensic samples can be detected by the presence of a-amylase, an enzyme that catalyses the hydrolysis of starch and glycogen. Alcohol dehydrogenase is used to monitor ethanol levels in forensic samples. RIA (Radioimmunosassay), ELISA (Enzyme linked immunosorbent assay) are being used to monitor the presence of agrochemicals and pharmaceuticals in biological samples. Assays relying on ELISA are also used to monitor

serum proteins and for the post-mortem confirmations of HIV infections. Moreover several polymorphic enzymes can be used to establish individual's identity. Several other enzymes like adenosine deaminase, adenylate kinase, carbonic anhydrase, glucose -6-phosphate dehydrogenase are important forensic markers.

Enzymes can be used for Aiding Digestion. Example: Amylases, Proteases and Lipase.They act as anti-clotting agents like Fibrinolytic and Thrombolytic. Examples: Urokinase and Streptokinase.Enzymes can be used as surface disinfectants. Example: Trypsin.

INDUSTRIAL PURPOSE

Enzymes can be used in the textile industry. Example: Amylase as softening agent for starched clothes.They can also be used for Leather purpose. Example: Proteolytic purpose.Enzymes has the importance in the paper manufacturing. Examples: Endoxylanases for bleaching of Wood pulp.They can be used in the manufacturing of organic compounds. Example: Bacterial enzymes for the manufacturing of acetone, butanol, lactic acid etc.

Enzymes as Food and in food industry:-

Enzymes can be used in the meat packing industry. Example: Papain which is proteolytic in action, therefore hydrolyses peptide bonds thus for tenderizing meat and beef. Enzymes have their role in Manufacturing of cheese. Example: Rennin (chymosin) found in stomach, converts milk protein casein to curd like calcium paracaseinate.Papain is used to stabilize chill proof bear. Yeast enzymes are also used in beverage industry. Lactose is used to prevent the formation of lactose crystals in ice-cream preparations.

Use in Baking

The wheat flour used for bread has naturally occurring enzymes that modify the starch, protein and fiber of the flour when water is added. Yeast added to the mixture also has enzymes, which ferment the maltose over time, to make the dough rise. In bakeries, the quality of the wheat flour varies, as a consequence of natural variation, time of year or inconsistencies in milling. To improve consistency and efficiency, extra enzymes (like xylanase, □-amylase, protease, glucose oxidase and lipase) are used as supplements, enabling better handling of the dough and the control of certain characteristics in the finished bread.

Use in Alcohol

In the alcohol industry, fermentation depends on the action of enzymes synthesised by the yeasts and bacteria used in the production process. Beer brewing essentially involves the yeast action on barley, maize, sorghum, hops or rice. The yeast cells convert simple sugars into alcohol and carbon dioxide. However most sugar present is in the complex polysaccharide form such as starch and cannot readily be used. So these nutrients are "released" by malting in which enzymes are released, degrading starch and protein to simple reducing sugars and amino acids. The traditional malting process is an expensive inefficient way of manufacturing enzymes. So nowadays industrial enzymes such as amylases, glucanases and proteases are added to unmalted barley to produce the same products that malting would produce by more controlled means. Use of enzymes in the beverage industry allows it to be more economic and have consistent quality.

Use in Fruit Juices

Enzymes are used in the processing of fruit juices to maximize the production of clear or cloudy juice. Nearly all fruits contain pectin. The presence of soluble pectin in squeezed juice causes cloudiness. The addition of pectin degrading enzymes (pectin methyl esterase, polygalacturonase and pectin lyase) at the pressing stage increases the amount of juice produced and can reduce cloudiness. The desired flavour and colour of citrus juices especially orange depends on the insoluble, cloudy materials of the pressed juice. The pectin component is manipulated requiring a balance between pectin methyl esterase, to promote cloudiness by increasing the pectin/calcium complex formation and polygalacturonase, to break cloudiness by depolymerisation of the pectin. The application of enzymes in these processes is cosmetic.

Use in Washing Powders

Principally protease digests on organic stains such as grass, blood, egg and human sweat and lipases are effective on stains resulting from fatty products and amylases are effective on removing starchy food deposits. Some powders contain cellulase to brighten colours and soften fabrics. Protease and amylase are also effective in dishwasher detergents, to remove food particles. These detergents are environmentally friendly with fewer bleaching agents and phosphates, allowing the enzymes to do more work and have beneficial effects on public and

environmental health.

Use in the Textile Industry

Enzymes are used in the leather and the textile industries in finishing processes. Proteases help in the de-hairing of the animal hides and lipases are used for de-greasing. The correct application of a cellulase enzyme can give a smoother, glossier brighter fabric to cellulose fibres like cotton. This technique is known as bio-polishing. In the denim industry, cloth was traditionally stonewashed with pumice stones to fade the fabric. A small application of cellulaseminimises damage to the garments and also to machinery. This technique is known as bio-stoning and can ensure greater fading without high abrasive damage to fabric and accessories (buttons, rivets). The use of enzymes in this area of industry illustrates their valuable technological contribution. Enzymes are also used in contact lense solution and in pet toothpaste.

Immobilized Enzymes

Immobilized enzymes are made by the attachment of an enzyme to an insoluble support which allows its reuse and continuous use and thus eliminating the tedious recovery process. Immobilization stabilizes the enzyme; moreover two or more enzymes catalyzing a series of reactions may be placed in close proximity to one another. Adsorption, covalent linkage, crosses linking, matrix entrapment or encapsulations are different methods for making immobilized enzymes. Production of glucose syrups from starch by the use of immobilized enzymes is one of the most important processes of food industry.

13.10 SUMMARY

- Enzymes are powerful and specific biological catalysts.
- They catalyze almost every biochemical reaction.
- All known enzymes are proteins with the exception of a few catalytic RNAs. Many require nonprotein coenzymes or cofactors for their catalytic function.
- Enzymes are classified according to the type of reaction they catalyze. All enzymes have formal E.C. numbers and names, and most have trivial names.
- The part of the Enzyme that acts a Catalyst is called the Active Site. The rest of the Enzyme is much larger and is involved in maintaining the specific shape of the Enzyme.

- When a reaction involving an Enzyme occurs, a Substrate is turned into a Product. The Substrate can be one or more molecules. The Active Site of an Enzyme is Complementary to the Substrate it catalyses.
- One of the properties of enzymes that make them so important is the specificity they exhibit relative to the reactions they catalyze.
- Enzymes increase the rate of a reaction by lowering its activation energy.

- The Lock-and-key Hypothesis is a model of how enzymes catalyse substrate reactions. It states that the shape of the active sites of enzymes areexactly complementary to the shape of the substrate.
- The recent model of Induced-Fit Hypothesis states that the shape of active sites is not exactly complementary, but change shape in the presence of a specific substrate to become complementary.
- Several factors affect the rate of enzymatic reactions - temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators.
- They have enormous application in the field of medical science, forensics and industries.

13.11 GLOSSARY

Enzyme: mainly proteins (a very few are RNA) that function as biological catalysts

Denaturation: a structural change in a protein that results in a loss (usually permanent) of its biological properties

Substrate: a molecule that is the starting point for a biochemical reaction and that forms a complex with a specific enzyme

Catalysis: It is the increase in the rate of a chemical reaction due to the participation of an additional substance called a catalyst

Reversible reaction: A reversible reaction is a reaction where the reactants form products, which react together to give the reactants back.

Activation energy: energy a substrate molecule must have before it can undergo a chemical change

Active site: region of enzyme molecule where substrate molecule binds

Optimum temperature: Temperature at which enzyme functions most effectively

Optimum pH: pH at which enzyme functions most effectively

Inhibitor: a substance which slows or blocks enzyme action (a competitive inhibitor binds to the active site; a non-competitive inhibitor binds

Competitive inhibitor: An Inhibitor, which binds to the active site of an enzyme

Non-competitive inhibitor: An inhibitor, which binds away from the active site of an enzyme

Allosteric enzyme: The enzymes that are inhibited by the end products of another reaction

Immobilized enzymes: An enzyme that is attached to an inert, insoluble material, providing increased resistance to changes in conditions such as pH or temperature.

13.12 LONG ANSWER TYPE QUESTIONS

Give a detailed account of the classification of enzymes with example.

Describe the mechanism of catalysis and the factors affecting the rate of reactions catalyzed by enzyme.

Describe the sources and applications of enzymes in industries.

13.13 SHORT ANSWER TYPE QUESTIONS

Ques. 1 what are enzymes?

Answer. Enzymes are macromolecular biological catalysts. They accelerate, or catalyze, chemical reactions. The molecules at the beginning of the process upon which enzymes may act are called substrates and the enzyme converts these into different molecules, called products.

Ques. 2 Name all the classes of the enzyme?

Answer. Enzymes are divided into six classes namely 1.Oxidoreductases, 2.Transferases, 3. Hydrolases, 4. Lyases, 5.Isomerases, 6. Ligases

Ques. 3 what do you mean by activation energy?

Answer. It is the minimum quantity of energy, which the reacting species must possess in order to undergo a specified reaction.

Fill in the blanks:

1. Some enzymes require an additional chemical non-protein component called a _____.
2. Enzyme reactions are always (Irreversible/Reversible)_____.
3. Ligases are also called _____.
4. Oxidoreductases are a class of enzymes that catalyze_____ reactions.
5. Enzymes that bind metal ions tightly are referred to as _____.
6. Apoenzyme (protein portion) plus the cofactor (coenzyme, prosthetic group or metal-ion-activator) is called the _____.

Answer: Fill in the blanks:

1. Cofactors, 2. Reversible, 3. Synthetases, 4. Oxidation-reduction, 5. Metalloenzymes, 6. Holoenzyme.

13.14 MULTIPLE CHOICE QUESTIONS

1. Enzymes are mostly

   a. Carbohydrate b. Protein c. Fat d. Nucleic acids

2. Which of the following enzyme groups can catalyse oxidation reactions?

   a. Phosphorylase b. Isomerases

   c. hydrolases d. Oxidoreductase

3. The term apoenzyme is applicable to

   a. Simple enzyme b. Protein part of conjugate enzyme

   c. Organic cofactor of a conjugate enzyme d. Inorganic cofactor of a conjugate enzyme

4. Enzymes

   a. Don't require activation energy b. don't change requirement of activation energyc.

   c .Increase requirement of activation energy d. Lowest requirement of activation energy

5. "Lock and key" theory of enzyme action was proposed by

   a. Fischer b. Koshland

   c. Kuhne d. Arrhenius

6. Koshland's theory of enzyme action is known as

   a. Reduced fit theory b. Lock and key theory

   c. Induced fit theory d. Enzyme coenzyme theory

7. An organic substance bound to an enzyme and essential for its activity is called

   a. Isoenzyme b.Coenzyme

   c. Apoenzyme d. Holoenzyme

8. Zymogens are

   a. Active enzymes b. Solvent of enzymes

   c. Chemical precursor of enzymes d. enzyme inhibitors

9. Enzymes works by

   a. Decreasing activation energy b. increasing activation energy

   c. Inorganic catalyst d. None of these

10. Enzymes are

    a. Thermolabile b. Thermophile c. Thermostable d. All of these

Answer: 1.b, 2. D, 3.b, 4.d, 5,a, 6.c, 7.b, 8.c, 9.a, 10.a

13.15 REFERENCES

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