III B.Sc BIO-CHEMISTRY

SEMESTER – V ; PAPER – IIIA

BCT – 501 PHYSIOLOGY, CLINICAL BIOCHEMISTRY AND IMMUNOLOGY

UNIT – 1

DIGESTION & ABSORPTION OF CARBOHYDRATES:

- Carbohydrates are energy containing food stuff. The composition is starch maltose, lactose, Sucrose, isomaltose, glucose, fructose and some pentose sugars.
- Liquid food materials like milk, soup, fruit juice are the sources for carbohydrates.
- Digestion of carbohydrates takes place in four steps. **Digestion in Mouth, stomach, Duodenum, small intestine, large Intestine.

DIGESTION :

1) **Digestion in Mouth:**

The digestion of carbohydrates starts in mouth. Saliva secreted in mouth contains a carbohydrate splitting enzyme called salivary amylase hydrolysis of α – 1,4 glycosidic linkage. Optimum at pH 6.7.

alpha -amylase

Starch, glycogen, dextrins----------→ glucose + maltose

2) **Digestion in Stomach:**

No glycolytic enzymes present in stomach. Sucrose maybe Hydrolysed glucose, fructose by HCl at pH 2.0.

3) **Digestion in Duodenum:**

In duodenum, food mixed with pancreatic juice contains enzyme pancreatic amylase. The enzyme hydrolases alpha-1,4 linkage at optimum pH 7.1.

4) **Digestion in Small Intestine :**

Intestinal juice contains lactase, Maltase, surcrase, intestinal amylase, isomaltase and glycolytic enzymes.

Intestinal amylase:

This enzyme hydrolysis alpha-1,4 glycosidic linkage in poly and oligosaccharides molecules liberating free glucose molecules.

Lactase:

Also called as beta-galactosidase , lactose is hydrolysed to equimolar amount of glucose and galactose.

Lactase

Lactose----------→ glucose + galactose

pH 5 to 6

Sucrase:

Sucrase

Sucrose----------→ glucose + fructose

pH 5 to 7

Maltase :

Maltase

Maltose----------→ glucose + glucose

pH 3.8 to 6.2

Isomaltase:

Isomaltase

Dextrin ----------→ glucose + maltose

ABSORPTION :

Absorption Off carbohydrates takes place in small intestine.

Galactose > Glucose > Fructose > Mannose > Xylose > Arabinose.

The glucose and galactose are absorbed very fast. Fructose and Mannose are intermediate rate and the pentoses are absorbed slowly. Galactose absorbed more rapidly than glucose.

Mechanism

(1) **Simple diffusion** This is independent of sugar concentration gradient between intestinal human mucosa cells and blood plasma.

(2) **Active transport Mechanism**

Wilson and Crine suggested that to be actively transported sugar must have the following

- six numbered ring.
- one or more carbon atoms attached to C5.
- -OH groups at the C2 with the same sterio configuration as occurs in D-glucose.

Crine explained active transport by the presence of your carrier protein (transport protein) in the intestinal epithelial cells. Carrier protein is mobile, sodium dependent, energy dependent, specific for sugar.

Ping – Pong Mechanism :

Carrier protein exist in two principal confirmations depending on the salute concentration.

Two forms are 1. Ping State

2. Pong State

In the pong state it is exposed to high concentration or salute and molecules of salute bind to specific site on the carrier protein this occurs in lipid bilayer of this cell with high solute concentration.

In inner side a confirmation changes occurs to ping state and the state or discharge to side favouring new equilibrium.

The empty carrier protein the rivers to be original confirmation formed state to complete the cycle.

DIGESTION AND ABSORPTION OF PROTEINS

Eggs are a good dietary source of protein and will be used as our example to describe the path of proteins in the processes of digestion and absorption. One egg, whether raw, hard-boiled, scrambled, or fried, supplies about six grams of protein.

From the Mouth to the Stomach

Unless you are eating it raw, the first step in egg digestion (or any other protein food) involves chewing. The teeth begin the mechanical breakdown of the large egg pieces into smaller pieces that can be swallowed. The salivary glands provide some saliva to aid swallowing and the passage of the partially mashed egg through the esophagus. The mashed egg pieces enter the stomach through the esophageal sphincter. The stomach releases gastric juices containing hydrochloric acid and the enzyme, pepsin, which initiate the breakdown of the protein. The acidity of the stomach facilitates the unfolding of the proteins that still retain part of their three-dimensional structure after cooking and helps break down the protein aggregates formed during cooking. Pepsin, which is secreted by the cells that line the stomach, dismantles the protein chains into smaller and smaller fragments. Egg proteins are large globular molecules and their chemical breakdown requires time and mixing. The powerful mechanical stomach contractions churn the partially digested protein into a more uniform mixture called chyme. Protein digestion in the stomach takes a longer time than carbohydrate digestion, but a shorter time than fat digestion. Eating a high-protein meal increases the amount of time required to sufficiently break down the meal in the stomach. Food remains in the stomach longer, making you feel full longer

From the Stomach to the Small Intestine

The stomach empties the chyme containing the broken down egg pieces into the small intestine, where the majority of protein digestion occurs. The pancreas secretes digestive juice that contains more enzymes that further break down the protein fragments. The two major pancreatic enzymes that digest proteins are chymotrypsin and trypsin. The cells that line the small intestine release additional enzymes that finally break apart the smaller protein fragments into the individual amino acids. The muscle contractions of the small intestine mix and propel the digested proteins to the absorption sites. The goal of the digestive process is to break the protein into dipeptides and amino acids for absorption.

In the lower parts of the small intestine, the amino acids are transported from the intestinal lumen through the intestinal cells to the blood. This movement of individual amino acids requires special transport proteins and the cellular energy molecule, adenosine triphosphate (ATP). Once the amino acids are in the blood, they are transported to the liver. As with other macronutrients, the liver is the checkpoint for amino acid distribution and any further breakdown of amino acids, which is very minimal. Recall that amino acids contain nitrogen, so further catabolism of amino acids releases nitrogen-containing ammonia. Because ammonia is toxic, the liver transforms it into urea, which is then transported to the kidney and excreted in the urine. Urea is a molecule that contains two nitrogens and is highly soluble in water. This makes it a good choice for transporting excess nitrogen out of the body. Because amino acids are building blocks that the body reserves in order to synthesize other proteins, more than 90 percent of the protein ingested does not get broken down further than the amino acid monomers.

Very little protein makes it to the large intestine if you are not eating excessive amounts. If you have smelly flatulence, this may be a sign you are eating too much protein because the excess is making it to the colon where you gut microbes are digesting it and producing smelly gas.

Protein Absorption

In adults, essentially all protein is absorbed as tripeptides, dipeptides or amino acids and this process occurs in the duodenum or proximal jejunum of the small intestine. The peptides and/or amino acids pass through the interstitial brush border by facilitative diffusion or active transport. Active transport sodium and ATP to actively transport the molecule through the cell membrane. The R group determines the type of transporter used. Once passed through the membrane, the amino acids or peptides are released into the intestinal blood stream and are transported to the liver by the hepatic (liver) portal vein. This is known as the enterohepatic circulation.

In the liver, 50-65% remain and are used to synthesize protein, nitrogen containing compounds and form purine/pyrimidine bases. In some cases, they may be converted to energy. The liver regulates the amino acid levels in the blood. The amino acids that do not stay in the liver, pass through and are transported to the rest of the body to be taken up and utilized by other cells. Most branch chain amino acids pass through the liver.

DIGESTION AND ABSORPTION OF LIPIDS

Lipids are large molecules and generally are not water-soluble. Like Carbohydrates and protein, lipids are broken into small components for absorption. Since most of our digestive enzymes are water-based, how does the body break down fat and make it available for the various functions it must perform in the human body?

From the Mouth to the Stomach

The first step in the digestion of triacylglycerols and phospholipids begins in the mouth as lipids encounter saliva. Next, the physical action of chewing coupled with the action of emulsifiers enables the digestive enzymes to do their tasks. The enzyme lingual lipase , along with a small amount of phospholipid as an emulsifier, initiates the process of digestion. These actions cause the fats to become more accessible to the digestive enzymes. As a result, the fats become tiny droplets and separate from the watery components.

In the stomach, gastric lipase starts to break down triacylglycerols into diglycerides and fatty acids. Within two to four hours after eating a meal, roughly 30 percent of the triacylglycerols are converted to diglycerides and fatty acids. The stomach's churning and contractions help to disperse the fat molecules, while the diglycerides derived in this process act as further emulsifiers. However, even amid all of this activity, very little fat digestion occurs in the stomach.

Going to the Bloodstream

As stomach contents enter the small intestine, the digestive system sets out to manage a small hurdle, namely, to combine the separated fats with its own watery fluids. The solution to this hurdle is bile. Bile contains bile salts, lecithin, and substances derived from cholesterol so it acts as an emulsifier. It attracts and holds on to fat while it is simultaneously attracted to and held on to by water. Emulsification increases the surface area of lipids over a thousand-fold, making them more accessible to the digestive enzymes.Once the stomach contents have been emulsified, fat-breaking enzymes work on the triacylglycerols and diglycerides to sever fatty acids from their glycerol foundations. As pancreatic lipase enters the small intestine, it breaks down the fats into free fatty acids and monoglycerides. Yet again, another hurdle presents itself. How will the fats pass through the watery layer of mucous that coats the absorptive lining of the digestive tract? As before, the answer is bile. Bile salts envelop the fatty acids and monoglycerides to form micelles. Micelles have a fatty acid core with a water-soluble exterior. This allows efficient transportation to the intestinal microvillus. Here, the fat components are released and disseminated into the cells of the digestive tract lining.

Just as lipids require special handling in the digestive tract to move within a water-based environment, they require similar handling to travel in the bloodstream. Inside the intestinal cells, the monoglycerides and fatty acids reassemble themselves into triacylglycerols.

Triacylglycerols, cholesterol, and phospholipids form lipoproteins when joined with a protein carrier. Lipoproteins have an inner core that is primarily made up of triacylglycerols and cholesterol esters (a cholesterol ester is a cholesterol linked to a fatty acid). The outer envelope is made of phospholipids interspersed with proteinsand cholesterol. Together they form a chylomicron, which is a large lipoprotein that now enters the lymphatic system and will soon be released into the bloodstream via the jugular vein in the neck. Chylomicrons transport food fats perfectly through the body's water-based environment to specific destinations such as the liver and other body tissues.

ABSORPTION

Cholesterols are poorly absorbed when compared to phospholipids and triacylglycerols . Cholesterol absorption is aided by an increase in dietary fat components and is hindered by high fiber content. This is the reason that a high intake of fiber is recommended to decrease blood cholesterol. Foods high in fiber such as fresh fruits, vegetables, and oats can bind bile salts and cholesterol, preventing their absorption and carrying them out of the colon.

If fats are not absorbed properly as is seen in some medical conditions, a person's stool will contain high amounts of fat. If fat malabsorption persists the condition is known as steatorrhea. Steatorrhea can result from diseases that affect absorption, such as Crohn's disease and cystic fibrosis.

COMPOSITION OF BLOOD

Blood is a fluid connective tissue that consists of plasma, blood cells and platelets. It circulates throughout our body delivering oxygen and nutrients to various cells and tissues. It makes up 8% of our body weight. An average adult possesses around 5-6 litres of blood. **Types of Blood Cells :**

We have seen blood consist of cells known as formed elements of blood. These cells have their own functions and roles to play in the body. The blood cells which circulate all around the body are as follows:

Red blood cells (Erythrocytes)

RBCs are biconcave cells and without nucleus in humans; also known as erythrocytes. RBCs contain the iron-rich protein called haemoglobin; give blood its red colour. RBCs are the most copious blood cells produced in bone marrows. Their main function is to transport oxygen from and to various tissues and organs.

White blood cells (Leucocytes)

Leucocytes are colourless blood cells. They are colourless because it is devoid of haemoglobin. They are further classified as granulocytes and agranulocytes. WBCs mainly contribute to immunity and defence mechanism.

Types of White Blood Cells

There are five different types of White blood cells and are classified mainly based on the presence and absence of granules.

- Granulocytes
- Agranulocytes

Granulocytes

They are leukocytes, with the presence of granules in their cytoplasm. The granulated cells include- eosinophil, basophil, and neutrophil.

Eosinophils

- They are the cells of leukocytes, which are present in the immune system.
- These cells are responsible for combating infections in parasites of vertebrates and for controlling mechanisms associated with allergy and asthma.
- Eosinophil cells are small granulocyte, which are produced in the bone marrow and makes 2 to 3 per cent of whole WBCs. These cells are present in high concentrations in the digestive tract.

Basophils

- They are the least common of the granulocytes, ranging from 0.5 to 1 per cent of WBCs.
- They contain large cytoplasmic granules, which plays a vital role in mounting a non-specific immune response to pathogens, allergic reactions by releasing histamine and dilates the blood vessels.
- These white blood cells have the ability to be stained when exposed to basic dyes, hence referred to as basophil.
- These cells are best known for their role in asthma and their result in inflammation and bronchoconstriction in the airways.
- They secrete serotonin, histamine and heparin.

Neutrophils

- They are normally found in the bloodstream.
- They are predominant cells, which are present in pus.
- Around 60 to 65 per cent of WBCs are neutrophils with a diameter of 10 to 12 micrometres.
- The nucleus is 2 to 5 lobed and cytoplasm has very fine granules.
- Neutrophil helps in the destruction of bacteria with lysosomes, and it acts as a strong oxidant.
- Neutrophils are stained only using neutral dyes. Hence, they are called so.
- Neutrophils are also the first cells of the immune system to respond to an invader such as a bacteria or a virus.
- The lifespan of these WBCs extend for up to eight hours and are produced every day in the bone marrow.

Agranulocytes

They are leukocytes, with the absence of granules in their cytoplasm. Agranulocytes are further classified into monocytes and lymphocytes.

Monocytes

- These cells usually have a large bilobed nucleus, with a diameter of 12 to 20 micrometres.
- The nucleus is generally of half-moon shaped or kidney-shaped and it occupies 6 to 8 per cent of WBCs.
- They are the garbage trucks of the immune system.
- The most important functions of monocytes are to migrate into tissues and clean up dead cells, protect against the bloodborne pathogens and they move very quickly to the sites of infections in the tissues.
- These white blood cells have a single bean-shaped nucleus, hence referred to as Monocytes.

Lymphocytes

- They play a vital role in producing antibodies.
- Their size ranges from 8 to 10 micrometres.
- They are commonly known as natural killer cells.
- They play an important role in body defence.
- These white blood cells are colourless cells formed in lymphoid tissue, hence referred to as lymphocytes.
- There are two main types of lymphocytes – B lymphocytes and T lymphocytes.
- These cells are very important in the immune systems and are responsible for humoral and cell-mediated immunity.

Platelets (Thrombocytes)

- Thrombocytes are specialized blood cells produced from bone marrow.
- Platelets come into play when there is bleeding or haemorrhage.
- They help in clotting and coagulation of blood. Platelets help in coagulation during a cut or wound.

BLOOD COAGULATION

Blood Coagulation is the process of forming a clot or thrombus in order to prevent excess loss of blood from the body. It is a gel-like mass which is formed by the platelets and fibrin in the blood. Process of Blood Coagulation

The mechanism which helps the body in order to prevent from constant loss of blood is known as hemostasis. The entire process is divided here into three major steps:

- The primary hemostasis involves the process of vasoconstriction, which response to the injury of the body in the vascular wall. Once injured, the vascular walls react immediately by reducing the amount of blood flow in the infected area.
- Next, the platelets play a key role in covering the injured area to stop the bleeding. They also activate a process which forms a fibrin clot known as the secondary hemostasis. This leads to release of stored granular contents which contain serotonin, ADP and thromboxane, which results in further activation of platelets in the blood plasma.
- Since the platelets alone could not secure the damages caused in the vessel walls, thus a blood clot should be formed necessarily. This formation of the blood clots depends on several clotting factors which activate each other in the clotting cascade.
- This cascade results in the formation of fibrinogen, which is a soluble plasma protein. These fibrin proteins finally stick together to form a clot. Platelet clots are termed as a white thrombus and if the red blood cells are also present, it is known as red thrombus.

Mechanism of Blood Coagulation

Blood coagulation or clotting is an important phenomenon to prevent excess loss of blood in case of injury or trauma. The blood stops flowing from a wound in case of injury. The blood clot or 'coagulum' is formed by a network of fibrin threads. In this network, deformed and dead formed elements (erythrocytes, leukocytes and platelets) get trapped.

The enzyme thrombin converts fibrinogen present in the plasma to fibrin. It is a cascade process of a series of enzyme catalysed reactions. Fibrinogen and various inactive blood clotting factors are present in the plasma. An injury stimulates platelets or thrombocytes to release various factors that initiate the blood clotting cascade. Calcium ions play an important role in blood coagulation.

Let's learn more in detail about the blood coagulation pathway.

Blood Coagulation Pathway

The process of blood coagulation leads to haemostasis, i.e. prevention of bleeding or haemorrhage. Blood clotting involves activation and aggregation of platelets at the exposed endothelial cells, followed by deposition and stabilisation of cross-linked fibrin mesh.

Primary haemostasis involves platelet aggregation and formation of a plug at the site of injury, and secondary haemostasis involves strengthening and stabilisation of platelet plug by the formation of a network of fibrin threads. The secondary haemostasis involves two coagulation pathways, the intrinsic pathway and the extrinsic pathway. Both pathways merge at a point and lead to the activation of fibrin, and the formation of the fibrin network.

Platelet Activation

The blood circulating in the blood vessel does not clot under normal circumstances. The blood coagulation process is stimulated when there is any damage to the endothelium of blood vessels. It leads to platelet activation and aggregation. When collagen is exposed to the platelets due to injury, the platelets bind to collagen by surface receptors. This adhesion is stimulated by the von Willebrand factor released from endothelial cells and platelets. This forms additional cross-linking and activation of platelet integrins, which facilitate tight binding and aggregation of platelets at the site of injury. This leads to primary haemostasis.

Blood Coagulation Cascade

The process of coagulation is a cascade of enzyme catalysed reactions wherein the activation of one factor leads to the activation of another factor and so on.

The three main steps of the blood coagulation cascade are as follows:

1. Formation of prothrombin activator
2. Conversion of prothrombin to thrombin
3. Conversion of fibrinogen into fibrin
4.

1. **Formation of prothrombin activator**

The formation of a prothrombin activator is the first step in the blood coagulation cascade of secondary haemostasis. It is done by two pathways, viz. extrinsic pathway and intrinsic pathway.

Extrinsic Pathway

It is also known as the tissue factor pathway. It is a shorter pathway. The tissue factors or tissue thromboplastins are released from the damaged vascular wall. The tissue factor activates the factor VII to VIIa. Then the factor VIIa activates the factor X to Xa in the presence of Ca^{2+}.

Intrinsic Pathway

It is the longer pathway of secondary haemostasis. It begins with the exposure of blood to the collagen from the underlying damaged endothelium. This activates the plasma factor XII to XIIa.

XIIa is a serine protease, it activates the factor XI to XIa. The XIa then activates the factor IX to IXa in the presence of Ca^{2+} ions.

The factor IXa in the presence of factor VIIIa, Ca^{2+} and phospholipids activate the factor X to Xa.

Common Pathway

The factor Xa, factor V, phospholipids and calcium ions form the prothrombin activator. This is the start of the common pathway of both extrinsic and intrinsic pathways leading to coagulation.

2. **Conversion of prothrombin to thrombin**

Prothrombin or factor II is a plasma protein and is the inactive form of the enzyme thrombin. Vitamin K is required for the synthesis of prothrombin in the liver. The prothrombin activator formed above converts prothrombin to thrombin. Thrombin is a proteolytic enzyme. It also stimulates its own formation, i.e. the conversion of prothrombin to thrombin. It promotes the formation of a prothrombin activator by activating factors VIII, V and XIII.

3. **Conversion of fibrinogen into fibrin**

Fibrinogen or factor I is converted to fibrin by thrombin. Thrombin forms fibrin monomers that polymerise to form long fibrin threads. These are stabilised by the factor XIII or fibrin stabilising factor. The fibrin stabilising factor is activated by thrombin to form factor XIIIa. The activated fibrin stabilising factor (XIIIa) forms cross-linking between fibrin threads in the presence of Ca^{2+} and stabilises the fibrin meshwork. The fibrin mesh traps the formed elements to form a solid mass called a clot.

TRANSPORT OF GASES IN BLOOD

What is Respiration?

Respiration is the process through which living organisms take in oxygen and give out carbon dioxide to release energy. So, naturally, respiration is a major and vital process of gas exchange. The transport of gases during respiration, both oxygen and carbon dioxide are carried out by the blood cells.

Transport of Oxygen during Respiration

During respiration, about 97% of oxygen is transported by Red Blood Cells in the blood and the remaining 3% gets dissolved in the plasma. Haemoglobin is a pigment present in the RBCs that gives blood its red colour. Oxygen binds with haemoglobin to form oxyhaemoglobin, which depends on the partial pressures of oxygen, carbon dioxide, H+ concentration and the temperature. One haemoglobin molecule can carry up to 4 molecules of oxygen. The partial pressure of oxygen, H+ concentration and low temperature are the ideal conditions for the formation of oxyhaemoglobin. These conditions are met in the alveoli. But in the tissues, opposite conditions exist and so oxygen is dissociated from the oxyhaemoglobin. Every 100mL of blood that gets oxygenated in the lung surface can deliver 5 mL of oxygen to the tissues on an average.

Transport of Carbon Dioxide during Respiration

Around 20-25% of carbon dioxide is carried by haemoglobin as carbamino-haemoglobin. 7% is in a dissolved state in the plasma and the remaining is carried as bicarbonate. Again, the binding of carbon dioxide with haemoglobin is related to the partial pressure of carbon dioxide, and the partial pressure of oxygen. As mentioned earlier, the partial pressure of carbon dioxide is high in the tissues and this is where more binding of carbon dioxide occurs.

UNIT – 2

ENDOCRINOLOGY

CLASSIFICATION OF HORMONES:

Hormones:

- Hormones are the chemical messenger produced in small amount by endocrine glands, secreted into blood stream to control metabolism and biological activities in target cell or organs.

Characteristics or properties of hormone:

- Low molecular weight
- Small soluble organic molecules
- Rate of diffusion is very high and are readily oxidized but the effect does not remains constant
- It is effective in low concentration
- Travels in blood
- It has its target site different from where it is produce and is specific to a particular target
- Hormones are non-specific for organisms and may influences body process of other individuals

Functions of hormones

- Regulatory and homeostasis functions
- Maintain consistency of interior of cell
- Permissive functions; movement of substance in and out of cell
- Integrative function; usually balance two system
- Developmental function; helps in development of foetus

Classification of hormone:

Hormones are classified as

A. On the basis of chemical nature
B. On the basis of mechanism of hormone action
   1. Group I hormone
   2. Group II hormone

A. **On the basis of chemical nature:**

1. **Protein hormones:** insulin, glucagon
2. **Steroid hormone:** sex hormones, glucocorticoids
3. **Aminoacids derivatives hormones:** epinephrine, nor epinephrine etc

B. **On the basis of mechanism of hormone action:**

1. **Group I hormone (lipophilic hormone):**

- These hormones are lipophilic in nature.

- They are mostly derivatives of cholesterol.
- These hormones binds to intracellular receptors
- Example: **Steroid hormones, Estrogen, androgen, glucocorticoids, cholcalciferol, thyroxine** etc

2. Group II hormones (water soluble hormone):

- These hormones binds to cell surface receptors and stimulates the release of certain molecules (secondary messengers) to perform biochemical functions

On the basis of secondary messengers group II hormones are of 3 types;

i. **Secondary messenger is cAMP:**

- eg. Adrenocorticotropic hormone, FSH, LH, PTH,ADH, calcitonin, glucagon,

ii. **Secondary messenger is phosphotidylinocitol/calcium or both:**

- eg. Acetylcholine, vasopressin, cholecystokinin, gastrin, gonadotropin releasing hormone, thyrotropin releasing hormone,
- Insulin, chorynoic somato mamotropin, epidermal growth factors, fibroblast growth factors, GH, prolactin

iii. **Secondary messenger is cGMP:**

- Atrial natriuretic peptide (ANP).

HYPOTHALAMUS

Hypothalamus lies below or inferior to the thalamus. It provides the anatomical connection between the nervous and endocrine systems. This connection is through the hypophysis (pituitary gland).

The hypothalamus is connected to the anterior lobe of pituitary gland by hypophysial portal veins, however, it is connected to the posterior lobe of pituitary gland mainly by axons of neurosecretory cells. The hormones of the hypothalamus influence the functioning of the pituitary gland. The hypothalamus is often called the control centre or 'supreme commander' of endocrine regulation.

Hormones of Hypothalamus:

Cells in the hypothalamus synthesize at least nine different hormones. The neurosecretory cells (neurons) of hypothalamus secrete hormones called neurohormones (= releasing factors) which are summarised below.

(i) Adrenocorticotropic Releasing hormone (ARH) or Corticotropin Releasing Hormone:
tropic hormone (ACTH).

(ii) Thyrotropin Releasing Hormone (TRH):
It stimulates the anterior lobe of the pituitary gland to secrete its thyroid stimulating hormone (TSH) or thyrotropin.

(iii) Growth Hormone-Releasing Hormone (GHRH):
It stimulates the anterior lobe of the pituitary gland to release its growth hormone (GH) or somatotrophin.

(iv) Growth Hormone-Inhibitory Hormone (GHIH):
This hormone is also called somatostatin (SS). It inhibits the secretion of growth hormone from the anterior lobe of the pituitary gland.

(v) Gonadotropin Releasing Hormone (GnRH):
It stimulates the anterior lobe of the pituitary gland to secrete two gonadotropic hormones: (follicle stimulating hormone (FSH) and luteinizing hormone (LH)).

(vi) Prolactin Releasing hormone (PRH):
It stimulates the anterior lobe of the pituitary gland to secrete its prolactin.

(vii) Prolactin Inhibitory Hormone (PIH):
It inhibits the secretion of prolactin from the anterior lobe of pituitary gland.

(viii) MSH Releasing Hormone (MSHRH):
It stimulates the intermediate lobe of the pituitary gland to secrete its melanocyte stimulating hormone (MSH).

(ix) MSH Inhibitory Hormone (MSHIH):
It inhibits the secretion of melanocyte stimulating hormone from the intermediate lobe of the pituitary gland.

PITUITARY GLAND

The pituitary gland is located just below the hypothalamus. The pituitary gland is situated in a depression the sella turcica of sphenoid bone of the skull. The pituitary gland is the smallest endocrine gland.

The gland is attached to the brain by a stalk the infundibulum which is continuous with the hypothalamus above. The pituitary gland is formed of two main lobes of different origin.

These lobes are the much larger anterior lobe or adenohypophysis or pars distalis and the smaller posterior lobe or neurohypophysis or pars nervosa. Adenohypophysis originates as Rathke's pouch from dorsal wall of stomodaeum in the embryo, but later its connection with the stomodaeum disappears.

The neurohypophysis originates as an outgrowth from the floor of the diencephalon. Thus the pituitary gland is dual in origin (from stomodaeum – foregut and diencephalon). A third lobe, called the intermediate lobe or pars inter-media is a part of adenohypophysis.

(A) Hormones of the anterior lobe:

The anterior lobe of the pituitary gland secretes the following hormones, most of them are trophic hormones.

(i) Growth hormone (STH or GH) or Somatotrophin (Soma— body, trophe— nourishment):
This hormone stimulates growth. Growth hormone promotes protein anabolism, the absorption of calcium from the bowel and the conversion of glycogen to glucose.

(ii) Thyroid stimulating hormone (TSH) or Thyrotropin:
This hormone controls the growth and activity of the thyroid gland. It influences the uptake of iodine, the synthesis of the hormones, thyroxine and tri-iodothyronine by the thyroid gland and the release of stored hormones into the blood stream.

(iii) Adrenocorticotropic hormone (ACTH):
This hormone stimulates the cortex of the adrenal gland to produce its hormones.

(iv) Prolactin hormone (PRL) or Mammotropin hormone (MTH) or Luteotropic hormone (LTH):
Prolactin is also called the "hormone of maternity" because its main physiological effect is to activate growth of breasts during pregnancy and secretion of mammary glands after child birth. The name luteotrophic hormone (LTH) refers to because it also stimulates the corpus luteum of the ovary to secrete progesterone hormone.

(v) Gonadotropic hormones:

These are as follows:

(a)Follicle-stimulating hormone (FSH):

It stimulates growth of ovarian follicles and their secretion of oestrogens in the female, and spermatogenesis (formation of sperms) in the male.

(b) Luteinizing hormone (LH):

In female it stimulates the corpus luteum of the ovary to secrete progesterone. In male it activates the Leydig's (interstitial) cells of the test is to secrete androgenes hence it may be called interstitial cell stimulating hormone (ICSH) in male.

(B) Hormones of the Posterior lobe:

It contains two hormones:

(i) Oxytocin or pitocin.

(ii) Antidiuretic hormone (ADH) or vasopressin. Once again it is reminded that the posterior lobe of the pituitary gland does not secrete any hormone. Its hormones are secreted in the hypothalamus.

(i) Oxytocin (OT):

Oxytocin promotes contraction of the uterine muscle and contraction of the myoepithelial cells of the lactating breast, squeezing milk into the large ducts behind the nipple. In late pregnancy the uterus becomes very sensitive to oxytocin.

The amount secreted is increased just before and during labour and by sucking of the baby. Because of its role, oxytocin is called "birth hormone" and "milk ejecting hormone". Milkmen inject synthetic oxytocin, called pitocin, into their cows and the buffaloes to get more milk.

(ii) Antidiuretic hormone (ADH) or Vasopressin or Pitressin:

This hormone has two main functions:

(a) Antidiuretic effect:

It increases the reabsorption of water in the distal convoluted tubule and collecting ducts of the nephrons of the kidneys. As a result, the reabsorption of water from the glomerular filtrate is increased,

Pituitary Disorders:

(a) Pituitary Dwarfism:

It is caused by the deficiency of growth hormones (GH) from childhood. It is characterised by small but well proportioned body and sexual immaturity. The dwarfs produced by the deficiency of growth hormone are different from those which are formed from the deficiency of thyroid hormone in having normal intelligence.

(b) Gigantism:

It is caused by excess of growth hormone from early age. It is characterised by large and well proportioned body. If size of pituitary gland increases, it affects (suppresses) optic chiasma and ultimately affects vision.

(c) Acromegaly (Aero- extremity, megaly- large):

It is caused by excess of growth hormone after adult size is reached. It is characterised by disproportionate increase in size of bones of face, hands and feet.

(d) Diabetes Insipidus:

It is caused by the deficiency of ADH. It is characterised by excessive dilute urine.

THYROID GLAND

The thyroid gland is the largest endocrine gland located anterior to the thyroid cartilage of the larynx in the neck. The gland is well supplied with blood vessels. It is bilobed organ. The two lobes are connected by a narrow structure called the isthmus. The microscopic structure of the thyroid gland shows thyroid follicles composed of cubical epithelium and filled with a homogenous material called colloid.

Hormones of Thyroid Gland:

The thyroid gland secretes three hormones. Thyroxine (tetraiodothyronine or T_4), and tri-iodothyronine or T_3 are secreted by the thyroid follicular cells. Thyrocalcitonin is secreted by the C-cells of the thyroid gland. This gland is stimulated to secrete its hormones by thyroid stimulating hormone (also called thyrotropin) secreted by the anterior lobe of pituitary gland.

(i) Thyroxine (T_4) and Triiodothyronine (T_3):

T_4 and T_3 contain four and three atoms of iodine respectively, therefore, they are named so. T_3 is secreted in smaller amounts but it is more active and several times more potent than T_4. T_4 is converted to T_3 by removal of one iodine in the liver, kidneys and some other tissues. Since both T_4 and T_3 have similar effects on the target cells, they are generally considered together under the name, thyroid hormone (TH). The thyroid gland is the only gland that stores its hormones in large quantity. T_4 and T_3 are synthesised by attaching iodine to tyrosine amino acid.

The functions of thyroxine (T_4) and tri-iodothyronine (T_3) are as follows.

(a) They regulate the metabolic rate of the body and thus maintain basal metabolic rate (BMR).

(b) They stimulate protein synthesis and, therefore, promote growth of the body tissues.

(c) They regulate the development of mental faculties.

(d) As they increase heat production, thus they maintain body temperature.

(e) They help in metamorphosis of tadpole into adult frog. If thyroid gland of the tadpole (larva) is removed, the larva fails to change into an adult.

(f) They increase action of neurotransmitters like adrenaline and noradrenaline.

(ii) Thyrocalcitonin (TCT):

It is secreted when calcium level is high in the blood. It then lowers the calcium level by suppressing release of calcium ions from the bones. Thus calciton has an action opposite to that of the parathyroid hormone on calcium metabolism. Calcitonin is a peptide which contains 32 amino acids.

Thyroid Disorders:

(a) Cretinism:

This disorder is caused by deficiency of thyroid hormone in infants. A cretin has slow body growth and mental development of reduced metabolic rate.

Other symptoms of this disorder are slow heart beat, lower blood pressure, decrease

in temperature, stunted growth, pot-belly, pigeon chest and protruding tongue and retarded sexual development. This disease can be treated by an early administration of thyroid hormones.

(b) Myxoedema or Gull's disease:

It is caused by deficiency of thyroid hormone in adults. This disease is characterized by puffy appearance due to accumulation of fat in the subcutaneous tissue because of low metabolic rate. The patient lacks alertness, intelligence and initiative. He also suffers from slow heart beat, low body temperature and regarded sexual development. This disease can be treated by administration of thyroid hormones.

(c) Simple Goitre:

It is caused by deficiency of iodine in diet because iodine is needed for the synthesis of thyroid hormone. It causes thyroid enlargement. It may lead to cretinism or myxoedema. This disease is common in hilly areas. Addition of iodine to the table salt prevents this disease.

(d) Hashimoto's disease:

In this disease all the aspects of thyroid function are impaired. It is an autoimmune disease in which the thyroid gland is destroyed by autoimmunity.

PARATHYROID GLAND

The parathyroid glands consist of four separate glands located on the posterior surface of the lobes of the thyroid gland. The cells of parathyroid glands are arranged in a compact mass and are of two types: small chief cells or principal cells and large oxyphil cells (or eosinophil cells). The cells are enclosed by a delicate connective tissue capsule. The chief cells are much more numerous than the oxyphil cells. The latter are absent in the young and appear a little before the age of puberty.

Hormones of Parathyroid Glands:

The chief cells of the parathyroid secrete a hormone called parathyroid hormone (PTH) or parathormone or also called Collip's hormone after the name of its discoverer. This hormone regulates the calcium and phosphate balance between the blood and other tissues. PTH inhibits collagen synthesis by osteoblasts and bone resorption by osteoclasts.

It mobilises the release of calcium into the blood from the bones. PTH increases calcium absorption from the intestines. It increases calcium resorption from the nephrons (and inhibits phosphate resorption) of the kidneys. Thus parathormone regulates the metabolism of calcium and phosphate.

Parathyroids are under the feeding back control of blood calcium level. A fall in blood calcium stimulates them to secrete parathormone, a rise in blood calcium inhibits parathormone secretion from them. Thus PTH has an effect that opposes the effect of calcitonin. The functions of oxyphil cells are unknown.

Parathyroid Disorders:

(i) Hypoparathyroidism (deficiency of PTH):

It causes the lowering of blood calcium level. This increases the excitability of nerves and muscles, causing cramps and convulsions. Sustained contractions of the muscles of larynx, face, hands and feet are produced. This disorder is called parathyroid tetany or hypocalcaemic tetany.

(ii) Hyperparathyroidism (excess of PTH):

Excess of PTH draws more calcium from the bones. It causes demineralisation, resulting in softing and bending of the bones. Some of the bone substance is replaced by cavities that are filled with fibrous tissues. This condition leads to osteitis fibrosa cystica or osteoporosis.

Because bones become deformed, they are easily fractured. Osteoporosis is common in women who have reached menopause (cessation of menstruation). An excess of parathormone also causes calcium to be deposited in the kidneys. Analysis of the content of kidney stones sometimes suggests the presence of a parathyroid tumour.

ADRENAL GLAND

Adrenal Glands are paired structures located on the top of the kidneys. Each adrenal gland has two parts external adrenal cortex and internal adrenal medulla. The cortex is surrounded by a fibrous capsule. Both adrenal cortex and medulla have different embryonic origin, structure and functions.

Types of Adrenal Glands:

(A) Adrenal Cortex:

Hormones:

All hormones of adrenal cortex are synthesized from cholesterol. Corticosteroids (corticoids—hormones of adrenal cortex) are grouped into three categories : mineralocorticoids, glucocorticoids and gonad corticoids.

(i) Mineralocorticoids:

These hormones are secreted by the cells of zona glomerulosa of adrenal cortex. As the name indicates, they are responsible for the regulation of mineral metabolism. Aldosterone (salt-retaining hormone) is the principal mineralocorticoid (90 to 95%) in humans.

Like all other hormones of the adrenal cortex, aldosterone is a steroid. Its main function is to regulate the sodium content of the body. It is secreted when the sodium level is low. It acts on the kidneys to cause more sodium to be returned to the blood and more potassium to be excreted. As the sodium concentration in the blood increases, water follows it by osmosis, so the blood volume also increases. Thus the effect of aldosterone is to increase both sodium and water in the blood.

(ii) Glucocorticoids:

As their name suggests, they affect carbohydrate metabolism, however, they also affect the metabolism of proteins and fats. Glucocorticoids include three main hormones: cortisol (= hydrocortisone), corticosterone and cortisone. Of the three, cortisol is the most abundant

(about 95%). It stimulates the liver to synthesize carbohydrates from non-carbohydrates such as amino acids and glycerol.

(iii) Gonad corticoids (Sex-corticoids):

They are also called sex hormones of adrenal glands. Large quantities of male than female sex-corticoids (sex hormones) are produced. These male sex hormones are called androgens which are important in the development of a male foetus.

Although the genetic sex is determined by the chromosomes in a fertilized egg, a male foetus develops normal male characteristics only if the foetal gonads and adrenal glands produce sufficient quantities of androgens.

Therefore , androgens stimulate the development of male secondary sexual characters like distribution of body hair. Female sex hormones secreted by the adrenal cortex are oestrogens which maintain the development of female secondary sexual characters.

Disorders of the Adrenal cortex:

(i) Addison's disease:

This disease is caused by the deficiency of mineralocorticoids and glucocorticoids. It is also caused by the destruction of adrenal cortex in disease such as tuberculosis. Its symptoms include low blood sugar, low plasma Na^+, high K^+ plasma, increased urinary Na^+, nausea, vomiting, diarrhoea and a bronze-like pigmentation of skin. Severe dehydration is also common in the person suffering from this disease.

(ii) Cushing's Syndrome:

It is caused by excess of cortisol which may be due to a tumour of the adrenal cortex. It is characterised by high blood sugar, appearance of sugar in the urine, rise in plasma Na^+, fall in plasma K^+, rise in blood volume, high blood pressure, obesity and wasting of muscles of thighs and pectoral and pelvic girdles.

(B) Adrenal Medulla:

Origin:

The adrenal medulla develops from the neuroectoderm of the embryo.

Structure:

The adrenal medulla consists of rounded groups of relatively large and granular cells. These cells are modified postganglionic cells of sympathetic nervous system which have lost normal processes and have acquired a glandular function.

These cells are called chromaffin cells or phaeochromocytes. These cells are connected with the preganglionic motor fibres of the sympathetic nervous system. Obviously, the adrenal medulla is simply an extension of the sympathetic nervous system, therefore, these are discussed together as sympatheticoadrenal system.

Hormones:

The medulla of the adrenal glands secretes two hormones: norepinephrine (noradrenaline) and epinephrine (adrenaline). Norepinephrine and epinephrine are derived from tyrosine amino acid.

(i) Norepinephrine (= Noradrenaline):
It regulates the blood pressure under normal condition. It causes constriction of essentially all the blood vessels of the body. It causes increased activity of the heart, inhibition of gastrointestinal tract, dilation of the pupils of the eyes and so forth.

(ii) Epinephrine (= Adrenaline):
It is secreted at the time of emergency. Hence it is also called emergency hormone.

It causes almost the same effects as those caused by norepinephrine, but the effects differ in the following respects:
First, epinephrine has a greater effect on cardiac activity than norepinephrine.

Second, epinephrine causes only weak constriction of the blood vessels of the muscles in comparison with a much stronger constriction that results from norepinephrine.

A third difference between the action of epinephrine and norepinephrine relates to their effects on tissue metabolism. Epinephrine probably has several times as great a metabolic effect as norepinephrine.

PANCREAS

The pancreas lies inferior to the stomach in a bend of the duodenum. It is both an exocrine and an endocrine gland. A large pancreatic duct runs through the gland, carrying enzymes and other exocrine digestive secretions from the pancreatic acinar cells to the small intestine. The

tissue of the pancreas has in addition to the acinar cells, groups of cells called islets of Langerhans, after the name of their discoverer (1869). These produce endocrine secretions.

Four kinds of cells have been identified in the islets:

(i) Alpha cells (about 15%) produce glucagon. Alpha cells are also called A-cells.

(ii) Beta cells (about 65%) produce insulin. Beta cells are also called B-cells.

(iii) Delta cells or D-cells (about 5%) produce somatostatin (SS), and

(iv) Pancreatic Polypeptide cells or PP cells or F-cells (15%), produce pancreatic polypeptide (PP). Beta cells are usually found towards the middle of the islet, the alpha cells towards the periphery of the islet and Delta (D) and F-cells are found scattered.

Hormones of Pancreas and Their Role:

(i) Glucagon:

It stimulates the liver to convert stored glycogen into glucose. Glucagon is also called an "anti-insulin" hormone.

(ii) Insulin:

(a) It is antagonistic to glucagon. Insulin converts glucose into glycogen in the liver and muscles.

(b) It promotes protein synthesis in tissue from amino acids.

(c) Insulin reduces catabolism of proteins. It is an anabolic hormone.

(d) It increases the synthesis of fat in the adipose tissue from fatty acids.

(e) Insulin reduces the breakdown and oxidation of fat.

(iii) Somatostatin (SS):

The same substance as growth inhibiting hormone from the hypothalamus, is produced not only by the pancreas and hypothalamus but also by some cells of the digestive tract. One of the actions of somatostatin seems to suppress the release of other hormones from the pancreas. It also appears to suppress the release of hormones from the digestive tract. **(iv)**

Disorders of Pancreas:

(i) Diabetes mellitus (Hyperglycemia):

The most common endocrine disorder of the pancreas is the diabetes mellitus, now recognized to exist in two forms — insulin-dependent and non-insulin-dependent.

The insulin-dependent diabetes mellitus (IDDM) is caused by a failure of the Beta-cells to produce adequate amount of insulin while the non-insulin-dependent diabetes mellitus (NIDDM) appears to involve failure of insulin to facilitate the movement of glucose into cells. In both disorders the blood glucose concentration is elevated above the normal range. Some of the glucose is excreted in the urine, and water follows the glucose, causing excessive urination and dehydration of body tissues. This causes excessive thirst (polydipsia). The cells are unable to utilize glucose and other carbohydrates for energy production. They utilize their proteins for it. The person becomes very weak. Degradation of fats increases, producing ketone bodies (ketosis). The latter are acidic and poisonous. Blood cholesterol level rises. Healing power is impaired. Administration of insulin lowers the blood-glucose level. It gives relief to the patient. A tendency towards non-insulin-dependent diabetes appears to be inherited as an autosomal recessive characteristic.

(ii) Hypoglycemia:
It occurs when the blood glucose level falls below normal. Theoretically, it may be caused by an excess of insulin, a deficiency of glucagon, or a failure of the secretion of the two hormones to completely regulate the blood sugar.

Some individuals have been found to have few or no Alpha cells and thus are deficient in glucagon, whereas others produce excess quantities of insulin usually because of a tumour of the beta cells.

The presence of excess insulin is more correctly referred to as hyperinsulinism. Symptoms of hypoglycemia include

weakness, profuse sweating, irritability, confusion, unconsciousness and convulsions. It needs urgent intake of sugar or glucose.

GONADS

The gonads are the sex glands; the ovaries and the testes. They produce ova and sperms respectively but also secrete hormones.

UNIT – 3

NUTRITIONAL BIOCHEMISTRY

Classification of nutrients

Food is the prime necessity of life the food we eat is digested and assimilated in body and used for its maintenance and growth. Food gives energy for doing work.

Discovery of nutrients:

Studies of scientists have shown that food contained several chemical constituents which are known as nutrients.

Nutrients are classified as,

- proteins
- carbohydrates
- fats
- Minerals
- Vitamins.

1) PROTEINS:

Proteins are required for growth in children and maintenance of bady weight in adults. 20% of body weight is derived from the dietary proteins. Proteins are made up of simple. Chemicals known as amino acids. Proteins are essential for cell and tissue growth, adequate intake of protein is particularly important during the periods childhood, adolescence, of pregnancy etc...

2) CARBOHYDRATES:

These are the main sources. for doing work. There all Occur in foods such as starch, sugarcane, glucose, fructose, Milk etc... About 50-70% of energy value (calorie value) in the average diet is provided by Carbohydrates They are cheapest source of energy in the body.

3) Fats:

Fats & oils Serve main source. of energy Contains essential fatty acids. Fat is essential for maintaining good health. Fat stores & provides energy when food intake is limited, aids in the absorption of fat - Soluble Vitamins (such as Vitamin - A, D, E, K). Fat is also a building of block the hormones.

4) Minerals:

The body contains about 24 Minerals, all of which are derived from diet. The important Minerals are Ca, P, K, Na, Cl, Mn, Fe, cu, I, Co, F and Zink.

Eg:

(a) Ca & P for the formation of bones & Teeth.

(b) Na, el, k, - for maintaining water balance in the body.

(c) Fe - for the formation of hemoglobin.

(d) I - for the normal function of Thyroid glands.

5) Vitamins:

Food Contain Certain chemical substances in Small amounts, called as Vitamins. These are essential the for growth of amount the body. The Lower Vitamins in the body leads to deficiency diseases.

Vitamins are 2 types :

(a) fat-Soluble Vitamins (A, D, E, K)

(6) Water-Soluble Vitamins (B,C)

Nutritional classification of foods:

Since food vary widely in their contents. Various nutrients. They are broadly grouped as,

(1) Energy yielding
(2) Body building
(3) protective foods.

1) Energy yielding:

Foods rich in carbohydrates & fats are called energy yielding foods. Cereals, roots, tubers, dried fruits are included in this group. Cereals Contain fair amount of proteins.

2) Body building foods :

Foods rich in proteins are called body building foods. Body building foods include Mick, Meat, eggs, pulses, nuts, low fat oilseeds, flour.

3) Protective foods :

Foods rich in proteins, Vitamins & Minerals. are termed an protective foods Milk, eggs, green leafy Vegetables & fruits are included in this group.

(1) Food rich in Vitamins, Minerals, & proteins of biological Value
Eg: Milk, Eggs.

(2) food rich with Certain Minerals & Vitamins.
Eg: Green Vegetables & fruits.

Calorific Values of foods and their determination by bomb calorimeter :

Lavosier and Laplace made pioneering Studies on energy Metabolism, during 18th century. They found a relationship between heat (Energy) produced and co_2 Output. Later Lavisier measured 0_2 Consumption increased during heavy exercise (or) work. In 1866, pettenkofer & Voit Measured heat Output & O_2 Consumed. During 1892-1902, Atwater clearly established, the relation between heat Output, O2 Consumed and co_2 evolved in humans.

The energy yielding factors:

- Carbohydrates
- Fats
- Proteins.

There are Oxidized in the body to produce (heat), CO_2 & Water as well as Urea in the case of protein.

Carbohydrates Or Proteins —O2 Oxidation——→ CO2 + Water + Heat

Proteins —O2——→ CO2 + Water + Heat + Urea.

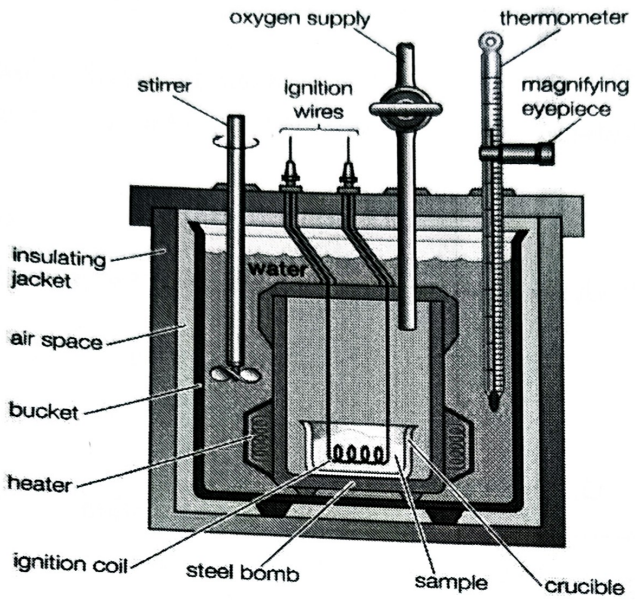
Energy Unit:

The energy Value of food Usually expressed in Kilo Calories (Kcal), Mega joules (MJ) term Used by international Union of nutritional Science. One Kilocalorie is the amount of energy required. to raise the temperature of One litre degree. Average Values of different food stuffs are given of water by One the heat Combustion for below:

- Carbohydrates ----------4.1 cal/grams
- Fat----------9.4 cal/grams
- Protein----------5.6 cal/grams.

Determination of Energy value of foods by Bomb Calorimeter:

The energy Value Value of foods by Bomb of food is determined by using Bomb Calorimeter. The caloric Value of a food stuff can be determined by measuring the heat produced when given amount in completely burnt in Oxygen. It is done in a bomb calorimeter where the Oxygen is put in under considerable pressure.



The One Commonly Used for the purpose in the "Atwater" bomb calorimeter. It consists of heavy steel bomb, with platinum (or) gold plated Copper Lining. It has a cover which is held tightly by a strong screw - Collar. A weighed amount of sample & the bomb is charged with an Oxygen Valve. The valve is then closed & the bomb ik immersed in a weighed amount of water.

The burning of the sample is set. by an electric spark and the heat liberated is measured by the rise in temp. of the surrounding water by means. of differential thermometer which can a read Upto One thousandth of a degree.

Gross energy value of food in pure forms by Bomb Calorimeter :

- 1gm of carbohydrates yields – 4.10 K Cal
- 1 gm of fat yields – 9.45 K Cal
- 1 gm of Protein yields – 5.65 K Cal.

Physiological Energy Value of food (PEV):

When the above foods Utilized in the body, it is not possible to accept the same amount (as above) of energy A Certain amount is lost during digestion. In the case of proteins, Certain

amount is lost during Metabolism as Urea. Hence, the physiological values of foods are given as follows:

Type of Food	GEV/ gm	Energy loss in digestion	Energy available after digestion per gm	Loss of energy in metabolism	PEV/ gm
Carbohydrates	4.10 Kcal	2%	4.0 Kcal	Nil	4.0 Kcal
Fats	9.45 Kcal	5%	9. 0 Kcal	Nil	9.0 Kcal
Proteins	5.65 Kcal	8%	5.2 Kcal	Nil	4.0 Kcal

BASAL METABOLISM (OR) BASAL METABOLIC RATE (BMR):

The basal Metabolic rate (BMR) is the of energy needed while resting. BMR is the of Calories that the body needs. Only need. amount Minimum number to function at rest. We may think, we energy to exercise (or) Complete tanks, but Our body has a specific energy need just to Complete basic functions like breathing & regulating Our hormone levels.

FACTORS EFFECTING BASAL METABOLIC RATE (BMR):

There are many factors that affected the BMR. These include

- **Genetics (race) :**

Some people are born with faster metabolism and some with slower metabolism. Indians and Chinese seem to have lower BMR than the Europeans. This may due to dietary differences between these races.

- **Gender:**

women have a lower BMR than men. Men have a greater muscle mass and lower body fat percentage.

- **Age:**

BMR reduces with age i.e., it is inversely proportional to raise children have higher BMR than adults.

- **Weight:**

The heavier the weight, the higher the BMR. Example include the metabolic rate of obese person is 25% higher than thin person.

- **Surface area:**

The BMR are is directly proportional to the surface area. BMR increases with the increase in body surface area.

- **Hormones:**

Thyroxine east is key BMR - regulator which speed up the metabolic activity of the body. The more thyroxine produced the higher the BMR. If too much thyroxine is produced, BMR can actually double (thyrotoxicosis). If too little thyroxine is produced (Myxoedoema), BMR may shrink to 30 - 40% of normal rate.

- **Exercise:**

Physical exercise not only influences body weight by burning calories, it also helps raise the BMR.

Significance of BMR:

The determination of BMR is the principal guide for diagnosis and treatment of thyroid disorders.

2. If BMR is less than 10% of the normal, it indicates moderate hypothyroidism. In severe hypothyroidism, the BMR may be decreased to 40 to 50 percent below normal.

3. BMR aids to know the total amount of food or calories required to maintain body weight.

4. The BMR is low in starvation, under nutrition, hypothalamic disorders, Addison's disease and lipoid nephrosis.

5. The BMR is above normal in fever, diabetes insipidus, leukemia and polycythemia.

SPECIFIC DYNAMIC ACTION OF FOODS (SDA):

◊ During the digestion of food the energy produced is converted into heat ingested foods increase the metabolic rate because of their specific dynamic action (SDA). SDA is the is the phenomenon of the extra heat production by the body over and above the calculated caloric value when a given food is metabolised by the body.

◊ Heat production increases by about 15% after a meal, starts within one hour of taking food becomes maximum in about 3rd hour this is called specific dynamic action of food (SDA) or thermic effect of food or calorigenic action of food.

◊ SDA of foods are the energy require for digestion, absorption, transport, metabolism and storage of foods in the body.

- SDA of protein : Mainly to meet the requirements for determination, synthesis of urea, buisynthesis of proteins etc...
- SDA of carbohydrates : For the conversion of glucose to glycogen.
- SDA of fat: for it's storage, mobilization and oxidation.

◊ SDA is different for different foodstuffs. Ingestion of protein rich food has more SDA value 30% than carbohydrate (6%) fat (12%) and mixed died (10%).

◊ So people living in hot climate should avoid protein food and who live in cold climate are recommended protein food (which helps to maintain the body temperature liberating extra heat).

ENERGY REQUIREMENTS & RECOMMENDED DIETARY ALLOWANCE (RDA) FOR PREGNANT AND LACTATING WOMEN:

Healthy pregnant or breastfeeding women need to get between 300 to 500 additional calories per day to meet their energy needs and support the healthy growth of their baby.

During pregnancy or while breastfeeding your baby, be sure to eat a variety of healthy foods.

Calcium

Calcium helps build strong bones and teeth, and plays an important role in helping the circulatory, muscular, and nervous systems work properly. Pregnant and breastfeeding women should get 1,000 mg of calcium a day. Healthy sources of calcium include low-fat dairy products, calcium-fortified orange juice and milk-alternatives, cereals, and kale.

Carbohydrates

Eating carbohydrates helps provide energy to support the growth and development of a baby and, after delivery, breastfeeding. The best sources of carbs are whole grains, fruits, and vegetables, white flour, and white rice etc...

Fiber

Fiber is a nutrient that can help ease the constipation that's common during pregnancy. Whole grains (like whole-wheat bread, whole-grain cereals, and brown rice) and fruits, vegetables, and legumes (beans, split peas, and lentils) are good sources of fiber.

Folic acid

Folic acid helps with the development of a baby's brain and spinal cord. It's also needed to make red blood cells and white blood cells. Women who get at least 400 micrograms (0.4 milligrams) of folic acid daily before conception and during early pregnancy can reduce the risk that their baby will be born with a neural tube defect (a birth defect involving incomplete development of the brain and spinal cord).

Pregnant women should get 600 micrograms (0.6 milligrams) of folic acid during the second and third trimesters. Breastfeeding women need 500 micrograms (0.5 milligrams). Good sources of folic acid include fortified breads and cereals. Folate is the natural form of this vitamin and is found in leafy green vegetables, citrus fruits, avocados, lentils, and beans.

Healthy fats

During pregnancy, fat is needed to support baby's growth & development. Healthy fats are found in olive oil, canola and other vegetable oils, nuts and seeds, avocados, and fatty fish like salmon.

Iodine

Iodine helps the body's thyroid gland make hormones that help with growth and brain development. Not getting enough iodine during pregnancy can put a baby at risk for thyroid problems, developmental delays, and learning problems. Pregnant and lactating women should use iodized salt in their cooking and eat foods high in iodine, like seafood and dairy products.

Iron

Eating a diet rich in iron and taking a daily iron supplement while pregnant or breastfeeding helps prevent iron-deficiency anemia. Women who don't get enough iron may feel tired and have other problems. Good dietary sources of iron include lean meats, poultry, and fish, fortified cereals, legumes (beans, split peas, and lentils), and leafy green vegetables.

Protein

Protein helps build a baby's muscles, bones, and other tissues, and supports growth, especially in the second and third trimesters of pregnancy. Healthy sources of protein include lean meat, poultry, fish, beans, nuts and nut butters, eggs, and tofu.

Vitamin A

Vitamin A helps develop a baby's heart, eyes, and immune system. Good sources of vitamin A include milk, orange fruits and vegetables (such as cantaloupe, carrots, and sweet potatoes), and dark leafy greens.

Vitamin B12

Vitamin B12 plays an important role in the formation of a baby's red blood cells, as well as brain development and function. Vitamin B12 is found in animal products like meat, fish, milk, and eggs.

Vitamin D

Vitamin D helps the body absorb calcium for healthy bones and teeth. Vitamin D is made when the skin is exposed to sunlight. Good food sources of vitamin D include fortified low-fat or fat-free milk, fortified orange juice, egg yolks, and salmon.

BIOLOGICAL VALUE (OR) NUTRITIVE VALUE OF PROTEINS:

The nutritive Value (or) biological Value of proteins can conveniently be discussed Under two heads.

1. Nutritional Significance of amino acids.
2. Methods of proteins Evaluation of the nutritive Value of proteins.

1) Nutritional Significance of Works aminoacids:

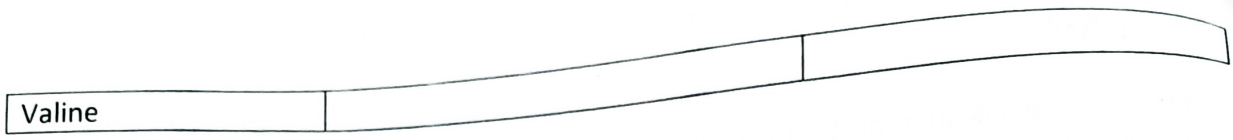
Works of Hopkins (1906) in England, Osborne & Mendel (1912) in America showed that when a rat was fed of Maize), it failed a diet Contains Zein (a protein of maize), it failed to grow. Chemical analysis showed that Zein did not contain tryptophan (or) Lysine. The two amino acids are important for growth Called on Essential amino acids.

Rose & Co-workers (1930-35) in USA Made a research by Mixing 14 amino acids in the diet. Each time Omitting (not include) One amino acid & fed the rats. They came to conclusion that 10 Out of 20 amino acids are Essential for growth of the rats.

But studies with human subjects showed that infants required 9 essential amino acids while adult did not require histidine and hence Only 8 essential amino acids required for growth & Maintenance of the body.

Hence essential amino acids defined. One which is necessary for growth & health These Cannot be synthesized on adequate amount in the body & must therefore be supplied by directory proteins.

Essential Amino Acids	Semi – Essential Amino Acids	Non – Essential Amino Acids
Histidine	Arginine	Glutamic acid
Lysine	Tyrosine	Aspartic acid
Tryptophan	Cysteine	Alanine
Phenylalanine	Glycine	Proline
Methionine	Serine	Hydroxy proline
Threonine		
Leucine		
Isoleucine		



Essential amino acids:

Amino acids which are not produced /Synthesized by Our body & are take-up as food supplements in diet are called Essential amino acids..

Semi-Essential amino acids:

Amino acids which are produced (Synthesized by Our bodies in Very Low quantities. These can taken in diet at the time of sick, injury, after surgery, pregnancy.

Non- Essential amino acids:

Amino acids which are produced I Synthesized by Our bodies & are not say taken up as food Supplements in diet are called Non- Essential amino acids.

2) **Evaluation of Nutritive Value of proteins:**

From the above, it is evident, that the nutritive Value of proteins will depends on their essential amino acid Composition.

On the basis, protein may be classified as follows:

(i) Complete protein:

A food is considered a complete protein when it Contains a essential amino acids that our body cannot produce On it's Own

Sources: Eggs, Meat, poultry, fish & dairy products

(ii) partially complete protein:

Proteinic food partially lacks One (or) Move essential amino acids partially complete protein provide normal Maintenance but will not support growth.

Sources: plant proteins, pear, Beans etc...

(iii) Incomplete protein:

Incomplete protein Completely lacks One (or) More essential amino acids. Incomplete proteins don't include all the essential amino acids.

Sources : Nuts & Whole grains like brown rice, whole wheat bread etc.... Beans and Zein etem

Methods for evaluation of Nutritive Value of proteins:

Two important Methods are available for evaluation of proteins:

1. Nitrogen Balance Method.
2. Growth Method

1. **Nitrogen Balance Method:**

This Method indicates the not amount. of nitrogen available to the body in the form of protein. It can be calculated through digestibility Coefficient (DC) and Biological value (BV) as follows:

$$\text{Digestibility Coefficient (D.C)} = \frac{\text{Protein intake (g)} - \text{protein lost in digestion}}{\text{Protein intake (g)}} \times 100$$

- Biological value (BV) refers to the percentage of digested protein utilized by body.

$$\text{Biological Value (B.V)} = \frac{\text{Protein digested (g)} - \text{Protein lost in metabolism}}{\text{Protein digested}} \times 100$$

Net Protein Utilization (NPU):

It takes into account of losses in digestion and metabolism.

$$\text{NPU} = \frac{\text{DC} \times \text{BV}}{100}$$

Net Available Protein:

It is calculated as follows:

$$\text{Net Available Protein} = \frac{\text{Protein \%} \times \text{NPU}}{100}$$

2.Growth Method:

This method measures the ability of proteins to promote the growth of rat. The nutritive value of protein in terms of PER (Protein Efficiency Ratio) can be calculated as follows:

UNIT – 4

CLINICAL BIOCHEMISTRY

Disorders of Blood Coagulation – Hemophilia

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

There are three forms of hemophilia :

- Hemophilia – A
- Hemophilia – B
- Hemophilia – C
- Hemophilia – A is the Most Common type of Haemophilia , and it is caused by a deficiency of factor VIII
- Hemophilia -B, which is also called as christmas disease, is caused by a deficiency of factor - IX.
- Hemophilia - C is a mild form of the disease that's XI caused by a deficiency of facter - X!.
- The Symptoms of hemophilia include,

  Blood in Urine, Stool.

  Excessive bleeding

  frequent nose bleeds.

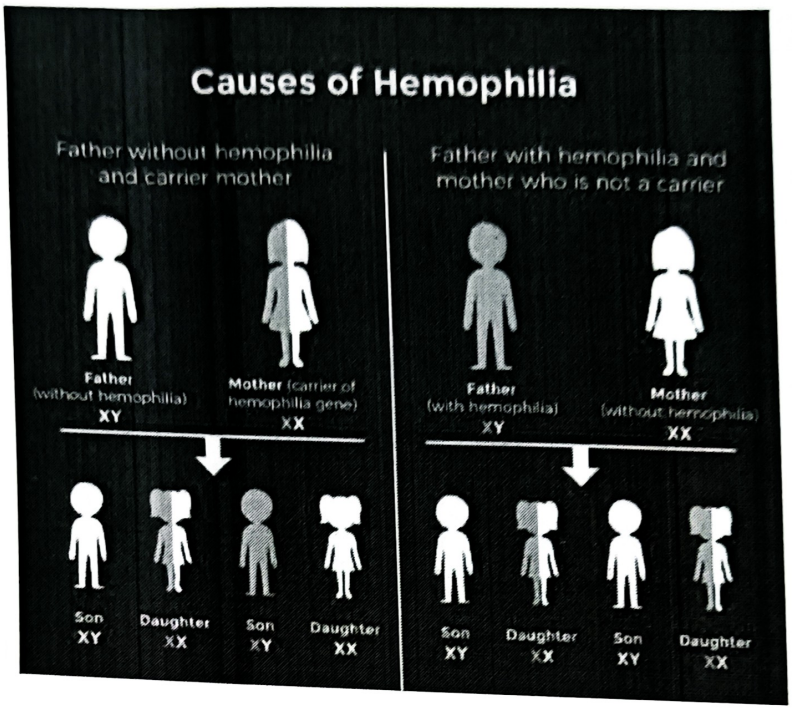
  pain in the joints.

Hemophilia is an inherited genetic Condition, it is passed down through families. It is genes. These genes are meaning caused by a defect in the located on the X-chromosome, making hemophilia an X-linked recessive disease

Each person inherits 2 Sex chromosomes parents females one y have 2 X-chromosomes. Males chromosome from their have One X and

Males inherit an X-chromosome from Mother and a 4 chromosome from their father. females receive an X-chromosome from each parent. Because the genetic defect that causes hemophilia in located on the x- chromosome, Can't fathers pam their sons. This also means that if x- chromosome with the altered a the disease to Male gets the gene from his Mother, he'll have hemophilia. A female who has the altered gene on One a Carrier" of hel X - chromosomes in typically called a carrier.

Hemophilia is diagnosed through a blood Test. Doctor will remove a small sample of blood from Vein and measure the amount of clotting factor present.



TYPES OF ANEMIA

Anemia is a condition in which you lack enough healthy red blood cells to carry adequate oxygen to your body's tissues. Having anemia, also referred to as low hemoglobin, can make you feel tired and weak.

Symptoms

Anemia signs and symptoms vary depending on the cause and severity of anemia. Depending on the causes of your anemia, you might have no symptoms.

Signs and symptoms, if they do occur, might include:

- Fatigue
- Weakness
- Pale or yellowish skin
- Irregular heartbeats
- Shortness of breath
- Chest pain
- Cold hands and feet

Types and Causes of anemia

Different types of anemia have different causes. They include:

- **Iron deficiency anemia.** This most common type of anemia is caused by a shortage of iron in your body. Your bone marrow needs iron to make hemoglobin. Without adequate iron, your body can't produce enough hemoglobin for red blood cells.

  Without iron supplementation, this type of anemia occurs in many pregnant women. It's also caused by blood loss, such as from heavy menstrual bleeding; an ulcer in the stomach or small bowel; cancer of the large bowel; and regular use of some pain relievers that are available without a prescription, especially aspirin, which can cause inflammation of the stomach lining resulting in blood loss. It's important to determine the source of iron deficiency to prevent recurrence of the anemia.

- **Vitamin deficiency anemia.** Besides iron, your body needs folate and vitamin B-12 to produce enough healthy red blood cells. A diet lacking in these and other key nutrients can cause decreased red blood cell production. Some people who consume enough B-12 aren't able to absorb the vitamin. This can lead to vitamin deficiency anemia, also known as pernicious anemia.

- **Anemia of inflammation.** Certain diseases — such as cancer, HIV/AIDS, rheumatoid arthritis, kidney disease, Crohn's disease and other acute or chronic inflammatory diseases — can interfere with the production of red blood cells.

- **Aplastic anemia.** This rare, life-threatening anemia occurs when your body doesn't produce enough red blood cells. Causes of aplastic anemia include infections, certain medicines, autoimmune diseases and exposure to toxic chemicals.

- **Anemias associated with bone marrow disease.** A variety of diseases, such as leukemia and myelofibrosis, can cause anemia by affecting blood production in your bone marrow. The effects of these types of cancer and cancer-like disorders vary from mild to life-threatening.

- **Hemolytic anemias.** This group of anemias develops when red blood cells are destroyed faster than bone marrow can replace them. Certain blood diseases increase red blood cell destruction. You can inherit a hemolytic anemia, or you can develop it later in life.

- **Sickle cell anemia.** This inherited and sometimes serious condition is a hemolytic anemia. It's caused by a defective form of hemoglobin that forces red blood cells to assume an abnormal crescent (sickle) shape. These irregular blood cells die prematurely, resulting in a chronic shortage of red blood cells.

Prevention

Many types of anemia can't be prevented. But you can avoid iron deficiency anemia and vitamin deficiency anemias by eating a diet that includes a variety of vitamins and minerals, including:

- **Iron.** Iron-rich foods include beef and other meats, beans, lentils, iron-fortified cereals, dark green leafy vegetables and dried fruit.
- **Folate.** This nutrient, and its synthetic form folic acid, can be found in fruits and fruit juices, dark green leafy vegetables, green peas, kidney beans, peanuts, and enriched grain products, such as bread, cereal, pasta and rice.
- **Vitamin B-12.** Foods rich in vitamin B-12 include meat, dairy products, and fortified cereal and soy products.
- **Vitamin C.** Foods rich in vitamin C include citrus fruits and juices, peppers, broccoli, tomatoes, melons and strawberries. These also help increase iron absorption.

HAEMOGLOBINOPATHIES – SICKLE CELL ANAEMIA

Hemoglobinopathy is a group of disorders in which there is abnormal production or structure of the hemoglobin molecule. It is passed down through families (inherited).
This group of disorders includes hemoglobin C disease, hemoglobin S-C disease, sickle cell anemia, and thalassemias.

SICKLE CELL ANAEMIA :

Sickle cell anemia is one of a group of disorders known as sickle cell disease. Sickle cell anemia is an inherited red blood cell disorder in which there aren't enough healthy red blood cells to carry oxygen throughout your body.

Normally, the flexible, round red blood cells move easily through blood vessels. In sickle cell anemia, the red blood cells are shaped like sickles or crescent moons. These rigid, sticky cells can get stuck in small blood vessels, which can slow or block blood flow and oxygen to parts of the body.

There's no cure for most people with sickle cell anemia. But treatments can relieve pain and help prevent complications associated with the disease. **Symptoms**

Signs and symptoms of sickle cell anemia usually appear around 5 months of age. They vary from person to person and change over time. Signs and symptoms can include:

- **Anemia.** Sickle cells break apart easily and die, leaving you with too few red blood cells. Red blood cells usually live for about 120 days before they need to be replaced. But sickle cells usually die in 10 to 20 days, leaving a shortage of red blood cells (anemia).

Without enough red blood cells, your body can't get enough oxygen, causing fatigue.

- **Episodes of pain.** Periodic episodes of pain, called pain crises, are a major symptom of sickle cell anemia. Pain develops when sickle-shaped red blood cells block blood flow through tiny blood vessels to your chest, abdomen and joints. Pain can also occur in your bones.

  The pain varies in intensity and can last for a few hours to a few weeks. Some people have only a few pain crises a year. Others have a dozen or more pain crises a year. A severe pain crisis requires a hospital stay.

  Some adolescents and adults with sickle cell anemia also have chronic pain, which can result from bone and joint damage, ulcers, and other causes.

- **Swelling of hands and feet.** The swelling is caused by sickle-shaped red blood cells blocking blood flow to the hands and feet.

- **Frequent infections.** Sickle cells can damage your spleen, leaving you more vulnerable to infections. Doctors commonly give infants and children with sickle cell anemia vaccinations and antibiotics to prevent potentially life-threatening infections, such as pneumonia.

- **Delayed growth or puberty.** Red blood cells provide your body with the oxygen and nutrients needed for growth. A shortage of healthy red blood cells can slow growth in infants and children and delay puberty in teenagers.

- **Vision problems.** Tiny blood vessels that supply your eyes can become plugged with sickle cells. This can damage the retina — the portion of the eye that processes visual images — and lead to vision problems.

Causes

Sickle cell anemia is caused by a mutation in the gene that tells your body to make the iron-rich compound that makes blood red and enables red blood cells to carry oxygen from your lungs throughout your body (hemoglobin). In sickle cell anemia, the abnormal hemoglobin causes red blood cells to become rigid, sticky and misshapen.

Both mother and father must pass the defective form of the gene for a child to be affected.

If only one parent passes the sickle cell gene to the child, that child will have the sickle cell trait. With one normal hemoglobin gene and one defective form of the gene, people with the sickle cell trait make both normal hemoglobin and sickle cell hemoglobin.

Their blood might contain some sickle cells, but they generally don't have symptoms. They're carriers of the disease, however, which means they can pass the gene to their children.

Prevention

If you carry the sickle cell trait, seeing a genetic counselor before trying to conceive can help you understand your risk of having a child with sickle cell anemia. They can also explain possible treatments, preventive measures and reproductive options.

Diagnosis

A blood test can check for the defective form of hemoglobin that underlies sickle cell anemia. In the United States, this blood test is part of routine newborn screening. But older children and adults can be tested, too.

In adults, a blood sample is drawn from a vein in the arm. In young children and babies, the blood sample is usually collected from a finger or heel. The sample is then sent to a laboratory, where it's screened for the defective hemoglobin.

LIVER FUNCTION TESTS

Liver function tests are blood tests used to help diagnose and monitor liver disease or damage. The tests measure the levels of certain enzymes and proteins in your blood.

Some of these tests measure how well the liver is performing its normal functions of producing protein and clearing bilirubin, a blood waste product. Other liver function tests measure enzymes that liver cells release in response to damage or disease.

Abnormal liver function test results don't always indicate liver disease. Your doctor will explain your results and what they mean.

Liver function tests can be used to:

- Screen for liver infections, such as hepatitis
- Monitor the progression of a disease, such as viral or alcoholic hepatitis, and determine how well a treatment is working
- Measure the severity of a disease, particularly scarring of the liver (cirrhosis)
- Monitor possible side effects of medications

Liver function tests check the levels of certain enzymes and proteins in your blood. Levels that are higher or lower than normal can indicate liver problems. Some common liver function tests include:

- **Alanine transaminase (ALT).** ALT is an enzyme found in the liver that helps convert proteins into energy for the liver cells. When the liver is damaged, ALT is released into the bloodstream and levels increase.

- **Aspartate transaminase (AST).** AST is an enzyme that helps metabolize amino acids. Like ALT, AST is normally present in blood at low levels. An increase in AST levels may indicate liver damage, disease or muscle damage.
- **Alkaline phosphatase (ALP).** ALP is an enzyme found in the liver and bone and is important for breaking down proteins. Higher-than-normal levels of ALP may indicate liver damage or disease, such as a blocked bile duct, or certain bone diseases.
- **Albumin and total protein.** Albumin is one of several proteins made in the liver. Your body needs these proteins to fight infections and to perform other functions. Lower-than-normal levels of albumin and total protein may indicate liver damage or disease.
- **Bilirubin.** Bilirubin is a substance produced during the normal breakdown of red blood cells. Bilirubin passes through the liver and is excreted in stool. Elevated levels of bilirubin (jaundice) might indicate liver damage or disease or certain types of anemia.
- **Gamma-glutamyltransferase (GGT).** GGT is an enzyme in the blood. Higher-than-normal levels may indicate liver or bile duct damage.
- **L-lactate dehydrogenase (LD).** LD is an enzyme found in the liver. Elevated levels may indicate liver damage but can be elevated in many other disorders.
- **Prothrombin time (PT).** PT is the time it takes your blood to clot. Increased PT may indicate liver damage but can also be elevated if you're taking certain blood-thinning drugs, such as warfarin.

Results

Normal blood test results for typical liver function tests include:

- **ALT.** 7 to 55 units per liter (U/L)
- **AST.** 8 to 48 U/L
- **ALP.** 40 to 129 U/L
- **Albumin.** 3.5 to 5.0 grams per deciliter (g/dL)
- **Total protein.** 6.3 to 7.9 g/dL
- **Bilirubin.** 0.1 to 1.2 milligrams per deciliter (mg/dL)
- **GGT.** 8 to 61 U/L
- **LD.** 122 to 222 U/L
- **PT.** 9.4 to 12.5 seconds

UNIT – 5

IMMUNOLOGY

TYPES OF IMMUNITY

Immunity is the ability of the body to defend itself against disease-causing organisms. Everyday our body comes in contact with several pathogens, but only a few results into diseases. The reason is, our body has the ability to release antibodies against these pathogens and protects the body against diseases. This defence mechanism is called immunity.

There are two major types of immunity:

1. Innate Immunity or Natural or Non-specific Immunity.
2. Acquired Immunity or Adaptive Immunity.

Innate Immunity

- This type of immunity is present in an organism by birth.
- This is activated immediately when the pathogen attacks. Innate immunity includes certain barriers and defence mechanisms that keep foreign particles out of the body.
- Innate immunity refers to the body's defence system.
- This immunity helps us by providing the natural resistance components including salivary enzymes, natural killer cells, intact skin and neutrophils, etc. which produce an initial response against the infections at birth prior to exposure to a pathogen or antigens.
- It is a long-term immunity in which our body produces the antibodies on its own. Our body has few natural barriers to prevent the entry of pathogens.

Types of Barriers

The four types of barriers are:

- Physical barrier

These include the skin, body hair, cilia, eyelashes, the respiratory tract, and the gastrointestinal tract. These form the first line of defence.

The skin does more than providing us with fair or dark complexions. Our skin acts as a physical barrier to the entry of pathogens. The mucus coating in our nose and ear is a protective barrier which traps the pathogen before it gets inside.

- Physiological barriers

We know that our stomach uses hydrochloric acid to break down the food molecules. Due to such a strongly acidic environment, most of the germs that enter our body along with the food are killed before the further process is carried on.

Saliva in our mouth and tears in our eyes also have the antibiotic property that does not allow the growth of pathogens even though they are exposed all day.

- Cellular barriers

In spite of the physical and physiological barriers, certain pathogens manage to enter our body. The cells involved in this barrier are leukocytes (WBC), neutrophils, lymphocytes, basophil, eosinophil, and monocytes. All these cells are all present in the blood and tissues.

- Cytokine barriers

The cells in our body are smarter than we give them credit for. For instance, in case a cell in our body experiences a virus invasion, it automatically secretes proteins called interferons which forms a coating around the infected cell and prevents the cells around it from further infections.

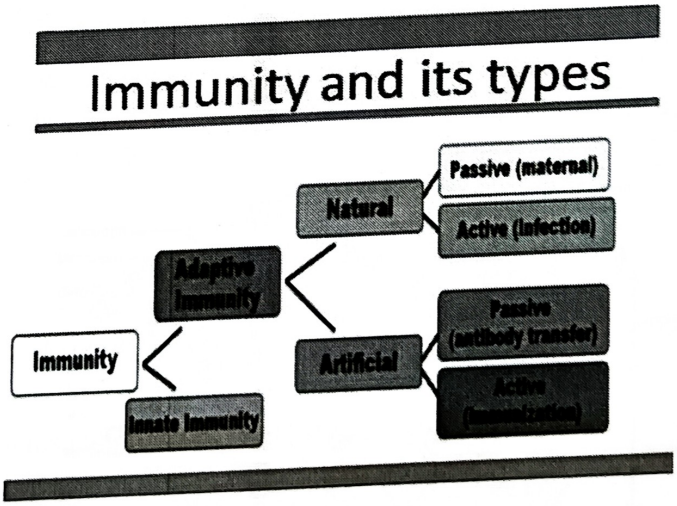
Acquired Immunity

- Acquired immunity or adaptive immunity is the immunity that our body acquires or gains over time. Unlike the innate immunity, this is not present by birth.
- The ability of the immune system to adapt itself to disease and to generate pathogen-specific immunity is termed as acquired immunity. It is also known as adaptive immunity.
- An individual acquires the immunity after the birth, hence is called as the acquired immunity.
- It is specific and mediated by antibodies or lymphocytes which make the antigen harmless.
- The main function of acquired immunity is to relieve the victim of the infectious disease and also prevent its attack in future.
- It mainly consists of an advanced lymphatic defence system which functions by recognizing the own body cells and not reacting to them.
- The immune system of our body identifies the pathogens which have encountered in the past. It is mainly caused when a person comes in contact with the pathogen or its antigen.
- Our body starts producing antibodies to engulf the pathogen and destroy its antigen.
- When it encounters for the first time, it is called a primary response. Once a body gets used to these pathogens, antibodies are ready to attack them for the second time and are known as naturally acquired immunity.
- The acquired immunity in our body has certain special features.
- **Passive immunity**
- Passive immunity is developed by antibodies that are produced outside the body. This immunity lasts for a short time. For example, antibodies present in a mother's breast milk provides a baby with temporary immunity against the diseases.

- **Active Immunity**
- Active immunity is a type of adaptive immunity, which is developed due to the production of antibodies in one's own body. This type of immunity occurs when we are in contact with the pathogen or its antigen. When the active immunity happens for the first time, it is called a primary response. Once a body experiences a pathogen for the first time, it keeps a few of the antibodies that attacked the pathogen just in case it attacks for the second time. This is known as natural active immunity.

Features of Acquired Immunity

- **Specificity**: Our body has the ability to differentiate between different types of pathogens, whether it is harmful or not, and devise ways to destroy them.
- **Diversity**: Our body can detect vast varieties of pathogens, ranging from protozoa to viruses.
- **Differentiate between self and non-self**: Our body has the unique ability to differentiate between its own cells and foreign cells. It immediately starts rejecting any foreign cell in the body.
- **Memory**: Once our body encounters a pathogen, it activates the immune system to destroy it. It also remembers what antibodies were released in response to that pathogen, so that, the next time it enters, a similar procedure is followed by the body to eliminate it.



ORGANS OF IMMUNE SYSTEM

The following points highlight the two main types of organs present in immune system of humans. The types are: 1. Primary Lymphoid Organs 2. Secondary Lymphoid Organs.

1. Primary Lymphoid Organs:

Primary lymphoid organs (PLO) are the major sites of lymphocyte development i.e. lympho-poiesis. Lymphocytes differentiate from lymphoid stem cells, proliferate and mature into functional cells called immuno-competent cells. In mammals, B-cell maturation occurs in the bone marrow and T-cell maturation occurs in the thymus.

Thymus:

i. Location:

Thymus is located in the thoracic cavity (in the mediastinum), just above the heart and beneath the breast bone

ii. Origin:

In mammals thymus develops from the endoderm of the third and fourth pharyngeal pouch.

iii. Structure:

The thymus is flat, bilobed, greyish lympho-epithelial organ. Each lobe is made of lobules separated from each other by strands of connective-tissue trabeculae and covered by a capsule. Each lobule consists of two compartments the outer compartment (cortex) is densely packed with immature T-cells (thymocytes) and the inner compartment (medulla) is sparsely populated with mature thymocytes which express CD44 (not found in the cortical thymocytes).

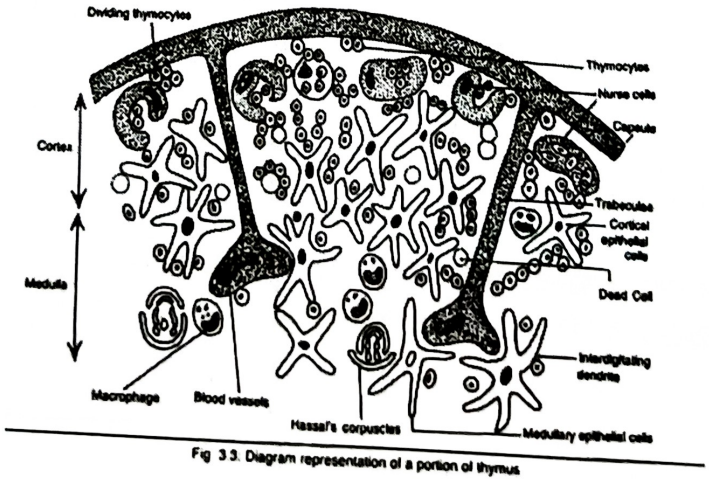


Fig 3.3. Diagram representation of a portion of thymus

There are basically four types of cells found in thymus— thymocytes, dendritic cells, epithelial cells and macrophages. Both the cortex and medulla of the thymus are crisscrossed by a three dimensional stromal-cell network.

Functions:

i. Out of four cell types, dendritic cells, epithelial cells and macrophages act in a combine manner, as a framework to assist in thymocyte maturation. Some thymic epithelial cells in the outer cortex, called nurse cells, have long membrane processes which surround as many as 50 thymocytes forming large multicellular complexes. Other cortical epithelial cells have long inter-connecting cytoplasmic processes that form a network.

The thymocytes are differentiated and matured into different types of T-cells under hormonal influence. Lymphoid progenitor cells formed in the bone marrow migrate to the thymus under the influence of specialized thymic environment.

Maturation of T-cells:

There are four types of hormones named as α_1-thymosin, β_4-thymsin, thymopoeitin and thymulin (and thymic factor and. thymic humoral factor). The T-cell progenitors formed in the bone marrow during hematopoiesis, begin to migrate to the thymus at the 9th week of gestation in humans.

Proliferation of mature T-cell:

After maturation is completed, the mature T- cells leave thymus via post capillary venules of cortico-medullary junctions. Again they will migrate into different target oriented secondary lymphoid tissues. Thymus is one of the most important primary lymphoid organ as because it is meant for generation of T- cells which have immense importance in cell mediated immune response.

Bone marrow:

i. **Location**:

It is found in the cavities of most bones in the body including the skull, ribs, sternum, femur and spine. In birds, no bone marrow is found to be present, instead a lymphoid organ named Bursa identified by Fabricius called Bursa of Fabricius, is present and performs the same duty of bone marrow of mammals.

Structure:

Bone marrow of different bones mainly consists of a sponge like reticular framework located between long trabeculae. The spaces in this framework are filled with fat cells, stromal fibroblasts and precursors of blood cells

Function :

The bone marrow is the main site of generation of all types of circulating blood cells in adult and is the principal site of B-cell maturation and proliferation. During foetal development, the generation of all blood cells, called haematopoiesis, occurs initially in blood island of yolk sac and para-aortic mesenchyme and later in the liver and spleen.

Gradually, these functions are shifted to bone marrow. All blood cells originate from haematopoietic stem cell and become committed to differentiate along particular lineages (erythroid, megakaryocyte granulocytic, monocyte and lymphocytic).

Bone marrow is not only the source of all blood cells but also provides the microenvironment for the antigen independent differentiation of B-cell. Besides this, bone marrow serves as a secondary lymphoid organ where mature, virgin, antigen reactive lymphocytes (T & B cell) may respond to antigen, trapped by antigen presenting cells, such as macrophages. Thus, like spleen, bone marrow may provide an antigen processing environment.

Bursa of Fabricius:

i. **Origin**:

The Bursa is a lympho-epithelial organ, present as an intestinal pouch in birds only.

ii. **Location**:

It is located just above the cloaca of a chick and opens directly in the cloaca.

Structure:

It is a hollow sac like structure (about 1 cm. in diameter) with a duct. It is lined with epithelial cells which cover outer cortical and inner medullary areas. The Bursa reaches its greatest size about one to two weeks after the chick's hatching and then it gradually atrophies.

Inside it, folds of epithelium extend into the lumen, a number of follicles of lymphoid cells remain scattered through out the folds. Each follicle is divided into cortex and medulla. The cortex contains lymphocytes, plasma cells and macrophages.

At the boundary between these regions i.e. cortico-medullary junction, there is a basement membrane and capillary network. Inside of which epithelial cells are present. Each follicle is directly connected with the epithelium covering the surface of the fold.

Function:

The Bursa of birds serves as the hemopoietic inducing micro-environment for progenitor B-cells. It serves as a maturation and differentiation site for the lymphocytes of the antibody forming system. These cells are called B-lymphocytes or B-cells. Several hormones have been extracted from the Bursa. The most important of this is bursin, a tripeptide (lys-his-glycylamide). Bursin activates B-cells but not T-cells.

2. Secondary Lymphoid Organs:

Besides the primary lymphoid organs, there are some other lymphoid organs which are referred to as secondary lymphoid organs. Lymph nodes and spleen are the most important and highly organized secondary lymphoid organs.

Importance of Secondary lymphoid organ:

In case of closed blood vascular system, blood remain always confined within the blood vessels. Lymph and lymphatic system bathe the tissue, tissue fluid and cells. As because lymphatic system represents an accessory route through which fluids or lymph can flow from interstitial spaces into the blood, which is comprised by tiny lymphatic vessels.

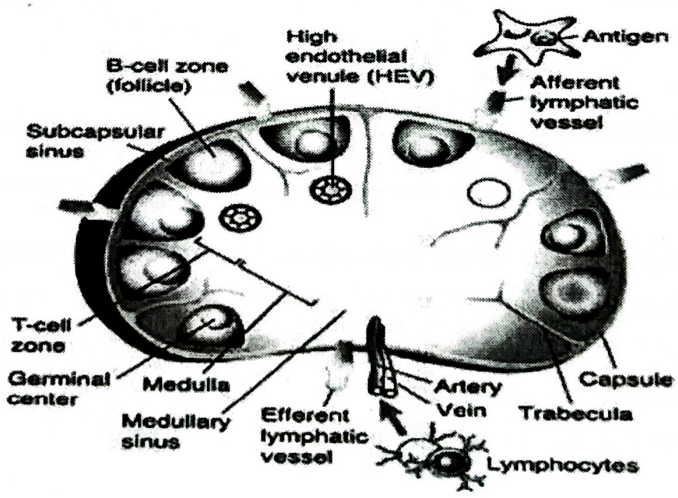
Lymph nodes:

i. **Location**:

Lymph nodes are located at major junctions of the network of lymph flow through lymphatic channels

Structure:

The lymph nodes of man are round or bean shaped structures placed on lymphatic vessels so that they can filter out any foreign material carried in the lymph. Lymph nodes consist of a fibrous reticular network filled with lymphocytes, macrophages and dendritic cells. Lymphatic sinuses penetrate the node.A sub-capsular sinus is located immediately under the connective tissue capsule of the node; other sinuses pass through the node but are most prominent in a



medulla. Afferent lymphatic vessels (those flowing into the node) enter the lymphatics node around its circumference, and efferent lymphatic vessels (those flowing out of the node) leave from depression (or hilus) on one side. The blood vessels supplying a lymph node also enter and leave via hilus.

The interior of a lymph node is divided into three concentric zones—an outer cortex, a para-cortex and a central medulla. Each of which provides a distinct micro-environment. The cells in the cortex are predominantly (B-cells) lymphocytes (arranged in nodules), macrophages and follicular dendritic cells arranged in primary follicles.

Spleen:

i. **Location**:

Spleen, the secondary lymphoid organ is located high in the left abdominal cavity. The spleen is specially adapted for filtering blood and trapping blood-borne antigens and respond to systemic infections.

Structure:

Spleen is one of the most important lymphoid organ remain surrounded by a capsule. From the capsule, a no. of projections called trabeculae remain extended to the interior to form a compartmentalized structure.

There are two types of compartments, named red pulp and white pulp.

These two pulps are separated by a diffused marginal zone:

i. Red pulp:

The red pulp of spleen consists of a network of sinusoids. Sinusoids are with huge macrophages and erythrocytes.

Function:

This splenic zone helps to destroy and remove old and defective red blood cells. Some of the macrophages within the red pulp contain engulfed red blood cells or iron pigments from degraded haemoglobin.

ii. White pulp:

This zone is with T-lymphocytes. It surrounds the periarteriolar lymphoid sheath (PALS).

Function:

The T-cell-rich-PALS mediate the initial activation of B and T cells. Here, dendritic cells capture antigen and present it with THelper (TH) cells. In terms these activated T-cells activate B cells. Activated B and TH cells together migrate to primary follicles present in the marginal zone. Primary follicles gradually develop characteristic secondary follicles after antigenic challenge and rapidly give rise to B-cells and plasma cells.

Mucosal-associated lymphoid tissue:

Besides lymph nodes, spleen mucosal associated lymphoid tissue (MALT) is also considered as secondary lymphoid organ.

i. Location:

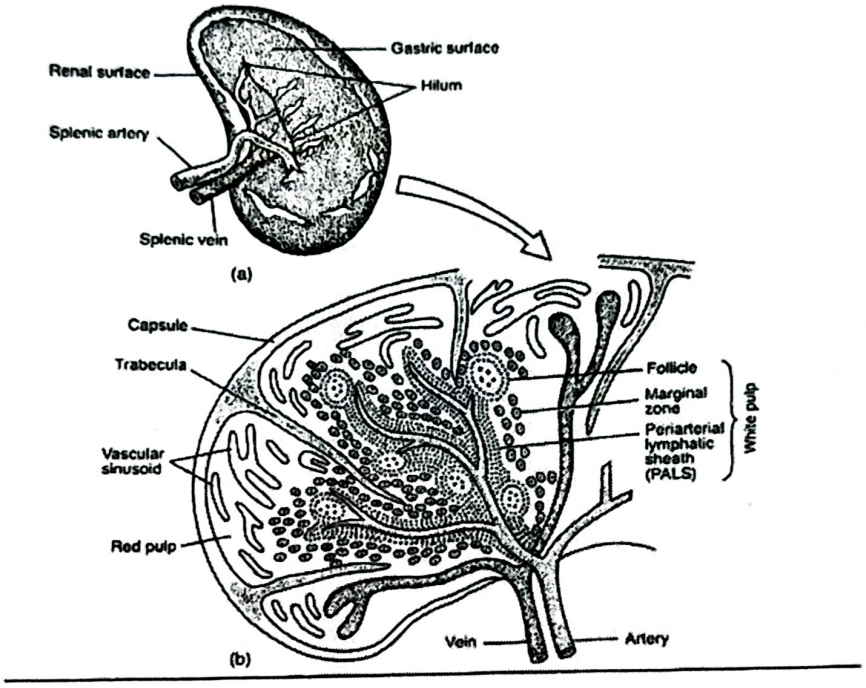
The digestive, respiratory and urogenital systems have a mucous membranes which are lined up by a vulnerable membrane with lymphoid tissues.

Structure:

Loose cluster of lymphoid cells are present with little organizations in the lamina propria of intestinal villi to organised structures such as the tonsils, appendix and Peyer's patches.

iii. Function:

MALTs are meant for the production of huge antibody-producing plasma cells which are very essential for the body defense



CELLS OF IMMUNE SYSTEM

1. **Lymphoid Cells:**

Lymphoid cells differentiate from lymphoidal stem cells or common lymphoid progenitors present in the primary or central lymphoid organs. In mammals, the primary or central lymphoid organs are thymus gland (bilobed and located in the thorax, overlying the heart and major blood vessels), fetal liver and adult bone marrow cells.

The lymphocytes which are produced in the thymus gland are called thymus derived lymphocytes or simply T cells or T lymphocytes. The lymphocytes which are produced in the bone marrow are called B cells or B lymphocytes.

2. **T cells:**

T cells or T lymphocytes show a lot of variations in their morphology, function and the presence of markers or antigen binding receptors on their surface. On the basis of their variations, T lymphocytes have been classified variously. Morphologically, T lymphocytes are of two types which can be distinguished by light microscopy using Giemsa staining.

Lymphocytes express a large number of different molecules or markers on their surface which can be used to differentiate cell population. Many of these molecules have now been identified and a system of nomenclature has been developed and such system is referred to as CD system in which the molecules are numbered as CD1, CD2 etc.

The term CD (cluster of differentiation) refers to group or cluster of monoclonal antibodies, each cluster binding specifically to a particular cell-marker. However, the definitive T cell lineage marker is T cell antigen receptor (TCR).

There are two defined types of TCR—TCR1 and TCR2.

3. **B Cells:**

B cell is an important type of lymphocytes of the immune system. These cells are produced in the bone marrow. B cells represent about 5-15% of the circulating lymphoid pool and are defined by the presence of surface immunoglobulin's which act as cell surface antigen receptors.

Each B cell specifically recognises a particular antigen, using a receptor molecule on its surface. Having recognised as specific antigen, the B cells divide and differentiate with the help of T cell into either memory cell or antibody forming cell (AFCs) or plasma cells which produce large amount of cytoplasmic immunoglobin molecules in a soluble form which can be secreted.

This is known as antibody. These antibody molecules are large glycoproteins found in the blood and tissue fluids, they bind to the antigen and inactivate them. The presence of antibodies also activates other part of immune system which, in turn, then eliminate the inactive antigen.

4. Natural Killer Cells:

These cells are mostly derived from the large granular lymphocytes. Most surface antigen of NK are shared with T cells or cells of the myelomonocytic series. NK cells are able to kill certain tumour cells and are also cytotoxic for virus infected cells.

NK cells may also release interferon-7 and other cytokines (immunological mediators) which may be important in the regulation of hemopoiesis and immune responses. NK cells have

other important surface molecules which are common to all leucocytes. These surface molecules are important for cell adhesion and intercellular communication.

5. **Mononuclear Phagocytes:**

Hemopoietic stem cells of bone marrow or fetal liver give rise to mononuclear phagocyte system which is found is many organs. Mononuclear phagocyte system includes the most important group of long-lived phagocytic cells.

The nucleus is never lobed and the cytoplasm is not granular and colourless. The main function of these cells is to engulf particles including infectious agents, internalize them and finally destroy them.

Hemopoietic stem cells differentiate into common myeloid progenitor which, ultimately, gives rise myeloid cells. The progenitor of the myeloid cell gives rise to pro-monocyte in the bone marrow. These differentiate into blood monocytes .

They migrate through blood vessel into various organs where they become phagocytic cells which are called macrophages. But in blood, monocytes are readily available than macrophages.

Macrophage or Monocyte possesses a well developed Golgi complex and many intracytoplasmic lysosomes. These lysosomes contain peroxidase and several acid hydrolases which are important in intracellular killing of microorganism.

6. Polymorphonuclear Granulocytes and Plateletes:

Most of the granulocytes are leucocytes whose cytoplasm is granular, containing specific chromophilic granules. The nucleus of the granulocytes is lobulated and contains 2-7 lobes. Due to presence of lobes in the nuclei these cells are called polymorph.

Granulocytes are formed within red bone marrow from myeloblasts at the rate of 80 million per minute and are short-lived (2-3 days). Granulocytes make up about 60-70% of the total normal blood leucocytes. They are also found to come out from blood capillaries to extravascular sites.

Granulocytes are of following types:

(a) Neutrophils:

Neutrophils constitute over 90% of the circulating polymorphs and are 10-20 µm in diameter. Neutrophil contain a multi-lobed nucleus. It has a life span of only a few days and is incapable of division so that its supply must be continuously replenished by the bone marrow.

(b) Eosinophil:

Eosinophil's comprise 2-5% of the blood leucocytes in healthy, non-allergic individuals and are 10-12 μm in diameter. The cytoplasm of eosinophil contains coarse granules with central crystalloids. These granules in mature eosinophil's are membrane-bound. The nucleus commonly possesses two to three lobes.

7. Basophils and Mast Cells:

Basophils [Fig. 21.6(h)] are found in very small numbers in circulation, i.e., less than 0.2% of leucocytes. The cell diameter varies from 8 to 10 μm. The cell nucleus is lobed and kidney-shaped.

In this cell the cytoplasm contains large membrane-bound basophilic granules easily stained by basophilic dyes like methylene blue and hematoxylin. In a number of properties the basophil is identical to the group of connective tissue cells known as mast cells which are static and are not found in the circulation. That is why basophils are sometimes called circulating mast cells.

The static mast cells of connective tissue are large, round or oval in shape. The cells have nuclei either oval or round in form. The cytoplasm contains coarse granules. These cells play a role in the formation of the anticoagulant heparin, a vasoconstrictor serotonin and an allergy causing chemical histamine.

8. Platelets:

Platelets which are found only in mammalian blood are non-nucleated round or oval, biconvex discs having various sizes and covered by unit membrane. The platelets originate from large megakaryocytes cells found in the bone marrow and apparently are portions of cytoplasm of these cells which are pinched off and enter the circulation. The life span of platelets is 3-5 days.

It takes part in blood coagulation. In addition to their role in blood clotting, platelets are also involved in the immune responses specially in inflammation. Following injury to endothelial cells, platelets adhere to and aggregate at the endothelial surface of damaged vascular tissue. They release substances that increase permeability and release factors that activate complement and hence attract leucocytes.

UNIT – 6

VACCINES AND IMMUNODIAGNOSTICS

TYPES OF VACCINES

- Vaccines are the substances Used to boostup the Immune system and also stimulate the production of antibodies in the body.
- Vaccines can effectively Controlling the spread of infectious human and animal diseases is by immunization.
- Vaccines Confer protective immunity to the immun -ized individuals."
- Small pox in the first human disease eradicated by the immunization Campaign.
- Some of the important Vaccines developed for human disease in the part include smallpox, diphtheria, Whooping Cough, Measles, polio, rabies, cholera, TB etc....

Types of Vaccines :

Vaccines can be grouped into 2 major classes:

(1) Traditional Vaccines.

(2) Modern Vaccines.

1) **Traditional Vaccines:**

Traditional Vaccines are again. divided into 4 types :

a. Live-attenuated Vaccines
b. Killed (or) Inactivated Vaccines
c. Toxoids
d. Sub Unit Vaccines

(a) Live attenuated Vaccine:

- These are typically produced by passaging Virulent strains another of the relevant pathogenic Organison in species until. non - Virulent Valiants arise.
- Attenuation is a process of rendering pathogen the to a harmless strain.
- The resulting non - Viralent strains are used to infect the recipient.
- This form of Vaccination typically induces long lasasting protection.
- Just 1 (or) 2 doses of vaccines can give a lifetime of protection against the pathogen and the disease it causes.

Advantages:

(1) Can induce high levels. of immunity.
(2) Can be produced inexpensively by simply growing the strain.
(3) Can be given Orally.

Disadvantages:

(1) there is a a Virulent possibility form reversion of Strain to
(2) Can produce. Serious and sometimes fatal disseminated infection in people who are Immunodeficient
Eg: Measles, Mumps etc...

(b) Killed (or) Inactivated Vaccines:

- These Vaccines are completely safe.
- However, the immunity given by these Vaccines, even when administered along with adjuvant (= Substance that increases the immunogenicity of the antigen) its often inferior of attenuated Vaccines. to that of attenuated vaccines.
- The pathogen Can be killed (or) Inactivated by chemical agents (treated with formaldehyde) (or) heat Killed (or) UV treatment.
- They do not offer lifelong immunity & need. topping Up Over time.
  Eg: Typhoid, cholera, Rabies.

(c)Toxoids:

- Some diseases are not bacterium itself.. that bacterium. but by directly caused by a toxin produced by that bacterium.
  Eg: Tetanus, Diphtheria
- The disease tetanus is not caused by the bacterium clostridium tetani but by neurotoxin produced by it.
- The neurotoxin produced by this bacterium is Tetano spasmin" (Exotoxin).
- These exotoxins, when purified & treated with formalin are rendered non-toxic.
- These non-toxic exotoxins are called as toxoids.
- Exotoxins causes life-threatening effect On nervous system & heart. But tonoids are not pathogenic.

(d) Sub-Unit Vaccines:

- These Vaccines Uke specific pieces of -gen like it's protein, Sugar the patho (or) Capsid and give a protective immune response.
- Because, there Vaccines Use Only Specific preces of the pathogon, they give a very strong Immune of response that targeting the they parts the pathogen.
  Eg: pneumococcus, Influenza

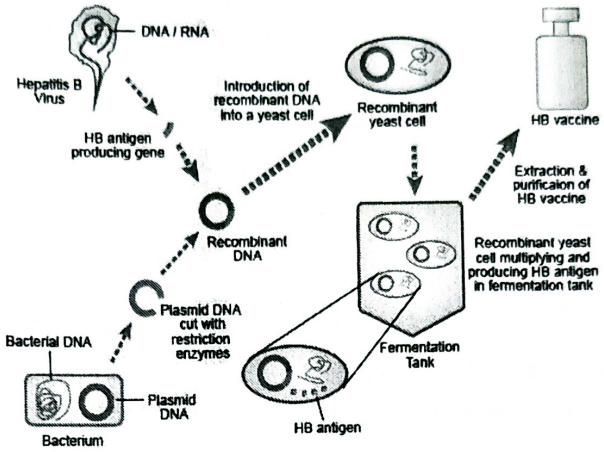
(2) Modern Vaccines:

- Newer strategies of Vaccines are developed for the production

- Modern Vaccines are produced by r-DNA Technology.
  Eg: Hepatitis B.
- Hepatitis - B. Virus (HBV) in One the Most Common of infectious disease..
- Hepatitis B is the swelling (Inflammation) of the liver due to the infection with the Hepatitis B. Virus (HBV).
- It produces chronic liver disorders.

General steps for Recombinant Hepatitis - B Vaccine production:

(1) HBA antigen producing gene is isolated from the HB virus by normal isolation process



(Like cell lysis, protein denaturation, precipitation Centrifugation. and drying).

(2) A plasmid DNA is extracted from a bacterium E. coli and in cut with restriction enzyme. EcoRI forming the plasmid Vector

(3) The isolated HBs antigen producing gene located and inserted into the bacterial plasmid Vector on forming the recombinant DNA.

(4) This recombinant DNA, Containing the target gene, is introduced into a yeast cell forming the recombinant yeast cell.

(5) The recombinant yeast cell Multiplies in the fermentation tank & produce the HBS antigens.

(6) After 48 hours, yeast cells are ruptured to free HBs Ag. The mixture in processed for extraction.

(7) The HBA antigens ale purified.

(8) HBs Ag are Combined with preserving agent and other ingredients and bottled. Now it in ready for Vaccination in humans.

ELISA

ELISA is the basic assay technique, known as enzyme-linked immunosorbent assay (also referred to as EIA: Enzyme Immunoassay) that is carried out to detect and measure antibodies, hormones, peptides and proteins in the blood.

Antibodies are blood proteins produced in response to a specific antigen. It helps to examine the presence of antibodies in the body, in case of certain infectious diseases.

ELISA is a distinguished analysis compared to other antibody-assays as it yields quantitative results and separation of non-specific and specific interactions that take place through serial binding to solid surfaces, which is normally a polystyrene multiwell plate.

Types Of ELISA

ELISA tests can be classified into three types depending upon the different methods used for binding between antigen and antibodies, namely:

- **Indirect ELISA** – Antigen is coated to the microtiter well
- **Sandwich ELISA** – Antibody is coated on the microtiter well
- **Competitive ELISA** – Microtiter well which is antigen-coated is filled with the antigen-antibody mixture.

Principle of ELISA

ELISA works on the principle that specific antibodies bind the target antigen and detect the presence and quantity of antigens binding. In order to increase the sensitivity and precision of the assay, the plate must be coated with antibodies with high affinity. ELISA can provide a useful measurement of antigen-antibody concentration.

ELISA Procedure

ELISA is one of the easiest blood tests that can be carried out. It is rapid, quick and requires a blood sample of the patient. The entire procedure of ELISA is mentioned below.

- An antibody is attached to a polystyrene plate which is a solid surface and is attracted or has an affinity towards bacteria, other antibodies and hormones.
- A microtiter coated with antigen is filled with this antigen-antibody mixture after which free antibodies are removed by washing.
- A second antibody specific to primary antibody is added which is usually conjugated with an enzyme.
- Free enzyme-linked secondary antibodies are removed by washing the plate.
- Finally, the substrate is added. The substrate is converted by the enzyme to form a coloured product, which can be measured by spectrophotometry.

HCG protein which indicates pregnancy is detected by ELISA. A combination of blood or urine sample and purified HCG linked to an enzyme is added to the system. If HCG is absent in the test sample, then only the linked enzyme binds to the solid surface.

The more the substance of interest is present, the more reaction takes place and less of linked enzyme binds to the solid surface. These reactions are indicated usually with a change in the colour of the solution.

Advantages Of ELISA

Following are some of the advantages of the ELISA technique:

- Results fetched from ELISA gives an accurate diagnosis of a particular disease since two antibodies are used.
- Can be carried out for complex samples as the antigen is not required to get purified to detect.
- It is highly responsive since direct and indirect analysis methods can be carried out.
- It is a rapid test, yields results quickly.
- Possible detection for ELISA ranges from the quantitative, semi-quantitative, standard curve, qualitative, calibration curve models etc.
- Easier to perform and uncomplicated process as compared to other assays which require the presence of radioactive materials.

Applications of ELISA

The applications of ELISA are discussed below:

1. The presence of antibodies and antigens in a sample can be determined.
2. It is used in the food industry to detect any food allergens present.
3. To determine the concentration of serum antibody in a virus test.

RIA

RIA Full Form: The radioimmunological assay (**RIA**) is based on the principle of all immunological assays, which is the recognition of an antigen present in a sample by antibodies directed against this antigen. The radioimmunoassay principle is very similar to that of competitive ELISA and quantifies small molecules, peptides, and proteins in biological samples.

Radioimmunoassay technique (RIA) is very sensitive in vitro technique used to measure the concentration of antigens (eg, hormone levels in the blood) through the use of antibodies directed against these antigens. Radioimmunoassay (RIA) is based on the principle of all immunoassays which is the recognition of an antigen present in a sample by antibodies directed against this antigen. The principle of radioimmunoassays is very similar to that of

competitive ELISA and allows quantification of small molecules, peptides and proteins in biological samples.

RIA is performed by using antibody-antigen binding and radioactive antigen. The basic principle of RIA is a competitive binding reaction, where the analyte (for example, antigen) competes with radio-labelled antigen for binding to the fixed antibody or the binding sites of the receptor. The binding of the unlabeled antigen to the fixed and limited amount of antibody causes displacement of radio-labelled antigen and results in decreasing the radioactivity of the antigen-antibody complex.